Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the suitability of the additional hygiene requirements applicable to raw milk intended for direct sale to the final consumer

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Abstract

Regulation (EC) No. 853/2004, laying down specific hygiene rules for food of animal origin, allows placing on the market raw milk for direct human consumption. Moreover, it determines that the Member States of the European Union can regulate by means of specific rules, prohibit or limit the placing on the market of raw milk intended for direct human consumption. The current situation in Spain allows placing on the market raw milk without any additional requirements to the aforementioned Regulation.

Despite being authorised, in terms of risk assessment, the scientific reports of the European Food Safety Authority (EFSA, 2015), and the AESAN Scientific Committee (AESAN, 2015) show that the consumption of raw milk involve a risk to the health of consumers.

AESAN (Spanish Agency for Food Safety and Nutrition) requests its Scientific Committee to prepare a report assessing whether the risk management measures related to raw milk intended for direct human consumption, are adequate to provide a high level of consumer protection. Or if, alternatively,

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it is necessary to prohibit the placing on the market of raw milk intended for direct human consumption in Spain. These proposals will be incorporated into the draft Royal Decree regulating certain conditions for applying the provisions of the European Union in the area of production hygiene and marketing of foodstuffs and regulating activities excluded from its scope.

The proposed risk management measures related to raw milk intended for direct human consumption (microbiological criteria, mandatory packaging of milk and indicating on the label: "Raw milk not heat-treated: boil before consumption" and "Store in refrigeration between 1 and 4 ºC") may be considered, provided all of them are met, adequate to provide a high level of protection to consumers of this product.

The evaluation of the growth kinetics of the pathogens considered at 4, 6 and 7 ºC based on bibliographic data show that with the predicted latency period, in the most unfavourable case, growth would be lower than authorised levels (taking *Listeria monocytogenes* as the reference microorganism). It is therefore recommended that the shelf life is set at 3 days.

There are numerous microbiological risks that may be present in raw milk, so raw milk is considered a dangerous food for the health of the consumer if the established criteria are not strictly fulfilled, especially refrigeration and boiling process, before consumption.

It is also recommended the inclusion of a warning to avoid that the consumption of raw milk without following the required measures poses a health risk.

Key words

Raw milk, *Campylobacter* spp., *Escherichia coli,* STEC, *Listeria monocytogenes*, *Salmonella*, pathogen, microbiological risk.

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1. Introduction

Regulation (EC) No. 853/2004 which establishes specific rules on hygiene in foods of animal origin, allows the marketing of raw milk for direct human consumption, that is to say, without prior treatment, and sets down the sanitary requirements that must be met (EU, 2004a). Additionally, it stipulates that Member States of the European Union may regulate, prohibit or limit, by means of specific regulations, the marketing of raw milk for direct human consumption. Until now Spain has not made use of this possibility, and therefore, the sale of raw milk directly to the consumer is currently authorised under this Regulation, for which the requirements included in it must be fulfilled.

Additionally, EU legislation allows Member States to establish more flexible (less strict) requirements regarding the sale of small quantities of raw milk directly to the end-consumer, or to the retailer by the producer. Spain has also not made use of this provision and, therefore, currently raw milk can be sold regardless of volume, provided it meets the requirements established by the European Union, that are applicable from 2006 onwards (BOE, 2006).

In some Member States of the European Union, the sale of raw milk is permitted if it complies with Regulation (EC) No. 853/2004, in others, additional criteria set at national must be fulfilled. In yet other countries, the sale of raw milk for direct human consumption is prohibited.

Currently Spain permits the sale of raw milk without any other requirement in addition to the aforementioned Regulation.

In spite of being authorised, when it comes to risk assessment, the scientific reports of the European Food Safety Authority (EFSA, 2015) and the AESAN Scientific Committee (AESAN, 2015) show that consuming raw milk constitutes a health risk for consumers.

For this reason, different Member States of the European Union such as France, Italy, Austria, Finland and Latvia have limited the marketing of raw milk by means of additional requirements regarding information to the customer, limiting sales systems and establishing microbiological criteria.

AESAN (Spanish Agency for Food Safety and Nutrition) requests its Scientific Committee to prepare a report assessing whether the risk management measures related to raw milk intended for direct human consumption, are adequate to provide a high level of consumer protection. Or if, alternatively, it is necessary to prohibit the placing on the market of raw milk intended for direct human consumption in Spain. These proposals will be incorporated into the draft Royal Decree regulating certain conditions for applying the provisions of the European Union in the area of production hygiene and marketing of foodstuffs and regulating activities excluded from its scope.

