SCIENTIFIC OPINION

Scientific Opinion on the public health risks related to the consumption of raw drinking milk[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Raw drinking milk (RDM) has a diverse microbial flora which can include pathogens transmissible to humans. The main microbiological hazards associated with RDM from cows, sheep and goats, horses and donkeys and camels were identified using a decision tree approach. This considered evidence of milk-borne infection and the hazard being present in the European Union (EU), the impact of the hazard on human health and whether there was evidence for RDM as an important risk factor in the EU. The main hazards were *Campylobacter* spp., *Salmonella* spp., shigatoxin-producing *Escherichia coli* (STEC), *Brucella melitensis*, *Mycobacterium bovis* and tick-borne encephalitis virus, and there are clear links between drinking raw milk and human illness associated with these hazards. A quantitative microbiological risk assessment for these hazards could not be undertaken because country and EU-wide data are limited. Antimicrobial resistance has been reported in several EU countries in some of the main bacterial hazards isolated from raw milk or associated equipment and may be significant for public health. Sale of RDM through vending machines is permitted in some EU countries, although consumers purchasing such milk are usually instructed to boil the milk before consumption, which would eliminate microbiological risks. With respect to internet sales of RDM, there is a need for microbiological, temperature and storage time data to assess the impact of this distribution route. Intrinsic contamination of RDM with pathogens can arise from animals with systemic infection as well as from localised infections such as mastitis. Extrinsic contamination can arise from faecal contamination and from the wider farm environment. It was not possible to rank control options as no single step could be identified which would significantly reduce risk relative to a baseline of expected good practice, although potential for an increase in risk was also noted. Improved risk communication to consumers is recommended.

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KEY WORDS

raw milk, food-borne, pathogen, public health, antimicrobial resistance, vending machine, control options

SUMMARY

Following a request from the European Food Safety Authority (EFSA), the EFSA Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on the public health risks related to the consumption of raw drinking milk (RDM). In particular, the BIOHAZ Panel was requested to identify the main microbiological hazards of public health significance that may occur in RDM from different animal species, to assess the public health risk arising from the consumption of RDM, to assess the likelihood of RDM being a significant source of antimicrobial resistant bacteria/resistance genes, to assess the additional risks associated with the sale of RDM through vending machines and via the internet and to identify and rank potential control options to reduce public health risks arising from consumption of RDM.

According to European Union (EU) legislation, "raw milk" is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40 °C or undergone any treatment that has an equivalent effect (Regulation (EC) No 853/2004). A top-down four-step decision tree was used to identify the main microbiological hazards associated with RDM of different milk-producing species in the EU. Microbiological hazards that can be transmitted to humans through milk and which were reported from cows, sheep and goats, horses and donkeys and camels in the EU were listed. Those hazards which could be transmitted via milk but were not reported from milk-producing animals in the EU were excluded from further consideration. Microbiological hazards identified as potentially transmissible through milk and present in the EU milk-producing animal population included the bacteria *Campylobacter* spp. (thermophilic), *Salmonella* spp., shigatoxin-producing *Escherichia coli* (STEC), *Bacillus cereus*, *Brucella abortus*, *Brucella melitensis*, *Listeria monocytogenes*, *Mycobacterium bovis*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Corynebacterium* spp., *Streptococcus suis* subsp. *zooepidemicus*, the parasites *Toxoplasma gondii* and *Cryptosporidium parvum* and the virus tick-borne encephalitis virus (TBEV). Those hazards transmissible via milk of one species and present in the EU were also considered to be potentially transmissible by milk of other species if present in the EU.

Evidence for RDM as an important risk factor for human infection in the EU was based on epidemiological evidence that the hazard has been associated with illness from the consumption of RDM in the EU, the extent of occurrence of the hazard in different milk-producing species in the EU, the prevalence of the hazard in milk bulk tanks or retail RDM in the EU, and expert opinion. Between 2007 and 2012 there were 27 reported outbreaks in the EU involving RDM. Of these, 21 were attributed to *Campylobacter* spp., predominantly *C. jejuni*, one to *Salmonella* Typhimurium, two to STEC and three to TBEV. Four of the 27 outbreaks were due to raw milk from goats, the rest being attributed to raw milk from cows. The published literature was also considered, which highlighted additional outbreaks of TBEV and outbreaks of *B. melitensis*, *M. bovis* and STEC, although some of these were prior to 2007. No outbreaks attributable to *L. monocytogenes* in RDM were reported between 2007 and 2012.

STEC, *Salmonella* spp. and *Campylobacter* spp. are essentially ubiquitous pathogens and are likely to be found in milk-producing animals and their milk throughout the EU, as indicated by prevalence data from raw milk testing. TBEV was also considered to be a main hazard based on outbreak data, together with evidence of spread in Europe and the virus being detected in raw milk. *B. melitensis* and *M. bovis* have been associated with outbreaks involving raw milk, but these are less common and more geographically restricted than the other pathogens and control programmes in Europe have generally been successful in reducing human disease from these pathogens.

For other hazards, epidemiological evidence of illness was either historical or limited to reports from outside Europe. *L. monocytogenes* infection is associated with a high mortality rate in vulnerable groups, and the organism was as frequent as *Campylobacter* and STEC in raw milk. The lack of robust epidemiological data (including outbreaks) linking listeriosis to consumption of raw milk in Europe meant that it could not be considered a main hazard. The ability of *L. monocytogenes* to grow at chill temperatures, coupled with its prevalence in raw milk, suggests that further study in relation to RDM

may be justified, particularly as several risk assessment models outside Europe have already been developed for this pathogen.

There is a clear link between drinking raw milk and human illness with *Campylobacter* spp., *S.* Typhimurium, STEC, TBEV, *B. melitensis* and *M. bovis*, with the potential for severe health consequences in some individual patients. Owing to the lack of epidemiological data, the burden of disease linked to the consumption of raw milk could not be assessed. Published quantitative microbiological risk assessment (QMRA) models from Australia, New Zealand, the USA and Italy, for *Salmonella* spp., *Campylobacter* spp., STEC O157 and *L. monocytogenes* in RDM from cows, were reviewed to identify their strengths and limitations. No QMRAs were available for RDM of other species. The risk estimates provided by the QMRA models reviewed cannot be extrapolated to the European situation as a whole. The outputs from the Australian and New Zealand risk assessments for STEC O157 and *Salmonella* spp. estimate a high level of milk contamination, which contrasts with the outputs from the risk assessment for these pathogens in RDM in one region of northern Italy, where the risk associated with STEC O157 was estimated as very low because of model uncertainty. Similarly, the Australian and New Zealand risk assessments predicted a higher risk for *Campylobacter* spp. than the risk assessment conducted in one region of northern Italy, largely as a result of differences in the extent of faecal contamination. From the model used in the Australian study it can be concluded that improving on-farm hygiene leads to a decrease in the number of predicted cases of illness due to *Campylobacter* spp., *Salmonella* spp. and STEC O157 from the consumption of RDM. A QMRA could have helped in further estimating the public health risks and evaluating the effect of the mitigation options in Europe for these hazards, but could not be undertaken because country and EU-wide data are limited.

Antimicrobial resistance has been reported in several EU countries in isolates of *Campylobacter* spp., *Salmonella* spp., STEC and *S. aureus* from raw milk or associated equipment such as milk filters, and may be significant for public health. Such isolates have been primarily associated with raw milk from bovine animals, which may reflect the more limited screening of milk from other species. Strains of *Campylobacter* spp., and particularly *C. jejuni*, exhibiting resistance predominantly to tetracyclines but also to some other antimicrobials have been reported in two Member States (MS). There have been no reports of antimicrobial resistance in isolates of *Salmonella* spp. from outbreaks associated with raw/unpasteurised in the EU in countries other than the UK. In the USA, there has been a report of a raw milk-associated outbreak caused by multidrug-resistant (MDR) *S.* Typhimurium, with a single fatality ascribed to resistance of the organism to antibiotics. Despite STEC O157 being the organism most commonly associated with RDM-related outbreaks of STEC gastrointestinal illness in several EU countries, little information is available about the occurrence of antimicrobial resistance in such outbreak strains. Antimicrobial resistance has been reported in a water buffalo raw milk-associated STEC O26 outbreak in one MS in 2008 and in raw milk-associated STEC outbreaks in the USA. Antimicrobial resistance in isolates of *L. monocytogenes* from raw milk and raw milk dairy products has only rarely been reported in EU countries.

Meticillin-resistant *Staphylococcus aureus* (MRSA) has not been isolated during outbreaks of infection associated with RDM in EU countries. Although not typically regarded as a food-borne pathogen, there have been increasing reports of the isolation of MRSA from dairy farms and bulk tank milk in several EU MS. Although identified in *E. coli* in bovine animals in some MS, extended spectrum beta lactamase (ESBL)/*Amp*C gene-carrying bacteria have not been reported in RDM in EU MS. In the USA, a range of *Salmonella* serovars with ESBL/*Amp*C genes have been identified in raw milk surveys.

Sale of RDM through vending machines is permitted in some EU MS, with considerable variation in the number of machines in different countries. There is little indication of RDM other than cow's milk being sold through vending machines. Although vending machines dispense drinking milk in a raw state, consumers are usually instructed to boil the milk prior to consumption. If consumers were to comply with these instructions, the microbiological risks associated with raw milk would be eliminated. The temperature of RDM in vending machines is generally kept below 4 °C and therefore

variability in milk temperature is more likely to arise between the farm and vending machine and between the vending machine and point of consumption by the consumer. One study in Italy demonstrated that temperature variability in the supply chain from farm to consumer could potentially result in the multiplication of *L. monocytogenes*, *S.* Typhimurium and STEC O157:H7.

Fresh and frozen RDM of different species (cows, goats, sheep and camels) is available via internet sales although there are no data on the microbiological or temperature controls for these milks from the bulk milk tank through to the point of consumption. The variability in temperature control and duration of storage by consumers would contribute to the multiplication of some pathogens if these are present in the milk.

The steps in the production to consumption chain for RDM present many opportunities for contamination by microorganisms, some of which may be transmissible to humans. Intrinsic contamination of milk can arise from systemic infection in the milk-producing animal as well as from localised infections, such as mastitis. Extrinsic contamination of milk can arise from faecal contamination and from the wider farm environment associated with collection and storage of milk. Observance of good animal health and husbandry, together with the application of good agricultural practices (GAPs) and good hygienic practices (GHPs), are essential to minimise opportunities for contamination of RDM with pathogens in the production to consumption chain for RDM. No single step could be identified which would provide a significant reduction in risk relative to a baseline of expected good animal health and welfare and good agricultural and hygienic practices. Therefore, it was not possible to rank control options with respect to risk reduction since any deviations from the expected "best practice" baseline are likely to result in an increase in risk.

The reviewed QMRA models identified on-farm hygiene control and maintenance of the cold chain as factors influencing the outcome of the models for some pathogens. Although *L. monocytogenes* is not considered to be one of the main hazards associated with RDM in the EU, the reviewed QMRAs from outside the EU do show that the risk associated with *L. monocytogenes* in raw cow's milk can be mitigated and reduced significantly if the cold chain is well controlled, the shelf-life of raw milk is limited to a few days and there is consumer compliance with these measures/controls.

The BIOHAZ Panel identified several recommendations arising from the opinion. There is a need for a better evidence base to inform future prioritisation and ranking approaches and studies should be undertaken to systematically collect data for source attribution for the hazards identified as associated with RDM and collect data to identify and rank emerging milk-borne hazards. Because of the diverse range of potential microbiological hazards associated with different milk-producing animals, hazard identification should be revisited regularly. There is a need for validated growth and survival models for pathogens in RDM of different milk-producing species, particularly in relation to the temperature and storage time of RDM from the producer up to the point of consumption. Finally, the Panel recommended that there should be improved risk communication to consumers, particularly susceptible/high risk populations, regarding the hazards and control methods associated with consumption of RDM.

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BACKGROUND AS PROVIDED BY EFSA

According to EU legislation, "raw milk" is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40 °C or undergone any treatment that has an equivalent effect (Reg. (EC) 853/2004[4]).

Reg. (EC) 853/2004 stipulates the microbial criteria in the EU for raw cows' milk as ≤ 100 000 CFU/mL for plate count at 30 °C and ≤ 400 000 CFU/mL for somatic cells. For raw milk from species other than cows a plate count at 30 °C of ≤ 1 500 000 CFU/mL is specified. In this Regulation, health requirements for production animals and hygienic requirements on milk production holdings (e.g. regarding premises and equipment, hygiene during milking, collection and transport, staff hygiene) are established as well.

Regulations (EC) 853/2004 and 854/2004[5] give provision for market sale of raw milk for human consumption. A Member State may, on its own initiative and subject to the general provisions of the Treaty, maintain or establish national rules that prohibit or restrict the marketing of raw milk or raw cream intended for direct human consumption. Some Member States including Germany, France, Holland, Belgium, Denmark and Italy in addition to England, Wales and Northern Ireland allow restricted sales of raw drinking milk directly to the consumer.

Responsibility for the production of safe food rests with the food business operator (FBO). There is no requirement for Member States to introduce national controls for raw drinking milk. Effective enforcement of the controls for raw drinking milk provides a level of public health protection, but cannot remove the inherent risk associated with the unpasteurised commodity. In practice, it is often difficult to take enforcement action to require immediate corrective action by the FBO based on non-compliance with the current microbiological criteria. This is because these standards relate to indicator organisms or somatic cells (see above) and there is no direct relationship between these and the presence of pathogens.

The consumer interest in raw drinking milk is growing in the EU and raw milk producers are using new routes of sale for raw milk through vending machines and internet sale. Consumption of raw drinking milk appears to be low among the general population, but in specific groups large amounts are consumed. Prevalent among this group of consumers is the belief that raw milk possesses particular healthy properties or attributes, in addition to the existing nutritional components. As a result of these perceived health benefits, raw milk is often consumed by individuals who may have lowered immunity such as the very young, very old or immunocompromised or to people with specific dietary needs.

Assessing the risk from consumption of raw drinking milk is important, since raw milk can often be contaminated with pathogens, either directly through organisms shed as a result of udder infection or indirectly through contamination during milking or subsequent handling. Indirect contamination may arise from (i) a cow's own faecal matter contaminating the udder and teats, (ii) faecal matter of other cows contaminating the udder, (iii) milking clusters contacting surfaces with faecal contamination, and (iv) post-milking environmental contamination.

In 2012, there were seven food-borne outbreaks reported by three MS which were strongly linked with the consumption of milk. Of these, six were associated with consuming raw milk and were reported by two MS. The remaining outbreak was linked to UHT milk. Of the raw milk associated outbreaks, five were caused by *Campylobacter* (four *C. jejuni* and one to an unspecified *Campylobacter* sp.) and one by STEC O157:H7. There is less information concerning sporadic cases of illness linked to consuming

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[4] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules on the hygiene of foodstuffs. L 139, 30.4.2004, p. 55-200.
[5] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. OJ l 226, 25.6.2004, p. 83-127.

raw milk as it is difficult to separate such exposures from other risk factors for example where cases are residents on farms.

Raw milk may be a source of bacteria that are resistant to antimicrobials, depending on the reservoir of antimicrobial resistant bacteria in the farm and animal environment. Antimicrobial-resistant strains of *Salmonella* spp. and *Campylobacter* spp. have been linked to the consumption of raw milk. Antimicrobial resistant bacteria may also transfer their resistance determinants to other bacteria.

The EFSA Scientific Network on Microbiological Risk Assessment discussed risks related to the consumption of raw drinking milk during its meetings in October 2012 and November 2013. The representative from Czech Republic reported results of a study on the microbiological quality of raw milk from vending machines. The study was carried out because of the rapid expansion of such vending machines and increased consumption of raw milk via this route. Pathogens isolated from raw milk samples were: *S. aureus* (56 % positive samples), *Campylobacter* spp. (4.6 %), *Salmonella* spp. (3.7 %), and *L. monocytogenes* (1.9 %). At least five Network members reported the existence of raw milk vending machines in their countries.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Biological Hazards Panel is requested to issue a scientific opinion on the public health risks related to the consumption of raw drinking milk. In particular, the Biological Hazards Panel is requested to:

1. Identify the main microbiological hazards of public health significance that may occur in raw drinking milk from different animal species;

2. Assess the public health risk arising from the consumption of raw drinking milk;

3. Assess the likelihood of raw drinking milk being a significant source of antimicrobial resistant bacteria/resistance genes;

4. Assess the additional risks associated with the sale of raw drinking milk through vending machines and via the internet;

5. Identify and rank potential control options to reduce public health risks arising from consumption of raw drinking milk.

ASSESSMENT

1. **Introduction**

1.1. **Legislative background and scope of the opinion**

Regulation (EC) No 853/2004 defines "raw milk" as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40 °C or undergone any treatment that has an equivalent effect. "Dairy products" are defined as processed products resulting from the processing of raw milk or from the further processing of such processed products. In Europe, the current regulatory microbial criteria for raw cow's milk are $\leq 100\,000$ colony-forming units (CFU)/mL for total bacterial plate count (at 30 °C) and $\leq 400\,000$ CFU/mL for somatic cells (Regulation (EC) No 853/2004). Raw milk from species other than cows has to comply with a total plate count at 30 °C of $\leq 1\,500\,000$ CFU/mL. In this Regulation, health requirements for production animals and hygienic requirements for milk production holdings (e.g. regarding premises and equipment, hygiene during milking, collection and transport, staff hygiene) are also established. Raw milk intended for human consumption must meet the requirements of the General Food Law and be free of pathogens.

The farm bulk tank milk, collected for processing into pasteurised milk, is systematically controlled for total bacterial count (TBC), somatic cell count, purity, presence of residues of veterinary drugs, fat and protein content and freezing point. Farms are obliged to comply with good manufacturing practices, which are regularly audited by an external control body as appropriate.

This opinion focuses on raw milk and not on milk or dairy products obtained after processing. Although several methods used to treat milk have the potential to reduce or eliminate safety concerns, they are not in the scope of this opinion because they change the intrinsic quality characteristics of the raw milk. One of the principal treatments of raw milk is heating. Several types of heat treatment can be applied: thermisation, pasteurisation and commercial sterilisation including ultra-high-temperature (UHT) treatment. Thermisation is typically used to reduce the vegetative microbial flora in milk, but it will not ensure elimination of bacterial hazards. Milk pasteurisation aims to inactivate vegetative bacterial organisms but will have little or no impact on bacterial spores. Commercial sterilisation is obtained by various heat treatments, the most common being UHT processing in combination with aseptic packaging or in-container sterilisation. During the UHT treatment, the milk is exposed to a brief, intense heating, normally to temperatures in the range 135–140 °C for a second or less (e.g. 135 °C/1 second). Other processes are extended shelf-life (ESL) and innovative steam injection (ISI). Some additional technologies, such as microfiltration, bactofugation, pulsed light or high pressure, can reduce the bacterial count of raw milk (Walkling-Ribeiro et al., 2011; Yang et al., 2012; Innocente et al., 2014).

In this opinion, only those microbial hazards associated with raw drinking milk (RDM) arising from transmission of zoonotic microorganisms or other microorganisms originating from the farm environment will be evaluated. Person-to-person transmission of non-zoonotic microorganisms (for example *Shigella* spp.) via milk will not be considered in this opinion. The risk evaluation considers the risk linked to the consumption of RDM from European herds, and focuses on the main hazards identified and the prevalence of these in the European Union (EU). The risk linked to the consumption of RDM produced outside the EU can differ from the European situation because of differences in the application of controls and, in some cases, because of a higher prevalence of certain pathogenic microorganisms (e.g. *Mycobacterium bovis*).

1.2. **Sale of raw drinking milk to consumers**

1.2.1. **Direct sale to consumers on-farm**

In many European countries, such as Germany, France, the Netherlands, Belgium, Denmark, Italy, Ireland and parts of UK, raw cow's milk can be sold at the farm directly to the consumer. Certain

other countries (Spain, Poland and Norway) do not allow the sale of raw milk to consumers. In a few countries, raw milk from goats, sheep and buffaloes is sold directly to the consumer at the farm. Information on the sale of RDM from animals other than cows, goats and sheep is very limited, but the sale of horse milk is permitted in the UK, France, Belgium and the Czech Republic and the sale of donkey's milk is permitted in France (information gathered from EFSA questionnaire, Appendix A).

The levels and pattern of raw milk consumption in Europe are poorly documented. The consumption of raw milk from sheep and goats is reported to be very limited, and in some cases is restricted to consumption on farms.

Farm milk production in the EU-27 in 2011 amounted to a total of 156 million tonnes (Eurostat[6]). Dairies collected 142 million tonnes, 98 % of which was cow's milk; 31 million tonnes was processed by industry as drinking milk. About 14 million tonnes is processed at the farm, and only a small fraction of the milk produced is sold as RDM. The vast majority of milk produced on EU farms (96.8 %) comes from cows, although in certain Member States (MS) in southern Europe significant quantities of milk are produced by sheep, goats and buffaloes. Five countries (Greece, Spain, France, Italy and Romania) produced about 92 % of the sheep milk in the EU. Italy is the biggest producer (88 %) of buffalo milk in the EU (Eurostat[4]).

1.2.2. Sale through vending machines

Traditionally, RDM is either consumed on-farm or sold directly from a farm shop or via local delivery. In some parts of Europe vending machines are used for dispensing RDM for sale, and these may be located on-farm or in retail settings. Information regarding the numbers of vending machines and sale of raw milk via this route varies between MS and these data are incomplete (see EFSA questionnaire, Appendix A). Some MS do not sell or permit the sale of RDM through vending machines (Denmark, Ireland, Greece, the Netherlands, Spain and the UK). Of those that do, Italy has the largest number of vending machines (1 066 in 2013), followed by Slovakia (182 in 2012), Austria (121 in 2013), France (93 in 2013), the Czech Republic (14 in 2013) and Lithuania (6 in 2013). Although available data are limited, they do not indicate any recent increase in the number of vending machines in the EU in recent years, with numbers in Austria, the Czech Republic and Italy being similar each year between 2009 and 2013. Where vending machines are permitted, they seem to be used exclusively for the sale of cow's milk, although the sale of goat's milk through vending machines occurs in the Czech Republic (information gathered from EFSA questionnaire, Appendix A).

1.2.3. Sale through the internet

Consumer interest in raw milk has increased over the past decade, in part stimulated by the availability and familiarity with internet search tools, social media including blogs, as well as the development of dedicated websites by some retailers selling RDM and other dairy products. This has probably led to a greater awareness of the location and availability of raw milk outlets and to alternative ways of purchasing raw milk other than visiting a farm or farm shop. No published studies were found which have investigated the sale of RDM via the internet in terms of either the trend in sales for different types of RDM or whether the milk was sold fresh or frozen, delivered at home by a delivery service. Google trends data indicate a rise in searches for the term "raw milk" and "buying raw milk" over the past 10 years. Internet searches have increased generally, and it is not possible to identify whether searches relating to raw milk were linked to seeking local outlets or websites where RDM could be purchased or for other reasons.

1.3. Composition of raw milk from different animal species

1.3.1. Physical and chemical composition of raw milk

The principal constituents of milk of different animals are summarised in Table 1. Milk composition varies depending on the species (e.g. cow, goat, sheep), the animal (breed, stage of lactation, digestive

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[6] http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Milk_and_dairy_production_statistics

tract fermentations, udder infections) and feed (grain, energy and dietary protein intake, seasonal and regional effects) (Fox et al., 1998; Silanikove et al., 2010; Butler et al., 2011; Chen et al., 2014). Although there are variations in milk composition, the milk from herds, which may vary in size, is mixed in bulk tanks at the farm and at an industrial level and provides a relatively consistent composition year round. Commercially sold cow's milk intended for heat treatment is usually standardised to a fat content of 3.5 %.

Milk can be described as an oil-in-water emulsion consisting of fat globules dispersed in a continuous serum phase. The milk fat globule membrane consists of a complex of proteins including enzymes, phospholipids, triacylglycerols and other minor components. It is a natural emulsifying agent which protects fat globules against coalescence, agglomeration and enzymatic action (Walstra and Jenness, 1984; Spreer, 1998). Unlike RDM, industrially commercialised milk is generally homogenised which results in a degradation of the milk fat globule membrane and a homogeneous dispersion of the fat molecules in the milk. Lactose, or milk sugar, is the principal carbohydrate in milk and the principal carbon source for most of the microorganisms that grow in milk.

Table 1: General composition of milk from different mammals (indicative values) (reprinted from Claeys et al., 2014[a])

| | Human | Non-ruminants | | Ruminants | | | | | | | |
		Horse	Donkey	Cow	Sheep	Goat	Buffalo	Camel	Llama	Yak	(Rein)deer
Total dry matter (g/L)	107–129	93–116	88–117	118–130	181–200	119–163	157–172	119–150	131	135–184	201–271
Proteins (g/L)	9–19	14–32	14–20	30–39	45–70	30–52	27–47	24–42	34–43	42–59	75–130
Casein/whey ratio (g/L)	0.4–0.5	1.1	1.28	4.7	3.1	3.5	4.6	2.7–3.2	3.1	4.5	~4–5
Fat (g/L)	21–40	3–42	3–18	33–54	50–90	30–72	53–90	20–60	27–47	53–95	102–215
Lactose (g/L)	63–70	56–72	58–74	44–56	41–59	32–50	32–49	35–51	59–65	33–62	12–47
Ash (g/L)	2–3	3–5	3–5	7–8	8–10	7–9	8–9	6.9–9	5–9	4–10	12–27
Energy (kJ/L)	2843	1 936–2 050	1 607–1 803	2 709–2 843	4 038–4 439	2 802–2 894	4 244–4 779	2 410–3 286	2 709–3 358	3 811–4 295	5 541–8 436

Sources: Mittaine, 1962; Arman et al., 1974; Guo et al., 2007; Shamsia, 2007, 2009; Souci et al., 2008; Hassan et al., 2009; Xi et al., 2010; Potocnik et al., 2011; Uniacke-Lowe, 2011; Medhammar et al., 2012; Salimei and Fantuz, 2012; Naert et al., 2013.

(a): Reprinted from Food Control, 42, Claeys WL, Verraes C, Cardoen S, De Block J, Huyghebaert A, Raes K, Dewettinck K and Herman L, Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits, 188–201, Copyright (2014), with permission from Elsevier.

1.3.2. Somatic cells

The somatic cell count of milk comprises the number of white blood cells and the number of epithelial cells present in the milk. White blood cells comprise mononuclear cells (macrophages and lymphocytes) and neutrophils, and make up 70 to 80 % of the somatic cells in milk of uninfected udder quarters; up to 99 % in mastitic udder quarters (Schukken et al., 2003). Epithelial cells are derived from the udder tissue itself, and represent a very small portion of the somatic cells present in raw milk. Somatic cell count directly reflects the inflammatory status of the mammary gland in an individual animal whereas herd somatic cell count is related to the inflammatory process and udder health status of the herd (Schukken et al., 2003). An increased somatic cell count can therefore indicate the presence in the herd of animals with a poor health status (subclinical mastitis) which can lead to lower milk production. Physiological parameters (e.g. parity, lactation stage and breed) could also be the origin of an increased somatic cell count. Whilst the somatic cell count may correlate positively with the total plate count, it is not indicative of the presence of pathogens in the milk (Griffiths, 2010).

For cow's milk it has been repeatedly shown that, on average, approximately 2×10^5 somatic cells/mL are present in milk derived from an uninfected udder (Schukken et al., 2003). In contrast, cows with intramammary infections can produce milk with more than 5×10^5 somatic cells/mL (Lam et al., 1997).

The somatic cell count in milk from sheep with a healthy udder should generally be around 2.5×10^5 somatic cells/mL, which is in the range of that reported for cow's milk from uninfected animals (Ten Hag, 2010). The somatic cell count of goat's milk is generally higher than for cow's milk (Droke et al., 1993; D'Amico and Donnelly, 2010) and this increases during lactation, from 2×10^5 somatic cells/mL to over 10^6 somatic cells/mL, although a high somatic cell count may already be present at the start of the lactation (Raynal-Ljutovac et al., 2007).

