



Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on the microbiological risks associated with the consumption of raw milk and raw milk-based products

Section of Food Safety and Nutrition

Elena Alonso Lebrero, José Manuel Barat Baviera, María Pilar Conchello Moreno, Ramón Estruch Riba, María Antonia Ferrús Pérez, Guillermina Font Pérez, Susana Guix Arnau, Arturo Hardisson de la Torre, Ángeles Jos Gallego, Ascensión Marcos Sánchez, Amelia Martí del Moral, Olga Martín Belloso, María Aránzazu Martínez Caballero, Alfredo Palop Gómez, Gaspar Pérez Martínez, José Luis Ríos Cañavate, Gaspar Ros Berrueto, Jesús Ángel Santos Buelga, Jesús Simal Gándara, Josep Antoni Tur Marí

Technical Secretary

Vicente Calderón Pascual

Reference number: AECOSAN-2015-004

Report approved by the Section of Food Safety and Nutrition of the Scientific Committee on plenary session
May, 20th 2015

Working group

María Antonia Ferrús Pérez (Coordinator)
José Manuel Barat Baviera
María Pilar Conchello Moreno
Susana Guix Arnau
Alfredo Palop Gómez
Jesús Ángel Santos Buelga

External collaborators: Antonio Herrera Marteache, María Rosario Martín de Santos, Antonio Martínez López

Abstract

At present in Spain, placing raw milk or cream on the market for direct human consumption is not restricted or prohibited if all requirements set out by Regulation (EC) No 853/2004 are met. However, direct supply of small quantities of raw milk from the producer to the end consumer or to local retail establishments that supply it directly to the end consumer is prohibited, according to Royal Decree 640/2006.

The Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) is looking into the possibility of modifying Royal Decree 640/2006. Consequently, they have requested information from the Scientific Committee on the microbiological risks associated with consuming raw milk and raw milk-based products.

The Committee was asked to draw up a report which was to take three factors into account: 1) the sale of raw milk and cream, including alternative ways of placing it on the market such as vending machines, 2) the production of raw-milk cheese aged for more than 60 days, which does not comply with somatic cell and aerobic germ criteria and 3) colostrum-related requirements.

The Scientific Committee has drawn a report concerning its assessment of the microbiological risks associated with raw milk and cream. Many of the recommendations requested relating to other matters can be gleaned from this report.

Based on the report drawn up, the Committee considers that raw milk may carry pathogenic microorganisms and the risk could be reduced, but not eliminated, through extreme hygiene practices. Pasteurisation is the only effective method that guarantees the elimination and control of pathogenic microorganisms in this foodstuff and its derivatives. Therefore, it is recommended that Article 3 (1a) of Royal Decree 640/2006 is left unchanged and the terms it refers to are retained. The same considerations should be taken into account for direct colostrum consumption.

In the same way, the microbiological hazards identified for raw milk are initially present in raw milk destined to/for other distribution channels, such as vending machines, together with some additional risks specific to such methods of placing the product in the market. Therefore, the Committee recommends that a series of preventative measures are established, including informing the consumer that boiling milk before consuming is obligatory.

Nevertheless, it is considered unlikely, though not out of the question, that pathogenic microorganisms could survive in cheese aged for more than 60 days. Safety in this case essentially depends on the level of hygiene in facilities and the microbiological quality of the initial milk.

Finally, the report recommends those consumers in high-risk or susceptible groups to avoid consuming raw milk and insists they should be appropriately informed about the risks associated with it as well as the preventative hygiene measures that should be taken.

Key words

Raw milk, raw cream, cheese, colostrum, microbiological risks.

1. Introduction

The Management Board of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has asked the Section of Food Safety and Nutrition of the Scientific Committee to report on the microbiological risks associated with the consumption of raw milk and raw milk-based products.

The request indicates that “the consumption of raw milk involves certain associated risks to humans due to the presence of pathogenic microorganisms”, but that “there are at present alternative methods of sale other than the traditional distribution channels, as for example the case of vending machines”.

The Committee was asked to draw up a report, which was to take three factors into account: 1) the sale of raw milk and cream, 2) the production of raw-milk cheese aged for more than 60 days, which does not comply with somatic cell and aerobic germ criteria and 3) colostrum-related requirements.

As the content of the request sets out clear differences between the areas to be considered, this report is submitted in two different parts, the first of which is linked to milk, cream and colostrum and the second to cheese. However, the microbiological bases on which the reports should be based may be considered to be similar and, therefore, useful for each part.

2. Sale of raw milk and cream and colostrum-related requirements

2.1 Introduction

2.1.1 Basis of the request

At present in Spain, placing raw milk or cream on the market for direct human consumption is not restricted or prohibited as the following article from Regulation (EC) No 853/2004 of the European Parliament and the Council, of 29 April 2004, laying down specific hygiene rules for food of animal origin has not yet been developed (EU, 2004a):

“8. A Member State may, of its own initiative and subject to the general provisions of the Treaty, maintain or establish national measures: a) prohibiting or restricting the placing on the market within its territory of raw milk or cream intended for direct human consumption”.

Therefore, raw milk and cream intended for direct human consumption that meets the requirements of Regulation (EC) No 853/2004 may be marketed.

Nevertheless, Regulation (EC) No 853/2004 and Regulation (EC) No 852/2004 (EU, 2004b) of the European Parliament and of the Council of 29 April 2004, on the hygiene of foodstuffs, are not applicable to the direct supply by the producer of small quantities of primary products to the end consumer or to local retail establishments. Therefore, Royal Decree 640/2006, of 26 May, laying down control measures for the application of community provisions on the hygiene, production and marketing of food products, established the following ban (BOE, 2006):

“Article 3. General conditions.

1. The competent authority may authorise the direct supply by the producer of small quantities of primary products to the end consumer or to local retail establishments that sell directly to the end consumer, except: a) raw milk”.

Consequently, there are two types of situation:

1. At present in Spain, placing raw milk or cream on the market for direct human consumption is not restricted or prohibited if all requirements set out by Regulation (EC) No 853/2004 are met.
2. However, direct supply of small quantities of raw milk from the producer to the end consumer or to local retail establishments that supply it directly to the end consumer is prohibited, according to Royal Decree 640/2006.

2.1.2 Terms of reference

Based on the facts, the Scientific Committee has been asked to express their opinion on the following areas:

With respect to situation 1) there are various methods for the sale of raw milk, such as vending machines, and it would be advisable to assess whether there are additional risks associated with certain methods of sale which are not covered by the established requirements, and where applicable, recommendations on specific conditions for the packaging and sealing equipment, for the preservation of the milk, consumer recommendations, aspects relating to the shelf-life, the possible risks from bulk selling, where the consumer is required to take their own packaging, etc.

With respect to situation 2) of the above paragraph, the AECOSAN plans to modify Royal Decree 640/2006, and one of the questions to be assessed is the possibility of permitting this type of sale, that is, the direct supply by the producer of small quantities of raw milk to the end consumer or to local retail establishments that sell directly to the end consumer. This is why the Scientific Committee has been asked to prepare a report on the microbiological hazards associated with raw milk and cream marketed in this way, and where applicable, what options are available to mitigate these hazards.

2.2 Microbiological hazards conveyed by raw milk and/or cream. Characterisation of the same and risk assessment

2.2.1 Concept of raw milk and cream

From a biological point of view, raw milk is the product obtained from the unmodified secretion of the mammary glands of female mammals, a few days after birth and intended to feed their young. It is a natural food, nutrient-rich, containing high quality proteins, lipids, essential vitamins and minerals.

The Spanish Food Code defines natural milk as “the complete, unaltered and unadulterated product, without colostrum, obtained from the regular, hygienic complete and continuous milking of healthy well-fed domestic females”, indicating that the generic name of milk applies solely and exclusively to natural cow’s milk (BOE, 1967).

For the effects of Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin, raw milk is understood as “the milk produced by the secretion of the mammary glands of production animals which has not been heated beyond 40 °C, or undergone any treatment having an equivalent effect”.

The DG SANCO (Directorate General for Health and Consumers Protection) of the European Commission has indicated that this definition includes different animals from the common dairy producing breeds (cows, sheep and goats), although in practice milk from other production animals can also be marketed (mares, donkeys, camels), including wild game (for example reindeer), provided that the production and processing procedures comply with the relevant requirements established in Regulations (EC) No 852/2004 and 853/2004 (DG SANCO, 2009).

On the other hand, the Spanish Food Code defines cream as the product with a high fat content separated from milk allowed to stand or by centrifugation, produced from cow's milk, or if from a species other than the cow, the name of the species should be added. By extension of that specified in Regulation (EC) No 853/2004, the definition of raw milk is understood to include the requirements demanded of raw milk, both from production and from the limits of heat treatment in order to be considered as raw cream.

2.2.2 Colostrum

Regulation (EC) No 853/2004 defines colostrum as "the fluid secreted by the mammary glands of milk-producing animals up to three to five days post parturition that is rich in antibodies and minerals, and precedes the production of raw milk". The Regulation also defines colostrum-based products as processed products resulting from the processing of colostrum or from the further processing of such processed products, indicating that pending the establishment of specific Community legislation, national criteria for colostrum, as regards plate count, somatic cell count or antibiotic residue shall apply.

2.2.3 Hazards related to the consumption of raw milk or cream

The risks associated with the consumption of raw milk and cream are related to the presence of contaminating agents of either a biological or a chemical nature. This report is limited to the risks caused by hazards of a biological nature.

The sources of biological contamination of raw milk and by extension of raw cream are numerous and varied; although, in general, it is accepted that the milk inside the mammary glands of healthy animals is practically sterile, the possibilities for contamination during the milking and subsequent processing determine that in conditions of maximum asepsis, freshly drawn milk may have values ranging from 5×10^3 to 5×10^4 cfu/ml. In addition, sometimes the microorganisms present in the animal itself may directly contaminate the milk (endogenous contamination) either due to a systemic infection or due to a localised infection in the udder.

Milk is an excellent growing medium for many microorganisms thanks to its high water content, an almost neutral pH and a huge variety of nutrients. Therefore, once the microorganisms are present they rapidly adapt to the environment and begin to grow; nevertheless, although it is a suitable medium for development, not all microorganisms are able to grow in raw milk, thanks to certain natural barriers present in the milk (immunoglobulins, lysozyme, lactoferrin, transferrin) which prevent their growth.

An excellent bibliographic review, carried out by Claeys et al. (2013), studies the risks and benefits linked to the consumption of raw cow's milk and lists the principal pathogenic microorganisms potentially present in raw milk, grouping them according to the source of contamination (Table 1). Similarly, this had been seen previously by Oliver et al. (2005) in a study in which they analysed the implications that milk-borne pathogens may have for public health and food safety. This well-documented study lays out the risks associated with both the consumption of raw milk and of unpasteurised dairy products.