2. Proposing additional hygiene requirements for the marketing of raw milk for direct sale to the end-consumer

Currently in Spain, and in accordance with Regulation (EC) No. 853/2004, the marketing of raw milk is permitted in the European Union. The AESAN therefore considers it necessary to establish requirements in addition to those currently laid down by law for the direct sale of raw milk to the consumer, ensuring its safe consumption or conversely, prohibiting its marketing.

The additional requirements for the marketing of raw milk for direct sale to the consumer proposed by the AESAN are:

1. The marketing of raw milk for direct sale to the consumer can be made by authorised establishments registered with the General Sanitary Registry of Food Businesses (RGSEAA in Spanish). In accordance with Regulation (EC) No. 852/2004 of the European Parliament and of the Council, of 29 April 2004 (EU 2004b), Regulation (EC) No. 853/2004 of the European Parliament and of the Council, of 29 April 2004 (EU, 2004a), national regulations with regard to the checks that operators in the dairy sector must carry out and microbiological safety criteria applicable to raw milk:

Table 1. Microbiological safety criteria proposed for the marketing of raw milk

Microorganism	Sampling plan (*)		Limit (**)	Reference analysis	Stages of application of criteria
	n	c	M		
Campylobacter spp.	5	0	Absence in 25 ml	UNE-EN ISO 10272-1	Product marketed during its shelf life
E. coli STEC O157	5	0	Absence in 25 ml	UNE-EN ISO 16654	Product marketed during its shelf life
Listeria monocytogenes	5	0	Absence in 25 ml	UNE-EN ISO 11290-1	Product marketed during its shelf life
Salmonella	5	0	Absence in 25 ml	UNE-EN ISO 6579	Product marketed during its shelf life

(*) n= number of units that make up the sample; c= number of sampling units with values higher than M.
(**) The indicated limits are applicable to every sampling unit analysed.
Interpreting results of the analysis:
Satisfactory: when all observed values indicate the absence of bacteria.
Unsatisfactory: when the bacteria is detected in at least one sampling unit.
Sampling frequency: the analysis of the safety criteria must be carried out at least once every month.

2. Raw milk must always be packaged for its sale to the end-consumer. The packaging must be closed immediately after filling, with a closing device that prevents contamination and must be designed so that once open, the state of the product can be easily judged.
3. Without prejudice to the established general rules on the labelling of food products, packages containing raw milk must clearly state the date of packaging and the following instructions: "Raw milk without thermal treatment: boil before consumption" and "Maintain refrigerated at temperatures between 1 and 4 ºC".
4. Food establishments that serve meals to vulnerable groups such as hospitals, daycare, schools and retirement homes, cannot use raw milk as an ingredient.
5. Owners of milk production holdings cannot directly supply raw milk from their holdings to the end-consumer, or to retail business establishments that directly supply end-consumers, except when they comply with the stipulations of the aforementioned sections 1 to 4.

3. Assessment of the adjustment of proposed microbiological criteria

3.1 General considerations of the risks associated with raw milk

The consumption of raw milk has been linked to food-borne diseases. In the European Union, from 2007 to 2012, it was associated with 27 outbreaks, 4 of them from goat's milk and the rest from

cow's milk (EFSA, 2015) while between 2005 and 2016 in the United States, it was associated with 152 outbreaks, 5 of them due to the consumption of goat's milk (Whitehead and Lake, 2018). After reviewing the main biological hazards associated with the consumption of raw milk, two previous reports published by AESAN (2015) and EFSA (2015) have been identified. These reports establish that the main biological agents associated to diseases transmitted by the consumption of raw milk in the European Union are *Campylobacter* spp. (thermophilic), *Salmonella* spp., *Escherichia coli* Shiga toxin-producing (STEC) and *Listeria monocytogenes*. Between 2007 and 2012 in the European Union, 21 of the 27 outbreaks linked to the consumption of raw milk were attributed to *Campylobacter* spp., for which reason it can be considered to be the most frequently involved etiologic agent, followed by *E. coli* STEC (EFSA, 2015) (Table 2, Annex 1). There are also studies on its effect on raw milk, which presents an important variability (Table 3, Annex 1).