Research has shown that the somatic cell count is lower in horse milk than in milk from goats, sheep and cows (Dankow et al., 2006). The average cell count for milk from horses was reported to be 4.1×10^4 cells/mL (Salimei and Fantuz, 2012). The count is highest at the start of lactation, after which it declines (Dankow et al., 2006). The somatic cell count of donkey milk was reported to range from 3×10^3 to 3.2×10^4 cells/mL (Salimei and Fantuz, 2012) and is in the same range as that for horses milk. Neither the lactation stage nor the season had a significant influence on the somatic cell count of donkey milk (Ivankovic et al., 2009). The relatively low somatic cell count of horse and donkey milk results from a shielded, more protected, udder, which renders it less exposed to injury and infection, as well as the frequency of the emptying of the udder by the foal, which can take place up to 60 times a day (Dankow et al., 2006).

Somatic cell counts in raw buffalo milk are usually lower than those commonly found in raw cow's milk. The somatic cell count in buffalo milk increases with lactation, and is higher in milk from buffaloes with mastitis (Moroni et al., 2006). Nagy et al. (2013) has reported average counts of 3.9×10^5 somatic cells/mL for milk from dromedary camels.

1.4. Microbial flora of raw milk

Milk can be contaminated by animal pathogens directly shed into the milk within the udder or by microorganisms from a variety of environmental sources, during or after milking, including the teat apex, milking equipment, air, water, feed, grass, soil and other environments (ICMSF, 2005; Quigley, et al., 2013; Moatsou and Moschopoulou, 2014). Typically, cow's milk contains a significant population of lactic acid bacteria that includes *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Enterococcus* spp. (Vacheyrou et al., 2011; Quigley et al., 2013). A number of other microorganisms can be present as a significant proportion of the bacterial flora of raw milk (Ercolini et al., 2009). These include psychrotrophs such as *Pseudomonas*, *Acinetobacter* and *Aeromonas* spp. (Raats et al., 2011). The bacterial flora of sheep and goat's milk is similar to that of cows and is also

typically dominated by lactic acid bacteria (Quigley et al., 2013). Milk from donkeys, horses and camels have has been less extensively studied but the composition of the microbial flora is unlikely to differ substantially from cows, sheep or goats (Benkerroum et al., 2003; Quigley et al., 2013).

The rich nutrient composition and neutral pH of raw milk makes it a good vehicle for the survival and growth of pathogenic and spoilage bacteria during storage. If milk is maintained properly chilled, the proliferation of many bacteria can be suppressed. Psychrotrophic bacteria such as *Pseudomonas* spp., *Listeria* spp. or *Yersinia* spp. can still multiply under these conditions. If there is poor temperature control then certain pathogenic bacteria may grow and/or produce toxins. Lactic acid bacteria present in raw milk may inhibit the multiplication of many other bacteria. In addition, the growth of lactic acid bacteria can result in a short shelf-life for raw milk, because of rapid degradation of the milk (acidification, coagulation) rendering it unacceptable for consumption before substantial pathogen proliferation.

1.4.1. Total bacterial count (TBC) in raw milk

The TBC of milk from cows is systematically measured in all European countries. The mean counts vary greatly between EU countries ranging from 3.6×10^3 to 7.3×10^4 CFU/mL, with most counties having a mean bacterial count around 2×10^4 CFU/mL. Approximately 97 % of the analysed milk samples have a bacterial count below 10^5 CFU/mL; 92–96 % below 5×10^4 CFU/mL (Denmark, France) (information gathered from EFSA questionnaire, Appendix A).

Raw milk from sheep and goats generally has a higher bacterial count than milk from cows. Data from literature indicate bacterial counts for goat's milk of between 2.51×10^4 and 3.9×10^5 CFU/mL (Verraes et al., 2014). By contrast, the milk from horses and donkeys generally has a lower bacterial count than milk from cows (Sarno et al., 2012). Bacterial counts of 5.2×10^3 CFU/mL have been reported in raw milk from dromedary camels (Nagy et al., 2013).

1.4.2. Intrinsic antimicrobial factors present in raw milk

In raw milk of all animal sources, different natural antimicrobial components/systems are present as lactoferrin, lysozyme, immunoglobulins and lactoperoxidase (LPO) (Conesa et al., 2008; Claeys et al., 2014). The average concentration of these components/systems differs between animal species, with lactoferrin and the immunoglobulins occurring in higher concentrations in colostrum than in milk. These antimicrobial components/systems primarily have a protective role at mucosal surfaces of the digestive tract in humans and animals. The activity to suppress the growth of bacteria in raw milk and to function as a milk preservative is very limited and, without the addition of supporting components, as is the case for the LPO system (see below), not of practical relevance. Lactoferrin has activity against bacteria, fungi and viruses (Orsi, 2004). With bacteria this is attributed to its iron-binding capability (bacteriostatic effect) and its capacity to bind the lipid A part of bacterial lipopolysaccharides in the Gram-negative membrane and probably to lipoteichoic or teichoic acid in Gram-positive bacteria (Gonzalez-Chavez et al., 2009) increasing membrane permeability (bactericidal effect). Lactoferrin also shows specific effects on biofilm development, bacterial adhesion and colonisation, intracellular invasion and the immunological protective response (Orsi, 2004). Lactoferrin has been shown to interact with the membrane of fungi and with virus particles or with the receptors for viruses (Orsi, 2004). Lactoferrin shows a synergistic bactericidal effect with lysozyme and immunoglobulin IgA (Ellison and Giehl, 1991).

Lysozyme acts as a 1,4-β-acetylmuramidase that hydrolyses the glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of the Gram-positive bacterial cell walls and has significant immunomodulatory effects (Ogundele, 1998). Immunoglobulins are the main immune components of the acquired immune system present in milk. There are important differences in the abundance of different immunoglobulin classes in milk among species and these can change during lactation (Stelwagen et al., 2008). LPO is one of the most abundant enzymes in milk (Sharma et al., 2013). Combined with hydrogen peroxide (H_2O_2) from bacteria and thiocyanogen (SCN_2) present in the milk, it forms the LPO system, exerting antibacterial,

antiviral and antifungal activity. The antimicrobial activity is based on the formation of hypothiocyanite ($OSCN_2$), damaging sulfhydryl groups of proteins in the cytoplasmic membranes of microorganisms. Supplementary addition of the LPO components to raw milk has been shown to increase LPO activity sufficiently to make it a potentially useful tool for controlling bacterial growth in raw milk and to increase the shelf-life of the milk (WHO/FAO, 2005).

2. Hazard identification

2.1. Methodology for hazard identification

A food-borne hazard is defined by the Codex Alimentarius Commission (CAC) as a "biological, chemical or physical agent or property of food with the potential to cause an adverse health effect" (CAC, 1999). In RDM, microbiological hazards can arise from infection in the milk-producing animal, faecal contamination and from microorganisms in the wider farm environment. A diverse range of microbiological hazards can potentially be associated with contamination of raw milk and these have been documented in the literature, particularly for milk from cows (Gilmour and Rowe, 1981; ICMSF, 2005; Claeys et al., 2013; Moatsou and Moschopoulou 2014; Verraes et al., 2014). Although different milk-producing animals are likely to share similar hazards associated with their milk, there are also differences between animals, particularly with respect to infections, some of which may be transmissible via milk. For this reason, it was considered important to initially consider the hazards associated with each of the main milk-producing species relevant in the EU. These were bovine animals, principally cows, small ruminants, including sheep and goats, solipeds, which included horses and donkeys, and camelids, which included camels. Although the number of milk-producing camels in the EU is small, it was considered important to include consideration of this group in the assessment as camel milk is becoming more widely available.

The aim was to identify the main microbiological hazards associated with RDM and which are currently relevant in the EU. The method adopted was to take a broad approach initially considering a wide range of potential hazards associated with each of the milk-producing species rather than focusing solely on those which are already well documented. This was deemed necessary as an initial step, particularly given the changing pattern of raw milk consumption, the diversity of milk types now being consumed in Europe and the potential appearance of infections affecting milk-producing animals, which might have implications for consumers of RDM.

2.2. Approach

The process of categorising hazards into main hazards is illustrated schematically in Figure 1 and consisted of four steps applied through a top-down approach with the aim of arriving at a list of the main hazards of public health significance with respect to the consumption of RDM in the EU.

As a starting point, the hazards presented in a previous EFSA scientific opinion that had addressed the food safety aspects of dairy cow housing and husbandry systems were considered (EFSA, 2009). The scientific opinion provided a list of the main biological hazards associated with dairy cow farming based on a review of several scientific publications available at the time (Roginski et al., 2002; Klinth-Jensen et al., 2004; Bohm et al., 2007; Cavirani, 2008; Buncic et al., 2009). Biological hazards had been identified in the group of opinions covering meat inspection for bovines, small ruminants and solipeds (EFSA BIOHAZ Panel, 2013a, b, d) and these were also considered in drawing up the preliminary list of hazards which may be associated with milk. Further, additional hazards included in the preliminary list were derived from reviews of the wider peer-reviewed scientific literature, textbooks and other technical documents up to September 2014. When all other evidence was lacking, the inclusion of hazards in the preliminary list was based on an expert opinion from the Panel on Biological Hazards (BIOHAZ) and other experts involved in the RDM working group. This led to the development of a preliminary longlist of microbiological hazards for the main milk-producing species considered in this opinion, i.e. bovines (cows), small ruminants (sheep, goats), solipeds (horses, donkeys) and camelids (camels) (see Appendix D).
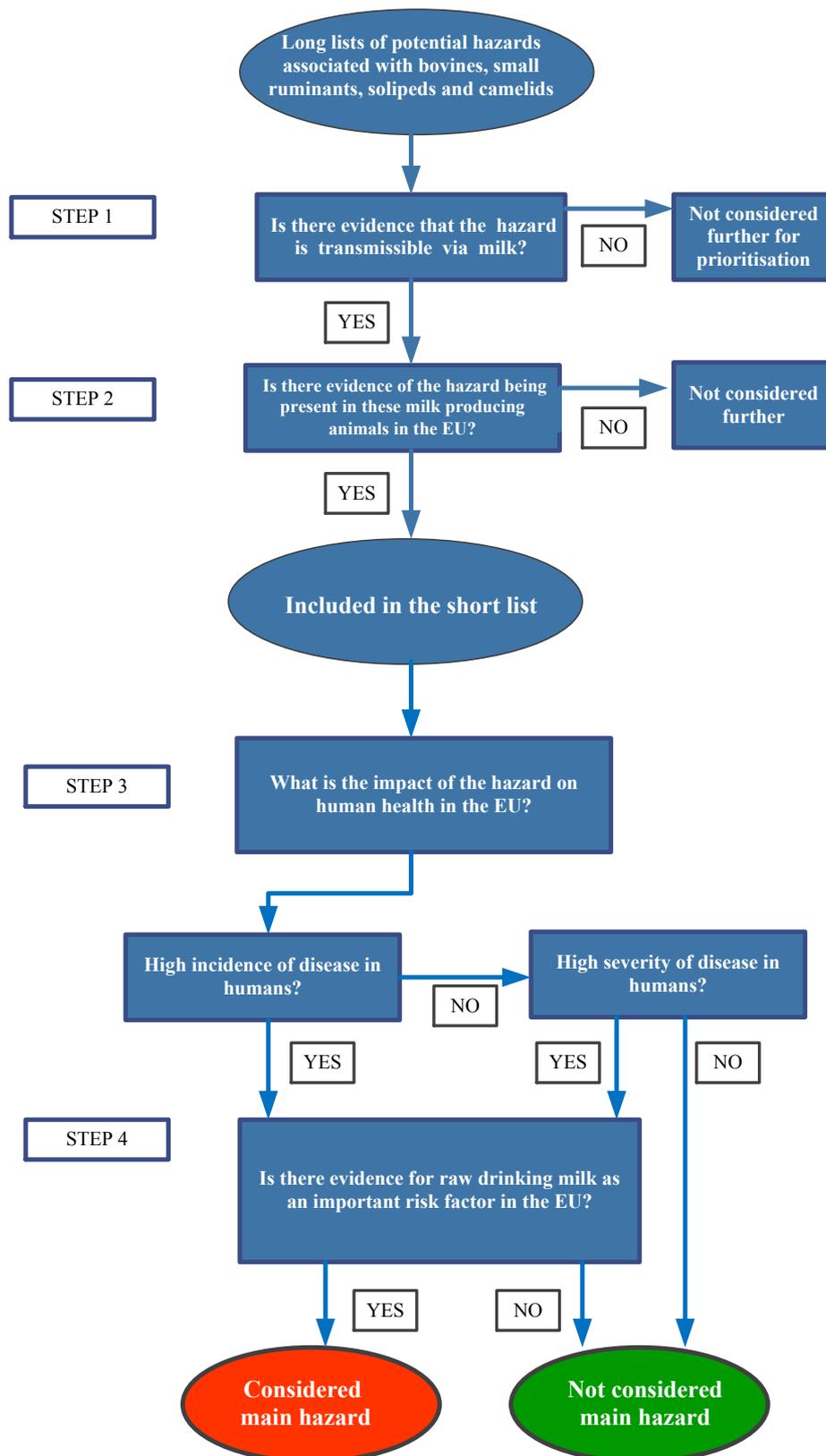
Figure 1: Decision tree used for prioritisation of microbiological hazards associated with raw drinking milk of bovines, small ruminants, solipeds and camelids in the EU

2.2.1. Step 1: is there evidence that the hazard is transmissible through milk?

The approach taken for each milk-producing species was to identify from the literature where there was evidence that the hazard identified in the preliminary longlist had led to illness through the consumption of milk of that species, irrespective of whether or not the milk was raw or pasteurised and regardless of the point of contamination. Evidence was considered to be one or more well-documented individual case reports, outbreaks, case–control studies, risk assessments, etc. reported in the literature and was not restricted to evidence from within the EU or specifically to RDM. Where no evidence of an association with human illness could be found in the literature then the hazard was not considered further. A consequence of this approach is that hazards which have been documented as occurring in a particular milk-producing animal or their milk but for which there is no evidence of an association with human illness are not considered further in the prioritisation with respect to milk from that species.

2.2.2. Step 2: is there evidence of the hazard being associated with the milk-producing animal population in the EU?

In step 2, it was considered whether or not there is evidence that the hazard is present in the EU population of one or more of the milk-producing species being considered. Evidence was considered to be one or more well-documented reports in the literature where the hazard was isolated from faeces, milk, the animal's environment or by serological reaction. Information was drawn from the published peer-reviewed and grey literature, including, where relevant, the EFSA report on Trends and Source of zoonotic agents (EFSA and ECDC, 2014). It is recognised that there are limitations and uncertainties associated with the approach adopted here. The available evidence supporting an association with illness is likely to be more extensive for milk from cows than from the other milk-producing animals since it accounts for the majority of drinking milk produced and consumed in the EU (Eurostat[4]).

Those hazards from the preliminary longlist for each milk-producing species that met both of the above criteria (steps 1 and 2) were included in a final shortlist to be considered at further steps of the hazard identification process. Hazards meeting the criteria were included in the final shortlist irrespective of whether they were associated with one of more of the milk-producing animal categories.

2.2.3. Step 3: what is the impact of the hazard on human health in the EU?

In step 3, consideration was given to the incidence of human illness from each of the microbiological hazards in the shortlist. If the incidence was below 10 per 100 000 population, then consideration was given to the severity of illness in terms of mortality. Hazards which showed a low incidence rate and low severity (below 0.1 % of deaths in confirmed cases) were not considered further as part of the assessment.

Data on human health were supplied by the European Centre for Disease Prevention and Control (ECDC) from The European Surveillance System (TESSy, covering the years 2009 to 2012 (see Appendix B)). Data supplied are reliable, albeit incomplete, since some countries did not report on certain diseases, and no corrections for under-ascertainment and under-reporting were made.

For some organisms, there were no TESSy data on the incidence of that infection in humans and/or severity as measured by percentage of deaths. In these cases, the literature was consulted for indications of incidence or severity through deaths associated with outbreak and sporadic cases in the EU. This ensured that hazards were not deemed to be less important because of a lack of EU collated data, such as in TESSy. If there was uncertainty about the incidence or severity of infection, then the default was to consider evidence of RDM as an important risk factor.

A consequence of using the top-down decision tree approach is that hazards which are of low incidence and low severity in the EU cannot be deemed to be main hazards. This does not mean that such hazards do not contribute to illness acquired through the consumption of RDM but merely that more detailed consideration in terms of risk is less appropriate at this time relative to hazards which

are considered to be main hazards associated with RDM in the EU. Hazards which were high in terms of disease incidence or severity were further considered in step 4 of the decision tree.

2.2.4. Step 4: is there evidence for raw drinking milk as an important risk factor in the EU?

Those hazards reaching step 4 were assessed as to whether or not there was evidence from the literature and other sources (e.g. outbreak data) that RDM is an important risk factor for infection in the EU. The following were considered in order of priority in deciding whether a hazard qualified as a main hazard or not:

(A)   epidemiological evidence that the hazard has been associated with illness from the consumption of RDM in the EU. This included outbreak and other data, where available;

(B)   the extent of occurrence of the hazard in different milk-producing species in the EU where available;

(C)   the prevalence of the hazard in milk bulk tanks or retail RDM in the EU where available; and

(D)   expert opinion.

The outcome of this step was that a hazard was considered to be either a "main hazard" or "not a main hazard" with respect to RDM in the EU. Those hazards where there was recent, well documented epidemiological evidence of illness in the EU were considered to be "main hazards" irrespective of any additional factors considered under B–D. For hazards where the epidemiological evidence was weaker (e.g. fewer outbreaks), then additional factors were taken into consideration as set out in B–D.

2.3. Results of hazard identification

2.3.1. Short list of microbiological hazards

Following the methodology explained in Section 2.1, the biological hazards included in the preliminary longlist of hazards are presented in Appendix D. Each of these hazards was assessed with respect to evidence in the literature of milk-borne transmission and, when such evidence was available, whether the hazard was present in the main milk-producing animal species in the EU. Weighting of such information was not undertaken until steps 3 and 4 of the decision tree.

The resulting shortlist of identified hazards is shown in Table 2 and consists of microbiological hazards which can be transmitted through the consumption of milk (step 1) and which occur in the main milk-producing animal species in the EU (step 2). For one hazard (i.e. Alkhumra haemorrhagic fever virus (AHFV), a tick-borne flavivirus associated with camels in Saudi Arabia) there was evidence of the hazard being transmissible via milk but no evidence was found that this hazard is present in milk-producing animals in the EU, and it was therefore excluded from further consideration in this opinion.

Table 2: Final (short)list of microbiological hazards where there is evidence that the hazard can be transmitted to humans through milk of different species and that the hazard is present in milk-producing animals in the EU

Microbiological hazards	Cows	Goats and sheep	Horses and donkeys	Camels
Bacillus cereus	Yes	No	Yes	No
Brucella abortus	Yes	No	Yes	No
Brucella melitensis	No	Yes	No	Yes
Campylobacter spp. (thermophilic)	Yes	Yes	Yes	No
Corynebacterium spp.	Yes	No	No	No
Listeria monocytogenes	Yes	Yes	Yes	No
Mycobacterium bovis	Yes	No	Yes	No
Salmonella spp.	Yes	Yes	Yes	No
Staphylococcus aureus	Yes	Yes	Yes	No
Streptococcus equi subsp. *zooepidemicus*	Yes	No	Yes	No
Shigatoxin-producing *E. coli* (STEC)	Yes	Yes	Yes	No
Yersinia enterocolitica	Yes	No	Yes	No
Yersinia pseudotuberculosis	Yes	Yes	Yes	No
Cryptosporidium parvum	Yes	No	No	No
Toxoplasma gondii	Yes	Yes	Yes	Yes
Tick-borne encephalitis virus (TBEV)	Yes	Yes	No	No

2.3.2. Categorisation of hazards into main hazards (results of step 3 and 4)

TESSy data, which were used in step 3 to highlight hazards of high incidence or high severity, were not available for all hazards in Table 2 (i.e. *B. cereus*, *Corynebacterium* spp., *S. aureus*, *S. equi* subsp. *zooepidemicus*) and information was sought from population-based studies and outbreak reports in Europe to provide information concerning incidence and or severity. The outcome of step 3 of the decision tree was that, based on disease incidence and/or severity data in humans, *B. cereus*, *B. abortus*, *S. aureus*, *Y. enterocolitica*, *Y. pseudotuberculosis* and *C. parvum* were not considered to be main hazards of concern. Although such hazards have been associated with illness linked to RDM and they occur in milk-producing animals in the EU their current impact in terms of human health was not considered sufficient for them to be considered further at this time with respect to RDM.

In terms of those hazards where disease data did indicate an important impact on human health, *Campylobacter* spp. and *Salmonella* spp. were considered because of their high incidence rate and STEC[7], *L. monocytogenes*, *Corynebacterium* spp., TBEV, *T. gondii*, *S. equi* subsp. *zooepidemicus*, *B. melitensis* and *M. bovis* were considered further because of the severity of their infections based on TESSy or other data. These hazards were further considered at step 4.

Step 4 in the decision tree considered epidemiological evidence that the hazard has been associated with illness from the consumption of RDM in the EU, presence of the hazard in different milk-producing species in the EU including any evidence of regional differences in the occurrence of the hazard, the prevalence of the hazard in milk and expert opinion.

2.3.2.1. Epidemiological evidence for illness associated with the hazard and raw drinking milk in the EU

Epidemiological evidence can relate to published outbreak reports, case–control studies, attribution studies and risk assessment studies, although in many cases epidemiological data in support of a hazard being transmissible to humans from RDM have come from outside the EU. In addition, epidemiological evidence may date back several decades and there are likely to have been significant changes in terms of animal health and hygiene control relating to raw milk production as well as the

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[7] Shiga toxin-producing *Escherichia coli* (STEC) is also known as verotoxigenic *E. coli*, verocytotoxigenic *E. coli*, verotoxin producing *E. coli* and verocytotoxin-producing *Escherichia coli* (VTEC).

taxonomic status of some hazards. Although outbreak data are an important source of evidence in support of hazard identification, not all outbreaks are reported centrally and the strength of evidence supporting a causal link to a food can vary. In addition, sporadic cases of illness associated with RDM are likely to go undetected unless the illness is severe, such as occurs in vulnerable groups.

Recent data on outbreaks in the EU relating to raw milk are presented in Table 3. Of the 27 reported outbreaks occurring in the EU between 2007 and 2012 in which there was a strong evidence of an association with consuming RDM, 21 were attributed to *Campylobacter* spp., one to *S.* Typhimurium, two to STEC and three to TBEV. Raw milk from goats was associated with four of the 27 outbreaks and the remainder were linked to milk from cows. It is of note that all the reported outbreaks were from the north of Europe.

Table 3: EU food-borne outbreaks reported to EFSA during 2007–2012 in which there was strong evidence of an association with raw drinking milk as the food vehicle

Year	Pathogen	Member State	Number of outbreaks	Number of cases
2007	*Campylobacter* spp.	The Netherlands	1	18
		Denmark	2	12
		Finland	1	4
		Germany	1	14
	Flavivirus (Tick-borne encephalitis virus (TBEV)) (raw goat's milk)	Hungary	2	32
2008	*Campylobacter* spp.	Austria	1	2
		Germany	1	45
		The Netherlands	1	8
	Shigatoxin-producing *E. coli* (STEC)	Germany	1	23
2009	No outbreaks associated with raw milk were reported			
2010	*Campylobacter* spp.	Slovakia	1	6
		Germany	3	42
	S. Typhimurium	Ireland	1	3
	Campylobacter jejuni (raw goat's milk)	The Netherlands	1	4
	Flavivirus (tick-borne encephalitis virus (TBEV)) (raw goat's milk)	Hungary	1	2
2011	*Campylobacter* spp.	Germany	3	32
		Sweden	1	13
2012	*Campylobacter* spp.	Denmark	1	11
		Germany	1	3
		Finland	2	22
	Shigatoxin-producing *E. coli* (STEC)	Finland	1	8

Source: Data on food-borne outbreaks included in this table were extracted from the EFSA zoonoses database on 10 November 2014.

Additional reports of outbreaks linked to RDM were also identified from the literature. TBEV is now endemic in 27 countries in Europe (Amicizia et al., 2013). In the majority of cases, human infections are caused by bites from an infected tick, although the virus can also be transmitted to humans through the consumption of raw milk and dairy products from viraemic livestock, mainly goats. In the Czech Republic, between 1997 and 2008, there were 7 288 cases of TBEV infection reported, of which 64 (0.9 %) were considered to be food-borne. These were mostly family outbreaks and included the involvement of unpasteurised goat's milk (Kriz et al., 2009). Kerbo et al. (2005) reported an outbreak from Estonia in 2005 linked to raw goat's milk. An outbreak involving three cases of TBEV infection associated with the consumption of raw goat's milk were reported from Slovenia in 2012 (Hudopisk et al., 2013). Four cases of TBEV infection which occurred in Hungary in 2011 were potentially linked with the consumption of raw milk from cows (Caini et al., 2012).

In 2006, there was an outbreak of brucellosis caused by *B. melitensis* in Spain, involving nine cases linked to the consumption of raw goat's milk (Ramos et al., 2008). *B. melitensis* has also been linked

to infections in Bulgaria and Greece in which raw milk or raw milk products have been implicated (Minas et al., 2007; Russo et al., 2009). A single food-borne outbreak with strong evidence was reported in France in 2012 (two human cases, implicated food vehicle: cheese) (Mailles et al., 2012) and four food-borne outbreaks for which there was weak evidence (involving 11 hospitalised cases) were reported in Greece in 2012, illustrating the health risk still associated with consumption of food contaminated with *Brucella*, although the evidence for RDM is probably not as strong as for raw milk products (EFSA and ECDC, 2014).

Human cases of *M. bovis* due to food-borne transmission are now very rare, and *M. bovis* can also be transmitted to humans through direct contact with infected animals. An outbreak of *M. bovis* infection associated with consuming raw milk from cows, affecting five family members on a farm in Ireland, was reported in 2005 (Doran et al., 2009). According to the EU Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012 (EFSA and ECDC, 2014), 25 MS provided information on human tuberculosis due to *M. bovis*. In total, 125 confirmed cases were reported by nine MS, while 16 MS reported zero cases. The number of confirmed cases reported in the EU decreased by 15.5 % compared with that reported in 2011. Most cases were reported in Germany, the UK and Spain, while the highest notification rate, 0.07 cases per 100 000 population, was reported in Ireland. The overall EU notification rate in 2012 was 0.03 cases per 100 000 population.

One outbreak of haemolytic–uremic syndrome (HUS) from STEC O26 was reported in southern Italy, and was linked to the consumption of water buffalo (*Bubalus bubalis*) dairy products, including raw milk (Lorusso et al., 2009).

Other hazards considered at step 4 were not represented in outbreak data in Table 3 and there is little, if any, recent evidence concerning the association of those hazards with the consumption of RDM in Europe. There are historical reports of cases of infection in Europe linked to raw milk involving *Corynebacterium* spp. (Hart, 1984; Barrett, 1986) and *S. equi* subsp. *zooepidemicus* (Barrett, 1986; Edwards et al., 1988), but for the other hazards (*L. monocytogenes*, *T. gondii*) the epidemiological evidence is essentially from outside Europe. In the case of *L. monocytogenes*, although there are reports of infections associated with the consumption of raw milk in the literature, the evidence in support of an association between illness and raw milk products is stronger than for RDM (Farina et al., 2008). On the basis of the above epidemiological information, *Campylobacter* spp., *Salmonella* spp., STEC, *B. melitensis*, *M. bovis* and TBEV are considered to be the main hazards associated with RDM in Europe. The outbreak evidence for *B. melitensis* and *M. bovis* is somewhat older than for the other pathogens.

2.3.2.2. Occurrence of pathogens in different milk-producing species in the EU

This criterion considered whether the hazard had been reported for more than one milk-producing species and information on the occurrence of the hazard in the milk-producing species in the EU. Such data are likely to reflect geographical and temporal distribution of studies on milk-producing animals, although some hazards are likely to be ubiquitous based on what is known about their occurrence in food animals, the environment and dairy products generally. Sixteen hazards were included in the shortlist in Table 2, 15 from cows, eight from sheep and goats, 11 from horses and donkeys and two from camels. *Campylobacter* spp., *Salmonella* spp. and STEC were reported in three animal species and *T. gondii* in all four. A summary of publications on the occurrence of selected pathogens in milk from cows, sheep, goats, horses, donkeys and camels is provided in Appendix C. Data available for many hazards are too sparse to draw any firm conclusions about the occurrence and distribution of hazards in milk-producing animals in the EU. Certain hazards are likely to be ubiquitous in the milk-producing bovine population (e.g. *Salmonella* spp., *Campylobacter* spp., STEC) and potentially other milk-producing species, although less information is available for species other than cows.