In accordance with these studies, *Salmonella* spp., *Campylobacter* spp., verotoxigenic *Escherichia coli*, *Yersinia enterocolitica* and *Listeria monocytogenes*, and enterotoxin poisoning produced by *Staphylococcus aureus*, are the most frequently identified agents in the outbreaks linked to the consumption of raw milk or derived products. The data provided by Claeys et al. (2013) summarise that between 1970 and 2010 in Europe a total of 36 outbreaks associated with the intake of raw milk were the subject of scientific publication (5 due to *Salmonella* spp., 18 due to *Campylobacter* spp. and 13 due to enteropathogenic *Escherichia coli*), while at global level 110 outbreaks were reported (39 due to *Salmonella* spp., 39 due to *Campylobacter* spp., 28 due to enteropathogenic *Escherichia coli* and 2 due to *Listeria monocytogenes*). Similar data are reported in various risk assessment studies conducted by the scientific committees of different food safety agencies. These include the epidemiological study submitted by Langer et al. (2012), in which they review all the outbreaks of food-borne infection associated with the consumption of milk in the United States between 1993 and 2006. This paper, in which 73 of the 121 outbreaks studied (60 %) were confirmed to have been caused by the consumption of raw milk or unpasteurised dairy products, concluded that there was a higher number of outbreaks (75 %) in those states in which the sale of unpasteurised milk was permitted than in those where the sale was not authorised (25 %).

Similarly, numerous investigations offer information about the frequency and levels of these microorganisms in raw milk and cream. A collection of these can be consulted in the document "Microbial Risk Assessment of Raw Cow milk" published by the Microbiological Risk Assessment Section of the Food Standards Australia New Zealand (FSANZ, 2009); the data submitted in this report are extremely variable between countries and indicate that the prevalence of *Campylobacter* in raw milk varies between 0 and 40 % of the samples analysed while *Escherichia coli* is found in between 0 and 33.5 %, reaching a prevalence value of 89.8 % in samples of raw milk in a study conducted in France. In the case of *Listeria*, the data collected in the report from the Australian and New Zealand Agency, range from 1 to 60 % (45.3 % in Spain in a study carried out by Dominguez Rodriguez et al. (1985)) and, for *Salmonella*, the values range between 0 and 11.8 %. Similar references had previously been cited in the revision performed by Oliver et al. (2005).

Other microorganisms, traditionally milk-borne, and responsible for transmissible zoonoses, including tuberculosis or brucellosis, currently have a low incidence. In the case of *Mycobacterium bovis*, a risk assessment study recently conducted by the Advisory Committee on the Microbiological Safety of Food of the FSA (Food Standards Agency) of the United Kingdom

(ACMSF, 2011) concludes that the risk of tuberculosis infection due to the consumption of milk or unpasteurised dairy products is very low (both for cow's milk and for other milk producing animals, in which transmission is possible but unlikely) although the presence of *Mycobacterium bovis* in cattle has increased. In the case of brucellosis, the reports of the results of epidemiological monitoring indicate that the prevalence of these zoonoses in Europe has decreased significantly with an incidence rate in 2012 of 0.07 cases per 100 000 inhabitants, although the evidence for the transmission of *Brucella* from milk and dairy products has always been considered as real (EFSA, 2014).

Another zoonotic agent involved in the possible milk-borne transmission is *Coxiella burnetii*, although in the majority of cases the disease in man is caused by the inhalation of dust contaminated with amniotic fluid or with fetal membranes of infected animals. This microorganism shows certain resistance to heat implying its possible presence when the heat treatment of the milk is not effective enough. In Spain in 2012 a total of 52 cases of Q Fever were reported in humans, and 643 cases (0.17/100 000) in the European Union (EU), with a significant decrease observed during the period between 2008 and 2012 (15 % from 2011 to 2012).

A number of scientific studies characterise these zoonotic agents and demonstrate how animal health measures, primary production hygiene and technological treatments have largely contributed to their control and decreased incidence.

This is not the case with those pathogenic microorganisms that may enter the milk as a result of extrinsic contamination, for which exclusion or control largely depends on the use of extreme measures of hygiene, among which the use of cold plays a predominant role. These microorganisms in many cases come from faecal sources and in others are very ubiquitous, with a high capacity for contaminating the milk from the moment of milking, and are able to actively multiply, even if the milk is stored at low temperatures.

The majority of the risk assessment studies associated with the consumption of raw milk and unpasteurised dairy products refer to *Campylobacter*, *Salmonella*, *Escherichia coli* and *Listeria monocytogenes*, agents known to be involved in the majority of outbreaks associated with the consumption of these foods. Nevertheless, and as shown in tables 1 and 2, the list of possible infectious agents transmitted by raw milk or derived products is long, and the severity of the damage caused is, on occasions, high, given the consumption of this food by particularly sensitive consumers.

A recent report written by the European Food Safety Authority (EFSA, 2015) establishes a list of microbiological hazards identified as potentially transmissible through raw milk, and present in milk-producing animals in the EU. Of these, based on the severity of the disease produced, the main hazards are *Campylobacter* spp., *Salmonella* spp., shigatoxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*, *Corynebacterium* spp., the tick-borne encephalitis virus (TBEV), *Toxoplasma gondii*, *Streptococcus equi* subsp. *zooepidemicus*, *Brucella melitensis* and *Mycobacterium bovis*. Of these, *Campylobacter* spp., *Salmonella* spp. and STEC are the most widely distributed in the EU, and *Campylobacter* spp. is the principal producer of outbreaks associated with the consumption of raw milk.

2.2.3.1 *Campylobacter* spp.

Campylobacter spp. may be directly present in milk as a result of clinical or subclinical mastitis or indirectly, through faecal contamination. *Campylobacter jejuni* is the species of *Campylobacter* most frequently detected in milk due to its regular presence in the intestinal tract of cattle and, although the concentrations in milk are generally low, they are sufficient to cause infection, as rates of 500 to 800 cells are able to cause it (Robinson, 1981) (EFSA, 2014). Although this microorganism does not grow in raw milk due to its specific microaerophilic requirements, the ICMSF (International Commission on Microbial Specifications for Foods) indicates that raw milk or underpasteurised milk is one of the vehicles identified as the cause of campylobacteriosis in humans (ICMSF, 1998). Spain's report on zoonosis and antimicrobial resistance for 2012 indicates that campylobacteriosis is the most frequently occurring food zoonosis both in Spain and in all the countries of the EU together, and in 2012, a total of 5 488 cases (47.5 cases per 100 000 inhabitants) were reported (MAGRAMA, 2012). In the EU the number of confirmed cases in 2012 was 214 268 (notification rate of 55.49 cases per 100 000 inhabitants) (EFSA, 2014), with particular indications that raw milk was involved in several outbreaks of campylobacteriosis. In the United States in 2013, the CDC (Centers for Disease Control and Prevention) reported the appearance of several outbreaks between 2011 and 2013, caused by *Campylobacter jejuni* and associated with the same dairy in the State of Pennsylvania (Weltmann et al., 2013); in addition in Kansas in 2007, an outbreak was reported, affecting 67 people who ate fresh cheese made from unpasteurised milk (Hunt et al., 2009).

2.2.3.2 *Salmonella* spp.

Salmonella spp. is found in the intestinal tract of the majority of animals and consequently the contamination of raw milk by cattle faeces is possible. Studies by different authors indicate contamination frequencies in raw milk of between 0 and 11.8 %. In the EU, there has been a significant fall in the number of cases in recent years; the number of cases of salmonellosis confirmed in 2012 was 92 916 (prevalence of 22.2 cases per 100 000 inhabitants) with a drop compared to 2011 of 4.7 % (EFSA, 2014). In Spain, the number of notifications of cases of salmonellosis has stabilised at 4 200 cases/year (4 216 in 2012) (MAGRAMA, 2012) and studies carried out by the official control bodies in their zoonosis control programmes did not detect the presence of *Salmonella* in milk and dairy products in 2012 in Spain (MAGRAMA, 2012). Similarly, the notifications recorded in the CDC advise of the appearance, on occasions, of outbreaks of salmonellosis related with the consumption of unpasteurised milk in Utah (Hall et al., 2010), cheese in Illinois (Austin et al., 2008) or contaminated unpasteurised milk and cheese in Pennsylvania (Lind et al., 2007).

2.2.3.3 *Escherichia coli*

Escherichia coli is a microorganism normally present in the digestive system of healthy animals and therefore its presence in raw milk may indicate contamination of a direct or indirect faecal nature. Similarly, this microorganism may be excreted with milk in the case of mastitis. The

virulence properties, mechanisms of pathogenicity and the clinical syndrome caused by certain strains and serotypes of *Escherichia coli* have determined its classification in different groups (enteropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative and enterohemorrhagic). Some of the strains are able to produce verotoxins (VTEC). Among the human pathogenic VTEC strains the most frequent is serotype O157:H7 (VTEC O157). This serotype and other less frequently occurring ones have often been isolated from raw cow's milk, with contamination rates of between 1 and 33.5 %. In 2012 in the EU, 5 671 cases of food-borne infections caused by *Escherichia coli* were reported, and in those cases reported for which food origin was detected, no one was related to milk. The most recent notifications reported in the CDC linked to food-borne infections of *Escherichia coli* and the consumption of raw milk are from 2006 in various zones of California, due to the consumption of unpasteurised milk and colostrum (Schneider et al., 2008), and from 2005, when an infection by this microorganism was associated with the consumption of raw milk in the states of Washington and Oregon (Bhat et al., 2007).

2.2.3.4 *Listeria monocytogenes*

Listeria monocytogenes can be isolated from a variety of sources including the floor, silage, wastewater, food production related environments, raw meat and human and domestic animal faeces.

This microorganism is estimated to be present in raw milk and dairy products in percentages ranging from 3 to 4 % (FSANZ, 2009), although some authors offer figures of up to 25 % in cheeses (Terplan, 1988). Oliver et al. (2005) summarise the figures from studies that estimate values between 1 and 12.6 % in milk bulk tanks. In Spain, the report on zoonosis and antimicrobial resistances (MAGRAMA, 2012) indicates that in 2012, the percentage appearance of *Listeria monocytogenes* in analysed samples was 0.68 % for milk and 0.77 % for dairy products. The EFSA (2014) report on trends of zoonoses in the EU, for 2012, states that more than 99 % of the samples analysed of soft and semi-soft cheeses collected at processing that complied with the stipulated criteria produced negative results in the search for *Listeria monocytogenes*, whereas in the same period levels of between 3.4 and 7.2 % were detected among cheese samples for which there was information about the processing type. In addition, this report observed levels in raw milk intended for direct consumption (between 1.9 and 4.4 %) in the tests carried out in five member states.