In Spain, there have been different studies on the prevalence of these agents in tanks of raw milk and different products. The oldest data on the presence of *L. monocytogenes* in Spain was published by Lucas Domínguez and associates in 1985, showing values of 45 % of the presence of this pathogen in samples of raw milk from a single farm (Domínguez-Rodríguez et al., 1985). Later sampling data that include a larger number of farms have confirmed the presence of *L. monocytogenes* in 3.6 % of samples of raw cow's milk (Gaya et al., 1998), in 2.19 % of samples of raw sheep's milk (Rodríguez et al., 1994) and 2.56 % of samples of raw goat's milk (Gaya et al., 1996). More recent data confirm similar values for the presence of *L. monocytogenes* in raw milk, ranging between 3 % in sheep's milk and 6.8 % in cow's milk in Navarre (Vitas et al., 2004) and 6.1 % in cow's milk in Galicia (Vilar et al., 2007). With regard to the prevalence of *Salmonella* in raw milk, this prevalence at first appears to be significantly less, as Gaya and associates (Gaya et al., 1987) were unable to isolate *Salmonella* in raw goat's milk, in spite of identifying multiple enterobacteria. Nor could Cortés and associates isolate *Salmonella* or *Campylobacter* in raw goat's milk or faeces, in more recent studies (Cortés et al., 2006). Another study on milk and dairy products stored at room temperatures sampled in restaurants in Spain was also unable to isolate salmonella in spite of exceeding the limit of mesophilic bacteria (Sospedra et al., 2009).

3.2 Does the microbiological criteria for each of them guarantee a high level of protection with regard to said pathogens?

On this basis and with regard to the enquiry "Evaluating whether risk management measures for the direct sale of raw milk to the end-consumer provide a sufficiently high level of protection to consumers or whether its marketing should be banned in Spain", the Scientific Committee makes the following assessment:

The proposed criteria focus on the main microbiological risks linked to food-borne diseases from the consumption of raw milk in the last few years in Europe. The sampling plan and proposed limits lead to a low prevalence of said pathogens in the product. In the case of large batches, a sampling could be carried out for each batch. Considering the level of statistical significance achieved with the established microbiological criteria, if a monthly sample of any of the included microorganisms were to be taken during a year, the upper limit of the confidence interval would be 4.8 % of positive samples

(confidence level 0.95) (BIOQURA Network calculator, 2019). Therefore a very low level of incidence may be expected. The possibility of increasing "n" size in the sampling plan given the health risk of some of the pathogens under consideration, in accordance with the recommendations of the International Commission on Microbiological Specifications for Foods (ICMSF) has also been evaluated.

These microbiological criteria, while ensuring a low level of contamination from said pathogens, would not be sufficient to guarantee a high level of consumer safety without additional measures, given the presence of *Salmonella*, *E. coli* STEC, *Campylobacter* and other pathogens that may be present and constitute a risk to consumer health.

Other measures stipulated for marketing, including the indication, "Raw milk without thermal treatment: boil before consumption" and "Maintain refrigerated at temperatures between 1 and 4 ºC" would reduce the microbiological risks associated with said product, given that:

- The thermal treatment to be applied before consumption (where the milk would reach a temperature of 100 ºC) would effectively nullify all biological risks present, both those highlighted within the microbiological criteria, and those included in Section 4, except some sporulated microorganisms (some strains of *Bacillus cereus*). It has been established that a thermal treatment of 72 ºC for 2 minutes would be sufficient to inactivate vegetative pathogenic bacteria (Rosnes et al., 2012), therefore, following the recommendation of boiling the product would be more than sufficient to comply with this requirement.
- Nevertheless, it is necessary to take into account the temperature at which milk is kept from the moment it is bought, to its storage at home and in companies where it is sold. Various studies have demonstrated the differences and even the abuse of temperature during these stages and the consequent effect in the growth of *Listeria monocytogenes*, for which reason it is necessary to establish a shelf life and verify said temperature.
- A growth in *L. monocytogenes/L. Innocua* at 4 ºC under ideal conditions (Table 4) has been predicted at www.ComBase.cc and it has been observed that the latency period adjusted according to Baranyi and Roberts (1994) is 99.6 hours at 4 ºC, and considering a temperature higher than that which is indicated (7 ºC), it would not reach a growth of 100 CFU/ml in 72 hours. The rest of the pathogens included in the microbiological criteria display a growth rate of 0 at this storage temperature, for which reason, *L. monocytogenes* is the microorganism to be considered in this case. Taking into consideration the uncertainty associated with the growth rate of *L. monocytogenes* (Combase.cc), establishing a shelf life of 3 days (to be calculated from milking) seems to be appropriate. During this period, there would be limited or no growth of *L. monocytogenes*. Maintaining milk refrigerated between 1 and 4 ºC during the recommended shelf life (3 days) would not allow a significant proliferation of any sporulated microorganism that might have survived thermal treatment (*B. cereus*).
- It is important to establish hygiene criteria stipulating somatic cells to be less than or equal to 300 000 ml (geometric mean of a sample per month for 3 months) and bacteria at 30 ºC less than or equal to 50 000 bacteria per ml (geometric mean of two samples per month for at least 2 months) in the case of cow's milk. For sheep's milk, a value less than or equal to 250 000 bacteria at 30 ºC per ml must be demanded (geometric average of 2 months with at least two