The status regarding freedom from brucellosis in cattle, sheep and goats in 2012 is reported in the EU summary report on zoonoses, zoonotic agents and food-borne outbreaks in 2012 (EFSA and ECDC,

2014). Among MS there are regional differences in *Brucella* spp. infection of livestock, with some Mediterranean or Eastern European countries, or part of them, not having acquired the status of officially free areas. In 2012, as in previous years, MS with the status officially free of bovine brucellosis (officially brucellosis free, OBF) as well as officially free of ovine and caprine brucellosis caused by *B. melitensis* (officially *B. melitensis* free, ObmF) reported low numbers of human cases, whereas the non-OBF/non-ObmF MS, such as Greece, Portugal and Spain, accounted for 67.7 % of all confirmed cases in 2012. The highest notification rates were observed in Greece (1.09 cases per 100 000 population), Portugal (0.36), Sweden (0.14), Spain (0.13) and Norway (0.08), but, while the majority of cases were domestically acquired in the non-OBF/non-ObmF MS, the majority of cases in Sweden and Norway, as in other OBF and ObmF countries, were travel associated.

2.3.2.3. Prevalence of hazard in bulk milk tanks or retail raw drinking milk in the EU

A key criterion is the likely extent of contamination of raw milk with pathogens, although available data are more comprehensive for cow's milk and for certain pathogens. The point of sampling is also an important consideration, with most studies reporting data from the testing of bulk tank milk samples rather than RDM sold in containers or dispensed from vending machines. Some studies have also examined milk filters, which tend to give a higher positivity than milk taken from bulk tanks. The methodology employed has included molecular detection methods as well as traditional culture-based techniques. Most data relate to detection rather than enumeration, for which very few published data are available, other than for certain hazards and microbial indicators.

With respect to the presence of pathogens in raw milk from cows, no statistically based European prevalence data are available. Studies of the frequencies of occurrence of pathogens in raw milk in the EU have been published in the international scientific literature, and these provide an indication of the range of prevalence values in different EU countries. An overview is presented in Appendix C, summarising these data collected, which were available from international literature for the European countries (raw milk from cows) and for outside Europe (raw milk from sheep, goats, donkeys and horses). It should be noted that prevalence figures can vary according to the sampling and methodological approaches used and the period when the studies were undertaken, which spans several decades. Variation can also be explained by geographical differences, the season in which the samples were taken, the size of the farm, the density of the animal population and regional differences. Prevalence data were available for *Salmonella* spp., STEC, *Campylobacter* spp. and *L. monocytogenes* and from a single study reporting data for TBEV (Appendix C). Despite extensive searching, EU data were not readily available for the other hazards considered at step 4. The prevalence of the four bacterial hazards ranged from 0 to 12 %, with the ranges being largest for *Campylobacter* (0–12 %) and *L. monocytogenes* (0–10.1 %) compared with STEC (0–5.7 %) and *Salmonella* spp. (0–2.9 %). Prevalence figures for TBEV in raw milk are based on a single study in Poland, which involved screening raw milk from cows (11.1 % of 63 samples), sheep (22.2 % of 27 samples) and goats (20.7 % of 29 samples) (Cisak et al., 2010). No prevalence data were found for *B. melitensis* or *M. bovis* in raw milk in the EU. Official data report that, in 2012, there was one *Brucella*-positive finding in a sample of raw milk reported by one MS.

2.3.3. Outcome of the decision tree

Table 4 provides a summary of the outcome of applying the four-step decision tree to microbiological hazards associated with RDM in the EU. The main bacterial hazards identified, in terms of recent outbreak data, were STEC, *Salmonella* spp. and *Campylobacter* spp. These hazards are essentially ubiquitous pathogens and are likely to be found in milk-producing animals and their milk throughout the EU, as indicated by prevalence data from raw milk testing. TBEV was also considered to be a main hazards based on outbreak data, together with evidence of spread in Europe and the virus being detected in raw milk. *B. melitensis* and *M. bovis* have been associated with outbreaks involving raw milk but these are much older and less frequent than for the other hazards. These pathogens are less common now than in the past and control programmes in Europe have generally been successful in reducing human disease from these organisms. Whilst they could still be regarded as within the

category of main hazards associated with raw milk they are unlikely to justify further detailed consideration for risk assessment at this time.

Other hazards listed in Table 4 are not considered to be "main hazards" since epidemiological evidence of illness was either historical or limited to reports from outside Europe. *L. monocytogenes* has a high mortality rate for vulnerable groups and the organism was as frequent as *Campylobacter* and STEC in surveys of raw milk. The lack of robust epidemiological data (including outbreaks) linking listeriosis to consumption of raw milk in Europe means that listeriosis cannot be considered a main hazard at this time. The ability of *L. monocytogenes* to grow at chill temperatures, coupled with its prevalence in raw milk, suggests that further studies in relation to RDM may be justified, particularly as several risk assessment models have already been developed for this pathogen outside Europe.

In the current hazard identification, the outcome was not dissimilar to the findings of a detailed review undertaken in 2008. Jaros et al. (2008) conducted a systematic review of the world literature (up to August 2008) which examined microbiological hazards associated with RDM and raw milk products (Jaros et al., 2008). The review provided moderate evidence to support a causal link between consumption of raw milk/raw milk products and *Campylobacter* spp., *Salmonella* spp. and STEC infections. It also provided some evidence to support a causal link between infection with *B. melitensis*, *L. monocytogenes*, *M. bovis* and TBEV and the consumption of raw milk products.

A recent quantitative risk assessment study on raw milk in New Zealand considered *Campylobacter* spp., *L. monocytogenes*, STEC (with a particular focus on *E. coli* O157) and *Salmonella* spp. as important hazards because of their significance to public health and likelihood of occurrence in New Zealand (Soboleva, 2013). A semi-quantitative risk assessment was also undertaken for *M. bovis* in raw milk and milk products because of the potential severity of the hazard in vulnerable groups (Ryan and Soboleva, 2013). A qualitative risk assessment for this hazard in RDM and raw milk products has also been undertaken in the UK (ACMSF, 2011).

Table 4: Summary of the information relating to the identification of the main hazards associated with raw drinking milk in the European Union (EU)

Hazard	High notification rate in humans? (high: ≥ 10/100 000)[a]	High severity (% deaths) in confirmed cases? (high: ≥ 0.1 % in more than one year)[a]	Evidence for RDM as an important risk factor in the EU[b]	Main hazard for RDM in the EU
Bacillus cereus	No[c]	No[c]	NA	No
Brucella abortus	No	No	NA	No
Brucella melitensis	No	Yes	Yes[d]	Yes[d]
Campylobacter spp.	Yes	No	Yes	Yes
Corynebacterium spp.	No[e]	Yes[e]	No	No
Listeria monocytogenes	No	Yes	No	No
Mycobacterium bovis	No	Yes	Yes	Yes
Salmonella spp.	Yes	No	Yes	Yes
Staphylococcus aureus	No[f]	No[f]	NA	No
Streptococcus equi subsp. *zooepidemicus*	No[g]	No[g]	No	No
Shigatoxin-producing *E. coli* (STEC)	No	Yes	Yes	Yes
Yersinia enterocolitica	No	No	NA	No
Yersinia pseudotuberculosis	No	No	NA	No
Cryptosporidium parvum	No	No	NA	No
Toxoplasma gondii	No	Yes	No	No
Tick-borne encephalitis virus (TBEV)	No	Yes[h]	Yes[d]	Yes[d]

NA: not applicable as the hazard did not reach step 4 because of low incidence or severity; RDM: raw drinking milk.
(a): Assessment based on data in Appendix B (data provided by ECDC extracted from TESSy, covering the years from 2009 to 2012).
(b): Evidence for RDM as an important risk factor for human infection in the EU. Evidence based on (A) epidemiological evidence for illness associated with RDM, (B) extent of occurrence of hazard in different milk-producing species in the EU, (C) prevalence of the hazard in bulk milk tanks or retail RDM in the EU and (D) expert opinion.
(c): No TESSy data. Incidence considered as low with mortality being very rare (Haagsma et al., 2006).
(d): Hazards which are restricted to certain parts of Europe, although in the case of TBEV the range appears to be expanding.
(e): No TESSy data. Incidence data considered as low but mortality rates of 5–10 % reported in a multicentre European study (Wagner et al., 2011).
(f): No TESSy data. Incidence considered as low with mortality from food-borne infection being very rare (Haagsma et al., 2006).
(g): No TESSy data. Incidence considered as low but severity high. Bordes-Benitez et al. (2006) reported mortality of 33.3 %. Edwards et al. (1988) reported mortality of 64 % in milk/dairy product associated outbreaks.
(h): Lindquist and Vapalahti (2008) reported mortality figures of 2 % in Europe.

2.4. Limitations in hazard identification

The use of a top-down approach using a decision tree to inform hazard identification is helpful when a large number of potential hazards are being considered. The main advantage of the decision tree approach is that it is able to categorise pathogens when limited information is available and the approach can be clearly communicated. Owing to the structure of decision trees it may not be possible to include certain factors which can have a significant impact on the final outcome. For example, significant risk factors, such as the extent of initial concentration and the extent of growth during storage, are not included in this process. In addition, arbitrary limits were defined in order to split data into an arbitrary number of categories for answering the questions in the decision trees. The above limitations can be misleading if applied in risk ranking. Moreover, uncertainty and variability can be qualitatively described, but it is not easy to reflect these aspects in the outputs from a decision tree approach. Owing to these limitations, it should be recognised that the purpose here is not to try and rank the hazards, but to identify the main hazards which would justify more detailed consideration.

2.5. Concluding remarks

- A top-down decision tree approach was used to identify microbiological hazards associated with RDM from cows, sheep and goats, horses and donkeys, and camels, which are the main milk-producing species in the EU.

- Microbiological hazards reported in the reviewed published literature as potentially associated with milk-producing animals were identified and listed. Those hazard/milk combinations for which there was no evidence of transmission via the consumption of milk or for which there was evidence of transmission but not for presence in milk-producing animals in the EU were excluded from further specific consideration.

- Microbiological hazards identified as potentially transmissible through milk and present in the EU milk-producing animal population were the bacteria *B. cereus*, *B. abortus*, *B. melitensis* *Campylobacter* spp. (thermophilic), *Corynebacterium* spp., *L. monocytogenes*, *M. bovis*, *Salmonella* spp., *S. aureus*, *S. equi* subsp. *zooepidemicus*, STEC, *Y. enterocolitica*, *Y. pseudotuberculosis*, the parasites *C. parvum* and *T. gondii* and the virus TBEV.

- Fifteen hazards were associated with cows, eight with sheep and goats, 11 with horses and donkeys and two with camels. This may, in part, reflect the greater volume of cow's milk consumed relative to milk of other species.

- Hazards were further categorised based on the assessment of the following: (i) the magnitude of human health impact based on incidence of confirmed human cases reported to ECDC, (ii) the severity of the disease in humans based on fatalities and (iii) evidence that RDM is an important risk factor for the disease in humans in the EU.

- The main microbiological hazards identified as relevant in the EU were *B. melitensis*, *Campylobacter* spp., *M. bovis*, *Salmonella* spp., STEC and TBEV. Of these, *Campylobacter* spp., *Salmonella* spp. and STEC were considered to be more widely distributed in the EU than the other hazards and *Campylobacter* spp. were the leading cause of outbreaks.

- Based on the limited data available and expert opinion, microbiological hazards which are not regarded as main hazards with respect to raw milk consumption in the EU were *B. cereus*, *B. abortus*, *Corynebacterium* spp., *L. monocytogenes*, *S. aureus*, *S. equi* subsp. *zooepidemicus*, *Y. enterocolitica*, *Y. pseudotuberculosis*, *C. parvum* and *T. gondii*.

- To provide a better evidence base to inform future prioritisation and ranking approaches, studies should be undertaken to systematically collect data for source attribution for the hazards identified as associated with RDM and collect data to identify and rank emerging milk-borne hazards.

- Because of the diverse range of potential microbiological hazards associated with different milk-producing animals, hazard identification should be revisited regularly.

3. Assess the public health risk arising from the consumption of raw drinking milk

Owing to limited data in Europe, a meaningful quantitative microbiological risk assessment (QMRA) for the main hazards identified in the previous section could not be undertaken. Instead, a review of existing QMRAs for drinking raw milk was undertaken to identify the strengths and limitations of the approaches which might inform the development of QMRA for RDM in the EU in the future.

3.1. Review of published microbiological risk assessments

A critical review of the QMRA literature in relation to the main hazards in RDM can enable the importance of the main sources of contamination to be identified. It can also highlight the critical points along the milk production and supply chain where contamination is likely to be most important. This includes information on consumer habits in relation to transport, storage and consumption of RDM. Considering the published QMRAs for the main hazards can enable data gaps and potential control options to be identified.

A literature search was performed in order to retrieve all the published QMRAs on RDM. The search term used was "risk AND assessment AND milk AND (raw OR unpasteurised)" for title/abstract. The search was not limited in time and gave 52 results in PubMed. Among the found articles, only those QMRAs relating to microbiological hazards identified in the previous section were considered. The main hazards *Campylobacter* spp., *Salmonella* spp., STEC and *L. monocytogenes*, were included in the review. Published risk assessments for products derived from raw milk, such as certain cheeses, were excluded because their production involves additional steps to the production of RDM. Although risk assessments were identified for *M. bovis* these were qualitative or semi-quantitative in nature and, since the focus was on QMRA, these were excluded from further detail consideration.

The literature review identified four QMRAs which were appropriate for review. These were country or region specific and all related to raw milk from cows. A single QMRA study in Europe estimated the risk of illness arising from consumption of RDM in northern Italy. The other three studies were from the USA, Australia and New Zealand (see Table 5 for details).

3.1.1. Model structure

In addition to the evaluation of the risk associated with the consumption of raw milk, the risk assessment studies aimed to identify where in the production and supply chain the microbiological hazards may be introduced, reduced or increased.

Table 5: Specific microbiological hazards considered in the published quantitative microbiological risk assessments

Article	Country/region	Scenarios considered	*Campylobacter*	STEC	*L. monocytogenes*	*Salmonella* spp.
FSANZ (2009)	Australia	1a. Farm gate consumption 2. Off-farm sales 3. Retail outlet sales	✓ *Campylobacter* spp.	✓	✓	✓
Latorre et al. (2011)	USA	1b. Farm gate sales 2. Off-farm sales 3. Retail outlet sales			✓	
Giacometti et al. (2012a)	Northern Italy	Best storage condition Worst storage condition	✓ *C. jejuni*	✓		
Soboleva (2013)	New Zealand	1a. Farm gate consumption 1b. Farm gate sales 2. Off-farm sales 3. Retail outlet sales	✓ *Campylobacter* spp.	✓	✓	✓

The multiple pathways by which raw milk can reach the consumer were considered in the four risk assessments (Figure 2). In many European countries, raw milk may be directly consumed at the farm (farm gate consumption scenario) or can be sold at the farm and subsequently consumed at home (farm gate sales scenario). Domestic consumption incorporates transport from the farm to the home and then storage in the domestic refrigerator. Some milk producers establish raw milk vending

machines allowing the automatic dispensing of raw milk from a bulk reservoir. These machines may be placed near the farm or at other locations, such as in a car park or at the entrance to a supermarket. Purchasing raw milk at a vending machine and consuming it at home represents the off-farm sales scenario. In New Zealand, there also exists another possible pathway: the purchase of raw milk from a retail outlet, such as a convenience store or supermarket (retail outlet sales scenario). Raw milk may also be ordered via the internet with delivery to the consumer by the farm or a courier.

In Giacometti et al. (2012a), two scenarios are considered: the best condition scenario, in which raw milk is maintained at a constant temperature of 4 °C, and the worst-case scenario, using temperatures and durations representing the worst observed scenario of handling raw milk before consumption. The worst conditions observed in a preliminary survey were 7.0 °C ± 0.5 for 5 hours (maximum temperature registered during the transport from farm to the vending machine and transport duration), then at 11 °C ± 0.5 for 22.5 hours (maximum temperature registered in vending machines and maximum storage time established by law for raw milk), 30 °C ± 0.5 for 30 minutes (worst air temperature during the simulation of transport of raw milk from vending machines to the home in summer) and 12 °C ± 0.5 for 68 hours (data obtained by Beaufort et al. (2008) as a simulation of home storage).
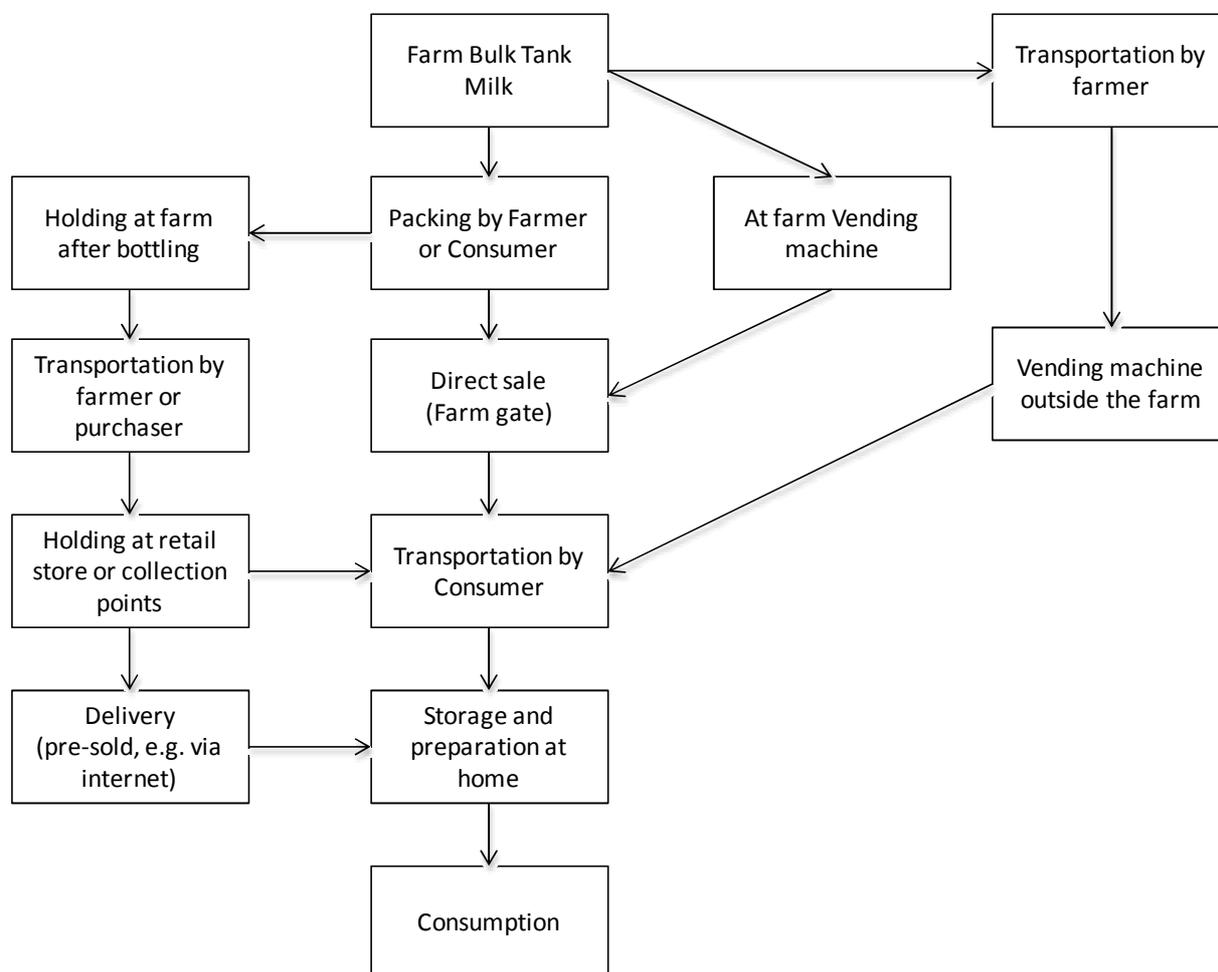
Figure 2: Potential supply pathways for raw drinking milk from cows from farm to consumer completely or partially included in the reviewed risk assessments

In the four reviewed risk assessments, the models calculate the risk for each pathogen independently. From the farm gate to the consumer, the change in concentration of pathogens in the milk are described based on available microbial predictive models in combination with the probability distributions for the temperature of the milk at each step in the food chain, and the duration of each

step. Monte Carlo simulations were performed to assess the pathogen concentration at the time of consumption using this series of distributions. All the models assume that contamination arises only at the farm level, i.e. no contamination occurs during transport to or from vending machines or during storage and use by the consumer. At the end of the food chain, the consumer drinks a serving of milk, which may be raw or boiled, if consumers follow advice which accompanies sales of RDM from vending machines. The exposure dose in terms of the number of pathogen cells in that serving is determined from the final concentration of pathogens in the milk. This is based on the accumulated growth or survival of the pathogen calculated at each step together with the quantity of raw milk consumed. A dose–response model is then applied to calculate the probability of illness from that number of pathogen cells present. The overall structure of the model used in the risk assessments is shown in Figure 3.
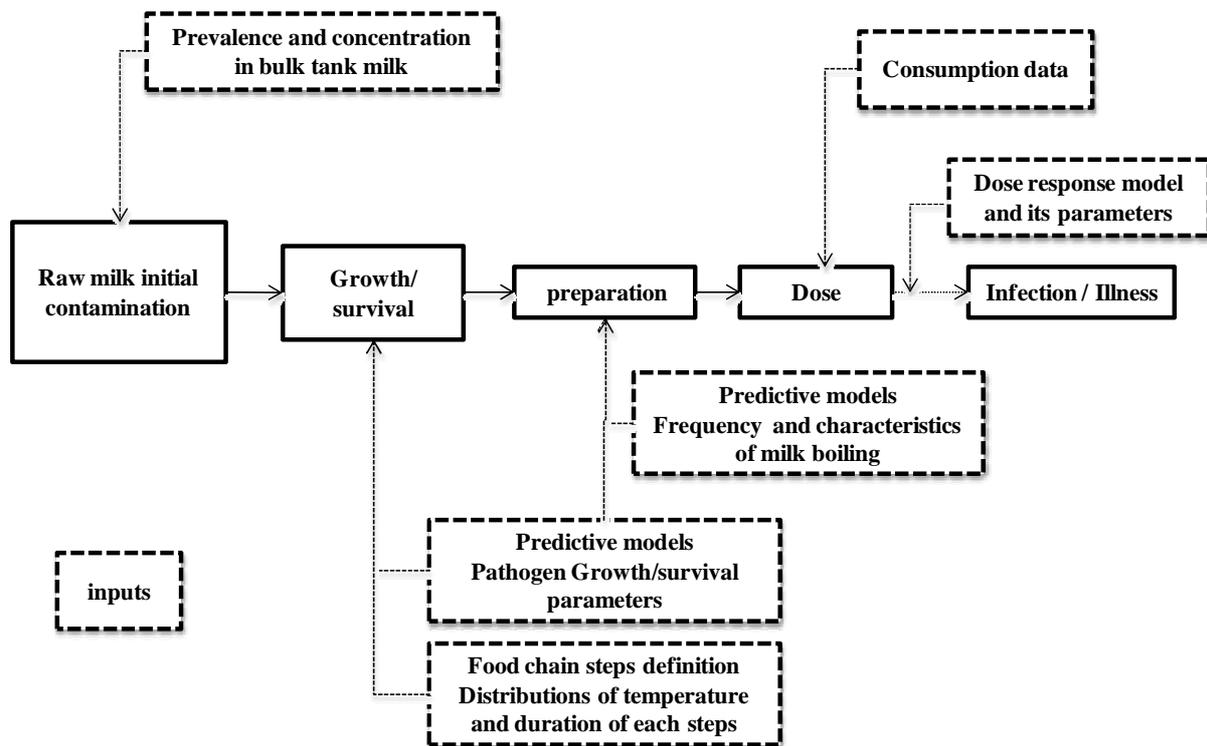
Figure 3: Overall structure of the risk assessment models and inputs (boxes with dotted lines are inputs)

3.1.2. Models inputs

3.1.2.1. Raw milk initial concentration

<u>Empirical-based approach</u>

Farm bulk tanks are the usual focal point when considering microbiological hazards associated with RDM production. Each bulk tank contains milk pooled from many individual mammary halves/quarters (depending on the animal species); a bulk tank corresponds, generally, to a single herd and to several milkings. The concentration of pathogens in quarter or half milk and in bulk tank milk can be variable and uncertain. In the absence of national surveillance data, prevalence was estimated in the models using combinations of prevalence surveys, other published data and industry data. In order to apply these relatively small scale surveys to randomly selected farms at a national level, it must be assumed that those farms sampled in the survey are representative of the national population of farms.

The occurrence of pathogens in raw bulk tank milk may be estimated using available data, although this will vary depending on survey designs and isolation methods. The available prevalence data cannot, in general, be assumed to be representative of the EU as a whole. In a risk assessment undertaken by Giacometti et al. (2012a), the prevalence of *C. jejuni* and STEC O157:H7 in dairy herds was estimated from a collection of 378 in-line milk filters from 27 farms, located in northern Italy, where the contamination with the two pathogens was assessed qualitatively (presence or absence). The representativeness of the 27 farms to the total dairy farm delivering RDM was not discussed in their paper. Latorre et al. (2011) assessed the occurrence of *L. monocytogenes* in bulk tank milk using reported data from one study in France (Meyer-Broseta et al., 2003) and data collected from different countries reviewed in the Food and Drug Administration–United States Department of Agriculture (FDA–USDA) Food Safety and Inspection Service (FSIS) listeriosis risk assessment for ready to eat foods (US FDA, 2003).

In order to assess the concentration of *C. jejuni* and STEC O157, Giacometti et al. (2012a) used a simplified model that links the proportion of positive samples and an estimate of the average concentration of these pathogens in raw milk. This model has an important hidden assumption which states that all the dairy farms share the same mechanism of raw milk contamination leading to a lognormal distribution of the concentration of pathogens. The probability of one sample of raw milk being positive for a pathogen can be derived using a Poisson distribution:

$$P^+ = 1 - e^{-(C \cdot v)} \qquad [1]$$

where C is the mean concentration of the pathogen in raw milk and v is the sample volume. Inverting formula [1], the value for C is obtained as:

$$C = -\frac{\ln(1-P^+)}{v} \qquad [2]$$

From this relationship, the distribution of the pathogen mean concentration is directly simulated: (i) generating a value from beta(a,b) distribution describing the prevalence in raw milk; (ii) substituting the generated value into formula [2] to calculate a simulated value of C. Assuming that the concentration of the pathogen in raw milk (C) has a lognormal distribution, $\log N(\mu, \sigma)$, μ was estimated with the mean of simulated C values, while σ was calculated to match the fraction of positive samples (i.e. with values greater than the minimum detectable level 0.004 CFU/mL) actually observed in a survey of in-line milk filters (Giacometti et al., 2012a). The way in which the parameters of the lognormal distribution were estimated is a major drawback of this model. In fact, the authors did not show the uncertainty associated with their estimates. The estimated mean and standard deviation (SD) parameters of the lognormal distribution were, respectively, –4.4 log CFU/mL and 0.86 log CFU/mL for *E. coli* O157:H7 and –4.1 log CFU/mL and 0.83 log CFU/mL for *C. jejuni*.

In Latorre et al. (2011), the *L. monocytogenes* risk assessment was based on data from North America and western Europe, and assessed the distribution of the initial concentration of *L. monocytogenes* in raw bulk tank milk as having a minimum of 0.04 CFU/mL and a maximum of 150 CFU/mL. The cumulative probabilities for 0.04, 10 and 100 CFU/mL were 0.926, 0.972 and 0.999, respectively. In the *L. monocytogenes* risk assessment carried out by the Food Safety Authority of New Zealand (FSANZ, 2009), the concentration of *L. monocytogenes* in bulk tank milk was assessed using survey data from Fenlon et al. (1995) based on bulk milk tanks from Scottish farms. In this survey, samples were taken at roughly monthly intervals and analysed using direct plating and enrichment techniques. Samples that were positive by direct plating had the count recorded, while samples positive after enrichment were recorded as presence only. The enrichment method has a limit of detection of 1 CFU/10 mL. Censored regression was used to estimate the mean and SD of the base 10 logarithm of the direct plated and enrichment results. The resulting normal distribution had a mean of 0.196 and a SD of 0.677, and was used as the concentration for *L. monocytogenes* in the bulk milk tank. The cumulative probabilities for 0.04, 10 and 100 CFU/mL were 0.009, 0.883 and 0.996, respectively.