Some cases of listeriosis have been associated with the consumption of soft cheeses and unpasteurised milk. In 2012, of the 32 surveys conducted in the EU and listed by the ECDC (European Centre for Disease Prevention and Control) in their annual report (ECDC, 2013), four of these were related to *Listeria monocytogenes* and in two of these cheese the bacterium was identified as the cause of the infection. Similarly, the CDC lists various multistate outbreaks linked to the consumption of dairy products in the United States, the last of which occurred in March 2014 (CDC, 2014). Cartwright et al. (2013) in the summary of cases of listeriosis in the United States reported by the food-borne infection monitoring system, indicate that among the 28 outbreaks registered in this period of time, in which 359 people were ill and 38 died, dairy products,

specifically fresh cheeses (Mexican style), were involved in four of the outbreaks and in three of these it was proven that this food had been manufactured from unpasteurised milk; pasteurised milk was also involved in two other outbreaks.

2.2.3.5 Tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV) is a serious disease that, since 2012, must be reported in the EU and which today is considered to be endemic in the majority of European countries (Amicizia et al., 2013). In 2012, in the EU a total of 2 560 cases (0.52/100 000) were reported (ECDC, 2014). The disease attacks the nervous system and appears as meningitis or meningoencephalitis in the majority of cases. The mortality rate in Europe ranges between 1 and 4 %. Although in most cases humans are infected by a tick bite, food-borne transmission as a result of consuming raw milk from infected animals is also possible. In countries such as the Czech Republic, food-borne transmission amounts to less than 0.9 %.

Although the majority of outbreaks have been associated with the consumption of goat's milk, cases have also been recorded of transmission through cow's and sheep's milk and the infectious virus has been isolated from dairy products including yoghurt, butter and cheese.

Table 1. Pathogenic microorganisms potentially present in raw cow's milk, sources of contamination and levels of presence in certain microorganisms in the same

Agent	Intrinsic contamination	Mastitis	External contamination (faecal, skin, etc.)	Environmental contamination	Frequency of appearance
Pathogenic bacteria					
<i>Salmonella</i> spp.	X (S. Dublin)	Rare	X	X	0-2.9 %
<i>Brucella abortus</i>	X	Rare		X	
<i>Mycobacterium bovis</i>	X		X	X	
<i>Coxiella burnetti</i>	X		X	X	
<i>M. avium</i> subs. <i>Paratuberculosis</i>	X		X	X	
<i>Listeria monocytogenes</i>	X	X	X	X	2.2-10.2 %
Verotoxigenic <i>E. coli</i>			X	X	0-5.7 %
<i>Campylobacter</i> spp.			X	X	0-6 %
<i>Corynebacterium pseudotuberculosis</i>	Rare	Rare			
<i>Yersinia</i>		X (only pseudotuberculosis)	X	X	
<i>Bacillus cereus</i>				X	
Enterotoxigenic <i>S. aureus</i>		X		X	
<i>Arcanobacter pyogenes</i>		X			
<i>Streptococcus zooepidemicus</i>		X			
Leptospira	X			X (urine)	
Virus					
Rift Valley Fever	X				
Tick-borne encephalitis virus	X				
Parasites					
<i>Cryptosporidium parvum</i>			X	X	

Source: (Claeys et al., 2013).

Table 2. Summary of microbiological risks associated with the consumption of raw milk

Microorganism	Possibility of contamination from udder (mastitis or endogenous contamination)	Severity (in accordance with ICMSF (2002))	Involved in FBD
<i>Bacillus cereus</i>		Moderate	++
<i>Campylobacter jejuni/coli</i>	X	Severe	++
<i>Clostridium perfringens</i>		Severe	+
<i>Coxiella burnetti</i>	X	--	+
<i>Cryptosporidi parvum</i>		Severe	+
Enterohemorrhagic <i>E. coli</i>	X	Severe	++
<i>Listeria monocytogenes</i>	X	Severe	++
<i>Salmonella</i> spp.	X	Serious	++
<i>Staphylococcus aureus</i>	X	Moderate	++
<i>Streptococcus</i>	X	--	+
<i>Toxoplasma gondii</i>	X	--	++
<i>Yersinia enterocolitica</i>	X	Serious	+

Source: (FSANZ, 2009).

2.2.3.6 Influence of raw milk storage temperature from the producer to the consumer in the multiplication of the most frequent pathogens

A review of the survival of the principal food pathogens according to the storage temperature is given in the EFSA report on the risks related to the consumption of raw milk (EFSA, 2015).

Salmonella is a microorganism which, although considered mesophyllic, has a huge capacity for adaptation at low temperatures, and strains able to grow at chill temperatures have been described. Studies of different foods have demonstrated that 2-4 °C is the limiting temperature (Li et al., 2013).

Campylobacter is unable to grow at temperatures below 30 °C. The microorganism survives best at chill temperature rather than at room temperature, and has been recovered from milk after several weeks at 4 °C (Habib et al., 2013), although there are huge differences in the capacity for survival according to the strain studied (EFSA, 2015). In modelling studies in milk Giacometti et al. (2012a) propose decimal reduction times of approximately 600 hours at 4 °C.

The limiting temperature of growth for *Escherichia coli* is commonly accepted to be 6 °C (Hudson, 2011) (Soboleva, 2013). However, Giacometti et al. (2012a) propose generation times of approximately 45.1 hours at 4 °C.

Listeria monocytogenes may grow at temperatures of above 0 °C, with generation times of 43 and 6.6 hours at 4 °C and 10 °C, respectively. Temperatures below 0 °C permit their survival, or slightly reduce the count, depending on the product and other ambient factors (Ryser and Buchanan, 2013). In a recent review of the *Agence Federale pour la Securite de la Chaine Alimentaire* from Belgium (AFSCA, 2015), however, the limiting temperature to growth was defined as -0.4 °C, with generation times of 11.4 hours at a pH of 6.5 and a water activity of 0.997.

2.2.4 Hazards related to colostrum

The majority of the pathogenic bacteria we have indicated as present in raw milk have also been isolated from the colostrum and, therefore, these bacteria may pose a health risk if they are able to survive the colostrum processing to make it fit to be used as a food. Initially the high content of immunoglobulins and lactoferrin are shown as being able to inhibit many microorganisms (Jayarao and Henning, 2001) (Godeden et al., 2006) (Claeys et al., 2013). Nevertheless, the main microorganisms recognised as pathogens borne by milk and its derivatives (*Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Clostridium* spp.) have also been isolated in colostrum (Headrick et al., 1998) (Heuvelink, et al., 1998) (Kendall, 2003), and although they are identified in different links of the production chain, but with greater frequency at the level of raw milk, outbreaks of infections caused by the consumption of colostrum can also be found in literature (Schneider et al., 2008).

In the elaboration of colostrum-derived products of a high nutritional quality, raw colostrum obtained under the maximum conditions of hygiene is required, as traditional pasteurisation processes may denature many bioactive proteins, resulting in a loss of its beneficial effects and of its physiological function (Gapper et al., 2007). This fact makes necessary, on occasion, to use methods to obtain colostrum-derived products that do not affect the biological wealth of the proteins. Therefore, if the initial hygienic quality is inadequate, the use of alternative methods may not destroy the pathogenic and/or spoilage microorganisms present in raw colostrum (Domínguez et al., 1997).

2.2.5 Assessment of the risk derived from the consumption of raw milk and cream in derived dairy products

The information provided in the above section justifies the numerous studies of the assessment of the risks associated with the consumption of raw milk and derived products.

At the end of this report, in appendix I, we provide a non-exhaustive list of different reports. We hereby draw attention to the main conclusions that can be extracted from these reports:

1. The consumption of raw milk has always posed risks for Public Health associated with the potential presence of pathogenic microorganisms in the milk. The presentation of outbreaks of milk-borne disease led, at the time, to the extended use of pasteurisation of the liquid milk and the effectiveness of this in eliminating the majority of the pathogenic microorganisms was increased with the improvement of conditions of hygiene during the bottling of the milk.
2. There is evidence to link these risks with the following pathogenic microorganisms: *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes* and some serovarieties of *Salmonella*. This evidence is classified as moderate in some reports (Jaros et al., 2008) and high in other studies for at least two of the four pathogens indicated (NSCFS, 2006) (FSAI, 2009) (AFSCA, 2012). In addition, this evidence shows differences with respect to the severity of the different agents, the most severe being shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes*, particularly for especially sensitive populations in the case of this last agent.

3. Assessments of a quantitative nature have shown that the risk associated with *Campylobacter* spp. is higher in the milking environment, whereas the risks related to *Escherichia coli*, *Salmonella* and *Listeria monocytogenes* are more evident in the subsequent treatment on the farm, in the storage and in transport (FSANZ, 2009).
4. In addition, some reports indicate that this evidence is also true, although less strong, with respect to other pathogens with a possible presence in raw milk including *Coxiella burnetti*, *Shigella*, *Staphylococcus*, *Yersinia*, *Cryptosporidium*, and other emerging microorganisms.
5. Existing prevalence data do not permit to obtain quantitatively feasible for brucellosis, as animal health controls have achieved a significant drop in this zoonosis for milk-producing animals, but the evidence for transmission of *Brucella* from raw milk and derived dairy products has been accepted and documented by the scientific community.
6. Risk assessment studies carried out with respect to the transmission of *Mycobacterium bovis* through unpasteurised milk and dairy products (ACMSF, 2009) indicate that, although the risk of infection has changed due to the increase of the presence of this agent in cattle, the risk with respect to unpasteurised milk and dairy products is very low.
7. The recent report from the EFSA (2015) lists the group of microorganisms potentially present in milk in the EU and also includes parasites such as *Cryptosporidium parvum*, *Toxoplasma gondii* and the tick-borne encephalitis virus (TBEV). This report concludes that the most relevant risks in the EU are from *Brucella melitensis*, *Campylobacter* spp., *Mycobacterium bovis*, *Salmonella* spp., STEC and TBEV, and *Campylobacter* spp., *Salmonella* spp. and STEC are more widely distributed.
8. The risk of food-borne infection from the consumption of raw milk is particularly significant for those groups who may be considered as vulnerable, including children, the elderly, pregnant women and consumers with a weakened immune system (AFSCA, 2011, 2013, 2014).
9. Epidemiological analyses carried out by the CDC indicate that the occurrence of outbreaks of food infections linked to the consumption of unpasteurised milk or dairy products is related to the control of its sale, as in those geographical areas in which its sale is permitted, the occurrence of outbreaks is higher than in those areas in which it is not permitted.
10. The majority of health administrations and food safety agencies have established recommendations related to the consumption of raw milk dairy products made from raw milk directed towards the risk posed with respect to the agents mentioned and maintaining the opinion that possible benefits from the consumption of milk that has not been heat-treated do not compensate for the harm caused by the possible appearance of these illnesses.
11. Numerous organisations and associations recommend pasteurisation for all dairy products consumed by humans, including the following: In the United States, the Center for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the American Academy of Pediatrics (AAP), the American Academy of Family Medicine (AAFM), the American Veterinary Medical Association (AVMA) and the National Association of Public Health Veterinarians (NAPHV); in Europe the Food Safety Authority of Ireland (FSAI),

the *Agence Fédérale pour la Sécurité de la Chaîne Alimentaire* of Belgium (AFSCA), the Norwegian Scientific Committee for Food Safety of Norway (NSCFS) and the *Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail* of France (ANSES).