samples per month). In this regard, Regulation (EC) No. 853/2004 allows at least 400 000 somatic cells per ml and up to 100 000 CFU/ml of bacteria in raw milk for producers. Greater hygiene in the direct sale of raw milk to consumers is extremely important and must be included in the Regulation, which would mean that only milk production holdings with high overall hygiene can sell this milk. Somatic cells in milk are indicators of both clinical and sub-clinical mastitis, and bacteria such as *L. monocytogenes* have been identified as agents of mastitis in cattle (Fedio et al., 1990) and small ruminants (Fthenakis et al., 1998); in addition to *Campylobacter* (Gudmundson and Chirino-Trejo, 1993) and enterobacterias (Ruegg, 2017). In this way, raw milk samples that have tested positive for verotoxigenic *E. coli* have higher values of somatic cells than raw milk samples that have tested negative for these pathogens (Steele et al., 1997). Various authors have also highlighted the utility of somatic cell recounts, as an overall indicator of hygiene in milk production, as according to Ruegg (2003), the majority of farms with high values of somatic cells in cold tanks were operated by livestock farmers whose approach may be classified as quick and dirty. This author also points out that high values of somatic cells are linked to a greater risk of traces of antibiotics. This last aspect has also been highlighted by other researchers (Schukken et al., 2003). Finally, given that one of the most significant causes of mastitis is *S. aureus* (Ruegg, 2017) and that the enterotoxins of this microbial agent are thermostable, a low number of somatic cells would also indicate a lower probability of the presence of substances that may not be inactivated by consumers' heating/boiling the milk. On the other hand, using the total recount of bacteria is directly justified as all the pathogens that may be present contribute to increasing said indicator and the lower the number, lesser the probability of the presence of pathogens. This has been clearly shown in the case of *L. monocytogenes* (Steele et al., 1997). Including these criteria with regard to the count of bacteria and somatic cells in raw milk from commercial holdings that sell directly to the consumer is taken into consideration in Catalunya (Generalitat, 2018) as well as in different regions of Italy such as Veneto (Veneto, 2005, 2012, 2017), Piamonte and Emilia-Romagna.

Studies carried out in Italy show that 40 % of consumers do not follow the instructions for thermal treatment and consume raw milk (Giacometti, 2015). Therefore, consumers must be adequately informed of the need to comply with these instructions.

Table 4. Growth prediction of *L. monocytogenes/L. innocua* at the temperatures indicated and in optimal pH (considered pH 7 although it may vary in milk) and a_w (considered 0.997) conditions through the Combase tool for predicting microbial kinetics

Temperature (ºC)	pH	a_w	Concentration (CFU/ml)	Latency (hours)	Time (hours) till 100 CFU/ml
4	7	0.997	10	99.6	158
6	7	0.997	10	67.7	104
7	7	0.997	10	54.6	85

Source: (www.combase.cc) (Baranyi and Tamplin, 2004).

4. Other biological risks associated with raw milk

4.1 Are there additional risks with sufficient evidence as a threat to consumer safety, that are not included in the microbiological criteria?