The differences in concentration estimates between FSANZ (2009) and Latorre et al. (2011) risk assessments can be explained by choice of input data and statistical models.

Modelling approach

There are multiple routes of transmission which can result in the contamination of raw milk by pathogens including faecal contamination, foremilk, udder infection and environmental sources (see Section 6). Amongst these pathways, faecal contamination is often considered to be the most likely contamination source and the quantitative models used in Soboleva (2013) and FSANZ (2009) for multiple pathogens focused on this route.

The approach chosen in the FSANZ (2009) risk assessment to calculate the contribution of faecal contamination of teats is similar to the one proposed by Clough et al. (2009). The likelihood of milk contamination by the other sources is considered negligible: lactating dairy animals carry pathogens in their intestinal tracts, excrete it in their faeces, which in turn soils the teats, and the milk could be subsequently contaminated during the milking process. The total number of pathogenic bacteria contaminating the milk depends on the number of lactating cows within a herd, the within herd pathogen prevalence, the concentration of pathogens in faeces (CFU/g), the amount of faecal material present on the teat prior to cleaning, teat cleaning efficacy, the mass of faecal material transferred from teat to milk (mg/l) and the volume of milk produced per day by each lactating animal. The number of positive animals was assessed using survey data on the prevalence of animals shedding pathogens in their faeces. Several data sources were used to estimate both prevalence and concentration of pathogens (*Campylobacter* spp., STEC and *Salmonella* spp.) in faeces: data from Australia and New Zealand, North America, the UK and Ireland, continental Europe and Scandinavia were considered. The mass of faecal material transferred to milk was assessed using the experiments performed by Vissers et al. (2007) at 11 dairy farms to estimate the amount of "dirt" (faecal, soil and bedding material) transferred from a teat during milking. In these experiments, spores of butyric acid bacteria (BAB) were used as a surrogate to estimate the amount of transferred material. The mean amount of dirt transferred varied from 1 to 1.160 mg/l of individual milk. The amount of dirt transmitted to milk per farm ranged from 3 to 300 mg/l. In the FSANZ (2009) risk assessment, the amount of dirt transmitted to milk used for the simulations was on average 3.04 mg/l for cows with low teat soiling and 25.1 mg/l for cows with high teat soiling, with a probability of teats being lightly soiled equal to 0.337.

The teat cleaning efficiency was modelled as a Pert distribution with a minimum value of 0.9, a most likely value of 0.93 and a maximum value of 0.99. This teat cleaning efficiency was used for *Campylobacter* spp., STEC O157 and *Salmonella* spp.

In the MPI risk assessment model (Soboleva, 2013), available data on the concentration of pathogens in milk were judged to be insufficiently accurate and the concentration of pathogens in raw milk was estimated using a simulation model. For all pathogens included in this risk assessment (*Campylobacter* spp., *L. monocytogenes*, *Salmonella* spp. and STEC O157) it was assumed that some proportion of the TBC distribution relates to each of the pathogens in question. Under the assumption that most of the TBC originates from faecal material, the number of pathogens is calculated by multiplying the TBC by the proportion found in faecal material. Based on raw milk survey data collected in New Zealand in 2011–2012, for each farm, TBC was modelled as a mixture of two negative binomial distributions: the first representing the background contamination inevitable in routine milking and the second the consequence of a major contamination event, such as dropping a milking cluster into faecal material.

Under the assumption that the presence of a pathogen in raw milk represents a contamination event which is likely to have originated from faecal contamination from a single cow, the probability of major contamination was modelled as a function of both herd prevalence and animal prevalence. In the case of a major contamination event, the pathogen concentration distribution within the TBC was determined by the distribution of pathogens within faecal material sampled from a single animal.

Background contamination is assumed to derive from many cows through mixing either in the bulk milk tank or in the environment. Thus, the probability of background contamination is modelled as a function of on-farm prevalence, and the proportion of a pathogen within the TBC was determined by the distribution of pathogens within a pooled sample of faecal material sampled from all animals on the farm.

For each pathogen the probability of this arising from background contamination or a major contamination event are estimated and the two concentration distributions (background and major event) are assessed (Table 6). It is worth noting that multiple data sources were used to derive event probability and concentration: for *Campylobacter* spp., New Zealand data were used and for STEC O157, UK data were used. For *L. monocytogenes*, multiple European data sources were utilised. For *Salmonella* spp., data from a UK study on *S.* Typhimurium were used for on-farm prevalence and counts, and a national US study was used for the proportion of positive farms.

Table 6: Predicted initial concentration of pathogens in bulk tank milk based on different contamination scenarios (Soboleva, 2013)

Pathogen	Contamination	Proportion of bulk tank milk	Concentration distribution $\log_{10}$ CFU/mL (minimum, most likely, maximum)[a]
Listeria monocytogenes	Absence	0.348	0
	Background contamination	0.620	−2, −1, 1
	Major contamination	0.032	−3, 1, 3
Campylobacter spp.	Absence	0.076	0
	Background contamination	0.883	−2, 0.25, 2
	Major contamination	0.041	−5, 1.5, 4
STEC O157	Absence	0.886	0
	Background contamination	0.109	−4, −1, 0
	Major contamination	0.0055	−4, 0, 2
Salmonella spp.	Absence	0.581	0
	Background contamination	0.404	−5, −1, 3
	Major contamination	0.015	−5, −2, 6

(a): Data on minimum, most likely, maximum vales are derived from the graph of the distribution as shown in the publication.

Compared with the other risk assessment inputs, the distribution of *L. monocytogenes* in milk in the Soboleva (2013) risk assessment allows for higher concentrations than the distribution used by Latorre et al (2011). Concentrations higher than 100 CFU/mL have less than a 4 % chance of occurring.

The predicted distributions for STEC O157 and *C. jejuni* in raw milk in the Soboleva (2013) risk assessment allow much higher concentration than the ones estimated by Giacometti et al. (2012a). Concentrations higher than 1 CFU/g are expected to be common, which is not the assumption of the model of Giacometti et al. (2012a).

3.1.2.2. Growth and survival during storage

Growth models

STEC O157

In the FSANZ (2009) risk assessment, the growth rate of STEC was considered to be identical to that of non-pathogenic *E. coli* and was described by the extended square-root model, including factors such as temperature, water activity (a_w), pH and lactic acid concentration, proposed by Ross et al. (2003) and based on experimental results from (Salter et al., 1998). In Soboleva (2013), a minimum growth temperature of 6 °C was assumed based on data from Hudson (2011), and model generation times (GTs) were estimated based on data for *E. coli* O157:H7 growing in broth media (Marks et al., 1988).

In Giacometti et al. (2012a), the GT of STEC O157 was assumed to be variable with a minimum of 34.2 hours, a maximum of 56 hours and a most likely GT of 45.1 hours at a temperature of 4 °C. It should be noted that this temperature is lower than the growth limit for STEC O157 reported elsewhere in the literature. The GT was calculated for the worst conditions of temperature abuse observed in one Italian region and was assumed to be variable with a minimum of 14.2 hours, a maximum of 17.8 hours and a most likely GT of 15.6 hours.

Salmonella spp.

The growth rate models for *Salmonella* spp. used in the FSANZ (2009) and Soboleva (2013) models are based on the same data from a study in broth culture using a mixed cocktail of *Salmonella* strains (Gibson et al., 1988). A quadratic surface response model including temperature, pH and added salt concentration was fitted to the specific growth rate data. Based on data from Hudson (2011) it was assumed that the number of *Salmonella* bacteria remained unchanged between milking and consumption.

Listeria monocytogenes

In all three reviewed risk assessments, the growth rate for *L. monocytogenes* was estimated using a square-root model of the maximum specific growth rate with temperature as the only dependent variable and one variable or constant parameter T_{min} which is the theoretical minimum temperature for growth, modelled as a normal distribution, N(–2.47, 1.26), to allow for variation in growth rates in milk between strains as suggested by Pouillot et al. (2003).

3.1.2.3.   Survival model

Campylobacter spp., including C. *jejuni*, are microaerotolerant bacteria and are unable to grow below 30 °C. Doyle and Roman (1982) performed an experimental study which examined the survival of eight different strains of *C. jejuni* in unpasteurised milk stored at 4 °C. This study showed that there are considerable differences in survival between different strains of *C. jejuni*. Experimental data were used to fit a log-linear mixed model with random effects for the intercept and slope in order to capture the between-strain variability. The same model was used in the FSNAZ (2009) and Soboleva (2013) risk assessments.

In Giacometti et al. (2012a), the decimal reduction time (DRT) of *C. jejuni* at 4 °C was assumed to be variable with a minimum of 225.1 hours, a maximum of 1023.5 hours and a most likely DRT of 624.3 hours. The DRT was calculated for the worst conditions of temperature abuse observed in one Italian region and was assumed to be variable with a minimum of 113.3 hours, a maximum of 151.9 hours and a most likely DRT of 132.6 hours. In Giacometti et al. (2012a) the estimated DRTs are lower than the ones predicted from data collected by Doyle and Roman (1982).

Storage time and temperature

Latorre et al. (2011) described in detail the different steps from milking to consumption. The duration and temperature at each step were determined using data obtained from the literature or through personal communication. The storage/display time at the farm store was assumed to be uniform from one to seven days, with a storage temperature varying from –6 to 15 °C. The duration of holding at the farm before distribution to retail was considered to be much shorter from 4–12 hours; it was assumed that storage/display at retail may take one to seven days with a temperature varying from –6 to 15 °C. Transport from farm to retail and from farm or retail to home was assumed to be from 2–6 hours and from 0.2 to 3 hours, respectively. Home storage was assumed to vary between 0.5 and 8.5 days.

Giacometti et al. (2012a) use two scenarios, one at 4 °C and another using temperature and duration representing the worst observed scenario of handling raw milk before consumption.

In the FSANZ (2009) risk assessment, more realistic temperature and duration distributions were used. The time–temperature profiles were assumed to be: for farm storage 1–24 hours at 2–10 °C; for milk

collection 1–6 hours at 4–6 °C; for storage: 0–96 hours at 1–5 °C; for product distribution 0–24 hours at 0–7 °C; for retail storage 12–96 hours at 0–6 °C; for domestic transportation 93 % of the time being shorter than 1.5 hours at 7–20 °C; for domestic storage the temperature varying from –2 to 10 °C (with duration unreported). In the Soboleva (2013) risk assessment, temperature and duration are based on available data collected in New Zealand and literature data from elsewhere. The temperature distribution used in the risk assessment assumes the temperature control integrity of the supply chain is maintained. Moreover, the overall period from RDM production to consumption is limited to the interval during which the milk is organoleptically acceptable for consumption. Durations are in the same order of magnitude as those used in the FSANZ (2009) risk assessment.

3.1.2.4. Consumption habits

Giacometti et al. (2012a) assumed that 57 % of consumers heat treat the milk before consumption. The log reduction of STEC O157:H7 and *C. jejuni* populations was assumed to vary between 2 and 6, with 4 as the most likely value. In all the other risk assessments, heat treatment was not considered.

The portion size was assumed to vary between 100 and 1 000 mL, with 250 mL as the most likely value in Giacometti et al. (2012a). Latorre et al. (2011) assumed the same distribution of raw milk portion size as that used by FDA–USDA FSIS in its risk assessment for pasteurised milk (US FDA, 2003).

In FSANZ (2009), the mean daily consumption for children and adults was assumed to be 536 mL and 397 mL, respectively. The range of daily raw milk intakes was 250–1 750 mL, based on data for pasteurised milk consumption from the 1995 National Nutrition Survey. In Soboleva (2013), consumption was based on data for pasteurised milk consumption from the 2009 Adult Nutrition Survey and 2002 National Children's Nutrition Survey. The distribution of cold milk serving sizes for adults and children was represented by lognormal distributions (adults: lognormal (205.7, 153.1); children: lognormal (203.2, 122.3)).

3.1.2.5. Dose–response models

Table 7 provides details of the dose–response models used in each of the reviewed QMRAs for different subgroups in the human population. Exponential models were used for *L. monocytogenes*, beta Poisson for *Campylobacter* spp. and *Salmonella* spp. and beta Poisson and exponential for STEC O157.

Table 7: Details of the dose–response models used for the pathogens in the risk assessment models

Pathogens	FSANZ (2009)	Soboleva (2013)	Latorre et al. (2011)	Giacometti et al. (2012a)
Listeria monocytogenes	Exponential model WHO/FAO (2004) r-value: susceptible population: 5.85×10^{-12} Chen et al. (2006) r-values: 1.31×10^{-8} for lineage I; 5.01×10^{-11} for lineage II	Exponential model WHO/FAO (2004) r-values: susceptible population: 1.06×10^{-12}; general population 2.37×10^{-14}	Exponential model WHO/FAO (2004) r-values: intermediate-age population: 8.5×10^{-16}; perinatal [a] population: 5.0×10^{-14}; elderly population: 8.4×10^{-15}	
Campylobacter spp.	Beta Poisson model: $\log_{10} \alpha = $ normal(-0.767, 0.180) $\log_{10} \beta = $ normal(1.681, 0.742); correlation coefficient = 0.6455; probability of disease known infection = beta (8.855, 23.254)	Beta Poisson model: $\alpha = 0.145$; $\beta = 8.007$; probability of disease known infection = 0.33 For populations with an increased immunity parameters $\alpha = 0.145$ and $\beta = 50.000$		Beta Poisson model: $\alpha = 0.145$; $\beta = 7.589$; probability of disease known infection = 0.33
Salmonella spp.	Beta Poisson model p(illness)| $\log_{10} \alpha = $ normal(-0.871, 0.89); $\log_{10} \beta = $ normal(1.727, 0.227) Correlation coefficient: 0.892	Beta Poisson model $\alpha = 0.1324$; $\beta = 51.45$		
STEC O157	Beta Poisson model (with N_{50} re-parameterisation): $\log_{10} N_{50}$ discrete distribution with three equally weighted values of 2.38, 3.17, 4.48; $\alpha = 0.266$	Beta Poisson model (Strachan et al. 2005) $\alpha = 0.224$; $\beta = 4.88$		Exponential model r-values (Delignette-Muller and Cornu (2008)): 0–5 years age group: 1.2×10^{-3} > 5 years age group 2.4×10^{-4}

(a): Perinatal population: pregnant women and their foetuses or newborns.

3.1.2.6. Model outputs

Listeria monocytogenes

In Soboleva (2013), the predicted number of cases listeriosis per 100 000 daily servings of RDM from cows from all supply pathways was less than 10^{-7}. In Latorre et al. (2011), the estimated median number of listeriosis cases per year in the elderly population associated with the consumption of RDM from cows purchased directly from farms, farm shops and retail stores was 1.4×10^{-6}, 7.8×10^{-5} and 1×10^{-4}, respectively. For the perinatal population, i.e. pregnant women and their foetuses or newborns, the corresponding estimated median numbers of cases were 2.7×10^{-7}, 1.5×10^{-5} and 2.1×10^{-5} for milk purchased from farms, farm shops and retail stores, respectively.

In the FSANZ (2009) risk assessment, when milk is consumed at the farm (bulk tank) (scenario 1) the number of listeriosis cases per 100 000 daily servings is fewer than one. A greater number of predicted illnesses are observed for scenario 3 (domestic consumption after packaging, distribution and retail

sale) with a mean of 170 illnesses compared with 16.7 illnesses for scenario 2 (raw milk domestic consumption after farm gate purchase) when using a dose–response model representing a high virulence strain (Chen et al., 2006) (lineage I).

Campylobacter jejuni

In scenario 1 (consumption of RDM from the on-farm bulk milk tank), the average number of predicted illnesses was about 19 for *Campylobacter* spp., decreasing to 5 and < 1 illnesses per 100 000 daily servings in scenarios 2 and 3 (see previous paragraph) (FSANZ, 2009). This decrease in predicted cases is a result of the apparent inactivation of *Campylobacter* spp. in chilled raw milk. The decrease in illnesses in scenario 3 compared with scenario 2 is because of the longer supply chain for RDM that has passed through packaging, distribution and retail stages. In the farm gate purchasing scenario, the expected number of illnesses was about 140, with decreases to 90 and 30 cases per 100 000 servings respectively for these scenarios including farm gate vending machines, and retail (Soboleva, 2013). When the model is applied to a population with acquired immunity, the number of estimated cases was reduced by a factor of around 5.

In Giacometti et al. (2012a), the median risk of *Campylobacter* spp. infection per serving of RDM was calculated as 6.64 and 3.48 infections per year (per 10 000 to 20 000 consumers) for the best and worst storage temperature conditions, respectively. This equates to rates of campylobacteriosis of 0.123 and 0.064 per 100 000 daily servings, respectively. These figures are much lower than the ones estimated in Soboleva (2013) and FSANZ (2009) risk assessments.

Salmonella spp.

In the FSANZ (2009) risk assessment scenario 1 (consumption of RDM from the on-farm bulk milk tank) the average number of predicted illnesses for children was about 17 for *Salmonella* spp., increasing to 55 and 153 illnesses per 100 000 daily servings in scenarios 2 and 3 (FSANZ, 2009). In adults, the average number of predicted illnesses was 15, 59 and 130 per 100 000 daily servings for scenarios 1, 2 and 3, respectively. In the Soboleva (2013) risk assessment, the expected number of cases of salmonellosis was around 8 for all the purchasing scenarios: 7.8 (95 % confidence interval (CI) 6.3 to 9.3) for both consumption of RDM from the on-farm bulk milk tank and domestic consumption after farm gate purchase, and 7.0 (95 % CI 5.4 to 8.0) for domestic consumption after packaging, distribution and retail sale.

STEC

In the FSANZ (2009) risk assessment scenario 1 (consumption of raw milk from the on-farm bulk milk tank) the average number of predicted illnesses per 100 000 daily servings because of STEC O157 was 17 for both children and adults. In scenarios 2 and 3, this increases to 50 and 97 for children and 39 to 78 for adults (FSANZ, 2009). In the Soboleva (2013) risk assessment, the predicted number of cases because of STEC O157 was around 70 for all the purchasing scenarios, except for the scenario 4 (retail) where the predicted number of cases was 56.

In Giacometti et al. (2012a), the median risk for HUS was calculated, based on an r-value of 1.2×10^{-3} for the zero to five years age group and 2.4×10^{-4} for the > five years age group, estimated by Delignette-Muller and Cornu (2008). Based on an estimate of 3.57 % of the consumers of RDM being younger than five years old, the expected number of cases per year based on 10 000 to 20 000 consumers and 5.25 million servings was less than one case for scenarios representing both worst- and best-case storage temperature conditions in both age groups.

Table 8 provides median probabilities of illness per serving for the microbiological hazards in each of the models and for the different scenarios. In the risk assessment conducted by Giacometti et al. (2012a), the probability of illness per serving for *Campylobacter* spp. was lower in the worst-case than in the best-case scenario. This reflects the poorer survival of *Campylobacter* spp. under variable temperature storage conditions than at 4 °C (Giacometti et al., 2012c).

Table 8: Median (5th percentile, 95th percentile) probability of illness per serving from the hazards in the reviewed risk assessments

Article	Country/region	Scenario	*Campylobacter* spp.	STEC	*Listeria monocytogenes*	*Salmonella* spp.
FSANZ (2009)	Australia	1a. Farm gate consumption	19×10^{-5} $(14 \times 10^{-5};$ $23 \times 10^{-5})$	16×10^{-5} $(12 \times 10^{-5};$ $22 \times 10^{-5})$		17.5×10^{-5} $(10 \times 10^{-5};$ $2 \times 10^{-5})$
		2. Off-farm sales	5×10^{-5} $(2 \times 10^{-5};$ $8 \times 10^{-5})$	49.5×10^{-5} $(40 \times 10^{-5};$ $56 \times 10^{-5})$		54×10^{-5} $(41 \times 10^{-5};71 \times 10^{-5})$
		3. Retail outlet sales	$0\ (0; 1 \times 10^{-5})$	96.5×10^{-5} $(85 \times 10^{-5};$ $110 \times 10^{-5})$		150.5×10^{-5} $(135 \times 10^{-5};$ $171 \times 10^{-5})$
Latorre et al. (2011)	USA	1a. Farm gate sales			Intermediate: 1.8×10^{-15} $(6.3 \times 10^{-17},$ $4.8 \times 10^{-11})$	
		2. Off-farm sales			Intermediate: 1.0×10^{-13} $(4.0 \times 10^{-16},$ $3.2 \times 10^{-8})$	
		3. Retail outlet sales			Intermediate: 1.4×10^{-13} $(5.5 \times 10^{-16},$ $4.0 \times 10^{-8})$	
Giacometti et al. (2012a)	Northern Italy	Off-farm sales, best scenario	1.23×10^{-6} $(1.30 \times 10^{-9};$ $5.29 \times 10^{-3})$	HUS Children (0–5-year-old): 1.08×10^{-7} $(9.36 \times 10^{-11};$ $4.83 \times 10^{-4})$ Older consumers (> 5-year-old): 2.16×10^{-8} $(1.87 \times 10^{-11};$ $9.66 \times 10^{-5})$		
		Off-farm sales, worst scenario	6.64×10^{-7} $(6.5 \times 10^{-10};$ $2.95 \times 10^{-3})$	HUS Children (0–5-year-old): 4.99×10^{-7} $(2.80 \times 10^{-10};$ $2.56 \times 10^{-3})$ Older consumers (> 5-year-old): 9.97×10^{-8} $(5.60 \times 10^{-11};$ $5.13 \times 10^{-4})$		
Soboleva (2013)	New Zealand	1a. Farm gate consumption	139.4×10^{-5} $(123.2 \times 10^{-5};$ $150.7 \times 10^{-5})$	70.5×10^{-5} $(66.2 \times 10^{-5};$ $75.7 \times 10^{-5})$	4.13×10^{-12} $(4.10 \times 10^{-12};$ $4.13 \times 10^{-12})$	7.8×10^{-5} $(6.3 \times 10^{-5};$ $9.3 \times 10^{-5})$
		1b. Farm gate sales	98.84×10^{-5} $(85.64 \times 10^{-5};$ $108.14 \times 10^{-5})$	70.0×10^{-5} $(65.9 \times 10^{-5};$ $75.1 \times 10^{-5})$	4.69×10^{-12} $(4.68 \times 10^{-12};$ $4.71 \times 10^{-12})$	7.8×10^{-5} $(6.3 \times 10^{-5};$ $9.3 \times 10^{-5})$
		2. Off-farm sales	124.74×10^{-5} $(112.24 \times 10^{-5};$ $130.84 \times 10^{-5})$	75.5×10^{-5} $(70.5 \times 10^{-5};$ $80.4 \times 10^{-5})$	4.55×10^{-12} $(4.53 \times 10^{-12};$ $4.56 \times 10^{-12})$	8.4×10^{-5} $(6.7 \times 10^{-5};$ $10.6 \times 10^{-5})$
		3. Retail outlet sales	30.54×10^{-5} $(18.54 \times 10^{-5};$ $41.74 \times 10^{-5})$	56.3×10^{-5} $(53.6 \times 10^{-5};$ $60.2 \times 10^{-5})$	9.95×10^{-12} $(9.88 \times 10^{-12};$ $9.98 \times 10^{-12})$	7.0×10^{-5} $(5.4 \times 10^{-5};$ $8.0 \times 10^{-5})$

3.1.3. Sensitivity analysis

Sensitivity analysis is a systematic evaluation of model inputs to identify the most important factors affecting the model outputs.

In the FSANZ (2009) risk assessment, a sensitivity analysis was performed to assess those on-farm factors that have the greatest influence on the concentration of pathogens in the bulk milk tank. In the case of *Campylobacter* spp., four factors were considered: herd size, teat cleaning efficiency, teat soiling and within-herd prevalence. The herd size had no influence on *Campylobacter* spp. concentration, while the teat cleaning efficiency had only a weak influence. The two main factors that influence the mean *Campylobacter* spp. concentration are the degree of teat soiling and the within-herd prevalence.

In Latorre et al. (2011), sensitivity analysis showed that the home refrigerator temperature was the most important parameter affecting the predicted number of cases of *L. monocytogenes* infection associated with RDM consumption.

In the Soboleva (2013) risk assessment, sensitivity analysis was performed in order to study the effect of temperature of milk at the time of purchase at the farm gate, the length of time raw milk might be stored before consumption and the pathogen concentration in the milk bulk tank. An increase in the storage temperature increased the predicted number of illnesses for STEC O157 and *Salmonella* spp., while the predicted number of *Campylobacter* infections decreased and predicted listeriosis cases remained unchanged.

From the model developed by Soboleva (2013) it can be concluded that improving on-farm hygiene, assuming that only background bacterial contamination is present, leads to a decrease in the number of predicted cases of illness due to *Campylobacter* spp., *Salmonella* spp. and STEC O157 arising from the consumption of RDM.

3.2. Strength and weaknesses of the risk assessment models

A direct comparison between the four reviewed articles is not possible because of differences in the model structure and pathways, in the assessment of the prevalence and level of contamination of bulk tank milk, in the growth/survival models used, in the choice of dose–response model and different data sources for prevalence and concentration of pathogens in the herd and contamination in the raw milk bulk tank.

Giacometti et al. (2012a) estimated the concentration of pathogens in milk from the proportion of positive raw milk samples, while both Soboleva (2013) and FSANZ (2009) derived the concentration in milk by modelling the transfer of pathogens from faecal material to the milk. Latorre et al. (2011) started from the initial concentration in the raw milk. Giacometti et al. (2012a) used data on pathogen prevalence in raw milk from a study in northern Italy. Soboleva (2013) and FSANZ (2009) utilised data from Europe, the USA and New Zealand. Latorre et al. (2011) used pathogen concentration in milk based on data from North America and Western Europe.

All the reviewed QMRAs suffer from important data limitations. Data on time and temperature profiles are scarce and, when present, they are not representative of the current situation in Europe. The parameters for transfer of pathogens from faecal material to milk are based on limited data on both faecal concentration and quantity of dirt and faecal material soiling the milk. The growth models or their parameters are not fitted to RDM, and therefore the predictions made from these models may not be reliable.

All the reviewed QMRAs performed complete or partial sensitivity analysis in order to identify important factors either for the contamination of bulk tank raw milk or for the risk of illness. FSANZ (2009) assessed the influence of on-farm factors on the concentration of pathogens in the bulk milk tank. Both the Latorre et al. (2011) and Soboleva (2013) studies assessed the importance of raw milk

storage temperature at different points of the chain on the risk of illness. Latorre et al. (2011) also studied the effect of increase in home storage temperature, while in Soboleva (2013) the effect of an increase in raw milk temperature at point of purchasing was assessed. Moreover, Soboleva (2013) assessed the effect of changing the contamination distribution in bulk tank milk.

Owing to the uncertainties associated with these data gaps and to the multiple origins of data sources, the reviewed QMRAs cannot be extrapolated to assess the risk associated with RDM consumption in Europe as a whole. In addition, the models consider the risk from individual pathogens in raw milk and not the potential for exposure to more than one hazard through raw milk consumption.

The sensitivity analyses performed in the reviewed QMRAs provide evidence that the risk of illness tends to be influenced by the same factors (e.g. faecal contamination, storage temperature and time) but differently for each pathogen. Therefore, the results of sensitivity analysis can be a starting point in identifying potential control options for the important and other pathogens associated with RDM production.

3.3.   Concluding remarks

- Of the 27 outbreaks occurring in the EU between 2007 and 2012, and reported to EFSA, where there was strong evidence of an association with consuming RDM, 21 were attributed to *Campylobacter* spp., one to *Salmonella* Typhimurium, two to STEC and three to TBEV. Raw milk from goats was associated with four of the 27 outbreaks and the remainder were linked to milk from cows. In addition, other reports involving TBEV, *B. melitensis*, *M. bovis* and STEC in raw milk in the EU were identified in the literature.

- There is a clear link between drinking raw milk and human illness with *Campylobacter* spp., *S.* Typhimurium, STEC, TBEV, *B. melitensis* and *M. bovis*, with the potential for severe health consequences in some individual patients. Owing to the lack of epidemiological data, the burden of disease linked to the consumption of raw milk could not be assessed.

- Published QMRA models from Australia, New Zealand, the USA and Italy, for *Salmonella* spp., *Campylobacter* spp., STEC O157 and *L. monocytogenes* in cow's RDM were reviewed to identify their strengths and limitations. No QMRAs were available for RDM of other species.