2.3 Additional risks associated with the sale of raw milk through alternative channels

At present, the consumer has access to alternative channels of sale for raw milk, for example vending machines (EFSA, 2015). The additional hazards due to the sale using this channel are essentially due to the circumstances in which the machines operate, the temperature during transport and storage and the consumer treatment practices.

2.3.1 Conditions of the vending machines

Any hygiene deficit in the design or operation of the vending machine will result in an increase in the microbial load, either due to the growth of the milk contaminants, or due to the access of exogenous contamination. The easy formation of biofilms in the equipment is a factor that significantly increases the risk (EFSA, 2015).

A study carried out in 2010 at 33 farms authorised for the sale of raw milk in Emilia-Romagna Region shows that 44.8 % of the milk samples exceed the limit established for the total bacterial count (>50 000 cfu/ml) and 18.8 % the somatic cell count (>300 000 sc/ml). The analysis of pathogenic microorganisms is a point of conflict, as when the references techniques are used for the detection of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7 and thermotolerant species of *Campylobacter* no positive results were obtained, but if alternative methods are used, 1 to 2 % of positive results are obtained (Giacometti et al., 2012b).

Another study carried out in the Piedmont Region of Italy by Bianchi et al. (2013) in which milk samples taken at authorised farms and vending machines were analysed using QPCR (real-time PCR), found that 3 % of the machine samples were contaminated with one of the pathogens studied, and in some samples (contaminated with *Escherichia coli* O157, *Campylobacter jejuni* and *Listeria monocytogenes*), the contamination was only observed in the samples from the vending machine and not from the producer farm.

In a study carried out by Tremonte et al. (2014), 30 samples of raw milk from three vending machines were analysed immediately after their delivery. In all the cases, the total mesophilic count was very close to or exceeded those authorised under Italian legislation (5.0 log cfu/ml).

To prevent the risks associated with the poor operation or design of these machines, the Belgian *Agence Fédérale pour la Sécurité de la Chaîne Alimentaire* (FASFC) requires producers to apply strict good hygiene practices, and to implement a self-control system based on HACCP (Hazard Analysis and Critical Control Points) principles. Many other supplementary measures are required, including the following (EFSA, 2015):

- Instructions must be given on the machine indicating that the milk must be boiled before consumption, and stored at between 2 and 4 °C.

- The machines must indicate at all times the temperature of the milk stored, using a thermometer which is clearly visible to the consumer.
- The placement of the vending machines in retirement homes and in schools is discouraged, if there is no guarantee that the milk will be boiled before consumption.
- The vending machine must be filled with milk, already cooled to 6 °C at the farm, and the temperature of the milk during transport must be between 0 and 4 °C. The design of the machine must ensure homogenous cooling of the stored milk at all times. Some types of vending machine are designed to automatically stop dispensing milk if the milk temperature is greater than 4 °C or if the system does not perform the cleaning operation correctly.
- No mixing of milk from different farms is allowed.
- The vending machine has to be refilled daily and the machine thoroughly cleaned prior to refilling. The residual milk, or milk which has not been cooled properly cannot be used for human consumption.
- The design of the machine must prevent exogenous contamination, and the presence of residual milk between dispensed portions.
- The machines must be subjected to manual or automatic cleaning processes which guarantee good hygiene practices.

In Italy, the sale of raw milk has been permitted since 2004, at provincial level or in neighbouring provinces. In addition to the general conditions, established under European regulations, in some cases additional criteria have been designed, such as those listed in Appendix II corresponding to the Emilia-Romagna Region (Regione Emilia Romagna, 2008). In these criteria, the total plate count at 30 °C and somatic cell count in cow's milk is lowered (100 000 cfu/ml and 400 000/ml in Regulation (EC) No 853/2004) and includes *Staphylococcus aureus*, *Escherichia coli* O157 and thermotolerant species of *Campylobacter*, in addition to *Listeria monocytogenes* and *Salmonella*.

Milk must be chilled to <4 °C as soon as possible after milking and transported and stored in the vending machine at this temperature. It must be replaced every day.

2.3.2 Temperature during transport and storage of the raw milk

With regard to possible temperature abuse and considering the data obtained by Giacometti et al. (2012c), it can be assumed that during transport from the farm, storage in the vending machine and storage in the home, the milk is at temperatures of between 4 and 7 °C and during the transport from the machine to the home there may be an increase of up to 11 °C. With the data shown in table 3, and using a dynamic growth model in the Combase (www.combase.cc) simulator in accordance with the following profile, the greatest risk is from the growth of *Listeria monocytogenes*, which may increase in count by more than 1 log ufc. The rest of the microorganisms considered would not grow in these conditions, although counts of *Salmonella* and *Escherichia coli* would not decrease either.

Table 3. Conditions during transport and storage

Time (h)	Temperature (°C)	Situation reflected
0	4	Milk in tank at farm
2	7	Transport of milk to the machine (assuming a maximum of 2 hours travel time)
2.5	4	Lowering of temperature in vending machine
24	4	Milk remains in machine
24.5	11	Half hour unrefrigerated transport to home
30	7	4-5 hours until the temperature stabilises in the consumer's refrigerator
96	7	Storage in refrigerator at home

2.3.3 Conditions of treatment by the consumer

In the case of vending machines, consumers are advised to boil the milk before consumption. In some countries, such as Italy, one of the countries with the highest number of vending machines, and following a case of HUS (Hemolytic-Uremic Syndrome) attributed to the consumption of raw milk (Scavia et al., 2009), the sign "Milk must be boiled before consumption" is obligatory and the shelf-life is limited to 3 days after sale (Giacometti et al., 2012c) (Tremonte et al., 2014).

In the work of Tremonte et al. (2014) mentioned above, the conditions to which the milk is subjected after delivery were simulated and the total counts taken after storing the raw milk from the vending machines for 72 hours, the maximum permitted time under Italian legislation. During this time, a significant increase was observed in the counts ($P < 0.05$) in all the samples.

Giacometti et al. (2013) carried out a study in which they interviewed consumers in order to determine their behaviour with respect to the storage and treatment of the raw milk and measured the temperatures in the different possible situations. From these studies, it was concluded that 82 % of the consumers did not use any means of cold conservation (insulated cold bags) to carry the milk home and 4 % only used them in summer, and 43 % of the consumers did not boil the milk before consumption (23 % consumed the milk without heating and the remaining 20 % heated the milk without reaching the boiling point). On the basis of these parameters, they defined the ideal conditions (conservation at 4 °C) and the worst possible conditions (highest temperatures reached during the transportation from the farm, recorded on the vending machines and during transport to the home and the time during which the milk was at these temperatures). Analysis of the milk samples demonstrated the absence of pathogenic microorganisms, but the exposure studies carried out by inoculating samples with different pathogens (around 2 log cfu/ml) and storing the samples at 4 °C, indicated that the count of *Listeria monocytogenes* increased slightly (0.38 log cfu/ml in 96 hours or 69 hours 41 minutes of generation time) and the rest of the pathogens decreased. When the inoculated samples were stored in the worst conditions of temperature abuse identified, all the pathogens (except *Campylobacter*) increased their counts by more than 1 log cfu/ml. Subsequent boiling of the milk totally eliminated the microorganisms studied.

In this context, it is important to underline that many consumers not only ignore the requirement to boil the raw milk from vending machines, but also frequently give untreated milk to children (D'Ascenzi et al., 2010) (Giacometti et al., 2012c).

In addition, the use of microwave ovens is widespread as a means of heating due their ease of use and speed; however there is little scientific information available about their efficiency in the inactivation of pathogen microorganisms or the toxins which are potentially present in raw milk.

Some studies indicate differences in the sanitising effect of the raw milk depending on the power and treatment time (Tremonte et al., 2014). Consequently, and until there is a scientific assessment of the influence of the variables in the process, this heat treatment procedure cannot be recommended as an alternative to traditional boiling.

2.3.4 Influence of the packaging used by the consumer for collection and transport

If the packaging is provided by the producer (disposable), it will not pose a significant additional risk, provided that it complies with the handling and storage requirements (cleaning and disinfection, storage protected from environmental contamination, avoid humidity in storage zone, etc.).

When the consumer provides the packaging (containers used more than once), the risk may increase, especially given the possible existence of traces of food if the container has not been thoroughly cleaned. In these cases the container may act as a source of contamination by spoiling and pathogenic microorganisms (Wildbrett, 2000) (Tucker and Forsythe, 2012).

No studies have been made about the contamination due to the use of bottles provided by the consumer and therefore it is not possible to assess the risks derived from this practice. Studies of infant feeding bottles which have apparently been disinfected observed total microorganism contaminants of up to 10^4 cfu/ml (Redmond et al., 2009).

A paper published by Tacki et al. (2013) on breast milk seems to indicate that the use of glass bottles permits the antibacterial properties of the milk to be conserved better than in polyethylene bags. In addition, and also with regard to breast milk, some papers have suggested the possibility of contamination of milk stored in polypropylene bags due to possible breaks in the plastic, recommending that, where bags are used for the storage of the milk, the plastic should be strong, securely sealed to prevent exogenous contamination and stored in an area of the refrigerator where any possible damage may be reduced to a minimum (ABM, 2010).

As in the previous case, the risk essentially depends on the consumer's attitude and observation of certain hygienic practices when handling the milk. The consumer should be warned of the need to only use containers provided by the producer or, if these are not available, to use their own containers which are used solely for the collection and transport of the milk, and which have been adequately cleaned before each use, and which are kept closed during refrigeration, in addition to other basic measures.

2.4 Additional risks of consuming frozen raw milk

Freezing foods helps to reduce the microbial load, due to cold-related injuries, osmotic stress or mechanical damage of the cells during the freezing process. Nevertheless, the damage is

variable according to the type of microorganism, the product, the characteristics of the freezing process, etc, and therefore freezing does not guarantee the destruction of the pathogens present in the product. In light of the above, freezing the milk cannot be considered an effective measure for eliminating microorganisms.