- There may be risks associated with other pathogens that are transmitted by this product, such as the Tick-borne encephalitis virus (TBEV), *Corynebacterium* spp., *Toxoplasma gondii*, *Streptococcus equi* subsp. zooepidemicus, *Brucella melitensis* and *Mycobacterium bovis* (AESAN, 2015), that have caused food-borne diseases linked to raw milk in the European Union, although their incidence is currently quite low. A 2014 outbreak in Finland with 43 cases where the agent was *Yersinia pseudotuberculosis* (Pärn et al., 2015) has also been described.
- The presence and proliferation of *Staphylococcus aureus* may cause the formation of the toxin, which is highly resistant to heat and is not inactivated by boiling. In a study carried out by Zuflauf et al. (2018), *S. aureus* was detected in raw cow's milk in 24.7 % of analysed samples.
- Antimicrobial resistance, considered to be a public health risk, was also detected in samples of *Campylobacter* spp., *Salmonella* spp., *E. coli* STEC and *S. aureus*, taken from raw milk or milking equipment in many countries of the European Union. Strains that are resistant to antimicrobials associated to a food-borne outbreak produced by raw buffalo's milk in the European Union and to *E. coli* STEC in the United States.
- In the case of milk from small ruminants (sheep and goats), there may risks other than those that have been identified. In this sense, *Brucella melitensis* might be important in the case of sheep's milk, although given its current low incidence, the risk may be deemed to be very low.
- There are emerging risks that may give rise to new biological hazards in raw milk. Cases of vaccination in cattle using live attenuated strains that have been activated have been described, that give rise of diseases that may be transmitted through raw milk.
- Risks such as Q fever caused by *Coxiella burnetti* associated with raw milk have also been described.
- Although to a lesser degree than that which has been described in the case of microbiological risks of bacterial origin, there have been also been descriptions of food-borne cases and outbreaks of viral and parasitic origin associated to the consumption of raw milk. In this regard, the first documented food-borne outbreak of viral origin was described in the second decade of the last century in the United Kingdom, caused by the consumption of milk contaminated with the polio virus. More recently, the presence of tick-borne encephalitis virus in milk from different species as well as cases and outbreaks associated with the consumption of raw milk contaminated by this agent (Brockmann et al., 2018) (Dorko et al., 2018) (Kerlik et al., 2018) (Klaus et al., 2019) (Król et al., 2019) have been described in different countries of the European Union (Germany, Slovakia and Poland). Likewise, beyond the borders of the European Union, the presence of antibodies and the RNA of the hepatitis E virus (Huang et al., 2016) (Long et al., 2017) (Demirci et al., 2019), the rotavirus (Yolken et al., 1985), and even human norovirus (Yavarmanesh et al., 2015) have been detected. With regard to parasites, cases of outbreaks related to the consumption of raw milk have also been described. There is ample proof of the presence and possible transmission of *Toxoplasma gondii* through the raw milk of different

mammalian species, or dairy products derived from unpasteurised raw milk (Boughattas, 2017) (Saad et al., 2018) (da Costa et al., 2019) (Gazzonis et al., 2019) (Iacobucci et al., 2019) (Pinto-Ferreira et al., 2019). Both the presence of *Cryptosporidium parvum* in raw milk as well as cases of cryptosporidiosis outbreaks linked to the consumption of raw milk in different countries including the United States, United Kingdom and Switzerland have been described (Palmer, 1990) (Baumgartner et al., 2000) (Rosenthal et al., 2015). Infections associated to other protozoa (for example, *Giardia dudonealis*, *Eimeria* spp. or *Cyclospora*) cannot be discarded, as their presence in milk from different species of mammals has been described.

Many of these microbiological risks may be considered especially serious for particularly susceptible consumers, given that, in case of suffering a food-borne illness, it would manifest with greater severity.

All of this proves that raw milk may be a vehicle for multiple diseases that may be transmitted to the consumer if food safety measures are not correctly applied, and therefore, it is important to establish additional restrictions for its use by populations at risk.

Conclusions of the Scientific Committee

The proposed risk management measures associated with raw milk for direct sale to the end consumer (microbiological criteria, compulsory packaging of milk and labelling "Raw milk without thermal treatment: boil before consumption" and "Maintain refrigerated at temperatures between 1 and 4 ºC)" can be considered, in case all of them are met, to provide a high level of protection to consumers of this product. Additionally, it is an improvement upon the current situation, where only compliance with Regulation (EC) No. 853/2004 is demanded.

Evaluating the growth kinetics of the discussed pathogens at 4, 6 and 7 ºC with regard to bibliographic data shows that the predicted latency period, would cause, in a worst-case scenario, a growth under authorised levels (taking *Listeria monocytogenes* as the reference microorganism). For this reason, it is recommended that a shelf life of 3 days be established.