- There were marked differences in the estimates of public health risk for the same hazards in the reviewed QMRAs, with some estimates differing by several orders of magnitude.

- Owing to important data gaps, model uncertainties and the broad origin of data sources used, risk estimates provided by the QMRA models described in the reviewed articles cannot be extrapolated to the European situation as a whole.

- From the model used in the Australian study, it can be concluded that improving on-farm hygiene leads to a decrease in the number of predicted cases of illness attributed to *Campylobacter* spp., *Salmonella* spp. and STEC O157 from the consumption of RDM.

- A QMRA could have helped in further estimating the public health risks and evaluating the effect of the mitigation options in Europe for these hazards, but could not be undertaken because of limited country and EU-wide data.

4.   Assess the additional risks associated with the sale of raw drinking milk through vending machines and via the internet

RDM is consumed on-farm as well as sold to consumers, usually through on-farm sales or local delivery. In some countries, particularly Italy, vending machines are used to sell raw milk to consumers. Although such milk is sold in a raw state, consumers are instructed to boil the milk dispensed from these machines prior to consumption. Certain other EU countries also instruct consumers to boil RDM before consumption (Austria, Belgium, Croatia, the Czech Republic, Italy, France (vulnerable groups), Lithuania, the Netherlands and Slovakia) (information gathered from

EFSA questionnaire, Appendix A). Depending on the level of compliance with these instructions this practice would have a significant impact on the microbiological hazards associated with RDM.

The growth of the internet in recent years has enabled consumers to gain better awareness and access to businesses selling fresh or frozen RDM. RDM is a perishable commodity and this will impact on how the milk is normally sold to consumers via internet sales. Measures aimed at ensuring good temperature control during transport and delivery are important as are subsequent consumer handling, shelf-life and temperature control. These factors are likely to be important considerations with respect to additional risks arising from the sale of RDM via this route.

4.1. Additional risk associated with the sale through vending machines

4.1.1. Operation of vending machines

In some EU countries, milk producers set up raw milk vending machines which allow the automatic dispensing of raw milk from a bulk reservoir. The machines can be placed near the farm or at another location, such as in a parking lot at the entrance to a supermarket. Because of the potential microbiological risks, the Belgian Food Safety Agency (FASFC) requires raw milk producers to apply strict good hygienic practices (GHPs) and to implement a self-control system based on Hazard Analysis and Critical Control Points (HACCP) principles. The producers must be authorised and registered for supplying raw milk through vending machines. They are also required to indicate on the vending machine that the raw milk is "to be boiled before consumption" and to "store between 0 °C and 4 °C". Because of the susceptibility of the elderly and young children to pathogens that may be present in milk, the FASFC discourages the placement of raw milk vending machines in retirement homes and in schools, if there is no guarantee that the milk will be boiled before consumption. The vending machine has to be filled with milk, already cooled to 6 °C at the farm and the temperature of the milk in the machine and between the tank and the nozzle has to be held between 0 °C and 4 °C and measured with a thermometer clearly visible to the consumer. Vending machine storage tanks are equipped with an agitator, which is automatically activated at pre-defined time intervals to ensure homogeneous cooling of the milk. No mixing of milk from different farms is allowed. The vending machine has to be refilled daily and the residual milk has to be removed and the machine cleaned prior to refilling. This residual milk or milk which has not been cooled properly cannot be used for human consumption. To reduce microbial growth, some types of vending machine automatically stop dispensing milk if the milk temperature is greater than 4 °C or the system does not perform the cleaning operation. To prevent possible environmental contamination of the nozzle, the milk dispensing chamber is equipped with a door that is automatically closed after dispensing the milk.

Depending on the type of machine, the customer can choose between a pre-set milk volume or the desired quantity of milk to purchase. The quantity of milk supplied to the customer is monitored by means of a volumetric counter. Since formation of biofilms in the milk processing environment can lead to an increased opportunity for microbial contamination of the milk (Austin and Bergeron, 1995; Latorre et al., 2010), the tubing associated with milk dispensing should be equipped with a mechanism which prevents emptying when the pump stops working. Milk is moved from the tank to the nozzle by means of pumps. The milk-dispensing chamber, where milk exits from the nozzle to enter the bottle, is the most critical part of the vending machine in terms of potential for microbial contamination. Residual milk can stagnate in the nozzle increasing the possibility of bacteria (derived from the milk or from the environment) surviving and multiplying. The so-called "splash area", composed of surfaces on which milk may splash or flow along requires particular attention to avoid the build-up of milk residues and bacterial contamination.

Stainless steel is the main material used in vending machine construction because of its durability, resistance to corrosion and easiness to clean. Other materials used can include glass, elastomers (also known as rubbers) and plastics. Milk vending machines should be subject to internal and external cleaning procedures as part of GHP. Internal surface cleaning may be performed manually or automatically with a rinsing programme. For surfaces coming into contact with milk, an automatic

rinsing programme is preferred and it consists of rinsing with appropriate cleaning and disinfecting agents which are active against a wide range of microorganisms. After cleaning, the pipes are rinsed with water and the milk tanks need to be thoroughly cleaned each time they are to be refilled.

There are few published surveys monitoring the presence of food-borne pathogens in raw milk from vending machines. In the Piedmont Region of Italy, in 2010–2011, 618 raw milk samples were collected from 112 dairy herds and 131 raw milk vending machines: 2 samples tested positive for *Salmonella* spp. (0.3 %), 9 for *Campylobacter* spp. (1.5 %) and 10 for *L. monocytogenes* (1.6 %) (Bianchi et al., 2013). In this study, the only factor that appeared to be significantly associated with a positive microbiological result was the previous occurrence of positivity from the same herd. Other factors (sampling site, vending machine site, average daily temperature, herd size, season) were not associated with the presence of pathogens. Other surveys have reported the isolation of pathogens from raw milk sold through vending machines (Giacometti et al., 2012b; Serraino et al., 2013; Gasperetti et al., 2014). Tremonte et al. (2014) analysed 30 samples collected from vending machines in southern Italy for indicator microorganisms. In all of the samples, the total mesophilic count (TMC) was around 5 log CFU/mL and increased to over 6 log after 72 hours of storage at 4 °C; counts of *Pseudomonas* spp. were also around 5 log CFU/mL at the time of sale, and increased up to 8 log CFU/mL after 72 hours of storage at refrigeration temperature.

4.1.2. Temperature control in vending machines and subsequent transport and storage

Refrigeration of raw milk in vending machines will not prevent changes in the microbiological composition of the milk and certain pathogens which are capable of multiplying at low temperatures may be able to grow particularly where there is prolonged storage. Consumer handling and storage practices can also potentially have an impact on the safety of raw milk from vending machines. Giacometti et al. (2012a) interviewed 100 consumers concerning their practices for transporting and handling of raw milk obtained from vending machines in northern Italy. They found that only 18 % of consumers used insulated bags to transport raw milk to home. Although it is recommended that RDM purchased from vending machines in Italy is boiled prior to consumption, it was found that only 57 % followed the recommendation, 23 % consumed the milk without heating and 20 % heated the milk in a microwave but without reaching the boiling point (Giacometti et al., 2012a).

In further studies in Italy, the duration of transportation of milk from farm to vending machines varied widely from 10 minutes to five hours (Giacometti et al., 2012c). The highest temperature of the bulk tank milk prior to loading was 7.0 °C, although milk delivered to the vending machines was as high as 11.4 °C. During simulated transportation from the vending machine to home the maximum increase in milk temperature was 5.5 °C (from 6.2 °C to 11.7 °C) after 30 minutes. Figures 4 to 7 show the impact of constant (4 °C) and a variable temperature over four days on the multiplication of four pathogens inoculated into raw cow's milk. There was little if any change in counts of *L. monocytogenes*, *C. jejuni*, *S.* Typhimurium or STEC O157:H7 at 4 °C but a 1–1.5 log increase for these pathogens was observed under variable temperature conditions. The study assumes that the consumer may store the milk for up to three days, which may be an underestimation of the general storage duration of consumer. Domestic refrigerators can vary considerably in overall temperature, as well as temperature in different parts of the refrigerator. For example, milk may be stored in the door which is likely to be at a higher temperature than the core of the refrigerator (James and James, 2007; Cibin et al., 2013). Tremonte et al. (2014) examined raw milk vending machines in southern Italy and found that the milk temperature ranged from 3.5 to 4.1 °C, which was in line with Italian legislation.
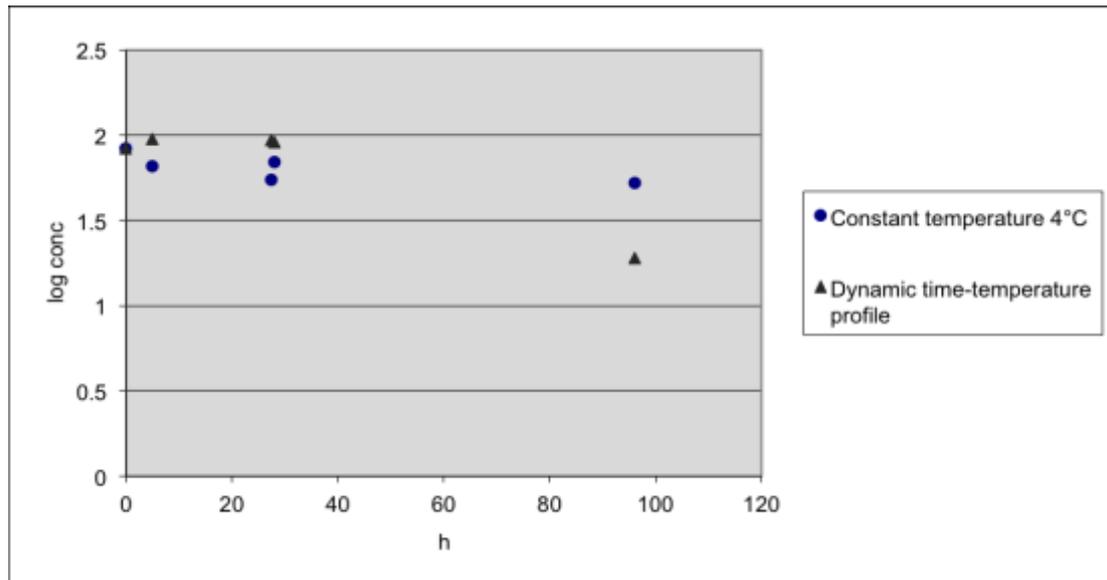
Figure 4: Counts of *C. jejuni* in spiked raw cow's milk during storage at 4 °C and at variable temperatures (mean ± SD log CFU/mL) (Giacometti et al., 2012c)
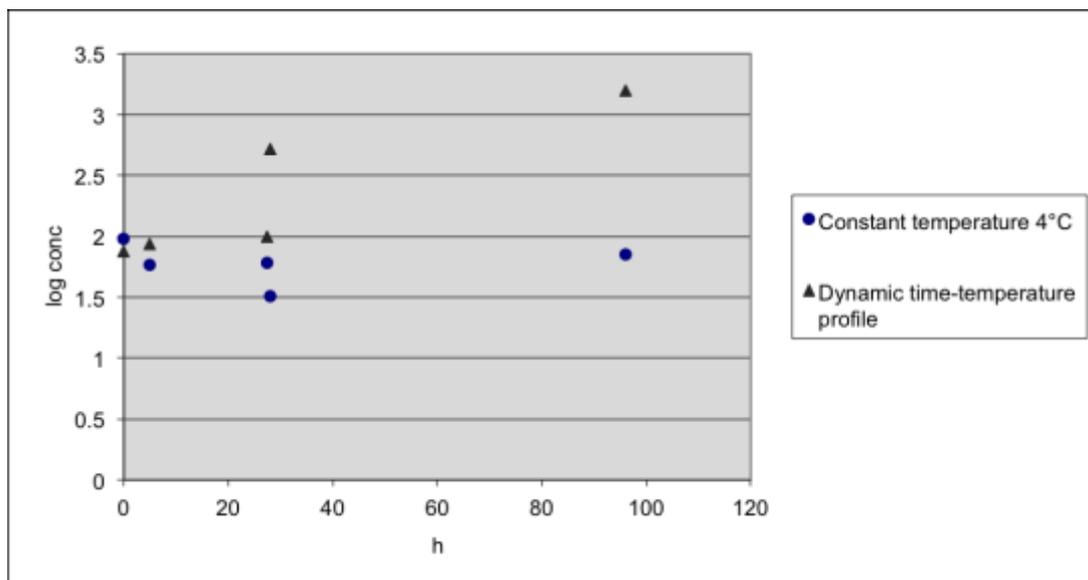
Figure 5: Counts of *S.* Typhimurium in spiked raw cow's milk during storage at 4 °C and at variable temperatures (mean ± SD log CFU/mL) (Giacometti et al., 2012c)
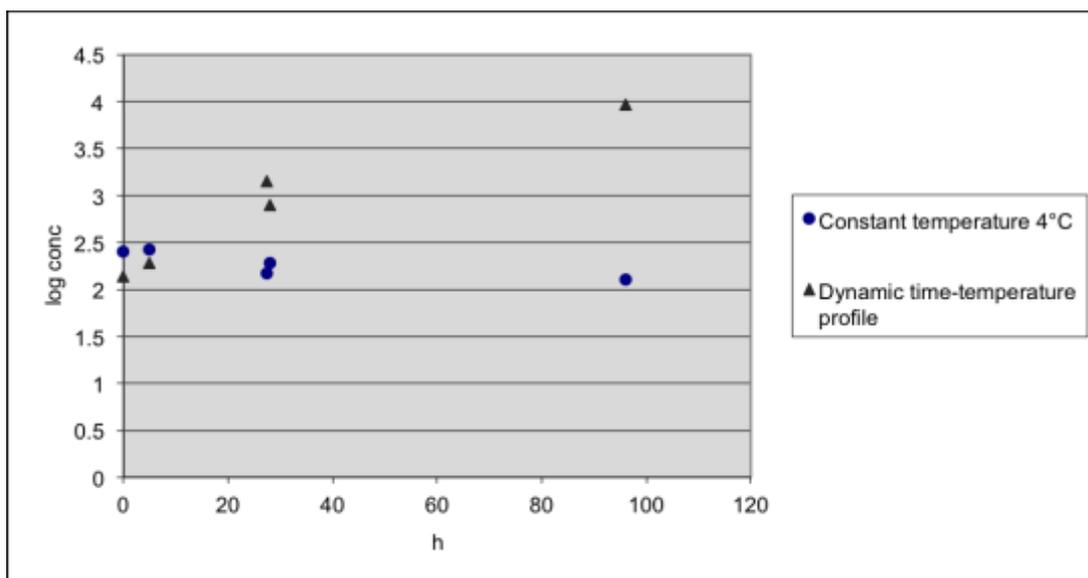
Figure 6:   Counts of STEC O157:H7 in spiked raw cow's milk during storage at 4 °C and at variable temperatures (mean ± SD log CFU/mL) (Giacometti et al., 2012c)
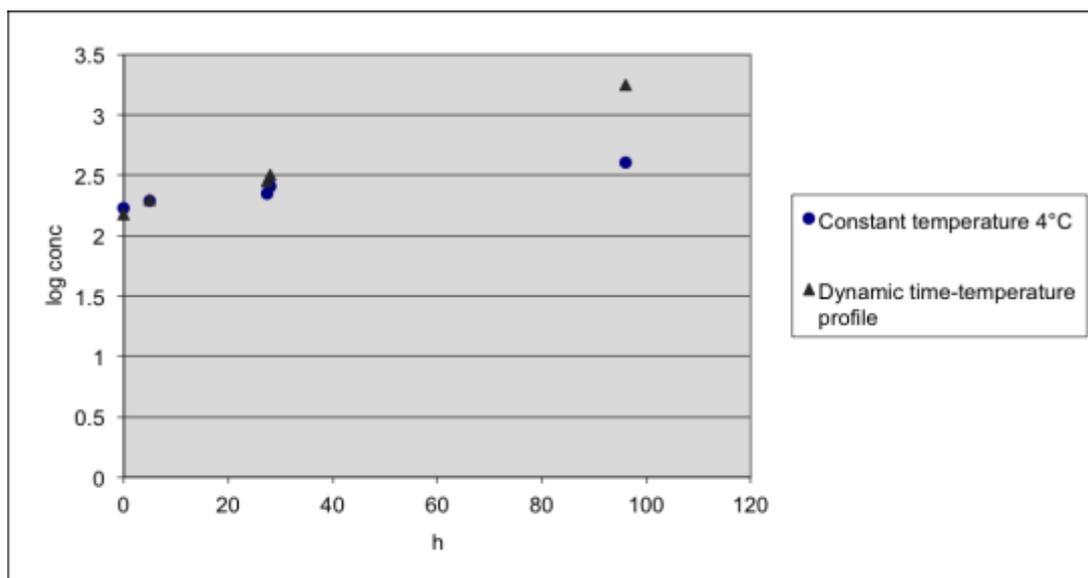
Figure 7:   Counts of *L. monocytogenes* in spiked raw cow's milk during storage at 4 °C and at variable temperatures (mean ± SD log CFU/mL) (Giacometti et al., 2012c)

4.2.   Additional risk associated with the sale through the internet

In many European countries, consumers can purchase raw milk from dairy farms through websites. The system of delivery, once the order has been placed online, varies widely. In some cases, the producer is directly responsible for the shipment, performing the delivery by their own means to the buyer's address. In this case, the distance between the farm and the place of delivery can be relatively short (approximately in the same town or city). In other cases, the shipment is performed by a courier, and the milk can be transported over long distances and with a lengthy travel time which may exceed 24 hours. In some situations, the milk will be transported using refrigerated vehicles, but in others cases the milk is transported by means of insulated boxes, which may not ensure the maintenance of refrigeration temperatures.

Such conditions, particularly when the maintenance of the cold chain is not guaranteed, can negatively affect the microbiological status of the milk as demonstrated by a study which examined human milk ordered via the internet (Keim et al., 2013). Most (74 %) internet purchased milk samples were colonised with Gram-negative bacteria or had a total aerobic plate count of $> 10^4$ CFU/mL. They exhibited higher mean total aerobic, total Gram-negative, coliform and *Staphylococcus* spp. counts than samples from the milk bank. Growth of most species was positively associated with days in transit (total aerobic count ($\log_{10}$ CFU/mL) $\beta = 0.71(95 \%$ CI: 0.38 to 1.05)). The authors concluded that human milk purchased via the internet exhibited high overall bacterial growth and frequent contamination with *Salmonella* spp. and *S. aureus*, which reflected poor collection, storage or shipping practices, thus posing a risk for babies fed with such products.

Frozen RDM can also be purchased via the internet. In addition to the risk of potential temperature abuse during transport, there is also the possibility that consumers may thaw the milk under unsuitable conditions, for example at room temperature, which may permit the growth of pathogens if these are present in the milk

4.3. Concluding remarks

- Sale of RDM through vending machines is permitted by some EU MS, with Italy having the highest number of these machines. There is little indication of milk from species other than cows being sold through vending machines.

- Although vending machine milk is dispensed in a raw state, consumers are usually instructed to boil the milk prior to consumption. If consumers comply with these instructions, the microbiological risks associated with raw milk would be eliminated.

- The temperature of raw milk in vending machines is expected to be kept below 4 °C, and variability in milk temperature is more likely to arise between the farm and vending machine and between the vending machine and point of consumption.

- Fresh and frozen RDM of different species (cow, goat, sheep, horse, donkey and camel) is available via internet sales, although there are no data on the microbiological or temperature controls for these milks from bulk tank through to the point of consumption.

- In the case of raw milk sold via the internet, the temperature must be controlled and correctly maintained during all steps from the farm to the consumer.

- In case of frozen raw milk, instruction should be given to consumers about appropriate conditions for thawing the milk.

- The variability in temperature control and duration of storage by consumers suggest that multiplication of pathogens may occur if these are present in the milk.

- There is a need for microbiological and temperature monitoring data for RDM sold through different routes together with more information on consumer handling and storage practices with respect to fresh and frozen raw milks from different species.

5. The likelihood of raw drinking milk being a significant source of antimicrobial-resistant bacteria/resistance genes

The potential risks to human health posed by bacteria in raw milk which exhibit resistance to antimicrobials or possess genes encoding resistance to such antimicrobials can be linked either to the presence of pathogens exhibiting such resistance or to non-pathogenic bacteria such as commensal *E. coli*, with resistance-encoding genes which can be transmitted to pathogenic bacteria after consuming the milk. Therefore, the possibility of raw milk destined for human consumption being a significant source of bacteria exhibiting antimicrobial resistance, whether pathogens or non-pathogens, is directly related to the presence of antimicrobial-resistant organisms in those animals and their environment. Furthermore, the presence of antimicrobial-resistant organisms, or of antimicrobial resistance genes in such organisms, is linked to the organism (pathogen or non-pathogen), the country

of origin and the antimicrobial practices in the relevant milk-producing animal species in such countries.

Whatever the organism, factors which complicate comparative studies of antimicrobial resistance between raw milk-producing species include differences between countries in methods for testing of food-borne pathogens and commensals for antimicrobial resistance, in the antimicrobials used for test and in the interpretation of breakpoints between isolates from animals and food, and from cases of human infection (EFSA BIOHAZ Panel, EFSA CONTAM Panel and EFSA AHAW Panel, 2012). The farm environment, including presence of other animal reservoirs, may also pose a potential source of antimicrobial-resistant organisms. The importance of such sources will depend on animal husbandry practices including hygiene associated with milking and storage of milk, and also the use of antimicrobials in the milk-producing animals and in other production animals when present on the farm.

5.1. Bacteria which may exhibit antimicrobial resistance in animals used for the production of raw drinking milk by animal species.

For animal species, the most significant pathogens which exhibit antimicrobial resistance are: (i) dairy cattle: thermophilic *Campylobacter* spp., STEC, *Salmonella* spp., meticillin-resistant *Staphylococcus aureus* (MRSA) (EFSA BIOHAZ Panel, 2013a) and extended spectrum beta lactamase (ESBL)/*Amp*C gene-carrying bacteria are also considered a hazard as defined as a potential source of antimicrobial resistance genes; (ii) sheep and goats: thermophilic *Campylobacter* spp., STEC; *Salmonella* spp., MRSA (EFSA BIOHAZ Panel, 2013b) and, as with bovine animals, ESBL/*Amp*C gene-carrying bacteria are also considered a hazard; and (iii) solipeds: as for sheep and goats (EFSA BIOHAZ Panel, 2013c).

A further organism which may be considered is *L. monocytogenes*, which has also been reported as a contaminant of raw milk (see above), and which has caused fatal infections in cases linked to the consumption of raw milk in the USA (Oliver et al., 2009).

5.1.1. Dairy cattle

5.1.1.1. *Campylobacter* spp.

Despite the importance of *Campylobacter* spp. in outbreaks of infectious intestinal disease associated with raw milk, reports of antimicrobial resistance in isolates of *Campylobacter* spp. in raw milk associated with cattle are rare.

For EU countries, in a study in 2010 on the occurrence of *Campylobacter* bacteria in 150 bulk milk samples of bovine origin tested from selected regions of Poland, *C. jejuni* was isolated from seven (4.6 %) samples. Of seven isolates tested, five (71.5 %) were resistant to doxycycline and six (85.8 %) to tetracyclines and ciprofloxacin (Wysok et al., 2011). In Italy, in a study in 2012 (Serraino et al., 2013), the presence of *Campylobacter* spp. and *Arcobacter* spp. in dairy herds used for the production and sale of raw milk was investigated at a water buffalo dairy farm. In addition, the susceptibility of isolates to ciprofloxacin, tetracycline, chloramphenicol, ampicillin, erythromycin and gentamicin was evaluated. Of 52 isolates from 49 milk filters sampled from 12 farms, (85.7 %), 16 of the isolates were identified as *Campylobacter* spp.—*C. jejuni* (6), *C. hyointestinalis* subsp. *hyointestinalis* (8), *C. concisus* (1) and *C. fetus* subsp. *fetus* (1). All *Campylobacter* isolates were susceptible to macrolides, which are the first-choice drugs for the treatment of campylobacteriosis should antibiotic treatment be considered necessary, although resistance to fluoroquinolones and tetracyclines was also detected. Isolates of *Arcobacter* spp.—*A. butzleri* (22) and *A. cryaerophilus* (14), were also made, within which resistance to ampicillin and chloramphenicol was detected. The authors concluded that the small number of isolates tested for antimicrobial susceptibility precluded any epidemiological considerations but stated that the high occurrence of *Campylobacter* spp. and *Arcobacter* spp. in dairy farms producing and selling raw milk represented an emerging hazard for human health. In particular, the presence of *C. jejuni* in in-line milk filters and raw milk confirmed that raw milk consumption is a

significant risk factor for human infection in Italy. Further studies have indicated a significant level of resistance to ciprofloxacin (62.8 %), tetracyclines (55.9 %) and nalidixic acid (55.2 %) in isolates of *C. jejuni* from a variety of animal-related sources, including raw milk, and also from human faeces, although the precise sources of such strains are not provided (Di Giannatale et al., 2014). Outside the EU, in a study in Quebec, Canada, of *C. jejuni* isolates obtained between 2000 and 2003 from a variety of sources, which included 33 isolates from raw milk, ciprofloxacin resistance was reported to be almost absent in isolates from water, chicken, and raw milk (Levesque et al., 2007).

5.1.1.2. *Salmonella* spp.

In England and Wales, *Salmonella* spp. were the causative organisms in 35.7 % of outbreaks associated with raw/unpasteurised milk between 1992 and 2000 (Gillespie et al., 2003). In all cases, the serovar was Typhimurium. Although neither phage types nor resistance profiles of the causative *S.* Typhimurium strains were reported in the above study, it is highly likely that the strains exhibited antimicrobial resistance, as over this period over 90 % of isolates of *S.* Typhimurium from cases of human infection in England and Wales showed resistance to at least one antibiotic, and over 70 % of the isolates were multiresistant (to four or more antibiotics) (Threlfall et al., 1999; Threlfall, 2002). No other reports of antimicrobial resistance isolates of *Salmonella* spp. from outbreaks associated with raw/unpasteurised milk of bovine origin in other EU countries have been identified.

In countries outside the EU, an outbreak of illness caused by raw milk contaminated with multiple antimicrobial-resistant *S.* Typhimurium occurred in Arizona, USA, in early 1983 (Tacket et al., 1985). One of the cases involved a 72-year-old woman who died with salmonella enteritis and sepsis that had not responded to treatment with chloramphenicol. The *S.* Typhimurium isolates from this patient, from other ill persons and from raw milk were resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, sulphonamides and tetracyclines. The authors concluded that this outbreak demonstrated the ability of drug-resistant *Salmonella* spp. to spread from the animal to the human reservoir via raw milk and, in a suitable host, to produce a fatal infection.

As part of the National Animal Health Monitoring System (NAHMS) Dairy 2002 and 2007 surveys in the USA, *Salmonella* isolates recovered from bulk tank milk and in-line milk filters were tested to determine the prevalence of antimicrobial resistance and to further characterise resistant isolates (Van Kessel et al., 2013). Susceptibilities to 15 antibiotics were determined for 176 *Salmonella* isolates of 26 serotypes. Thirty isolates (17.0 %) representing six *S. enterica* serovars exhibited resistance to at least one antimicrobial agent (serovars Newport (14 of 14 isolates exhibited resistance), Dublin (7 of 7), Typhimurium (3 of 5), Kentucky (4 of 22), Anatum (1 of 13) and Infantis (1 of 2)). Twenty isolates (11.4 %), including all 14 Newport, three Dublin, two Typhimurium and one Infantis isolate displayed a multidrug-resistant (MDR), *bla* (CMY)-positive (MDR-*AmpC*) phenotype, which included resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines, plus resistance to amoxicillin–clavulanic acid and extended-spectrum cephalosporins. From these studies, the authors concluded that there is a low but appreciable risk of infection with MDR *Salmonella* spp. from consumption of non-pasteurised milk and dairy products in the USA.

Of note is that antimicrobial resistance has rarely been reported in raw milk-associated or raw milk product-associated outbreaks of serovars such as Dublin, which is the most common serovar causing diseases in cattle in the UK (DEFRA, 2011; DEFRA/PHE, 2013) and in several other EU countries (Rabsch et al., 2001). The reasons for this are not fully understood, but may be related to low levels of antibiotic usage in adult cattle in contrast to calves in some countries, or to virulence plasmid-mediated exclusion of incoming plasmids coding for antimicrobial resistance by incompatibility mechanisms (Chu and Chiu, 2006).