The recovery of the damaged microorganisms or the reproduction of the unaltered cells may start during the thawing process, especially in the case of psychrotrophic microorganisms such as *Listeria monocytogenes* (Ryser and Buchanan, 2013).

There is very little available published work on the effects of freezing on the microbiological quality of the milk. In experimental studies on human milk, the microbial load was observed to decrease after freezing.

This implies therefore that the microbiological risks associated with the consumption of frozen milk depend on the quality of the raw material, and that the additional risk of its consumption would be determined by the possible break in the cold chain during the storage and transport of the product, or due to incorrect thawing methods. The consumer should always observe the correct procedures for thawing any food product. These include:

- Thaw the product in its original container, keeping it refrigerated at all times until its consumption.
- Once thawed, consume within the next 24 hours.
- If thawed using heat (microwave), consume immediately.
- Never refreeze.

In the case of raw milk, this should be boiled after thawing and prior to consumption.

3. Manufacture of cheese aged for more than 60 days using raw milk

3.1 Introduction

3.1.1 Basis of the request

Regulation (EC) No 853/2004 establishes that:

“8. A Member State may, of its own initiative and subject to the general provisions of the Treaty, maintain or establish national measures:

- b) permitting the use, with the authorisation of the competent authority, of raw milk not meeting the criteria laid down in Annex III, Section IX, as regards plate count and somatic cell count of the manufacture of cheese with an ageing or ripening period of at least 60 days, and dairy products obtained in connection with the manufacture of such cheeses, provided that this does not prejudice the achievement of the objectives of this Regulation”.

This provision has been developed through Royal Decree 640/2006 and its subsequent modification (Royal Decree 1338/2011) which indicates:

“Article 5. Specific conditions with respect to the milk and dairy products.

1. Raw milk from animals that do not meet the requirements of point 2 of section I of chapter I of section IX of Annex III of Regulation (EC) No 853/2004 may be used in the following cases:
 - a) in the case of cows and buffaloes that do not show a positive reaction to tests for

tuberculosis or brucellosis, nor any symptoms of these diseases, after having undergone a heat treatment such as to show a negative reaction to the phosphatase test;

- b) In the case of sheep or goats that do not show a positive reaction to tests for brucellosis, or which have been vaccinated against brucellosis as part of an approved eradication programme, and which do not show any symptom of that disease. In this case, the milk should be used for the manufacture of cheese with a ripening period of at least 60 days or undergo a heat treatment such as to show a negative reaction to the phosphatase test;
- c) In the case of females of other species that do not show a positive reaction to tests for tuberculosis or brucellosis, nor any symptoms of these diseases, but belong to a herd where brucellosis or tuberculosis has been detected in herds regularly checked for that disease under a control plan that the competent authority has approved, and provided they undergo a treatment guaranteeing their harmlessness.

2. When the controls established in chapter II, of Annex IV of Regulation (EC) No 854/2004 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, at the farm of origin show that the raw milk exceeds the parameters established in Annex III, section IX, to Regulation (EC) No 853/2004, with regard to plate count and somatic cell count, the competent authority shall notify the producer that they have three months to correct the situation. After three months, if the producer continues to exceed these parameters, they shall suspend the delivery of raw milk, or, in accordance with an authorisation from the competent authority, deliver this milk, giving notification of the situation, to establishments that guarantee the requirements concerning its treatment and use as listed below:

- a) The manufacture of cheeses with a ripening period of at least 60 days and dairy products obtained in the manufacture of these cheeses, with the condition that the managers of the establishments that manufacture these cheeses conduct controls of the warehouse such that the time that each batch of products is stored in the warehouse is known and recorded in order to guarantee a minimum stay of 60 days; or
- b) The manufacture of dairy products or colostrum-based products from this milk or colostrum, after undergoing the heat treatment required in chapter II of Annex III, Section IX to Regulation (EC) No 853/2004.

This suspension or these requirements remain in place until the food business operator has proved that the raw milk again complies with the criteria”.

3.1.2 Terms of reference

Pursuant to article 5.2.a) of Royal Decree 640/2006, cheese, and those dairy products obtained in the manufacture of these cheeses, shall be manufactured from raw milk and with a ripening period of at least 60 days. In this respect, the Scientific Committee considers that the report should consider the following aspects:

- a) The effect of this ripening time of at least 60 days on other pathogenic microorganisms especially *Listeria monocytogenes*, *Salmonella*, coagulase positive staphylococci and its staphylococcal toxins and *Escherichia coli*, in order to assess the microbiological safety of these cheeses and the dairy products obtained in the manufacture of these cheeses.
- b) With respect to article 5.2.b), the Scientific Committee has been requested to assess which heat treatments are effective for guaranteeing the microbiological safety of the dairy products (milk which undergoes heat treatment is also considered to be a dairy product) manufactured from this raw milk, that is, raw milk that exceeds the parameters established in Annex III, Section IX to Regulation (EC) No 853/2004, as regards plate count and somatic cell count.

3.2 Effect of ripening time of at least 60 days on other pathogenic microorganisms

The presence of pathogenic microorganisms in cheese depends on the microbiological quality and the heat treatment of the milk, the hygiene of the installations, the quality of the cultures, the handling during processing and the temperature during the storage, transport and distribution of the cheese.

According to the *Codex Alimentarius* (2004), the ripening or ageing process consists in the "Holding of the cheese for such time, at such temperature, and under such conditions as will result in the necessary biochemical and physical changes characterising the cheese in question". When applied as a microbiological control measure, the multifactorial, complex system developing in cheese (pH, antagonistic flora, decreased water activity, metabolism of bacteriocins and organic acids) is utilised to influence the microenvironment in and on the food and consequently the composition of the microflora present".

During the ripening a series of changes occurs to the physical, chemical and organoleptic properties of the cheese, including fermentations, lipolysis and proteolysis. It is assumed that the pathogenic microorganisms will disappear during the cheese ripening due to the low pH, the decreased water activity and the competition with other microorganisms, especially the lactic or endogenous microbiota added as a starter. Considerable literature exists in this respect and an exhaustive revision is beyond the objective of this report. In 1949, the Food and Drug Administration (FDA) had already established that cheese manufactured from raw milk had to undergo a minimum ripening period of 60 days prior to marketing. This rule was subsequently followed in the legislation of many other countries, including Spain.

However, the ripening process is dynamic and highly complex, and depends on factors including the quantity and type of initial microbiota, the storage time, temperature, ventilation, humidity, salt and pH, all of which affect the microbial development. Not all varieties of cheese have the same composition, pH or water activity (Martín Peñas, 2013) (BEDCA, 2015). In Spanish cheese ripened for more than 60 days, such as *Idiazabal*, *Manchego* or *Roncal* average values of 0.94 can be found for water activity, of 2.3-2.8 g/100 g for NaCl and a pH of around 5.4-5.7 (Marcos et al., 1983).

In addition, not all the pathogens are equally sensitive to the stress conditions produced during the ripening process. *Salmonella* spp. is able to adapt to pH conditions of up to 3.99, and survives in foods with water activity levels as low as 0.93 (Li et al., 2013). *Listeria monocytogenes* has minimum growth levels at a pH of 4.2 and water activity of 0.90 to 0.93, although they are able to survive in even more restrictive conditions (FSAI, 2005). Enterohemorrhagic *Escherichia coli* is also able to grow at a pH of 4.0-4.5 (Meng et al., 2013). With respect to the enterotoxin-producing *Staphylococcus aureus*, its resistance to environmental factors is not high. However it has a high osmotolerance, surviving at levels of less than 0.86 (Seo and Bohach, 2013). *Campylobacter* spp. does not appear able to survive in the conditions occurring in ripened cheeses (Habib et al., 2013).

Defining the behaviour of all these pathogens during the ripening process is extremely complicated, as the interaction of all the limiting factors (pH, water activity, presence of competing microbiota, etc.) has synergic effects, which are difficult to predict. At present there are no predictive models for the behaviour of these microorganisms which adjust to the complexity of the transformations that appear during the cheese ripening process. In addition, for the majority of the principal food pathogens, it has been shown that exposure to sublethal conditions of stress cross-protects their resistance to other adverse environmental conditions (Doyle and Buchanan, 2013).

This is why it is extremely difficult to provide a response to the question asked by this Committee. Therefore we have focussed on the possibility that, if present, the pathogens may survive a ripening process of 60 days or more.

There is evidence that the main food pathogens are able to survive this process. Most of this evidence comes from experimental work in which raw milk is experimentally inoculated and subjected to a ripening process of more than 60 days. D'Amico et al. (2010, 2014) demonstrated the presence of viable cells of *Salmonella* and *Escherichia coli* O157:H7 in Cheddar or Gouda type cheeses inoculated at levels of 20 cfu/ml after more than 200 days. Other studies confirm the capacity for survival of *Escherichia coli* O157:H7 during curing times equal to or higher than 60 days (Schlesser et al., 2006).

Escherichia coli, Shigatoxin-producing *Escherichia coli* (STEC E. coli) and *Listeria monocytogenes* are able to survive the ageing process for 60 days (Lin et al., 2006) (Peng et al., 2013a,b) (Valero et al., 2014) and it has been shown that a ripening period of 60 days is insufficient to eliminate *Listeria monocytogenes* when the microorganism reaches the product during the processing (D'Amico et al., 2008).

Lastly, the presence of unacceptable levels of pathogens in commercially cured cheeses has occasionally been documented (Almeida et al., 2007).

There is no reference to *Campylobacter* in this type of cheese. However it is agreed that its inability to survive in the environmental conditions occurring in ripened cheeses makes the risks in these foods extremely low (Habib et al., 2013).

It can therefore be concluded that the survival of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* in cheese ripened for more than 60 days is unlikely

but cannot be rejected. Safety in this case essentially depends on the level of hygiene in facilities and the microbiological quality of the initial milk.

3.3 Assessment of the effectiveness of heat treatments

According to Regulation (EC) No 853/2004; Chapter II: Requirements relating to milk products; III. Criteria relating to raw cow's milk states:

- "1. Food business operators manufacturing dairy products must initiate procedures to ensure that, immediately before processing:
 - a) Raw cows' milk used to prepare dairy products has a plate count at 30 °C of less than 300 000 per ml; and b) processed cows' milk used to prepare dairy products has a plate count at 30 °C of less than 100 000 per ml.
2. When milk fails to meet the criteria laid down in paragraph 1, the food business operator must inform the competent authority and take measures to correct the situation".

According to the *Codex Alimentarius* (2004), the result of applying any microbial control measure depends significantly on the microbial load (including the concentration of microbiological hazards) in the material subjected to it and the heat treatments to which it is subjected. These treatments determine a log reduction of the microbial load, starting from already established initial values, such that the level after treatment varies with respect to the initial levels. Exceeding these initial values would imply therefore that the treatment is insecure, and would require the modification of the treatment parameters (temperature, time...) or the development of new treatments.