There are numerous microbiological risks that may be present in raw milk (both bacterial and viral), and even possible emergent risks have been identified. For this reason, raw milk is deemed to be a food-item that poses a health risk to the consumer if the established criteria are not met, especially with regard to refrigeration and boiling milk before consumption. It is recommended that a warning be included in the label, to avoid that consuming raw milk without following the measures required poses a health risk.

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Annex I

Table 2. Campylobacteriosis outbreaks associated to the consumption of raw milk where the species is mentioned

Raw milk Species	Incidents	Year	Country	Reference
Goat	4 cases	1983	United States	Harris et al. (1987)
Goat	3 cases	1991	United States	Marler (2007)
Goat	11 cases, 3 hospitalised	2005	United States	Real Raw Milk Facts (2012)
Cow	18 cases	2007	Netherlands	EFSA (2015)
Cow	2 outbreaks, 12 cases	2007	Denmark	EFSA (2015)
Cow	4 cases	2007	Finland	EFSA (2015)
Cow	14 cases	2007	Germany	EFSA (2015)
Cow	2 cases	2008	Austria	EFSA (2015)
Cow	45 cases	2008	Germany	EFSA (2015)
Cow	8 cases	2008	Netherlands	EFSA (2015)
Cow	6 cases	2010	Slovakia	EFSA (2015)
Cow	3 outbreaks, 42 cases	2010	Germany	EFSA (2015)
Goat	4 cases	2010	Netherlands	EFSA (2015)
Cow	15 cases	2010	United States	Real Raw Milk Facts (2012)
Cow	10 cases	2010	United States	Real Raw Milk Facts (2012)
Cow	12 cases	2010	United States	Real Raw Milk Facts (2012)
Cow	3 outbreaks, 32 cases	2011	Germany	EFSA (2015)
Cow	13 cases	2011	Sweden	EFSA (2015)
Cow	5 cases	2011	United States	Real Raw Milk Facts (2012)
Cow	8 cases	2011	United States	Real Raw Milk Facts (2012)
Cow	18 cases	2011	United States	Real Raw Milk Facts (2012)
Cow	11 cases	2012	Denmark	EFSA (2015)
Goat	18 cases	2012	United States	Real Raw Milk Facts (2012)
Cow	3 cases	2012	Germany	EFSA (2015)
Cow	2 outbreaks, 22 cases	2012	Germany	EFSA (2015)
Cow	80 cases	2012	United States	Real Raw Milk Facts (2012)

Table 3. Prevalence of microorganisms in raw milk production

Raw milk	Incidence	Location of Toxin in farm	Country	Toxic effects	Observations	Reference
***Escherichia coli* O157**						
Buffalo (*Bubalus bubalis*)	1/160 (0.6 %)	Faeces in farm	Italy	No HUS	*Verotoxin:* O26 VTEC For mozzarella production *Resistence*: Vancomycin, teicoplanin, ampicillin, spiramycin and erythromycin	Lorusso et al. (2009)
Bovine	20 cases in children	No data	Italy	HUS. 2 children, serious neurological sequelae	*Verotoxin:* O26:H11. *E. coli* O26 (Stx2) with a high chance of progressing to HUS in children	Germinario et al. (2016)
Cow	78 outbreaks with a single agent, 13 (17 %) by *E. coli*. 1 multiple outbreak: STEC O157:H7 and *Campylobacter*	No data	United States: 2007-2012	A total of 106 affected patients. 34 % patients between 5 and 19 years old and 28 % in children between 1-4	*E. coli* serogroups: O157 (10 outbreaks), O111 (1 outbreak), O26:H11 (1 outbreak), O157:H7 y O121 (1 outbreak)	Mungai et al. (2015)
Cow	0-5.7 % Outbreaks between 1970-2010: 13 (Europe) and 28 (world). According to the EFSA (2009, 2010): between 0-2 %	Bulk milk tank. The concentration depends on the farming method used, etc.	Europe	Diarrhoea, vomiting, nausea, fever, abdominal cramping, etc. In severe cases, HUS. Chronic intoxication can produce reactive arthritis or even death	-	Claeys et al. (2013)
Cow	No data	No data	Austria (2003)	2 cases of HUS (11-month old boy and 28-month old girl)	*Verotoxin:* O26:H Consuming cold raw milk in hotel buffets (*not permitted in restaurants*). The law demands that milk be boiled before consumption and the hotel did not inform its consumers that the milk had not been boiled	Mylius et al. (2018)
Cow	1 sample out of a total of 297 displays Non-O157:H7 STEC	Milk storage	New Zealand	-	99 % of samples display < 100 CFU/ml and 1 sample >1000 CFU/ml. Positive sample of Non-O157:H7 STEC (STEC without Shiga toxins 1 and 2, or eae genes or Hyl A)	Hill et al. (2012)