5.1.1.3. Shigatoxin-producing *E. coli* (STEC)

Despite STEC O157 being the organism most commonly associated with raw/unpasteurised cow's milk-related outbreaks of gastrointestinal illness in many European countries (Gillespie et al., 2003; EFSA, 2007), little information is available about the occurrence of antimicrobial resistance in isolates

of STEC associated with such outbreaks. In the USA, antimicrobial resistance has been identified in some raw cow's milk-associated STEC infections between 2000 and 2009 (for a review, see Oliver et al., 2009), and in a 2009 study of isolates of STEC from raw milk samples in Iran, 23.1 % of isolates tested were resistant to tetracyclines (Mohammadi et al., 2013). Resistance to antibiotics in STEC has little clinical relevance, as for the most part antibiotics are contraindicated for treatment of infections with these organisms.

5.1.1.4. *Listeria monocytogenes*

Antimicrobial resistance in isolates of *L. monocytogene*s from raw milk has only rarely been reported in EU countries. In a study in Northern Ireland, reported in 2001, only two sporadic isolates of 45 tested showed resistance to tetracyclines when tested for resistance to ampicillin, gentamicin, streptomycin, sulphonamides, erythromycin, chloramphenicol, cephalothin and tetracyclines (Harvey and Gilmour, 2001). In non-EU countries, resistance to tetracycline and penicillin has been reported in isolates in Malaysia in 2012 (Jamali et al., 2013) and multiple resistance has recently been reported in *Listeria* isolates from raw milk in Nigeria (Uwanibe et al, 2014).

5.1.1.5. *Staphylococcus aureus*, including meticillin-resistant *Staphylococcus aureus* (MRSA)

Although *S. aureus* is a food-borne pathogen, infection by the oral route has not been confirmed for MRSA, which is regarded as a possible contaminant of raw milk. In this respect, MRSA has as yet not been isolated from outbreaks of infection associated with raw/unpasteurised milk in EU countries. Nevertheless, there have been increasing reports of the isolation of MRSA from dairy farms and bulk tank milk in several EU MS (Gindonis et al., 2013; Paterson et al., 2014).

In a recent study in Germany, the prevalence of MRSA infection among dairy herds, based on testing of bulk tank milk samples, was 4.4 % (Kreausukon et al., 2012), and regional studies performed in southern Germany reported a comparable, while slightly lower, prevalence of 2.2 % (Friedrich et al., 2011). Similarly, studies on milk samples from dairy herds in southwest Germany indicated a prevalence of between 5.1 and 16.7 %, and MRSA was also isolated from bulk tank milk samples from the farms under investigation (Spohr et al., 2011). MRSA belonging to sequence type (ST) 398 has also been shown to occur in dairy herds in other MS, such as Belgium (Vanderhaeghen et al., 2010) and MRSA from milk samples have also been reported in the USA (Haran et al., 2012). There was no information provided as to whether the bulk milk tank samples in the studies quoted above were to be consumed raw.

Studies assessed by EFSA have indicated that, among the different cattle production lines, veal calves have the highest MRSA burden and thus monitoring of MRSA in cattle primarily targets veal calf populations (EFSA, 2012). In addition, raw milk and derived raw milk products which may be contaminated with MRSA could be monitored in those MS where consumption of these products is most frequent (EFSA, 2012).

5.1.1.6. Extended spectrum beta lactamase (ESBL)/*Amp*C gene-carrying bacteria

ESBL/*Amp*C gene-carrying *Salmonella* serovars Newport, Dublin, Typhimurium and Infantis have been identified in the USA in bulk tank milk and in-line milk filter surveys undertaken as part of the NAHMS Dairy 2002 and 2007 (see above). ESBL/*Amp*C gene-carrying *Escherichia coli* have been reported in calves and dairy cattle in the UK (Teale et al., 2005; Liebana et al., 2006) and more recently in veal calves in the Netherlands (EFSA BIOHAZ Panel, 2011; Hordijk et al., 2013) and at lower levels in dairy cattle (CVI, 2013). Similarly *bla*CTX-M-15 and *bla*TEM-1 *E. coli* isolates belonging to ST88 (ST88; and *Klebsiella pneumoniae* subsp. *pneumoniae* isolates carrying *bla*SHV-12 and *bla*TEM-1 have recently been reported from cases of bovine mastitis in the UK (Timofte et al., 2014).

5.1.1.7. Other bacteria

Information on the occurrence of resistance to antibiotics in other potential bacterial pathogens which have been isolated from raw milk of bovine origin is minimal in the EU.

5.1.2. Other bovine animals

To our knowledge, there are only limited data on antimicrobial resistance in raw milk from bovine animals other than dairy cattle. Of note is a report of STEC O26, a known shigatoxin-producing organism, in water buffalo (*B. bubalis*) in southern Italy, where such animals are intensively reared, following an outbreak of HUS in which the consumption of typical dairy products, including raw milk, was considered to be a common risk factor (Lorusso et al., 2009). Of 160 analysed samples, a single STEC O26 isolate, showing the stx1+/stx2+/eae-/hlyA+ genotypic profile, showed resistance to glycopeptides, macrolides and penicillins.

5.1.3. Other animals—small ruminants, horses and donkeys and camels

Within the EU, the only reports of antimicrobial resistance in raw milk from small ruminant sources (sheep and goats) are in isolates of *Staphylococcus* spp. in raw milk and cheese in northern Italy, in which 10 % of isolates carried the *tet*(K) resistance gene (Ruaro et al., 2013), in suspect *E. coli* O157 isolates from milk from goats and cows in the Lombardy region of Italy (Picozzi et al., 2005), and in *E. coli* O157 isolates from bovine, caprine and ovine milk in Greece in 2009, in which high levels of resistance to tetracyclines, streptomycin and sulphonamides were observed (Solomakos et al., 2009). One isolate of MRSA was found in 601 *S. aureus* isolates obtained from milk from 229 dairy sheep farms in Spain (Ariza-Miguel et al., 2014). To the best of our knowledge, there have been no reports of antimicrobial resistance in bacterial isolates from raw milk from solipeds or camels in the EU.

Although fewer data are available for animals, other than dairy cattle, on the basis of the above reports we conclude that a high incidence of antimicrobial resistance is less likely in organisms from raw milk from animals other than dairy cattle, with the possible exception of water buffalo in southern Italy (see above).

5.2. Outbreaks of milk-related antimicrobial resistance infections which have been caused by breakdowns in pasteurisation or sterilisation

Although not raw milk-associated per se, several substantive milk-associated outbreaks of infection involving antimicrobial-resistant strains of *Salmonella* spp. have been reported, both in the EU (Walker et al., 2000) and the USA (Ryan et al., 1987). These outbreaks provide examples of the dangers to public health that can occur should milk containing antimicrobial-resistant *Salmonella* spp. be distributed either without treatment or after post-treatment contamination.

5.3. Concluding remarks

- Antimicrobial resistance has been reported in several EU countries in isolates of *Campylobacter* spp., *Salmonella* spp., STEC and *S. aureus* from raw milk or associated equipment such as milk filters and may be significant for public health. Such isolates have primarily been associated with raw milk from bovine animals which may reflect the more limited screening of milk from other species.

- Strains of *Campylobacter* spp., and particularly *C. jejuni*, exhibiting resistance predominantly to tetracyclines but also to some other antimicrobials have been reported in two MS, but such strains are not known to have caused human infections linked to consumption of raw milk.

- There have been no reports of antimicrobial resistance in isolates of *Salmonella* spp. from outbreaks associated with raw/unpasteurised milk in the EU in countries other than the UK. In the USA, there has been a report of a raw milk-associated outbreak caused by MDR *S.* Typhimurium, with a single fatality ascribed to resistance of the organism to antibiotics.

- Despite STEC O157 being the organism most commonly associated with RDM-related outbreaks of STEC gastrointestinal illness in several EU countries, little information is available about the occurrence of antimicrobial resistance in such outbreak strains. Antimicrobial resistance has been reported in a water buffalo raw milk-associated STEC O26 outbreak in one MS in 2008 and in raw milk-associated STEC outbreaks in the USA.

- Antimicrobial resistance in isolates of *L. monocytogene*s from raw milk and raw milk dairy products has only rarely been reported in EU countries.

- MRSA have not been isolated from outbreaks of infection associated with raw milk in EU countries. Although not typically regarded as a food-borne pathogen, there have been increasing reports of the isolation of MRSA from dairy farms and bulk tank milk in several EU MS.

- Although identified in *E. coli* in bovine animals in some MS, ESBL/*Amp*C gene-carrying bacteria have not been reported in raw milk in EU MS. In the USA, a range of salmonella serovars with ESBL/*Amp*C genes have been identified in raw milk surveys.

- There are only very limited data on the occurrence of antimicrobial resistance in raw milk-associated pathogens from non-bovine animals in the EU.

6. Description of potential control options to reduce public health risks arising from consumption of raw drinking milk

Important steps in the hygienic production of milk have been described in detail in the literature (Leaver, 1983; Castle and Watkins, 1984; Castle, 1985; FAO, 1989). Whilst heat treatment (e.g. high temperature short time pasteurisation) is well established as a measure to control microbiological hazards present in raw milk, such treatments are outside the scope of this opinion as they change the intrinsic characteristics of the raw milk. Likewise, other processing methods which do not involve heat treatment, such as ultrafiltration or high pressure (Walkling-Ribeiro et al., 2011; Yang et al., 2012; Innocente et al., 2014), are also outside the scope of this opinion and therefore were not be considered further as potential control options.

The Codex Alimentarius code of hygienic practices for milk and milk products emphasises the importance of good agricultural practices (GAP), GHP and good animal husbandry practices at the farm level whilst recognising that there are limitations to the full application of HACCP principles at the level of primary production (CAC, 2004). Such guidance, when fully implemented provides a baseline of against which the impact of any additional control options (where available) can be considered.

Contamination of milk with microbiological hazards can arise from a number of different sources involving intrinsic contamination from infection in the animal prior to milking or extrinsic contamination arising from environmental contamination of the milk with faecal material either directly from the animal at the time of milking, or indirectly from the milking equipment, farm environment or at the point of use (Cousins and Bramley, 1981; Moatsou and Moschopoulou, 2014). The potential sources of on-farm contamination and their relationship to each other are shown in Figure 8.
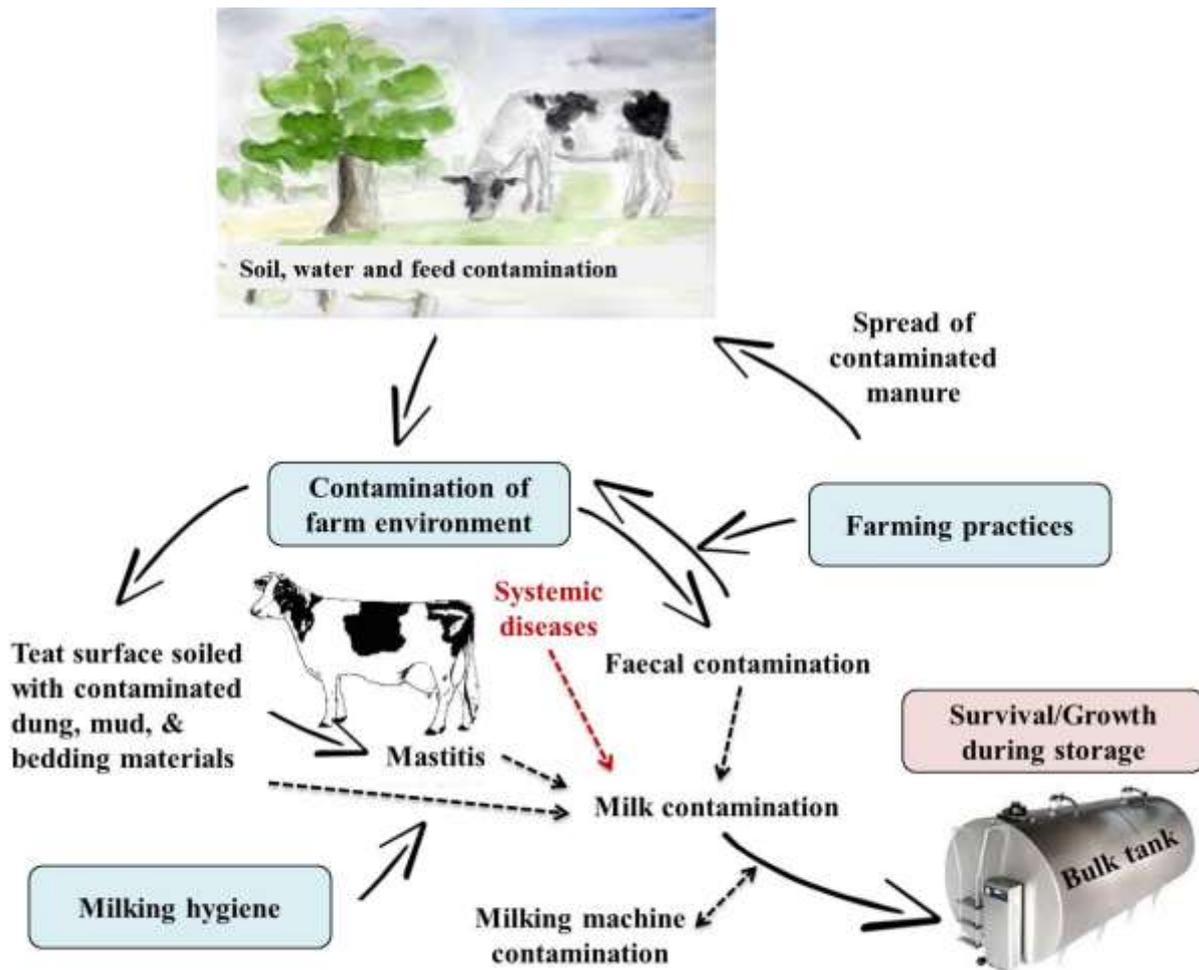
Figure 8: Schematic diagram to show potential on-farm sources of contamination with microbiological hazards associated with the production of raw drinking milk. The solid arrows are considered to be the main source of contamination.

Intrinsic contamination can arise from contamination of the milk through systemic disease in the animal which can result in the hazard being secreted in the milk. In general, unless there is an intramammary infection or an animal has a systemic disease, milk in the mammary gland at the site of its production should not contain microorganisms or their toxins. As milk is drawn from the udder, contamination can occur from the udder surface during milking or from milking equipment (Cousins and Bramley 1981; Castle, 1985; FAO, 1989). This contamination could be of human or animal origin or from soil or faecal microorganisms that contaminate the surface of the udder and, in particular, the teat skin surface or the epithelial lining of the teat canal which is the duct that conveys the milk from the mammary gland to the teat orifice (Castle, 1985).

Pathogens present in milk can originate from clinically healthy animals or from environmental contamination occurring during collection and storage of milk. Although steps can be taken to minimise the occurrence of pathogens and spoilage organisms in raw milk is not possible to eliminate them completely. At the same time, a poor level of animal health or hygiene control can potentially lead to an increase in risk from RDM. The following sections consider the impact of on-farm hygiene controls and distribution retail and storage on the main microbiological hazards associated with RDM and highlighting areas where risk may reduce or increase. Nada et al. (2012) observed that an improvement in the quality of raw milk from cows through implementation of GAP on farms with respect to a range of factors including feeding, animal health and welfare, sanitation, milking and maintenance of equipment and the cooling and storage and transport of raw milk. Piepers et al. (2014)

found management practices influence bacteria counts and coliform counts in raw milk but that information relating to milking, animal health and dry cow management suggest that there are other unidentified factors which may also be important in contamination of the milk. Jorritsma and Hofste (2011) found that less hygienic on-farm practices were risk factors for persistent presence of salmonella antibodies in bulk tank milk in the Netherlands.

6.1. On-farm hygiene controls

6.1.1. Prevention or eradication of systematic infectious disease

Whilst microorganisms should largely be absent in milk from healthy animals at the time of secretion, for certain systemic diseases infectious agents can be localised in the mammary gland or associated lymph nodes and consequently can be present in the milk. Bovine tuberculosis (bTB) and brucellosis are classic examples of zoonotic milk-borne diseases. The control of these diseases is complex and multifactorial, but, at a minimum, the requirements prescribed for obtaining the status of officially free from the disease have to be complied with in order to control the risk of transmission of such diseases through the consumption of raw milk. Other zoonotic infections, such as *Salmonella* spp., can also lead to systemic infectious disease with the potential to be transmitted to humans through milk or milk products.

6.1.1.1. Brucellosis

The control of brucellosis is based on surveillance in herds using serological tests, as well as milk screening tests such as the milk ring test which plays an important role in efforts to eliminate the disease. Individual animal testing, both for trade and disease control purposes, is also practised. In endemic areas, vaccination is often used to reduce the incidence of infection whereas in officially free areas, vaccination is not permitted.

6.1.1.2. Tuberculosis due to *Mycobacterium bovis*

Considerable efforts towards control and eradication of bTB have been made at both national and European levels for decades (e.g. 59 % of the farms were bTB positive in Germany in 1952 (Meyn, 1952) and one out of four herds was infected in France in 1954 (ANSES, 2011)). The control measures put in place by national and European regulations were triggered by the zoonotic character of the pathogen, economic losses in affected farms and the need to develop safer trade across Europe. Despite these efforts, control and eradication of bTB remains a challenge in several European regions or countries. This is, in particular, because of the complex interactions between the pathogen, the hosts and the local environment. As a result, the effect of individual intervention measures may not always be predictable; similar control measures as laid down by EU legislation may result in different outcomes if applied to different epidemiological situations.

6.1.1.3. Tick-borne encephalitis

TBEV is the most common tick-transmitted disease in Central and Eastern Europe and Russia (Rieille et al., 2014). This zoonotic disease is endemic to a wide area, from Alsace-Lorraine and Scandinavia to northeast China and northern Japan. Several animals, principally small rodents, deer, sheep and goats, act as these ticks' source of infection. There is no systematic control programme for TBEV in goats applied in Europe, and the control measures should be based on protecting the animals against tick bites through the use of suitable repellents. There are a variety of strategies for control of ticks and tick-borne diseases. Treatment with synthetic chemicals known as acaricides, including ixodicides, still provide the most widely used means to control hard ticks with the aim of preventing pathogen transmission. Control of ticks with acaricides can either be directed against the ticks on the host or against the free-living stages of ticks in the environment.

Impact of step

Eradicating systemic diseases solve only a fraction of the problem since the milk can be contaminated by one of the main identified hazards from the environment without observing infections or clinical

signs in milking animals. However, there is a potential for the risk to increase if systemic infection and the appropriate management regimes of control it are not fully implemented. This is likely to apply to all milk-producing animals to a varying degree depending on the pathogen, whether the infection can affect more than one species and the size and scale of the operation.

6.1.2. Mastitis control programmes

Mastitis-causing organisms, of which *Staphylococcus* and *Streptococcus* spp. are predominant (Lejeune and Rajala-Schultz, 2009), and of which some could be pathogenic for humans, can also be excreted into the milk. Among these pathogens are *Salmonella* spp. (rarely), *B. abortus* (rarely), *L. monocytogenes*, *C. pseudotuberculosis* (rarely), *Y. pseudotuberculosis*, enterotoxin-producing *S. aureus*, and *S. equi* subsp. *zooepidemicus* (Barrett, 1986; Edwards et al., 1988; Francis et al., 1993; Bleul et al., 2002; Shwimmer et al., 2007; Lejeune and Rajala-Schultz, 2009). The milk produced by animals with subclinical mastitis is not noticeably different from the milk produced by uninfected animals and frequently such milk is added to the collection or storage tank on a farm. Milk from cows with clinical mastitis, typically has an altered appearance (i.e. it may content flakes, clots or blood, or may have changed colour) and is withheld from human consumption (Barrett, 1986; Edwards et al., 1988; Francis et al., 1993; Bleul et al., 2002; Shwimmer et al., 2007; Lejeune and Rajala-Schultz, 2009).

Mastitis is considered to be one of the most common diseases in dairy cattle (Halasa et al., 2007). The vast majority of intramammary infections results from bacteria gaining entry to the mammary gland through the teat canal. Whether a quarter becomes infected depends on the level of exposure to pathogens and the efficiency of the bovine defence mechanism. Besides the systemic defence of the cow, the condition of the teat is of paramount importance in the defence against infections as the teat acts as primary defence mechanism (Elias, 2007). Proper milking procedures, proper maintenance and use of milking equipment, and the maintenance of a clean, dry and comfortable environment are three key factors in the holistic approach to prevent and control mastitis and achieve optimal milk quality. Proper milking procedures include, among others, wearing gloves during milking, sufficient pre-stimulation of the udder, avoidance of over milking, post-milking teat dipping, post-milking standing time and milking cows with mastitis last (Plozza et al., 2011; Sandrucci et al., 2007; Watters et al., 2013). Proper maintenance and use of milking equipment covers the settings of the vacuum level, the pulsator rate, the pulsator ratio and yearly inspection of these settings, good fit between teat and teat cup liner, in time renewal of liners and other equipment (Schmidt et al., 1963; Osteras and Lund, 1988; Barkema et al., 1999).

In addition to ensuring an optimal milking technique and a good quality cow environment, other management factor also play an important role, e.g. good record keeping, appropriate management of clinical mastitis during lactation, effective dry cow management, culling of chronically infected cows, regular monitoring of udder health, status, periodic review of the mastitis control programme (Eberhart, 1986; Green et al., 2007). The main reasons for the use of anti-infectious agents in dairy cattle are the control of bovine mastitis and dry cow therapy. Cows with clinical mastitis are, according to normal practice, treated with intramammary preparations.

Somatic cell counts can be used as a monitoring tool for measuring intramammary infections and milk quality at cow and herd level (Schukken et al., 2003). Monitoring somatic cell counts at herd level requires longitudinal data over time, establishing a threshold value above which further investigation and action is required in relation to the udder health of cows. This threshold should be based on mean somatic cell counts obtained for the herd. Typically, a threshold between 200 000 to 250 000 somatic cells/mL raw milk is obtained. This method has limitations since clinical mastitis may not always lead to an increased somatic cell count (reviewed by Schukken et al., 2003). Therefore, care has to be taken in interpreting the results.

Impact of step

No additional risk reductions steps are anticipated over and above the expected application of animal health controls, GAP and GHP, but potential for the risk to increase if infection, external contamination and cleaning and disinfection regimes are not well implemented or controlled. This is likely to apply to all milk-producing animals, but will vary depending on the size and scale of the operation including the degree of automation.

6.1.3. Reduce shedding of pathogens

There are a number of infections that may be present in animals and remain completely asymptomatic yet have potentially serious public health implications. Healthy animals can harbour human enteric pathogens such as pathogenic *E. coli*, *Salmonella* spp., *Cryptosporidium* spp., *L. monocytogenes* and *Campylobacter* spp. Although reports often document cattle, sheep or goats as reservoirs, live poultry, rodents, wild birds and other domestic and wild animals can also be potential sources of these organisms. The widespread occurrence of such pathogens, their persistence in environmental sources, their ability to infect or re-infect milk-producing animals, as well as their wide host range, including wildlife, makes complete eradication of such pathogens an unrealistic objective. One practical option is to reduce the level and/or the prevalence of pathogens in faecal shedding. This may be achieved by limiting farm-to-farm spread of pathogenic organisms, animal to animal spread within a farm or proliferation within an individual animal. In theory, possible interventions can be grouped into three general categories:

- exposure reduction interventions: related to water quality, feed hygiene, environmental exposure, animal density, wildlife exclusion;

- exclusion interventions: related to feed component management that may modify the competitiveness or survival of pathogens in the gastrointestinal tract, use of probiotics or prebiotics;

- direct interventions targeting the pathogen: specifically target and kill pathogenic bacteria through the use of, for example, bacteriophages or vaccination.

For STEC O157, different interventions are expected to decrease prevalence and concentration in faeces. In their systematic review on STEC O157, Sargeant et al. (2007) evaluated interventions including application of probiotics, vaccination, antimicrobials, sodium chlorate, bacteriophages and other feed additives. There was evidence of efficacy for the probiotic combination *Lactobacillus acidophilus* NP51 (NPC 747) and *Propionibacterium freudenreichii* and for sodium chlorate in feed or water. The effectiveness of vaccination varied among studies and among vaccine protocols and there was no consistent evidence to suggest that antibiotic use was associated with a decrease in faecal shedding of STEC O157, or that the current use of antimicrobials was associated with increased faecal shedding. There were an insufficient number of studies available to assess the effectiveness of bacteriophages and several other feed additives. While the results suggest that some interventions may be efficacious, there are knowledge gaps in understanding the efficacy of pre-harvest interventions for STEC O157 that require further targeted research.

Salmonella spp. is commonly found in dairy cattle populations and salmonellosis often occurs close to parturition linked to concurrent disease, dietary stress and the natural depression of immunity at this time of the cow's cycle. Decreased dietary intake influences the growth of ingested salmonellae in the rumen with acidity from high concentrations of volatile fatty acids inhibiting the growth of *Salmonella* spp. Feeding after starvation causes salmonellae to multiply and a few infected cows can result in substantial environmental contamination. *Salmonella* spp. can persist in cattle in a subclinical form, often for extended periods of time (Cobbald et al., 2006). Where interventions are implemented, these should be prioritised and focused on minimising the source of infection and maximising host immunity. To reduce persistence of *Salmonella* spp. on the farm, disinfection procedures and infection control must be developed to address bedding material, feed and feed refusals. Equipment and personnel who handle cattle with clinical illness should also be targets for disease-control measures.

Campylobacter spp. are common in cattle and these organisms have been reported from a wide range of other milk-producing animals. Studies in Europe and elsewhere have examined the prevalence and faecal shedding of *Campylobacter* spp. (De Rycke et al., 1986; Hakkinen and Hanninen, 2009; Klein et al., 2013; Ramonaitė et al., 2013). In the USA, Wesley et al. (2000) detected *C. jejuni* and *C. coli* in the faeces of healthy dairy cows using polymerase chain reaction (PCR). Faecal samples from cows from 80.6 % of farm operations (n = 31) and 37.7 % of individual dairy cattle (n = 2 085) were positive for *C. jejuni*. *C. coli* was detected in 19.4 % of farm operations and 1.8 % of individual cows (n = 2 085). Farm management factors were correlated with prevalence in herds in which > 25 % of cows were positive for *C. jejuni*. Application of manure with broadcast spreaders, feeding of whole cottonseed or hulls or alfalfa and accessibility of feed to birds were identified as possible risk factors for *C. jejuni* infection. Stanley and Jones (2003) observed that *Campylobacter* colonisation and shedding rates were higher among young animals with the shedding of *Campylobacter* spp. in adult animals appearing to be seasonal. Stored and land-dispersed slurries were identified as a reservoir for scavenging birds and flies and a source for runoff. Sproston et al. (2011) examined the temporal variation and host association in the *Campylobacter* population in a longitudinal farm study in Scotland involving cattle and sheep. The average *Campylobacter* concentrations shed by cattle (600 CFU/g) were similar to concentrations shed by sheep (820 CFU/g) in their faeces. In New Zealand, Rapp et al. (2012) found significant variation between individual dairy cows in *C. jejuni* faecal concentration with the median concentrations for carriage in 35 dairy cows varying by up to 3.6 $\log_{10}$ per gram of faeces.

Animals with clinical and subclinical listeriosis infection can excrete *L. monocytogenes* through faeces. Moreover, *L. monocytogenes* from contaminated feed (such as silage) may pass through an animal's digestive tract without causing infection. The prevalence of *L. monocytogenes* faecal shedding in cattle varies considerably over time, from 0 to 100 %, and can be associated with contamination of silage with the bacteria. *L. monocytogenes* faecal shedding in cattle can occur as a group of cases or as an isolated sporadic case. *L. monocytogenes* subtypes associated with human infections are commonly isolated from cattle faeces and silage and a single cow can harbour more than one *L. monocytogenes* subtype on any given day (Husu, 1990; Sanaa et al., 1996; Ho et al., 2007). The risk of raw milk contamination by *L. monocytogenes* from faecal contamination increases during housing of animals when the number of faecal shedders is highest because cows are grouped together and fed silage (Sanaa et al., 1993; Sanaa et al., 1996). *L. monocytogenes* can be present in considerable numbers in poor quality silage and pockets of silage that spoil and do not develop a pH of around 4 may permit growth of *L. monocytogenes*. Contamination of silage in silos appears to be highest at the edges and within some samples; concentrations of exceeding 10^6 viable *Listeria*/g of silage can be found (Sanaa et al., 1993, 1996).