For the use of milk that does not comply with the requirements as raw material, the application of a heat treatment should be required that guarantees that during its transformation, the milk complies with the requirements demanded by the Regulation.

To obtain this guarantee it will be necessary to analyse the product after the heat treatment to confirm that the reduction has reached the levels required by the legislation.

With regard to the destruction of pathogenic microorganisms, on considering this question, it should be noted that the total plate count at 30 °C, is not indicative of the presence or absence of pathogenic microorganisms in the milk (EFSA, 2015).

Pasteurisation provides a significant reduction in the level of microorganisms: the pasteurisation processes are designed to reduce by at least 5 log units the pathogens considered to be most resistant to heat treatment (Yousef and Balasubramanian, 2013). However, as milk is a heat-stable food, more severe heat treatments can be applied, without fear of significantly affecting its quality characteristics. Therefore, for the pasteurisation of milk a minimum heat treatment is applied of 71.7 °C for 15 seconds or an equivalent time/temperature combination.

Depending on the time and temperature profile applied, the majority of the vegetative forms, including the pathogenic microorganisms are inactivated in most of the pasteurisation processes, with few exceptions (streptococci and micrococcus), whereas the spore forms survive these processes (AFSCA, 2015). *Campylobacter*, *Salmonella*, EHEC and *Listeria monocytogenes* have

been shown to be inactive after a suitable pasteurisation process (Doyle and Buchanan, 2013), and *Listeria monocytogenes* is the non-spore-forming pathogenic bacteria most resistant to heat of those that can be transmitted through milk. The data published indicate that the initial population of *Listeria monocytogenes* is reduced by approximately 4 log cycles after a treatment of 6.32 minutes at 62 °C (Cava-Roda et al., 2012) or 5 cycles after 2.5 min at 65 °C (Chhabra et al., 2002).

Given that the pasteurisation treatments at temperatures of 62-65 °C last around 30 minutes, reductions of more than 12 cycles are obtained in both cases. In addition, the predictive microbiological databases such as Combase (www.combase.cc), which permit the modelling of the growth and inactivation of microorganisms in different conditions, based on the data existing in the bibliography, predict for *Listeria monocytogenes* an inactivation of 12 log cycles after 14 minutes at 62 °C, 5 minutes at 65 °C or 1.5 minutes at 68 °C in a medium with pH 7 and water activity of 0.997.

In these conditions, pasteurisation may be sufficient to provide microbiological safety in the majority of samples of milk that do not meet the requirements but it is not possible to guarantee that it is valid on its own if the initial microbial load is higher than 5 log units, for the reasons given above. In these cases, more severe heat treatments must be applied.

Conclusions of the Scientific Committee

1. Scientific studies recognise that raw milk may transport pathogenic microorganisms, in many cases from the faeces of animals considered to be healthy and from the materials and environment related with the process for obtaining and storing the milk. The strict application of good hygiene practices during milking and the subsequent handling of raw milk may reduce but not eliminate the risk of milk contamination, and therefore pasteurisation is the only efficient method of guaranteeing the elimination and control of the pathogenic microorganisms in this food and its derivatives.

Therefore, and based on the information above, this Committee considers that modification of article 3, section 1a) of the Royal Decree 640/2006, should not take place, maintaining it in the terms referred to in the same. Similar considerations should be taken into account for the direct consumption of colostrum, given that the risk of the presence of pathogenic microorganisms in colostrum exists, although it is less due to its composition.

In addition and in regard to the requirements related to the maximum plate count at 30 °C, somatic count and antibiotic residue, of the studies consulted it emerges that, in maintaining the same types of risks for public health, colostrum should be considered in the same way as raw milk, with regard to obligatory limits.

2. The microbiological dangers identified for raw milk are initially present in raw milk destined for other distribution channels, such as vending machines, together with some additional risks specific to such methods of placing the product in the market:

- The principal microbiological hazard is the presence of *Listeria monocytogenes*, as given its psychrotrophic nature it is able to multiply during refrigerated storage.

- The reference methods (ISO) for the microbiological analysis of raw milk may not be sufficiently sensitive for detecting certain pathogenic microorganisms present in low concentrations.
 - Temperature abuse, especially during the transport of the milk from the farm and from the vending machine to the consumer's home, may increase the risk derived from the presence of *Listeria monocytogenes* and, to a lesser extent, of *Salmonella* and verotoxigenic *Escherichia coli*.
 - Treatment of the milk by boiling at the consumer's home is an effective control measure, but depends on the consumer's attitude.
 - There is no scientific data that permits the assessment of the additional risk of contamination supposed by the use of containers provided by the consumer, but in any case it does not appear that this will reduce the risk of contamination; to the contrary, it may increase this risk. In the case of the sale of raw milk through vending machines, preventive measures to minimise the risks should be established, such as:
 - Ensure the compliance of good hygienic practices and self-control systems by the producer.
 - Maintain the cold chain throughout the process, ensuring that the temperature never goes above 4 °C.
 - Establish a maximum storage time for the milk after purchase of 72 hours.
 - Always boil the milk before consumption. In this respect, the consumer should be provided with clear and accessible instructions and information about the need for this practice.
3. The survival of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* in cheese ripened for more than 60 days is unlikely but cannot be rejected. Safety in this case essentially depends on the level of hygiene in facilities and the microbiological quality of the initial milk.
4. Lastly, consumption of raw milk by those consumers belonging to high risk or susceptible sectors of the population (pregnant women, infants, immunocompromised patients or the elderly) should be avoided, by means of information campaigns or legal provisions. For this population, and in the event that the raw milk is consumed, special emphasis should be placed on the need to strictly observe the hygiene measures for handling, in particular boiling the milk before consumption.

References

- ABM (2010). Academy of Breastfeeding Medicine Protocol Committee. ABM Clinical Protocol #8: Human Milk Storage Information for Home Use for Full-Term Infants (Original Protocol March 2004; Revision #1 March 2010). *Breastfeeding Medicine*, 5 (3), pp: 127-130.
- ACMSF (2011). Advisory Committee on the Microbiological Safety of Food. Food Standards Agency. Risk assessment: the possible health risks to consumers associated with *m. bovis* and unpasteurised milk and milk products (ACM/1047a).
- AFSCA (2011). Agence fédérale pour la Sécurité de la Chaîne alimentaire. Avis 15-2011: Evaluation des risques et bénéfiques de la consommation de lait cru de bovins et de l'effet du traitement thermique du lait cru sur ces risques et bénéfiques (Dossier Sci. Com. 2010/25 auto-saisine).
- AFSCA (2012). Agence fédérale pour la Sécurité de la Chaîne alimentaire. In: EFSA. Annual Report on the Microbiological Risk Assessment Network. Supporting Publications 2012: EN-369.

- AFSCA (2013). Agence fédérale pour la Sécurité de la Chaîne alimentaire. Avis 11-2013: Evaluation des risques et bénéfices de la consommation du lait cru d'espèces animales autres que les vaches (dossier Sci. Com. 2012/12: autosaisie).
- AFSCA (2014). Agence fédérale pour la Sécurité de la Chaîne alimentaire. Lait cru à chauffer avant consommation: Brochure informative à l'attention des consommateurs.
- AFSCA (2015). Agence fédérale pour la Sécurité de la Chaîne alimentaire. Avis 02-2015. Concerne: Evaluation des risques microbiologiques de la consommation des produits laitiers à base de lait cru (dossier Sci Com 2014/06: auto-saisie).
- Almeida, G., Figueiredo, A., Rôla, M., Barros, R.M., Gibbs, P., Hogg, T. and Teixeira, P. (2007). Microbiological characterization of randomly selected Portuguese raw milk cheeses with reference to food safety. *Journal of Food Protection*, 70 (7), pp: 1710-1716.
- Amicizia, D., Domnich, A., Panatto, D., Lai, P.L., Cristina, M.L., Avio, U. and Gasparini, R. (2013). Epidemiology of tick-borne encephalitis (TBE) in Europe and its prevention by available vaccines. *Human Vaccines and Immunotherapeutics*, 9 (5), pp: 1163-1171.
- Austin, C., Saathoff-Huber, L., Bordson, M. et al. (2008). Outbreak of multidrug-Resistant Salmonella enterica serotype Newport Infections associated with consumption of unpasteurized Mexican-Style Aged Cheese. Illinois, march 2006-april, 2007. *Morbidity and Mortality Weekly Report*, 57 (16), pp: 432-435.
- BOE (1967). Decreto 2484/1967, de 21 de septiembre, por el que se aprueba el texto del Código Alimentario Español. BOE 248 de 17 de octubre de 1967, pp: 14180-14187.
- BOE (2006). Real Decreto 640/2006, de 26 de mayo, por el que se regulan determinadas condiciones de aplicación de las disposiciones comunitarias en materia de higiene, de la producción y comercialización de los productos alimenticios. BOE 126 de 27 de mayo de 2006, pp: 19999-20002.
- BEDCA (2015). Base de Datos Española de Composición de Alimentos. Available at: <http://www.bedca.net/bdpub/> [accessed: 18-05-15].
- Bhat, M., Denny, J., MacDonald K. et al. (2007). *Escherichia coli* O157:H7 Infection associated with drinking raw milk. Washington and Oregon, Nov-Dec. 2005. *Morbidity and Mortality Weekly Report*, 56 (8), pp: 165-167.
- Bianchi, D.M., Barbaro, A., Gallina, S., Vitale, N., Chiavacci, L., Caramelli, M. and Decastelli, L. (2013). Monitoring of foodborne pathogenic bacteria in vending machine raw milk in Piedmont, Italy. *Food Control*, 32, pp: 435-439.
- Cartwright, E.J., Jacson, K.A., Johnson, S.D., Graves, L.M., Silk, B.J. and Mahon, B.E. (2013). Listeriosis Outbreaks and Associated Food Vehicles, United States, 1998-2008. *Emerging Infectious Diseases*, 19 (1), pp: 7-15.
- Cava-Roda, R.M., Taboada, A., Palop, A., López-Gómez, A. and Marín-Iniesta, F. (2012). Heat resistance of *Listeria monocytogenes* in semi-skim milk supplemented with vanillin. *International Journal of Food Microbiology*, 157, pp: 314-318.
- CDC (2014). Center for Disease Control and Prevention. Multi-state outbreak of Listeriosis linked to Roos Food Dairy Products. Available at: <http://www.cdc.gov/listeria/outbreaks/cheese-02-14/index.html> [accessed: 18-05-15].
- Chhabbra, A.T., Carter, W.H., Linton, R.H. and Cousin, M.A. (2002). A predictive model that evaluates the effect of growth conditions on the thermal resistance of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 78, pp: 235-243.
- Claeys, W.L., Cardoen, S., Daube, G., de Block, J., Dewentinc, K., Dierick, K., de Zutter, L., Huyghebaert, A., Imberechts, H., Thiange, P., Vandeplass, Y. and Herman, L. (2013). Raw or heated cow milk consumption: Review of risks and benefits. *Food Control*, 31, pp: 251-262.
- Codex Alimentarius (2004). Código de prácticas de higiene para la leche y los productos lácteos. CAC/RCP 57-2004. Available at: http://www.codexalimentarius.org/input/download/standards/10087/CXP_057s.pdf [accessed: 18-05-15].