Table 3. Prevalence of microorganisms in raw milk production

Raw milk	Incidence	Location of Toxin in farm	Country	Toxic effects	Observations	Reference
***Escherichia coli* O157**						
Cow and sheep	5 cases	Bacteria was present in cow faeces and in the surroundings, both within the stable and outside: on the floor of the milking room, feeding table, drinking troughs, and cattle shelters	Finland (Turku)	Hospitalised children 4 (67 %) with HUS	*Verotoxin:* SF O157	Jaakkonen et al. (2017)
Goat	0.75 % (1 sample of 133) bulk milk	Milk tank	United States (Vermont)	No data	Study where *E. coli* O157: H7 was isolated in a sample of goat's milk (0.75 %) by enrichment, which suggests a level of contamination <1 CFU/ml	D'Amico et al. (2008)
Not specified	16 cases (1992-1993)	Illegal sales	United States (ORegon)	1 hospitalisation. No HUS	-	Keene et al. (1997)
Not specified	Period 1983-2012. Total: 335 outbreaks, 2 caused by raw milk	No data	United Kingdom (England and Wales)	No data	-	Adams et al. (2016)
Not specified	255 outbreaks, 16 caused by dairy products (11 %). Of those outbreaks, 13 were caused by raw milk (81 %)	No data	United States: 2003-2012	140 sick, 52 hospitalised and 22 diagnosed with HUS	25 % of those sick were children below the age of 5. 47 % of those sick between 5-19 years of age	Heiman et al. (2015)
Not specified	14 cases	Specific cases that are a cause for concern: manually storing raw milk in bottles from the bulk storage tank, lack of hand soap and poor functioning of hot water taps in the hand washing basin of the storage room where milk is occasionally bottled by hand, faulty valves and the presence of a biofilm within the mobile tank	United States (Connecticut). 2008	Diarrhoea, bloody diarrhoea, vomiting, fever, abdominal cramping, etc. In severe cases, HUS and TTP	5 patients (36 %): hospitalisation 1 patient (20 %): TTP 3 patients (21 %): HUS that requires plasmapheresis	Guh et al. (2010)

Table 3. Prevalence of microorganisms in raw milk production

Raw milk	Incidence	Location of Toxin in farm	Country	Toxic effects	Observations	Reference
Not specified	Outbreaks between 2001-2010: of 530 cases, 19 (3.6 %) were due to *Escherichia coli* O157 serogroup, and 12 cases (2.3 %) due to Non-O157 STEC	No data	United States (Minnesota)	-	Study of Minnesota Health System patients with enteric infections due to STEC (including serogroups O157 and Non-O157). 1 patient was co-infected with STEC O157 and *Campylobacter* spp. and 1 with Non-O157 STEC and *Campylobacter* spp. 4 patients (21 %): had HUS. Of them, 1 child (11 months) died (**)	Robinson et al. (2014)
***Campylobacter* spp.**						
Cow	78 outbreaks with a single agent, 62 (81 %) by *Campylobacter* spp.	No data	United States: 2007-2012	A total of 756 affected patients. Principal age range affected: 40 % patients between 20-29 years, 32 % between 5-19 years and 14 % among children between 1-4 years of age	The number of outbreaks of infections due to *Campylobacter* spp. doubled in 6 years: from 22 (2007-2009) to 40 (2010-2012)	Mungai et al. (2015)
Cow	0-6 % Outbreaks between 1970-2010: 18 (Europe) and 39 (world)	Bulk milk tank. The concentration depends on the farming method used, etc.	Europe	Diarrhoea, vomiting, nausea, fever, abdominal cramps, etc. In exceptional cases, severe clinical symptoms such as Guillain-Barré syndrome. Chronic intoxication can produce reactive arthritis or even death	*Campylobacter jejuni* and *C. coli*	Claeys et al. (2013)
Cow	1 sample (0.34 %) of a total of 296 displays *Campylobacter*	Milk tank	New Zealand	-	-	Hill et al. (2012)