Impact of step

No additional risk reductions steps are anticipated over and above the expected application of GAP and GHP but there is potential for the risk to increase if external contamination and cleaning and disinfection regimes are not well controlled. This is likely to apply to all milk-producing animals but will vary depending on the size and scale of the operation including the degree of automation.

6.1.4. Housing management

Considering the current milking techniques, even with optimal and time-consuming cleaning methods, milk contamination cannot entirely be prevented (Magnusson et al., 2006). Therefore, hygienic management practices to reduce contamination with pathogens in the cow's environment e.g. a clean and comfortable cubicle with good quality and quantity of bedding material are a prerequisite for udder health and optimal milk quality (Osteras and Lund, 1988; Barkema et al., 1998; Elbers et al., 1998; Schukken et al., 1990; van Gastelen et al., 2011).

Housing management can impact on cleanliness of livestock including milk-producing animals and attention to good building design layout and construction are important considerations in cleanliness and udder health (Castle and Watkins 1984; Castle 1985). There will be less opportunity for

contamination of the milk if the animals are clean and with clean teats. Organic particles from bedding materials used for housing animals can adhere to the teat surface and these may have a high counts of psychrotrophic bacteria, coliforms and *Bacillus* spores even when the bedding appears clean and dry (Cousins and Bramley, 1981).

A cleanliness score for cows was found to be related to the occurrence of *L. monocytogenes* in milk (Sanaa et al., 1993). A farm classified as dirty (based on the observed average score of cleanliness) was almost six times more likely to have milk contaminated with *L. monocytogenes* than a farm classified as clean (Sanaa et al., 1993). The score of cleanliness is related to the frequency of cleaning the exercise area and the removal and quantity of bedding material in the bedding area. Insufficient hygiene in cow housing increases environmental exposure to pathogens including *L. monocytogenes*, and constitutes an important environmental factor impacting on the incidence of *L. monocytogenes* in milk. In a case–control study, cleanliness of the cows and adequate frequency of cleaning the exercise area were observed as strong risk factors for milk contamination (the odds ratios were, respectively, 5.9 and 3.9 (Sanaa et al., 1993)). The relationship between housing management and milk contamination observed with *L. monocytogenes* could be extrapolated to other pathogens found in the farm environment.

Impact of step

No additional risk reductions steps are anticipated over and above the expected application of GAP and GHP but potential for the risk to increase if external contamination and cleaning and disinfection regimes are not well controlled. This is likely to apply to all milk-producing animals but will vary depending on the size and scale age and design of the operation including the degree of automation.

6.1.5. Milking hygiene

At the farm level, microbial contamination of bulk tank milk occurs from three main sources: bacterial contamination from the external surface of the udder and teats, from the surface of the milking equipment and from mastitis organisms from within the udder (Murphy and Boor, 2000). Pre-milking udder hygiene (e.g. washing with clean water and drying using single use hand towels) reduces milk contamination by bacteria present on the udder. The process of drawing and disposing of foremilk removes microorganisms which may have entered the teat canal.

Good milking hygiene is essential for udder health of cows and for the production of good quality raw milk. Dirty teats and udders are possible sources of bacteria and poor udder preparation prior to milking can increase the numbers of bacteria in the milk. Pre-milking teat disinfection has been associated with a reduction in the total aerobic count and coliform count (Galton et al., 1986; Pankey, 1989; Piepers et al., 2014) and total bacteria and anaerobic spore counts (Rasmussen et al., 1991). Schreiner and Ruegg (2003) reported that dirty cows were 1.5 times more likely to be infected with a major mastitis pathogen than clean cows.

Current recommended procedures for pre-milking udder preparation range from use of a water hose wash, manual drying, wet paper towel wash plus paper towel dry, to pre-dipping alone plus paper towel dry. Elmoslemany et al. (2010) explored the association between herd management practices and bacterial levels in raw milk using longitudinal data collected from all Prince Edward Island dairy herds over a period of two years. In their study, they considered pre-milking udder preparation with various combinations of pre-dip, washing and drying using single or multiple towels.

Pre-dipping followed by drying the teats with a single-use towel was associated with the lowest bacterial counts than other methods of teat preparation. Pre-dipping the teats with an approved disinfectant is considered the most effective way of teat decontamination, and drying of the teats before milking is considered the most important step in a teat-cleaning regime. Using water to wash the teats without drying was associated with an elevated total aerobic plate count. Water contaminated with bacteria on the udder and teat surfaces can enter the teat cup liners and increase bacterial contamination of the milk (Galton et al., 1982). Higher bacterial counts were observed when the same

towel was used for drying multiple cows after washing than when a single towel was used for each cow. Sharing the same towel between cows can increase the risk of transmission of mastitis pathogens among animals and it reduces the efficiency of drying the teats. The efficiency of a commercial disinfectant towel in reducing the total aerobic plate count was related to the method of use. When used alone, it was associated with the highest bacterial counts. When followed by drying, the effect was no different to pre-dipping and drying. These results indicate that the use of a medicated towel alone does not adequately destroy or remove bacteria from the teats. In addition, these results indicate that manual drying is an important step in reducing the bacterial load on the teats. The effect of manual drying may be related to physical action on the teat surface and scrubbing of the teat ends (Rasmussen et al., 1991).

Zucali et al. (2011) investigated the effects of season, cow cleanliness and milking routine on bacteria and somatic cell count of bulk tank milk in intensive dairy farms in Lombardy, Italy. The microbiological quality of milk was influenced by cow cleanliness. Milking operation routine influenced bacterial counts of bulk tank milk. Farms that had a comprehensive milking scheme including two or more operations among fore-stripping, pre-dipping and post-dipping, had lower contamination of teats prior to cluster attachment and lower bacterial counts in bulk tank milk than farms that carried out one operation or none at all. The authors concluded that implementing and maintaining a few simple hygienic practices in terms of barn cleaning and milking procedures (fore-stripping, pre-dipping and post-dipping) can significantly improve the microbiological quality of cow's milk under intensive farming conditions in northern Italy where animals are kept in the barn all year.

Piepers et al. (2014) examined risk factors associated with bacterial and coliform counts in raw bulk milk in Flemish dairy herds. A decrease in bacterial counts was related to a number of factors including increased frequency of cubicle cleaning, use of an automatic cluster removal system in the milking parlour and when pre-milking teat disinfection was used. Nagy et al. (2013) observed a reduction in total viable count in camel milk following the reintroduction of pre-milking disinfectant wipes for pre-milking teat preparation (Nagy et al., 2013).

Impact of step

No additional risk reduction steps are anticipated over and above the expected application of GAP and GHP but there is potential for risk to increase if external contamination and cleaning and disinfection regimes are not well controlled. This is likely to apply to all milk-producing animals but will vary depending on the size and scale of the operation, including the degree of automation.

6.1.6.   Cleaning of milking machines

Bacteria require food, moisture and a favourable temperature to multiply. Milk and milk residues provide all of these requirements which mean that the milking machine is a potential source of bacteria for contamination of the milk (Cousins and Bramley, 1981). Water and water quality are important in ensuring effective cleaning as part of a cleaning programme as well as avoiding the introduction of extrinsic bacterial contamination.

The surfaces of milking and cooling equipment which are in contact with milk can be an important source of milk contamination as there may be a build-up of milk residues associated with the equipment (Castle and Watkins, 1984). Cleaning and disinfection procedures (e.g. circulation cleaning, acidified boiling water) can effectively decrease bacterial contamination of milk from this source although failures can occur and this can result in pathogenic bacteria being present on the milking equipment surface. The more frequent failures come from the use of inadequate water temperatures, over-dilution of detergent sanitisers, inadequate contact time with soiled surfaces and tubes being in poor condition. Rinses and swabs of milking equipment have shown that lower bacterial contamination is found where recommended methods of cleaning and disinfection are followed and high rinse counts could be related to poor condition of equipment and build-up of milk residues

(Cousins and Bramley, 1981). Maintenance of equipment is important particularly for teat-cup liners, milk tubes, jointing sleeves and gaskets (Castle, 1985).

Effective cleaning relies on the four key elements of a cleaning routine to be working effectively: thermal energy, chemical energy, kinetic energy and time. Daily cleaning procedure consists of three stages: a rinse with cold or tepid water (38 °C), a warm detergent wash and a final rinse with clean water. Because of milking machine design, cleaning and disinfection of milk contact surfaces may not be fully effective which can result in milk residues and bacteria not being completely removed from the equipment and these can accumulate between milking. Efficient monitoring of the efficacy of cleaning and disinfection procedures can be obtained by rinsing the equipment with a sterilised liquid followed by microbiological analysis (Cousins and Bramley, 1981).

Impact of step

No additional risk reductions steps are anticipated over and above the expected application of animal health controls, GAP and GHP, but potential for the risk to increase if external contamination and cleaning and disinfection regimes are not well controlled. This is likely to apply to all milk-producing animals but will vary depending on the size and scale of the operation.

6.2. Post milking storage on-farm

The bulk milk tanks is the key point of storage of raw milk on the farm and attention to hygiene and temperature control is critical because of the large volume of milk being stored which may derive from one of more milking. Most farm tanks have smooth stainless steel surfaces which are easier to clean than milking machines although other parts of the tank (e.g. valves, gaskets, agitator, dipstick) have been associated with contamination problems (Cousins and Bramley, 1981; Castle, 1985).

Cleaning of bulk tanks is important particularly to prevent the build-up of deposits on surfaces in contact with the milk. Cleaning may be a three-stage process utilising a cold water rinse, a cold or warm water spray with disinfectant and a subsequent cold water rinse. Because hot water is suitable for use on refrigerated tank walls, they may be cleaned with mechanical or hand sprays using cold disinfectant solutions or by manual brushing (Castle and Watkins, 1984; Castle, 1985). Potential areas for contamination are outlet ports and valves which may superficially appear clean but can act as sites for the build-up of bacteria. Panes et al. (1979) found that 9 % of bulk tank milk outlet plugs in the UK had TBCs of $> 1 \times 10^9$ CFU per plug with such sites being difficult to clean.

Impact of step

No additional risk reductions steps are anticipated over and above the expected application of GHP but there is potential for risk to increase if storage temperature, external contamination and cleaning and disinfection regimes are not well controlled. This is likely to apply to all milk-producing animals.

6.3. Distribution, retail and storage

There are many routes by which consumers may purchase RDM ranging from farm gate sales, farmers markets, vending machines, through home delivery and ordering directly from the farm. Such milk may be supplied as fresh or frozen and in small or large volumes. Producers may supply pre-packaged product or in some cases consumers may provide their own containers for the milk.

The temperature profile and duration of storage once RDM has been bottled and left the control of the producing farm are likely to be important if pathogens are present. Such studies are important to understand the impact on microbial contaminants which may be present in the milk and may represent a risk to human health.

In the case of vending machines, dispensing may be prevented if the milk temperature is greater than 4 °C or if the machine does not perform the cleaning operations. Particular attention should focus on preventing possible environmental contamination during and after supplying raw milk. To ensure good

hygiene in the filling area, different cleaning solutions can be applied: ultraviolet (UV)-based cleaning system, disinfectant washing system, vaporised cold water washing system, vaporised hot water washing system. Moreover, the nozzle can be concealed and projected with a system for eliminating foam during distribution or to prevent dropping.

6.3.1. Influence of storage conditions/time on hazards prior to consumption

RDM has a high water activity ($a_w > 0.99$) and near neutral pH (pH 6–7) (Chen et al., 2014) and temperature control is important in shelf-life and maintaining microbiological stability. Raw milk can be contaminated by a diverse range of microorganisms arising from intrinsic contamination of the milk from the animal or extrinsic contamination from the udder, milking equipment as well as the wider farm and, if there is poor handling, the distribution, retail and consumer environment (Cousins and Bramley, 1981; Vacheyrou et al., 2011; Mallet et al., 2012).

With respect to the presence of pathogens in raw milk from cows, no statistically based European prevalence data are available. Studies of the frequencies of occurrence of pathogens in raw milk that have been published in the international scientific literature and an overview is presented in Appendix C, summarising data for selected pathogens for European countries (raw milk from cows) and for the world (raw milk from sheep, goats, donkeys and horses). These frequencies can vary according to the sampling and methodological approaches used and when the studies were undertaken which spans several decades. Variation might also be explained by geographical differences, the season in which the samples were taken, the size of the farm, the density of the animal population and regional differences in farm management (Verraes et al., 2014).

Very few studies have reported levels of pathogens present in raw milk, although there are indications that the levels present are usually low. Humphrey and Beckett (1987) determined *C. jejuni* levels of 16 ± 30 CFU/mL in nine of 111 bulk tank milk samples analysed. In other studies, levels of *L. monocytogenes* ranged from less than 1 to 60 CFU/mL and less than 1 CFU/mL for STEC O157:H7 (Waak et al., 2002; D'Amico et al., 2008). Van Kessel et al. (2004) found levels of *Salmonella* spp. and *L. monocytogenes* in 22 (*Salmonella* spp.) and 56 (*L. monocytogenes*) positive samples from 861 bulk tank samples tested at levels of 1 to 40 CFU/10 mL. Meyer-Broseta et al. (2003) developed a simulation model which showed that, when contamination of milk occurs, the concentration of *L. monocytogenes* in the bulk tank is usually less than 3 CFU/mL. Although not intended for drinking as raw milk, Jackson et al. (2012a) in the USA determined the prevalence rates and levels of presumptive *B. cereus*, STEC O157:H7, *L. monocytogenes* and *Salmonella* spp. in samples of commingled milk destined for pasteurisation. *B. cereus* was detected at 3.0 to 93 CFU/mL. *E. coli* O157:H7 at < 0.0055 to 1.1 CFU/mL. *Salmonella* spp. at < 0.0055 to 60 CFU/mL and *L. monocytogenes* at < 0.0055 to 30 CFU/mL.

The microbiological hazards present in raw milk will differ with respect to their growth potential, primarily because of temperature but also in relation to the presence and growth of other microorganisms present in the milk, as well as intrinsic antimicrobial components (Claeys et al., 2014). Bacterial multiplication is usually limited for at least 24 hours if milk is collected, cooled and stored at $< 4\,°C$ (Cousins and Bramley, 1981). Psychrotrophic bacterial pathogens such as *L. monocytogenes* and *Yersinia* spp. and certain strains of *B. cereus* may be capable of multiplying at these temperatures (Najdenski et al., 2012) whereas other bacteria require higher temperatures in order to grow, highlighting the importance of good temperature control for raw milk. *S. aureus* will multiply slowly in milk at 7 °C with enterotoxin production reported to only occur at 10 °C (Schmitt et al., 1990). Other pathogens (*B. melitensis*, *M. bovis* and *S. equi* subsp. *zooepidemicus*) are unlikely to be capable of growth under chill storage conditions. *Campylobacter* spp. (*C. jejuni*, *C. coli*) do not multiply below 30 °C and numbers have been reported to decline during storage of raw milk (Doyle and Roman, 1982). In the case of parasites and viruses, multiplication will not occur, so the consideration is whether the levels and/or infectivity will decline during storage. Tachyzoites of *T. gondii* have been reported to survive for seven days at 4 °C in milk from cows (ACMSF, 2013).

RDM is sometimes marketed as a frozen product. This will prevent multiplication of bacterial hazards as well as parasites and viruses and will impact on viability/infectivity to a varying extent depending on the hazard. Bacteria are known to vary in their response to freezing, with *C. jejuni* appearing to be more sensitive than *E. coli* O157:H7 (Lu et al., 2011). There is less information available for parasites and viruses, although in the case of *T. gondii*, the tachyzoite stage, which can potentially be present in milk, is reported to be susceptible to freezing (ACMSF, 2013). Freezing is unlikely to completely eliminate microbiological hazards in raw milk and the method of thawing and subsequent storage and handling are important considerations if multiplication of surviving bacterial hazards is to be prevented.

<u>Impact of step</u>

No additional risk reductions steps are anticipated over and above the expected from the application of GHP but there is potential for an increase in risk with respect to certain pathogens depending on storage temperature and duration. This is likely to apply to all milk-producing animals. Freezing milk is likely to reduce the levels of some pathogens (e.g. *Campylobacter*) but not others and this will not ensure the elimination of such hazards prior to consumption.

6.4. Concluding remarks

- The steps in the production to consumption chain for RDM present many opportunities for contamination by microorganisms, some of which may be transmissible to humans.

- Intrinsic contamination of milk can arise from systemic infection in the milk-producing animal as well as from localised infections such as mastitis. Extrinsic contamination of milk can arise from faecal contamination and from the wider farm environment associated with collection and storage of milk.

- Observance of good animal health and husbandry together with the application of GAPs and GHPs are essential to minimise opportunities for contamination of RDM with pathogens in the production to consumption chain for RDM.

- No single step could be identified which would provide a significant reduction in risk relative to a baseline of expected good animal health and welfare and GAPs and GHPs. Therefore, it was not possible to rank control options with respect to risk reduction and any deviations from the expected "best practice" baseline are likely to result in an increase in risk.

- The reviewed QMRA models identified on-farm hygiene control and maintenance of the cold chain as factors influencing the outcome of the models for some pathogens.

- Despite the general limitations in the reviewed QMRAs, they do show that the risk associated with *L. monocytogenes* in raw cow's milk can be mitigated and reduced significantly if the cold chain is well controlled, the shelf-life of raw milk is limited to a few days and there is consumer compliance with these measures/controls.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

TOR 1. Identify the main microbiological hazards of public health significance that may occur in raw drinking milk from different animal species

- A top-down decision tree approach was used to identify microbiological hazards associated with raw drinking milk (RDM) from cows, sheep and goats, horses and donkeys, and camels, which are the main milk-producing species in the European Union (EU).

- Microbiological hazards reported in the reviewed published literature as potentially associated with milk-producing animals were identified and listed. Those hazard/milk combinations for which there was no evidence of transmission via the consumption of milk or for which there

was evidence of transmission but not for presence in milk-producing animals in the EU were excluded from further specific consideration.

- Microbiological hazards identified as potentially transmissible through milk and present in the EU milk-producing animal population were the bacteria *Bacillus cereus*, *Brucella abortus*, *Brucella melitensis Campylobacter* spp. (thermophilic), *Corynebacterium* spp., *Listeria monocytogenes*, *Mycobacterium bovis*, *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus*, shigatoxin-producing *Escherichia coli* (STEC), *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, the parasites *Cryptosporidium parvum* and *Toxoplasma gondii*, and the virus tick-borne encephalitis virus (TBEV).

- Fifteen hazards were associated with cows, eight with sheep and goats, 11 with horses and donkeys and two with camels. This may, in part, reflect the greater volume of cow's milk consumed relative to milk of other species.

- Hazards were further categorised based on the assessment of the following: (i) the magnitude of human health impact based on incidence of confirmed human cases reported to ECDC, (ii) the severity of the disease in humans based on fatalities, and (iii) evidence that RDM is an important risk factor for the disease in humans in the EU.

- The main microbiological hazards identified as relevant in the EU were *B. melitensis*, *Campylobacter* spp., *M. bovis*, *Salmonella* spp., STEC and TBEV. Of these, *Campylobacter* spp., *Salmonella* spp. and STEC were considered to be more widely distributed in the EU than the other hazards and *Campylobacter* spp. were the leading cause of outbreaks.

- Based on the limited data available and expert opinion, microbiological hazards which are not regarded as main hazards with respect to raw milk consumption in the EU were *B. cereus*, *B. abortus*, *Corynebacterium* spp., *L. monocytogenes*, *S. aureus*, *S. equi* subsp. *zooepidemicus*, *Y. enterocolitica*, *Y. pseudotuberculosis*, *C. parvum* and *T. gondii*.

TOR 2. Assess the public health risk arising from the consumption of raw drinking milk

- Of the 27 outbreaks occurring in the EU between 2007 and 2012, and reported to EFSA, where there was strong evidence of an association with consuming RDM, 21 were attributed to *Campylobacter* spp., one to *Salmonella* Typhimurium, two to STEC and three to TBEV. Raw milk from goats was associated with four of the 27 outbreaks and the remainder were linked to milk from cows. In addition, other reports involving TBEV, *B. melitensis*, *M. bovis* and STEC in raw milk in the EU were identified in the literature.

- There is a clear link between drinking raw milk and human illness with *Campylobacter* spp., *S.* Typhimurium, STEC, TBEV, *B. melitensis* and *M. bovis*, with the potential for severe health consequences in some individual patients. Owing to the lack of epidemiological data, the burden of disease linked to the consumption of raw milk could not be assessed.

- Published quantitative microbiological risk assessment (QMRA) models from Australia, New Zealand, the USA and Italy, for *Salmonella* spp., *Campylobacter* spp., STEC O157 and *L. monocytogenes* in cow's RDM were reviewed to identify their strengths and limitations. No QMRAs were available for RDM of other species.

- There were marked differences in the estimates of public health risk for the same hazards in the reviewed QMRAs, with some estimates differing by several orders of magnitude.

- Owing to important data gaps, model uncertainties and the broad origin of data sources used, risk estimates provided by the QMRA models described in the reviewed articles cannot be extrapolated to the European situation as a whole.

- From the model used in the Australian study, it can be concluded that improving on-farm hygiene leads to a decrease in the number of predicted cases of illness attributed to *Campylobacter* spp., *Salmonella* spp. and STEC O157 from the consumption of RDM.

- A QMRA could have helped in further estimating the public health risks and evaluating the effect of the mitigation options in Europe for these hazards, but could not be undertaken because of limited country and EU-wide data.

TOR 3. Assess the likelihood of raw drinking milk being a significant source of antimicrobial-resistant bacteria/resistance genes

- Antimicrobial resistance has been reported in several EU countries in isolates of *Campylobacter* spp., *Salmonella* spp., STEC and *S. aureus* from raw milk or associated equipment such as milk filters and may be significant for public health. Such isolates have primarily been associated with raw milk from bovine animals, which may reflect the more limited screening of milk from other species.

- Strains of *Campylobacter* spp., and particularly *C. jejuni*, exhibiting resistance predominantly to tetracyclines but also to some other antimicrobials have been reported in two Member States (MS), but such strains are not known to have caused human infections linked to consumption of raw milk.

- There have been no reports of antimicrobial resistance in isolates of *Salmonella* spp. from outbreaks associated with raw/unpasteurised milk in the EU in countries other than the UK. In the USA, there has been a report of a raw milk-associated outbreak caused by multi-drug resistant (MDR) *S.* Typhimurium, with a single fatality ascribed to resistance of the organism to antibiotics.

- Despite STEC O157 being the organism most commonly associated with RDM-related outbreaks of STEC gastrointestinal illness in several EU countries, little information is available about the occurrence of antimicrobial resistance in such outbreak strains. Antimicrobial resistance has been reported in a water buffalo raw milk-associated STEC O26 outbreak in one MS in 2008 and in raw milk-associated STEC outbreaks in the USA.

- Antimicrobial resistance in isolates of *L. monocytogenes* from raw milk and raw milk dairy products has only rarely been reported in EU countries.

- Meticillin-resistant *Staphylococcus aureus* (MRSA) has not been isolated from outbreaks of infection associated with raw milk in EU countries. Although not typically regarded as a food-borne pathogen, there have been increasing reports of the isolation of MRSA from dairy farms and bulk tank milk in several EU MS.

- Although identified in *E. coli* in bovine animals in some MS, ESBL/*Amp*C gene-carrying bacteria have not been reported in raw milk in EU MS. In the USA, a range of *Salmonella* serovars with ESBL/*Amp*C genes have been identified in raw milk surveys.

- There are only very limited data on the occurrence of antimicrobial resistance in raw milk-associated pathogens from non-bovine animals in the EU.

TOR 4. Assess the additional risks associated with the sale of raw drinking milk through vending machines and via the internet

- Sale of RDM through vending machines is permitted by some EU MS, with Italy having the highest number of these machines. There is little indication of milk from species other than cows being sold through vending machines.

- Although vending machine milk is dispensed in a raw state, consumers are usually instructed to boil the milk prior to consumption. If consumers comply with these instructions, the microbiological risks associated with raw milk would be eliminated.

- The temperature of raw milk in vending machines is expected to be kept below 4 °C, and variability in milk temperature is more likely to arise between the farm and vending machine and between the vending machine and point of consumption.

- Fresh and frozen RDM of different species (cow, goat, sheep, horse, donkey and camel) is available via internet sales, although there are no data on the microbiological or temperature controls for these milks from bulk tank through to the point of consumption.

- In the case of raw milk sold via the internet, the temperature must be controlled and correctly maintained during all steps from the farm to the consumer.

- In case of frozen raw milk, instruction should be given to consumers about appropriate conditions for thawing the milk.

- The variability in temperature control and duration of storage by consumers suggest that multiplication of pathogens may occur if these are present in the milk.

- There is a need for microbiological and temperature monitoring data for RDM sold through different routes together with more information on consumer handling and storage practices with respect to fresh and frozen milk from different species.

TOR 5. Identify and rank potential control options to reduce public health risks arising from consumption of raw drinking milk.

- The steps in the production to consumption chain for RDM present many opportunities for contamination by microorganisms, some of which may be transmissible to humans.

- Intrinsic contamination of milk can arise from systemic infection in the milk-producing animal as well as from localised infections such as mastitis. Extrinsic contamination of milk can arise from faecal contamination and from the wider farm environment associated with collection and storage of milk.

- Observance of good animal health and husbandry together with the application of good agricultural practices (GAPs) and good hygienic practices (GHP) are essential to minimise opportunities for contamination of RDM with pathogens in the production to consumption chain for RDM.

- No single step could be identified which would provide a significant reduction in risk relative to a baseline of expected good animal health and welfare and GAPs and GHPs. Therefore, it was not possible to rank control options with respect to risk reduction and any deviations from the expected "best practice" baseline are likely to result in an increase in risk.

- The reviewed QMRA models identified on-farm hygiene control and maintenance of the cold chain as factors influencing the outcome of the models for some pathogens.

- Despite the general limitations in the reviewed QMRAs, they do show that the risk associated with *L. monocytogenes* in raw cow's milk can be mitigated and reduced significantly if the cold chain is well controlled, the shelf-life of raw milk is limited to a few days and there is consumer compliance with these measures/controls.

RECOMMENDATIONS

- To provide a better evidence base to inform future prioritisation and ranking approaches, studies should be undertaken to systematically collect data for source attribution for the hazards identified as associated with RDM and collect data to identify and rank emerging milk-borne hazards.

- Because of the diverse range of potential microbiological hazards associated with different milk-producing animals, hazard identification should be revisited regularly.

- The models reviewed here only involved raw cow's milk. There is a need for validated growth and survival models for pathogens in RDM of different milk-producing species, particularly in relation to the temperature and storage time of RDM from the producer up to the point of consumption.

- There is a need for improved risk communication to consumers, particularly susceptible/high risk populations, regarding the hazards and control methods associated with consumption of RDM.

DOCUMENTATION PROVIDED TO EFSA

1. Replies to EFSA questionnaire addressed to members of the EFSA Network on Microbiological Risk Assessment sent by EFSA on 11/04/2014

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APPENDICES

Appendix A. Questionnaire to Members of the EFSA BIOHAZ Network on Microbiological Risk Assessment[8]

The EFSA BIOHAZ working group on raw drinking milk is seeking information from Member States in support of its work to assess the public health risk associated with raw milk consumption in the EU. A link to the mandate is provided with this questionnaire.

The working group would be grateful if the questionnaire could be answered as fully as possible and responses provided to the EFSA BIOCONTAM contact point Michaela Hempen by 7 May 2014.

Please provide details of the person(s) completing the questionnaire in case there are any queries regarding the responses.

Thank you in advance for your co-operation.

    Name:

    Institution:

    Address:

    Telephone:

Q1. Please provide data on volume of total liquid milk production in your country in each of the following years. The data should relate to all liquid milk from each species irrespective of intended use (e.g. for heat treatment, raw milk for drinking, used for making raw milk products etc.).

Q2. Please provide data on the total volume of liquid milk consumption in your country in each of the following years. If you have any data which relates specifically to the volume of raw drinking milk consumed then please provide this as well. If such data are not available then please provide indirect data by giving an indication or range for possible consumption (e.g. not higher than x % of the total milk production; between x % and y % of the total volume of liquid milk production). Please explain the reasoning for this data and how it has been calculated.