- D'Amico, D.J., Druart, M.J. and Donnelly, C.W. (2008). 60-day aging requirement does not ensure safety of surface-mold-ripened soft cheeses manufactured from raw or pasteurized milk when *Listeria monocytogenes* is introduced as a postprocessing contaminant. *Journal of Food Protection*, 271 (8), pp: 1563-1371.
- D'Amico, D.J., Druart, M.J. and Donnelly, C.W. (2010). Behavior of *Escherichia coli* O157:H7 during the manufacture and aging of Gouda and stirred-curd Cheddar cheeses manufactured from raw milk. *Journal of Food Protection*, 73 (12), pp: 2217-2224.
- D'Amico, D.J., Druart, M.J. and Donnelly, C.W. (2014). Comparing the behaviour of multidrug-resistant and pansusceptible *Salmonella* during the production and aging of a Gouda cheese manufactured from raw milk. *Journal of Food Protection*, 77 (6), pp: 903-913.
- D'Ascenzi, C., Pedonese, F., Nicodemi, L., Nuvoloni, R., Forzale, F. and Rindi, S. (2010). Effectiveness of risk management indirectly raw milk selling at "E. Avanzi" center of Pisa University. *Ital. Journal of Food Safety*, 7, pp: 30-35.
- DG SANCO (2009). Directorate General Health and Consumers Affairs. Guidance document on the implementation of certain provisions of Regulation (EC) No 853/2004 on the hygiene of food of animal origin (SANCO/1732/2008 Rev. 7).
- Domínguez Rodríguez, L., Fernández Garayzabal, J.F., Vazquez Boland, J.A., Rodríguez Ferri, E. and Suarez Fernández, G. (1985). Isolation of micro-organisms of the species *Listeria* from raw milk intended for human consumption. *Canadian Journal of Microbiology*, 31 (10), pp: 938-941.
- Domínguez, E., Pérez, M.D. and Calvo, M. (1997). Effect of Heat Treatment on the antigen-binding activity of anti-peroxidase immunoglobulins in bovine colostrum. *Journal of Dairy Science*, 80 (12), pp: 3182-3187.
- Doyle, M.P. and Buchanan, R.L. (2013). In book: *Food Microbiology, fundamentals and frontiers*. 4th ed. ASM Press.
- EU (2004a). Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for the hygiene of foodstuffs. OJ L 139 of April 30th, 2004, pp: 1-151.
- EU (2004b). Regulation (EC) No 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs. OJ L 139 of April 30th, 2004, pp: 1-54.
- ECDC (2013). European Centre for Disease Prevention and Control. Annual Epidemiological Report reporting on 2011 surveillance data and 2012 epidemic intelligence data.
- ECDC (2014). European Centre for Disease Prevention and Control Annual Epidemiological Report. Emerging and vector-borne diseases.
- EFSA (2014). European Food Safety Authority. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2012. *The EFSA Journal*, 12 (2): 3547.
- EFSA (2015). European Food Safety Authority. Scientific opinion on the public health risks related to the consumption of raw drinking milk. *The EFSA Journal*, 13 (1): 3490.
- FSAI (2005). Food Safety Authority of Ireland. The control and management of *Listeria monocytogenes* contamination of food. Available at: www.fsai.ie/WorkArea/DownloadAsset.aspx?id=1234 [accessed: 12-02-14].
- FSAI (2009). Food Safety Authority of Ireland. Health Risks from Unpasteurized Milk. General Facsheet series, Issue n° 1.
- FSANZ (2009). Food Standards Australia/New Zealand. Microbiological *Risk Assessment of Raw Cow Milk*, pp: 13.
- Gapper, L.W., Copestake, D.E.F.J., Otter, D.E. and Indyk, H.E. (2007). Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements. A review. *Analytical and Bioanalytical Chemistry*, 389, pp: 93-109.
- Giacometti, F., Serraino, A., Bonilauri, P., Ostanello, F., Daminelli, P., Finazzi, G., Losio, M.N., Marchetti, G., Liuzzo, G., Zanoni, R.G. and Rosmini, R. (2012a). Quantitative risk assessment of verocytotoxin-producing *Escherichia coli* O157 and *Campylobacter jejuni* related to consumption of raw milk in a province in northern Italy. *Journal of Food Protection*, 75, pp: 2031-2038.

- Giacometti, F., Serraino, A., Finazzi, G., Daminelli, P., Losio, M.N., Arrigoni, N., Piva, S., Florio, D., Riu, R. and Zanoni, R.G. (2012b). Sale of Raw Milk in Northern Italy: Food Safety Implications and Comparison of Different Analytical Methodologies for Detection of Foodborne Pathogens. *Foodborne Pathogens and Disease*, 9, pp: 293-297.
- Giacometti, F., Serraino, A., Finazzi, G., Daminelli, P., Losio, M.N., Tamba, M., Garigliani, A., Mattioli, R., Riu, R. and Zanoni, R.G. (2012c). Field handling conditions of raw milk sold in vending machines: experimental evaluation of the behaviour of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Campylobacter jejuni*. *Italian Journal of Animal Science*, 11, pp: 132-136.
- Giacometti, F., Bonilauri, P., Serraino, A., Peli, A., Amatiste, S., Arrigoni, N., Bianchi, M., et al. (2013). Four-Year Monitoring of Foodborne Pathogens in Raw Milk Sold by Vending Machines in Italy. *Journal of Food Protection*, 76 (11), pp: 1902-1907.
- Godeden, S., McMartin, S., et al. (2006). Heat-treatment of Bovine Colostrum. II. Effect of heating duration on pathogen viability and Immunoglobulin G. *Journal of Dairy Science*, 89 (9), pp: 3476-3483.
- Habib, I., De Zutter, L. and Uyttendaele, M. (2013). *Campylobacter* species. In book: Food Microbiology, fundamentals and frontiers. Doyle, M.P. and Buchanan, R.L. 4th ed. ASM Press, Washington D.C. pp. 263-286.
- Hall, J.M., Rolfs, R.T., Herlihy, R.K. et al. (2010). *Salmonella* Newport Infections associated with the consumption of Unpasteurized Milk. Utah, april-june 2010. *Morbidity and Mortality Weekly Report*, 59 (26), pp: 817-818.
- Headrick, M.L., Korangy, S., Bean, N.H., Angulo, F.J., Altekruze, S.F., Potter, M.E. and Klontz, K.C. (1998). The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, through 1973-1992. *American Journal of Public Health*, 88 (8), pp: 1219-1221.
- Heuvelink, A.E., Bleumink, B., van den Biggelaar, F.L.A.M., Te Giffel, M.C., Beumer, R.R. and de Boer, E. (1998). Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in raw cow's milk in the Netherlands. *Journal of Food Protection*, 61, pp: 1597-1601.
- Hudson, J.A. (2011). Minimum growth temperatures of foodborne pathogens and recommended chiller temperatures. Client Report FW1104. A report for MAF Food Safety. ESR.
- Hunt, D.C., Bañez, M.C., Neisses, D., Hanssen, G. and Aghoghovbia, S.T. (2009). *Campylobacter jejuni* Infection associated with unpasteurized milk and cheese. Kansas, 2007. *Morbidity and Mortality Weekly Report*, 57 (51&52), pp: 1377-1379.
- ICMSF (1998). International Commission on Microbiological Specifications for Foods. *Campylobacter*. In book: Microorganismos de los alimentos. Características de los patógenos microbianos. Ed. Acribia, Zaragoza.
- Jaros, P., Cogger, N. and Frech, N (2008). A systematic review of the human disease evidence associated with the consumption of raw milk and raw milk cheeses. Report prepared for the New Zealand Food Safety Authority.
- Jayarao, B.M. and Henning, D.R. (2001). Prevalence of Foodborne Pathogens in Bulk Tank Milk. *Journal of Dairy Science*, 84 (10), pp: 2157-2162.
- Kendall, P. (2003). Health. Bacterial Food-Borne Illness. *Food and nutrition series*, No.9.300.
- Langer, J.A., Ayers, T., Grass, J., Lynch, M., Angulo, F.J. and Mahon, B.E. (2012). Nonpasteurized Dairy Products, Disease Outbreaks, in State Laws-United's States, 1993-2006. *Emerging Infectious Diseases*, 18 (3), pp: 23-29.
- Li, H., Wang, H., D'Aoust, J.Y. and Maurer, J. (2013). *Salmonella* species. In book: Food Microbiology: Fundamentals and Frontiers. Doyle, M.P. and Buchanan, R.L. 4th ed. ASM Press, Washington D.C. pp 225-261.
- Lin, C.M., Zhang, L., Doyle, M.P. and Swaminathan, B. (2006). Comparison of Media and Sampling Locations for Isolation of *Listeria monocytogenes* in Queso Fresco Cheese. *Journal of Food Protection*, 9, pp: 2048-2303.
- Lind, L., Reesser, J., Stayman, K., et al. (2007). *Salmonella typhimurium* infection associated with raw milk and cheese consumption in Pennsylvania, 2007. *Morbidity and Mortality Weekly Report*, 56 (44), pp: 1161-1164.