Table 3. Prevalence of microorganisms in raw milk production

Raw milk	Incidence	Location of Toxin in farm	Country	Toxic effects	Observations	Reference
Not specified	Outbreaks between 2001-2010: of 530 cases, 407 (77 %) due to *Campylobacter* spp.	No data	United States (Minnesota)	-	Study of Minnesota Health System patients with enteric infections due to *Campylobacter jejuni, C. coli* and *C. lari*. 9 patients were co-infected with *Campylobacter* spp. and *Cryptosporidium* spp.; 1 with *Campylobacter* spp. and O157 STEC; 1 with *Campylobacter* spp. and Non-O157 STEC; and 1 with *Campylobacter* spp. and *Salmonella* spp. (**)	Robinson et al. (2014)
Salmonella						
Cow	78 outbreaks with a single agent, 2 (3 %) by *Salmonella entérica* Typhimurium serotype	No data	United States: 2007-2012	A total of 13 affected patients. 38 % (5) were children between 1-4 years and 1 patient aged less than 1 year	-	Mungai et al. (2015)
Cow	0-2.9 % Outbreaks between 1970-2010: 5 (Europe) and 39 (world). According to the EFSA (2010): <1 %	<1 % in the bulk milk tank	Europe	Diarrhoea, vomiting, nausea, fever, abdominal cramping, etc. Chronic intoxication can produce reactive arthritis or even death	-	Claeys et al. (2013)
Not specified	Outbreaks between 2001-2010: of 530 cases, 39 (7.4 %) due to *Salmonella*	No data	United States (Minnesota)	-	Study of Minnesota Health System patients with enteric infections due to *Salmonella entérica* Typhimurium serotype, *S. entérica* Montevideo serotype and *S. entérica* Newport serotype. 1 patient was co-infected with *Salmonella* spp *and Campylobacter* spp. (**)	Robinson et al. (2014)

Table 3. Prevalence of microorganisms in raw milk production

Raw milk	Incidence	Location of Toxin in farm	Country	Toxic effects	Observations	Reference
Listeria monocytogenes						
Cow	2.2-10.2 % Outbreaks between 1970-2010: 0 (Europe) and 2 (world)	Bulk milk tank. The concentration depends on the farming method used, etc.	Outside Europe	Diarrhoea, vomiting, nausea, fever, abdominal cramping, etc. Chronic intoxication can produce reactive arthritis or even death	*L. monocytogenes* is frequently detected in raw milk, but its importance with regard to outbreaks due to the consumption of raw cow's milk is considerably low	Claeys et al. (2013)
Cow	4.8 % (3 out of 62 samples of bulk cow's milk) and in 2.3 % (3 out of 133 samples) if we consider the 3 types of milk studied (cow, goat, sheep)	The presence of 2 sub-types suggests the recontamination of milk from a separate source, rather than persistent contamination. The tank does not appear to be contaminated	United States (Vermont)	-	2 different serotypes were isolated (DUP-1030B and DUP-1045B) from 3 samples of cow's milk through enriching, which suggests a contamination level <1 CFU/ml (*)	D'Amico et al. (2008)
Cow	16 samples of a total of 295 display *Listeria* sp.	Milk tank	New Zealand	-	Of the 16 samples with *Listeria*, 4 % belongs to *L. innocua* and 0.68 % to *L. monocytogenes*. In the case of *L. monocytogenes*, the contamination level is 1 CFU/4 ml. *Listeria* contamination is due to housing cattle inside, shoddy silage methods and poor hygiene in the farm	Hill et al. (2012)

HUS: hemolytic uremic syndrome; TTP: thrombotic thrombocytopenic purpura; Population at risk: children, the elderly, pregnant women, immunosuppressed individuals.
(*) The authors point out that the annual incidence of *L. monocytogenes* may be underestimated, as the sampling was carried out in summer and *Listeria* spp. contamination appears to increase in winter months when the animals are stabled inside, crowded together and fed on silage.
(**) An increase in cases of enteric disease linked to raw milk consumption is noted during the summer months (June-August) in comparison to other seasons. This trend is in line with the general seasonal nature of enteric pathogens included in this study, and is also consistent with recent data on the seasonal incidence of *Salmonella* spp. in the sampling of bulk milk tanks, milch cows and agricultural surroundings and the seasonal trend of faecal dissemination of *E. coli* O157 STEC by milk cattle.