Q3. Please provide data on number of registered producers of raw drinking milk in your country in each of the following years.Where such data is not available please provide an estimate of the number of producers and explain the reasoning and data on which this estimate is based.

Q4. Please provide data on number of raw drinking milk vending machines in use in your country in each of the following years.Please also provide information on how the use of these machines is currently regulated.

Q5. Please provide data on mean and range for Total Bacterial Count (CFU/mL) of liquid raw milk in your country in each of the following years. The data should relate to all liquid milk from each species irrespective of intended use (e.g. for heat treatment, raw milk for drinking, used for making raw milk products etc.).

Q6. Please provide information on how raw drinking milk is sold to consumers in your country.Please also indicate where this occurs (farm shop, other shop, supermarket, home delivery, restaurant).

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[8] This questionnaire was sent to EFSA's Scientific Network on Microbiological Risk Assessment on 18/04/2014. Replies were received from 18 Member States and from Norway and Switzerland. More details on the information gathered through the questionnaire, other than what reported in this opinion, are available from EFSA upon request.

Q7. Please provide information on the shelf-life (days) for raw drinking milk sold in your country.This might, for example, be included in legislation, good practice guidance or set by the producer.

Q8. Please provide information on any food safety advice to consumers of raw drinking milk in your country.Information could include for example directions to boil, labelling of containers or published advice to consumers including vulnerable groups such as the very young, the elderly, pregnant women or anyone who is unwell.

Q9. Please provide data on any foodborne outbreaks involving raw drinking milk in your country in 2013.For each outbreak please indicate the pathogen(s) involved, number of cases, number hospitalised and number of deaths together with an indication of the supporting evidence (descriptive, epidemiological, microbiological etc.) and source of the information.

Appendix B. Data provided by the European Centre for Disease Prevention and Control (ECDC) extracted from The European Surveillance System – TESSy

Table 9: Data provided by ECDC extracted from The European Surveillance System – TESSy: incidence and severity in humans based on TESSy data from 2009 to 2012

Selected hazard	Incidence in humans (number of reported confirmed cases per 100 000 EU population [number of confirmed cases])				Severity in humans (percentage of affected individuals who died [number of reported deaths])			
	2012	**2011**	**2010**	**2009**	**2012**	**2011**	**2010**	**2009**
Bacillus cereus	No data	No data	No data	No data	No data	No data	No data	
Brucella abortus	0.002 [10]	0.005 [27]	0.002 [8]	0.002 [9]	0.000 [0]	0.000 [0]	0.000 [0]	0.000 [0]
Brucella melitensis	0.016 [81]	0.015 [76]	0.012 [60]	0.012 [60]	0.000 [0]	1.316 [1]	0.000 [0]	1.667 [1]
Campylobacter spp. (thermophilic)	42.542 [213 484]	43.970 [220 143]	42.384 [211 531]	39.755 [197 929]	0.015 [31]	0.020 [45]	0.014 [30]	0.010 [20]
Corynebacterium spp.	No data	No data	No data	No data	No data	No data	No data	No data
Listeria monocytogenes	0.329 [1 651]	0.332 [15 151]	0.333 [1663]	0.336 [1675]	11.993 [198]	7.637 [127]	10.884 [181]	7.642 [128]
Mycobacterium bovis	0.026 [131]	0.031 [154]	0.035 [173]	0.028 [139]	6.870 [9]	1.299 [2]	2.890 [5]	2.878 [4]
Salmonella spp.	18.340 [92 033]	19.325 [96 755]	20.399 [101 808]	22.158 [110 319]	0.070 [64]	0.063 [61]	0.063 [64]	0.040 [44]
Staphylococcus aureus	No data	No data	No data	No data	No data	No data	No data	No data
Streptococcus equi subsp. *zooepidemicus*	No data	No data	No data	No data	No data	No data	No data	No data
STEC	1.130 [5 671]	1.893 [9480]	0.737 [3 677]	0.725 [3609]	0.212 [12]	0.591 [56]	0.218 [8]	0.139 [5]
Yersinia enterocolitica	0.622 [3 119]	0.732 [3 667]	0.716 [3 571]	0.858 [4 271]	0.000 [0]	0.0007 [0]	0.000 [0]	0.023 [1]
Yersinia pseudotuberculosis	0.023 [115]	0.013 [64]	0.024 [118]	0.019 [96]	0.000 [0]	0.000 [0]	0.000 [0]	0.000 [0]
Cryptosporidium	1.908 [9 576]	1.138 [5 697]	1.323 [6 605]	1.608 [8 007]	0.010 [1]	0.018 [1]	0.045 [3]	0.012 [1]
Toxoplasma	0.123 [617]	0.145 [728]	0.208 [1 039]	0.245 [1 219]	0.486 [3]	0.824 [6]	1.251 [13]	2.051 [25]
Tick-borne encephalitis (TBEV)	0.418 [2 150]	No data	No data	No data	0.42 [9]	No data	No data	No data

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The views and opinions of the authors expressed herein do not necessarily state or reflect those of the ECDC. The accuracy of the authors' statistical analysis and the findings they report are not the responsibility of ECDC. ECDC is not responsible for conclusions or opinions drawn from these data provided. ECDC is not responsible for the correctness of these data and for data management, data merging and data collation after provision of the data. ECDC shall not be held liable for improper or incorrect use of these data.

Appendix C.   Frequencies of occurrence of pathogens in raw milk of different sources in the EU

Table 10:   Frequencies of occurrence of *Campylobacter* spp., *Salmonella* spp., STEC, *Listeria monocytogenes* and *Staphylococcus aureus* in raw milk of different sources in the EU published in international scientific literature

| Pathogen | Milk from cow | | References for cow's milk[a] | Sheep (%) (no of samples) | Goat (%) (no of samples) |
	Range	Individual studies (%) (no of samples)			
Campylobacter spp.	0–12 %	1.4 (69)	Desmasures et al. (1997) (France)	2.2 (90)[a]	
		0 (200)	Oosterom et al. (1982) (The Netherlands)		
		4.5 (904)	Beumer et al. (1988) (The Netherlands)		
		0.5 (209)	Messelhausser et al. (2008) (Germany)		
		0 (310)	Stephan and Buhler (2002) (Switzerland)		
		1.7 (1 097)	de Louvois and Rampling (1998) (UK)		
		6 (1 138)	Humphrey and Hart (1988) (UK)		
		1.6 (62) (*C. coli*)	Whyte et al. (2004) (Ireland)		
		12 (282)	Bianchini et al. (2014) (Italy)		
		3 (260)	Bardon et al. (2012) (Czech Republic)		
		0 (260)	Mallet et al. (2012) (France)		
		1.5 (618)	Bianchi et al. (2013) (Italy)		
Salmonella spp.	0–2.9 %	0 (143)	De Reu et al. (2004) (Belgium)	5 (240)[a]	
		2.9 (69)	Desmasures et al. (1997) (France)		
		0 (310)	Stephan and Buhler (2002) (Switzerland)		
		0 (149)	Hahn et al. (1999) (Germany)		
		0.5 (1 097)	de Louvois and Rampling (1998) (UK)		
		0.2 (1 138)	Humphrey and Hart (1988) (UK)		
		0.1 (589)	Rea et al. (1992) (Ireland)		
		0.8 (260)	Bardon et al. (2012) (Czech Republic)		
		0.8 (260)	Mallet et al. (2012) (France)		
		0 (27)	Amagliani et al. (2012) (Italy)		
		0.3 (618)	Bianchi et al. (2013) (Italy)		
			Foschino et al. (2002) (Italy)		

Table 10: Frequencies of occurrence of *Campylobacter* spp., *Salmonella* spp., STEC, *Listeria monocytogenes* and *Staphylococcus aureus* in raw milk of different sources in the EU published in international scientific literature (continued)

Pathogen	Range	Milk from cow Individual studies (%) (no of samples)	References for cow's milk[a]	Sheep (%) (no of samples)	Goat (%) (no of samples)
STEC (serotypes 026, O91, O103, O111, O157 and O145)	0–5.7 %	0.7 (143) (O157) 0 (1 011) (O157) 0 (140) 0 (13) (O157) 0.3 (788) 0 (500) 5.7 (35) 0 (209) 0.9 (420) (O157) 5 (56) (O157) 3.5 (56) (O26) 1.2 (950) (O157) 0 (277) (O157) 0 (100) 0 (310) (STEC) 0.4 (260)(O157) 0 (27) 0.2 (618)	De Reu et al. (2004) (Belgium) Heuvelink et al. (1998) (the Netherlands) Schouten et al. (2005) (the Netherlands) Raynud et al. (2005)(France) Coia et al. (2001) (UK) Mechie et al. (1997) (UK) Messelhausser et al. (2008) (Germany) McKee et al. (2003) (Ireland) Murphy et al. (2007) (Ireland) Solomakos et al. (2009) (Greece) Colombo et al. (1998) (Italy) Massa et al. (1999) (Italy) Stephan and Buehler (2001) (Switzerland) Bardon et al. (2012) (Czech Republic) Amagliani et al. (2012) (Italy) Bianchi et al. (2013) (Italy) Foschino et al. (2002) (Italy) Cupakova et al. (2012) (Czech Republic)	1 (100) (O157 STEC)[a] 12.7 (344) (STEC)[a] 0.84 (595) (O157)[a]	0.7 (286) (O157)[a] 1.7 (60) (O157)[a] 16.3 (344) (STEC)[a] 1.4 (73) (O157)[a] 0.65 (460) (O157)[a] 0.75 (49) (O157)[a] 1.7 (60) 1 sample (48) O156 VT1[a]

Table 10: Frequencies of occurrence of *Campylobacter* spp., *Salmonella* spp., STEC, *Listeria monocytogenes* and *Staphylococcus aureus* in raw milk of different sources in the EU published in international scientific literature (continued)

Pathogen	Range	Milk from cow Individual studies (%) (no of samples)	References for cow's milk[a]	Sheep (%) (no of samples)	Goat (%) (no of samples)
Listeria monocytogenes	**0–10.1 %**	6.3 (143)	De Reu et al. (2004) (Belgium)	1.8 (56) [a]	0.8 (480)[a]
		5.8 (69)	Desmasures et al. (1997) (France)	**3.3 (90)** [a]	2.6 (1 445)[a]
		3.2 (2 000)	Sanaa et al. (1993) (France)		2.1 (286)[a]
		0 (310)	Stephan and Buhler (2002) (Switzerland)		3.8 (150)[a]
		0.4 (4 046)	Bachmann and Sphar (1995) (Switzerland)		7.8 (450)[a]
		0.6 (340)			**0 (60)**[a]
		10.1 (149)	Hahn et al. (1999) (Germany)		
		3.6 (361)	Greenwood et al. (1991) (England & Wales)		
		4.4–9.4 (160) [b]	Fenlon et al. (1995) (Scotland)		
		1–3.8 (180) [b]	Fenlon and Wilson (1989) (Scotland)		
		4.9 (589)	Rea et al. (1992) (Ireland)		
		5.3 (113)	Harvey and Gilmour (1992) (Ireland)		
		3.8 (80)	Rodler and Korbler (1989) (Hungary)		
		10 (260)	Bardon et al. (2012) (Czech Republic)		
		0 (260)	Mallet et al. (2012) (France)		
		0 (27)	Amagliani et al. (2012) (Italy)		
		1.6 (618)	Bianchi et al. (2013) (Italy)		
			Foschino et al. (2002) (Italy)		

No data were reported for milk from donkeys and horses for the four pathogens included.

(a): The data for sheep and goat were taken from Verraes et al. (2014).

(b): Season dependent.

Appendix D. Evidence that the hazard is transmissible via milk and if present in the milk-producing animal population in the EU

Table 11: Preliminary longlist of hazards

Biological hazard	A. Hazard reported as transmissible via milk? (yes/no)
Actinobacillus spp.	No
Aeromonas spp.	No
Anaplasma spp.	No
Trueperella pyogenes	No
Arcobacter spp.	No
Bacillus anthracis	No
Bacillus cereus	Yes
Bartonella spp.	No
Borrelia burgdorferi	No
Brucella abortus	Yes
Brucella melitensis	Yes
Burkholderia spp.	No
Campylobacter spp. (thermophilic)	Yes
Chlamydophila abortus	No
Clostridium botulinum	No
Clostridium difficile	No
Clostridium perfringens	No
Corynebacterium spp.	Yes
Coxiella burnetii	No
Cronobacter spp.	No
Erysipelothrix rhusiopathiae	No
Extended spectrum and/or *Amp*C β-lactamases (ESBL/*Amp*C) gene-carrying bacteria	No
Fusobacterium necrophorum	No
Helicobacter pylori	No
Klebsiella pneumoniae	No
Leptospira spp.	No
Listeria monocytogenes	Yes
Mannheimia haemolytica	No
Meticillin-resistant *Staphylococcus aureus* (MRSA)	No
Moraxella canis	No
Mycobacterium avium (including subsp. *avium* and *paratuberculosis*)	No
Mycobacterium spp. (*M. bovis*, *M. caprae*)	Yes
Mycoplasma bovis	No
Pasteurella multocida	No
Ricketsia spp.	No
Rhodococcus equi	No
Salmonella spp. (non-typhoid)	Yes
Shigella sonnei	No
Staphylococcus aureus	Yes
Stenotrophomonas maltophilia	No
Streptococcus agalactiae	No
Streptococcus equi subsp. *zooepidemicus*	Yes
Shigtoxin-producing Escherichia coli (STEC)	Yes
Yersinia enterocolitica	Yes
Yersinia pseudotuberculosis	Yes
Yersinia pestis	No
Babesia caballi	No
Balantidium coli	No
Candida albicans	No

Biological hazard	A. Hazard reported as transmissible via milk? (yes/no)
Cryptococcus neoformans var. *neoformans*	No
Encephalitozoon cuniculi	No
Enterocytozoon bieneusi	No
Ascaris lumbricoides	No
Babesia divergens, *B. microti*	No
Coenurus cerebralis	No
Cryptosporidium parvum	Yes
Dicrocoelium dendriticum	No
Echinococcus granulosus	No
Fasciola hepatica	No
Giardia duodenalis	No
Gongylonema pulchrum ("gullet worm")	No
Linguatula serrata	No
Moniezia expansa	No
Neospora caninum	No
Sarcocystis spp.	No
Taenia saginata, *T. ovis*, *T. hydatigena*	No
Toxoplasma gondii	Yes
Trichinella spp.	No
Trichostrongylus spp.	No
Trichophyton verrucosum (ringworm)	No
Trypanosoma evansi	No
Alkhumra haemorrhagic fever virus (AHFV)	Yes
Astroviruses	No
Borna disease virus	No
Bovine enterovirus type 1 (BEV-1)	No
Bovine papillomavirus	No
Bunyaviridae Orthobunyavirus (California encephalitis virus)	No
Camelpox virus	No
Chandipura virus	No
Coronovirus	No
Crimean Congo haemorrhagic fever virus (CCHFV)	No
Encephalitis virus (tick-borne encephalitis virus (*Flaviviridae*))	Yes
Other *Flaviviridae—Flaviviruses* (West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus)	No
Hepatitis E virus	No
Foot and mouth disease	No
Influenza virus	No
Lyssavirus (rabies)	No
Monegavirales Paramyxoviridae Henipavirus (Nipah virus, Hendra virus)	No
Orfvirus	No
Parapox virus (pseudocowpox)	No
Rift Valley fever virus	No
Rotavirus	No
Togaviridae Alphavirus	No
Vesicular stomatitis viruses	No

Table 12: Evidence that the hazard is present in the EU animal (bovines, sheep and goats, horses and donkeys, camels) population

Biological hazard	Hazard has been reported associated with milk-producing animal population in the EU?[a] (yes/no[b])				Included in shortlist for priority ranking? (yes/no)	Supporting evidence
	Bovine animals	Sheep and goats	Horses and donkeys	Camels		
Bacillus cereus [a]	Yes	No	Yes	No	Yes	Carlin et al. (2006), Rajkovic et al. (2008), Efimochkina et al. (2012), Ruusunen et al. (2013)
Brucella abortus	Yes	No	Yes	No	Yes	Corbel (1997), Earhart et al. (2009), Mailles et al. (2012), EFSA and ECDC (2014)
Brucella melitensis	No	Yes	No	Yes[c]	Yes	Almuneef et al. (2004), Gaffuri et al. (2006), Sofian et al. (2008), Ramos et al. (2008), Earhart et al. (2009), Shimol et al. (2012), Mentaberre et al. (2013), EFSA and ECDC (2014)
Campylobacter spp. (thermophilic)	Yes	Yes	Yes	No	Yes	Desmasures et al. (1997), Heuvelink et al. (2009), Schoder et al. (2010), Quigley et al. (2013), Dhama et al. (2013)
Corynebacterium spp.	Yes	No	No	No	Yes	Hart (1984), Barrett (1986), Wiertz et al. (2013)
Listeria monocytogenes [a]	Yes	Yes	Yes	No	Yes	Carrique-Mas et al. (2003), Brugere-Picoux (2008), Colavita et al. (2011), EFSA and ECDC (2014)
Mycobacterium bovis	Yes	No	Yes	No	Yes	Coker et al. (2006), de la Rua-Domenech (2006), Cvetnic et al. (2007), Portillo-Gomez and Sosa-Iglesias (2011), Delaune et al. (2012)
Salmonella spp. (non-typhoid)	Yes	Yes	Yes	No	Yes	Mazurek et al. (2004), EFSA and ECDC (2014)
Staphylococcus aureus [a]	Yes	Yes	Yes	No	Yes	Murphy et al. (2010), Schoder et al. (2010), Kunz et al. (2011)
Streptococcus equi subsp. *zooepidemicus*	Yes	No	Yes	No	Yes	Jovanovic et al. (2008), Quigley et al. (2013)
Shigtoxin-producing Escherichia coli (STEC)[b]	Yes	Yes	Yes	No	Yes	Allerberger et al. (2001, 2003), McIntyre et al. (2002), Guh et al. (2010), Martin and Beutin (2011), Lynch et al. (2012)

Table 12: Evidence that the hazard is present in the EU animal (bovines, sheep and goats, horses and donkeys, camels) population (continued)

Biological hazard	Hazard has been reported associated with milk-producing animal population in the EU?[a] (yes/no[b])				Included in shortlist for priority ranking? (yes/no)	Supporting evidence
Yersinia enterocolitica	Yes	No	Yes	No	Yes	Barrett (1986), Ackers et al. (2000), Lee et al. (2003), Bernardino-Varo et al. (2013), Schmid et al. (2013)
Yersinia pseudotuberculosis	Yes	Yes	Yes	No	Yes	Prober et al. (1979), Stojek et al. (2010)
Cryptosporidium parvum	Yes	No	No	No	Yes	Djuretic et al. (1997), Baumgartner et al. (2000), Harper et al. (2002), Fretz et al. (2003)
Toxoplasma gondii	Yes	Yes	Yes	Yes	Yes	Jones et al. (2009), Mancianti et al. (2013), Bezerra et al. (2013), Mentaberre et al. (2013), EFSA and ECDC (2014)
Alkhumra haemorrhagic fever virus (AHFV)	No	No	No	No	No	
Tick-borne encephalitis virus (TBEV)	Yes	Yes	No	No	Yes	Kriz et al. (2009), Balogh et al. (2010), Cisak et al. (2010), Klaus et al. (2012)

(a): Reported as associated with the milk-producing animal species based on detection in faeces, milk, the animal environment or from serological testing.

(b): For the purposes of this assessment, no information was found concerning the occurrence of the hazard in this species in the EU since 2000. Whilst this may reflect the actual situation, variation in level of investigation of different hazards means that some hazards will be present but have not been actively looked for or, when they have, the studies have been few or limited in scope. In addition, certain hazards are likely to be ubiquitous in the environment generally and will be expected to be present in the environment of milk-producing animals in the EU.

(c): *Brucella* sp. based on serological testing. Assumed to be *B.melitensis*

Appendix E   Operation of vending machines

Information below is extracted from Brasca and Lodi (2006).

Raw milk vending machines are equipped with the following components:

- cooling system to maintain the raw milk temperature between 1 and 4 °C;

- milk tanks to store the raw milk intended to be sold;

- agitator to guarantee homogeneity of the stored raw milk;

- pump and tubing system for delivering milk (and for cleaning procedure);

- volumetric counter for the measurement of the supplied milk;

- milk-dispensing chamber with nozzle, door and liquid discharge management system;

- electronic display for basic information for the consumers.

The type of machine depends on where the farm producing the milk is located with respect to the vending machine and on the expected sales volume.

If the installation is on or very close to the farm, it is possible to sell milk directly from the farm milk tank. The milk line from the vending machine to the farm milk tank should not exceed few metres and must be insulated to prevent milk warming.

Increasing the farm distance and the sale volume requires a vending machine with higher capacity and/or more milk tanks. A vending machine can accommodate one to four milk tanks of varying capacity (from 50 to 200 L) with an automatic changeover from the first (empty) tank to the second (full) tank. The tank can be placed outside the vending machine and therefore must be equipped with a refrigeration system. The non-refrigerated tank can be placed inside the vending machine, where the internal temperature is kept constant with the use of a temperature monitoring system and cooling/warming mechanism. Tanks are manufactured in high-quality stainless steel and equipped with an agitator which is automatically activated at pre-defined time intervals to ensure homogeneous cooling.

Placing a vending machine far from the farm is more complex since milk must be refilled daily and a temperature between 0 and 4 °C during transport must be guaranteed. A pre-set milk volume is delivered or the quantity of milk can be set freely. The flow of milk can be stopped and restarted by pressing a button, for example if a customer needs to change containers.

Milk is moved from the tank to the nozzle by pumps. The most suitable type of pump for moving a liquid with suspended small particles (as milk) is a positive displacement pump, in which fluid is moved by trapping a fixed amount and forcing (displacing) that trapped volume into the discharge pipe. With positive displacement pump, the volume is constant through each cycle of operation and the fluid is moved without turbulence. There are many types of positive displacement pumps: piston pump, gear pump, lobe pump, peristaltic pump.

Tubing should be equipped with a mechanism to prevent their emptying if the pump stops working; otherwise, when residual milk comes in contact with oxygen, a microbial biofilm could be formed. Since biofilms may contain spoilage and pathogenic microorganisms, formation of biofilms in milk-processing environments leads to increased opportunity for microbial contamination of the milk.

To prevent microorganism adhesion or biofilm formation, stainless steel is the most commonly employed material because of its durability, resistance to corrosion and easiness to clean. Alternative materials include glass, elastomers (also known as rubbers) and plastics.

The amount of milk carried by the pump and supplied to the customer is monitored by means of a volumetric counter. The most common flow meters are turbine flow meters (translating the mechanical action of the turbine rotating in the liquid flow around an axis into a user-readable measure of flow), piston meters (a piston rotates within a chamber of known volume: for each rotation, a known amount of liquid passes through the piston chamber), magnetic flow meters (which use a magnetic field applied to the metering tube, which results in a potential difference proportional to the flow velocity perpendicular to the flux lines) and rotameters (which consist of a tapered tube, typically made of glass, with a float inside that is pushed up by fluid flow and pulled down by gravity).

The milk-dispensing chamber, from which milk exits, via a nozzle, to the bottle, is the most delicate part of the vending machine. Indeed, residual milk could be stagnant in the nozzle, increasing the possibility of bacteria (deriving from the milk or from the environment) surviving and multiplying.

To prevent possible environmental contamination of the nozzle, the milk-dispensing chamber must be equipped with a door that closes automatically after dispensing milk. Various cleaning solutions can be used to remove the remaining milk and guarantee the hygiene in the filling area: UV-based cleaning systems, disinfectant washing systems, vaporised cold water washing systems or vaporised hot water washing systems. Moreover, all parts which come into contact with milk in the filling area could be chilled to prevent growth of microorganisms.

Particular attention should be paid to the so-called "splash area", comprising all surfaces on which milk may splash or flow along.

Finally, the nozzle can be concealed and projected with a system to eliminate foam during distribution or to prevent dropping.

The vending machine is, both internally and externally, subject to cleaning procedures to guarantee minimum hygienic requirements. Internal surface cleaning can be performed manually or automatically with a rinsing programme.

For surfaces coming in contact with milk, an automatic rinsing programme is preferred and consists in rinsing with cleaning and disinfecting agents with a wide action range against microorganisms. After cleaning, the pipes should be rinsed with plain water.

Milk tanks need to be thoroughly cleaned each time they are refilled.

In addition, vending machines can be equipped with an alarm system that sends an SMS message when:

- the milk tank is empty, or as soon as the milk level drops to a pre-determined limit;
- a power cut occurs;
- the milk/internal temperature is out of an established range (to prevent frosting of milk as well as excessive warming);
- the agitator is not properly functioning.

To guarantee safety, some type of machines automatically stop the milk supplying in case of milk temperature greater than 4 °C or if the system did not perform the cleaning operations.

GLOSSARY

Acquired immunity: immunity acquired by infection or vaccination (active immunity) or by the transfer of antibody or lymphocytes from an immune donor (passive immunity).

Beta distribution: a family of continuous probability distributions defined on the interval [0, 1] parameterised by two positive shape parameters, denoted α and β, that appear as exponents of the random variable and control the shape of the distribution.

Commensal: (of an animal, plant, fungus, etc.) living with, on or in another, without injury to either.

Colony-forming unit (**CFU**): a rough estimate of the number of viable bacteria or fungal cells in a sample.

Dose–response relationship: the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor.

Exponential models: a model in which the number of microorganisms in a culture increases exponentially until an essential nutrient is exhausted. Typically the first organism splits into two daughter organisms, which then each split to form four, which split to form eight, and so on.

Generation time: the average time between two consecutive generations in the lineages of a population.

Lognormal distribution: a continuous probability distribution of a random variable whose logarithm is normally distributed.

Monte Carlo simulations: a computer-based method of calculating the probability of an event using values, randomly selected from sets of data repeating the process many times, and deriving the probability from the distributions of aggregated data.

Normal distribution: (also Gaussian) a very commonly occurring continuous probability distribution—a function that tells the probability that any real observation will fall between any two real limits or real numbers, as the curve approaches zero on either side. Normal distributions are extremely important in statistics and are often used in the natural and social sciences for real-valued random variables whose distributions are not known.

Perinatal population: pregnant women and their foetuses or newborns.

Pert distribution: a version of the beta distribution that requires the same three parameters as the triangle distribution, namely minimum (a), mode (b) and maximum (c). The Pert distribution is used exclusively for modelling expert estimates based on an expert's minimum, most likely and maximum guesses.

Psychrotrophic bacteria: bacteria that are capable of surviving or even thriving in a cold environment.

Shelf-life: the length of time that a commodity may be stored without becoming unfit for use or consumption.

Somatic cell count: an indicator of the quality of milk, with white blood cells known as leucocytes constituting the majority of somatic cells in question.

Thermophilic bacteria: bacteria that thrive at relatively high temperatures.

Virulence: the ability of an agent of infection to produce disease. The virulence of a microorganism is a measure of the severity of the disease it causes.

ABBREVIATIONS

AHFV	Alkhumra haemorrhagic fever virus
BAB	butyric acid bacteria
BIOHAZ	Biological Hazards
bTB	bovine tuberculosis
CAC	Codex Alimentarius Commission
CFU	colony-forming unit
CI	confidence interval
DRT	decimal reduction time
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESBL	extended spectrum beta-lactamase
ESL	extended shelf-life
EU	European Union
FASFC	Belgian Food Safety Agency
FBO	food business operator
FDA–USDA FSIS	Food and Drug Authority–United States Department of Agriculture Food Safety and Inspection Service
FSANZ	Food Safety Authority of New Zealand
GAP	good agricultural practice
GHP	good hygienic practice
GT	generation time
HACCP	Hazard Analysis and Critical Control Points
HUS	haemolytic–uremic syndrome
ISI	innovative steam injection
LPO	lactoperoxidase
MDR	multidrug-resistant
MRSA	meticillin-resistant *Staphylococcus aureus*
MS	Member State
NAHMS	National Animal Health Monitoring System
PCR	polymerase chain reaction
OBF	officially brucellosis free
ObmF	officially *B. melitensis* free
QMRA	quantitative microbiological risk assessment
RDM	raw drinking milk
SD	standard deviation
ST	sequence type

STEC	Shigatoxin-producing *Escherichia coli*
TBC	total bacterial counts
TBEV	tick-borne encephalitis virus
TESSy	The European Surveillance System
TMC	total mesophilic count
TOR	Term(s) of Reference
UHT	ultra-high-temperature