- MAGRAMA (2012). Ministerio de Agricultura, Alimentación y Medio Ambiente. Informe de Zoonosis y Resistencias Antimicrobianas (2012). Available at: http://rasve.magrama.es/Recursos/Ficheros/Historico/00_Informe%20de%20Zoonosis%20y%20RAM%202012.pdf [accessed: 18-05-15].
- Marcos, A., Millán, R., Esteban, M.A., Alcalá, M. and Fernández-Salguero, J. (1983). Chemical composition and water activity of Spanish cheeses. *Journal of Dairy Science*, 66, pp: 2488-2493.
- Martín Peñas, G. (2013). Tablas de composición de alimentos. Sociedad Española de Nutrición. Available at: <http://www.sennutricion.org/es/2013/05/10/tabla-de-composicin-de-alimentos-martin-pea> [accessed: 18-05-15].
- Meng, J., LeJeune, J.T., Zhao, T. and Doyle, M.P. (2013). Enterohemorrhagic *E. coli*. In book: Food Microbiology, fundamentals and frontiers. Doyle, M.P. and Buchanan, R.L. 2013. 4th ed. *ASM Press*. pp: 263-310.
- NSCFS (2006). Norwegian Scientific Committee for Food Safety. A qualitative assessment of the risks of transmission of microorganisms to human resulting from the consumption of raw milk and raw cream in Norway.
- Oliver, S.P., Jayarao, B.M. and Almeida, R.A. (2005). Food Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. *Foodborne Pathogens and Disease*, 2 (2), pp: 117-129.
- Peng, S., Hoffmann, W., Bockelmann, W., Hummerjohann, J., Stephan, R. and Hammer, P. (2013a). Fate of Shiga toxin-producing and generic *Escherichia coli* during production and ripening of semihard raw milk cheese. *Journal of Dairy Science*, 96 (2), pp: 815-823.
- Peng, S., Schafroth, K., Jakob, E., Stephan, R. and Hummerjohann, J. (2013b). Behaviour of *Escherichia coli* strains during semi-hard and hard raw milk cheese production. *International Dairy Journal*, 31, pp: 117-120.
- Redmond, E.C., Griffith, C.J. and Riley, S. (2009). Contamination of bottles used for feeding reconstituted powdered infant formula and implications for public health. *Perspectives in Public Health*, 129, pp: 85-94.
- Regione Emilia Romagna (2008). Giunta Regionale Direzione Generale Sanita' E Politiche Sociali Determinazione N.004418 Bologna 21/04/2008. Oggetto: Vendita Diretta Al Consumatore Di Latte Crudo Vaccino, Ovi-Caprino, Bufalino E Asinino Dell'azienda Di Produzione. Prot. N. (Vet/08/96913).
- Robinson, D.A. (1981). Infective dose of *Campylobacter jejuni* in milk. *British Medical Journal*, 282, pp: 1584.
- Ryser, E.T. and Buchanan, R.L. (2013). *Listeria monocytogenes*. In book: Food Microbiology: Fundamentals and Frontiers. Doyle, M.P. and Buchanan, R.L. 4th ed. *ASM Press*, Washington D.C. pp 503-545.
- Scavia, G., Escher, M., Baldinelli, F., Pecoraro, C. and Caprioli, A. (2009). Consumption of Unpasteurized Milk as a Risk Factor for Hemolytic Uremic Syndrome in Italian Children. *Clinical Infectious Diseases*, 48, pp: 1637-1638.
- Schneider, J., Mohle-Boetani, J. and Vugia, D. (2008). *Escherichia coli* O157:H7 infections in children associated with raw milk and raw colostrum from cows. California, 2006. *Morbidity and Mortality Weekly Report*, 57 (23), pp: 625-628.
- Schlesser, J.E., Gerdes, R., Ravishankar, S., Madsen, K., Mowbray, J. and Teo, A.Y. (2006). Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during the 60-day aging period of cheddar cheese made from unpasteurized milk. *Journal of Food Protection*, 69 (5), pp: 990-998.
- Seo, K.S. and Bohach, G.A. (2013). *Staphylococcus aureus*. In book: Food Microbiology, fundamentals and frontiers. Doyle, M.P. and Buchanan, R.L. 2013. 4th ed. *ASM Press*. pp. 547-576.
- Soboleva, T. (2013). Assessment of the microbiological risks associated with the consumption of raw milk. Ministry for Primary Industries (MPI) Technical Paper No: 2014/12. June 2013. Available at: <http://www.foodsafety.govt.nz/elibrary/industry/raw-milk-sales-2014/2014-12-microbiological-risks-assessment-consumption-of-raw-milk.pdf> [accessed: 18-05-15].
- Takci, S., Gulmez, D., Yigit, S., Dogan, O. and Hascelik, G. (2013). Container Type and Bactericidal Activity of Human Milk during Refrigerated Storage. *Journal of Human Lactation*, 29 (3), pp: 406-411.
- Terplan, G. (1988). *Listeria* in the dairy industry. Proceedings of Gehr's seminar on problems of foodborne Listeriosis, European Symposium. (7 Sept) European Symposium, Wiesbaden, Germany.

- Tremonte, P., Tipaldi, L., Succi, M., Pannella, G., Falasca, L., Capilongo, V., Coppola, R. and Sorrentino, E. (2014). Raw milk from vending machines: Effects of boiling, microwave treatment, and refrigeration on microbiological quality. *Journal of Dairy Science*, 97 (6), pp: 3314-3320.
- Tucker, P.H. and Forsythe, S.J. (2012). In book: Higiene de los alimentos. Microbiología y HACCP. Acribia, S.A. ISBN: 978-8420009865.
- Valero, A., Hernández, M., De Cesare, A., Manfreda, G., González-García, P. and Rodríguez-Lázaro, D. (2014). Survival kinetics of *Listeria monocytogenes* on raw sheep milk cured cheese under different storage temperatures. *International Journal of Food Microbiology*, 184, pp: 39-44.
- Weltman, A., Longerberger, A.H., Moll, M., Johnson, L., Martin, J. and Beaudoin, A. (2013). Recurrent Outbreak of *Campylobacter jejuni* Infections Associated with a Raw Milk Dairy-Pennsylvania, april-may 2013. *Morbidity and Mortality Weekly Report*, 62 (34), pp: 702-702.
- Wildbrett, G. (2000). In book: Limpieza y desinfección en la industria alimentaria. Acribia, S.A.
- Yousef, A. and Balasubramanian, V. (2013). Physical methods of Food Preservation. In book: Food Microbiology: Fundamentals and Frontiers. Doyle, M.P. and Buchanan, R.L. 4th ed. ASM Press, Washington D.C. pp: 737-763.

Annex I. Some scientific reports and opinions on the risk associated with the consumption of raw milk

1. Centers for Disease Control and Prevention (CDC):
 - a) Food Safety and Raw milk: <http://www.cdc.gov/foodsafety/rawmilk/raw-milk-index.html>
 - b) The ongoing Public Health Hazard of consuming raw milk. Robert V. Tauxe.
2. Consumer Foodsmart of New Zealand (Ministry for Primary Industries): <http://www.foodsmart.govt.nz/food-safety/high-risk-foods/raw-milk/rawmilk.htm>
 - a) MPI Technical Paper (New Zealand Gov.): Risk Profile
 - *Listeria monocytogenes* in raw milk (2014/6).
 - Assessment of the microbiological risks associated with the consumption of raw milk (2014/12).
 - Shiga toxin *Escherichia coli* in raw milk (2014/14).
 - *Campylobacter jejuni/coli* in raw milk (2014/15).
3. European Food Safety Authority (EFSA). Scientific opinion on the public health risks related to the consumption of raw drinking milk. *The EFSA Journal*, 13 (1): 3490.
4. Food and Drug Administration (FDA). The Dangers of Raw Milk: Unpasteurized Milk Can Pose a Serious Health Risk. <http://www.fda.gov/Food/ResourcesForYou/consumers/ucm079516.htm>
5. Food Standard Australia/New Zealand (FSANZ):
 - a) Microbiological risk assessment of raw cow milk (2009).
 - b) Microbiological Risk Assessment of Raw Goat Milk (2009).
6. New Zealand Food Safety Authority (NZFSA):
 - a) A systematic review of the human disease evidence associated with the consumption of raw milk and raw milk cheeses (2008).
7. Norwegian Scientific Committee for Food Safety (NSCFS):
 - a) Risk assessment of trade and consumption of raw milk and colostrum from other species (2007).
 - b) A qualitative assessment of the risks of transmission of microorganisms to humans resulting from the consumption of raw milk and raw cream in Norway (2006).
8. Agence Fédérale pour la Sécurité de la Chaîne Alimentaire (AFSCA):
 - a) Lait cru à chauffer avant consommation: Brochure informative à l'attention des consommateurs (2014).
 - b) Avis 1-2013. Objet: Evaluation des risques et bénéfices de la consommation du lait cru d'espèces animales autres que les vaches (dossier Sci Com 2012/12: auto-saisine).
 - c) Avis 15-2011: Concerne: Evaluation des risques et bénéfices de la consommation de lait cru de bovins, et de l'effet du traitement thermique du lait cru sur ces risques et bénéfices (dossier Sci Com 2010/25, auto-saisine).
9. Food Safety Authority of Ireland (FSAI):
 - a) Health risks for unpasteurized milk (2009).
10. Food Standard Agency (FSA):
 - a) Risk assessment: the possible health risks to consumer associated with *M. bovis* and unpasteurised milk and milk products. Documento ACM 1047/a.

Annex II. Requirements for raw milk in the Emilia-Romagna Region

Prescrizioni relative al latte crudo

Il latte crudo per poter ritenersi idoneo alla vendita diretta al consumatore finale non deve avere subito in alcun modo operazioni di sottrazione o addizione di un qualsiasi suo componente naturale. Il latte crudo deve possedere un punto crioscopico uguale o inferiore a -0,520 °C. Nell'azienda di produzione dovranno essere valutati in autocontrollo i criteri specificati nella seguente tabella.

Tipologia prodotto	Criterio	Limite	Modalità di calcolo	Frequenza controllo
Latte crudo vaccino	Tenore di germi a 30 °C	≤50 000/ml	Media geometrica mobile, calcolata su un periodo di due mesi*	Almeno due prelievi al mese
	Tenore di cellule somatiche	≤300 000/ml	Media geometrica mobile, calcolata su un periodo di tre mesi*	Almeno un prelievo al mese
Latte crudo proveniente da altre specie	Tenore di germi a 30 °C	≤500 000/ml	Media geometrica mobile, calcolata su un periodo di due mesi*	Almeno due prelievi al mese
Latte crudo di qualsiasi specie	<i>Staphylococcus aureus</i>	<500 ufc/ml*		Mensile
Latte crudo di qualsiasi specie	<i>Listeria monocytogenes</i>	Assenza/25 ml		Mensile
Latte crudo di qualsiasi specie	<i>Salmonella</i> spp.	Assenza/25 ml		Mensile
Latte crudo di qualsiasi specie	<i>Escherichia coli</i> 0157	Assenza/25 ml		Mensile
Latte crudo di qualsiasi specie	<i>Campylobacter termotolleranti</i>	Assenza/25 ml		Mensile
Latte crudo vaccino	Aflatossina M1	≤50 ppt		Mensile
Latte crudo di qualsiasi specie	Residui di antibiotici	<LMR riguardo ad una qualunque delle sostanze di cui agli allegati I e III del Reg CE 2377/90		Commisurata all'analisi dei pericoli

Source: (Regione Emilia Romagna, 2008). La "media mobile": media calcolata su un numero fisso di osservazioni, il cui valore cambia perché in ogni periodo entra nel range di calcolo la rilevazione più recente ed esce quella più vecchia. *In caso di superamento del limite eseguire un campionamento in 5 u.c. m=500 e M=2 000 c=2.