

Pathogens in Milk and Product Dairy: Study in Europe

Zainab Musadaq Al-Shalah

Al-Qasim Green University, College of Food Science, Babylon, Iraq.;

ABSTRACT:

Milk and milk products contain natural microbial flora and / or addition to the origin of the diversity of products placed on the French market. The origin of contaminations by pathogenic bacteria varies according to the nature of the product and its mode of production and processing. The contamination of milk and milk products by pathogenic germs can be of endogenous origin, and it then follows a mammary excretion of the animal sick; it can also be of exogenous origin, it is then a direct contact with infected herds or a contribution from the environment (water, personnel). The whole process of processing and processing milk can slow down the multiplication of the germs possibly present or, on the contrary, favor their development. For each of the main germs likely to be found in these products, the authors describe aspects of physiology and of bacterial ecology, the incidence in dairy products and consequences in public health. The most frequently mentioned germs are *Mycobacteria*, *Brucella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterobacteria*, including *Escherichia coli* producing toxins and *Salmonella*. Currently, control of these pathogenic bacteria in milk and derivatives requires the establishment of systems of control and surveillance that rely on regulations now become European. The means of prevention must take into account the data now well-known in the field of microbiology for milk and dairy products. More and more, the presence of pathogenic bacteria in a food will have to be examined from the perspective of risk analysis for the consumer with respect to these micro-organisms.

Keywords: *Brucella* , Contamination, *Escherichia coli*, Milk, *Listeria monocytogenes* , *Mycobacteria* , Prevention , Milk Products , Risk , *Salmonella*, Public Health, *Staphylococcus aureus*

Salmonella:

Salmonella are Gram-negative bacteria of the type aerobic-anaerobic option belonging to the family of *Enterobacteriaceae* and possessing all their characteristics biochemical. Provided with peritrichous flagella, they are generally mobile but some serovars are motionless as *S. Gallinarum pullorum* and others having lost their flagella. The genus Salmonella comprises two species : *Salmonella enterica* and *Salmonella bongori*; the enterica species is itself subdivided in six subspecies defined on the basis of characters biochemical and genotypic by hybridization results DNA / DNA (42). The subspecies thus differentiated are enterica (I), salamae (II), arizonae (IIIa), diarizonae (IIIb), houtenae (IV) and indica (VI). The vast majority of isolated strains in humans and warm-blooded animals belong to the subspecies enterica. Subspecies II, IIIa and IIIb are frequently found in the commensal flora from the gut of cold-blooded animals but can be as isolated from the environment or warm-blooded animals. The subspecies IV, VI and the bongori species are very rarely found and do not seem to have any preferential habitat. Each subspecies is subdivided into serovars on the base of somatic and flagella antigenic characters. The combination of these antigenic factors makes it possible define a complete antigenic structure, which according to the Kauffmann-White scheme characterizes a strain. The current nomenclature grants to keep for each serovar the name that had been assigned for the first time to a strain of identified antigenic formula; this denomination corresponds most often to an origin zoological or geographical area for strains belonging to the first subspecies. Serovars from other subspecies are designated only by their antigenic formula and the indication of the subspecies. To date, there are more 2,300 different serovars; new serovars are sometimes still isolated in France and recognized by the Center National Reference Standard for Salmonella and Shigella (Institute Pasteur, Paris). Although any isolated Salmonella is considered potentially pathogenic for humans, the determination of the serovar makes it possible to better characterize the strain and restore it in its ecological context or Epidemiological. Salmonella can multiply at temperatures between 5 ° C and 45 ° C with an optimum at 35 ° C-37 ° C and at pH 4.5-9 with an optimum optimum between 6.4 and 7.5. Most salmonella can develop in foods with water activity (Aw between 0.945 and 0.999). The potential oxidation reduction may also be a determining factor in the growth of this

microorganism.

Salmonella can be divided into three distinct groups:

- Those that are specifically adapted to humans and isolated exclusively in humans: this is the case of *S. Typhimurium*, *S. Paratyphi A* and *S. Sendai* responsible for typhoid or paratyphoid fevers; serovars specifically adapted to animal species, such as *S. Gallinarum pullorum* in poultry, *S. Abortusovis* in sheep, *S. Abortusequi* in horses, *S. Typhisuis* and *S. Choleraesuis* in pigs;

- The other so-called ubiquitous serovars belonging to the third group and that can infect humans as well which animal. They are most frequently isolated in the countries industrialized and are at the origin of most salmonellosis human and animal. The gut of animals is the most important reservoir in salmonella and contributes greatly to their spread in the environment where they can survive but without multiply. The contamination of man can be done directly by contact or, most the intermediary of contaminated food, a large number of food products being likely to be vectors. Many animal species as well as humans can host the micro-organism in a non-apparent way as those healthy carriers allowing even more easily this dissemination. The prevalence of contaminations by Salmonella in dairy herds is variable according to countries and publications. In California, more than 72.7% of dairy cows would show signs of salmonella infection, the observed serovars being *S. Typhimurium*, *S. Dublin*, *S. Montevideo*, *S. Newport* and *S. Anatum* (85). Few data are published for countries European. In France, according to data from the serotyping of the National Center for Veterinary Studies and (CNEVA) of Paris, salmonella of origin accounted for 18% of strains ten years ago inventories of animal origin, while currently they corresponding to 36% of this same population (9, 10, 11, 17). At the same time, the severity of salmonella infections requires the use of anti-infective treatments, although antibiotic resistance of the original strains cattle has also increased. In France and also in other European countries, *S. Typhimurium* is the most frequently found serovar in cattle. According to data from the inventory of Salmonella from CNEVA Paris (2014-2015), *S. Typhimurium* represents 66.6% of all isolated strains of origin bovine, whereas ten years ago serovar Dublin was still predominant (10, 57). Currently, this last serovar appears to be increasing again in cattle (9, 10, 11). The infected farms constitute a potential reservoir of contamination of milk and products derived from raw milk. However, it seems that the contamination occurs

more frequently from the middle outside, from the environment or by contact with animals infected at the time of milking as intramammary. According to the Salmonella inventory data, 443 strains were identified in dairy products during the 2014-2015, accounting for 3.6% of the total stem from food hygiene. This figure appears to be low, corresponds to a sharp increase in number of strains from milk and milk products, doubtless in connection with the Decree of 30 March 2014 which Mandatory search for Salmonella in milkshakes consumption and milk-based products when they are on the market. Table 1 summarizes the evolution of the number of isolated strains and their distribution in different categories of dairy products. The results presented depend heavily on the establishment of guidelines and monitoring or control plans in this sector. The recent data show that serovar Typhimurium is largely predominant and accounts for more than 35% of isolated strains, two thirds of which come from raw milk.

Table 1. Evolution of the number of isolated strains of Salmonella in milk and dairy products

Products analyzed					
Raw milk	15	44	134	94	257
Powdered milk	9	10	6	3	0
Cheese	1	19	60	47	156
Milk products	15	13	16	25	30
Total	40	86	216	169	443
isolated strains in hygiene alimentary	0.9	1.3	3.2	1.9	3.6

Is followed by serovar Montevideo with more than half of strains are isolated from cheeses (10, 63). Although Salmonella is the leading cause of food poisoning in France, milk and products are rarely responsible for salmonellosis. Raw milk is rather infrequently contaminated and this contamination is then most often of external origin. The pasteurized milk is usually free of all salmonella because they are eliminated during the pasteurization. Incidents can only occur by recontamination after pasteurization. Powders milk were responsible for several incidents; indeed, he has been shown that some salmonella could withstand the freeze-drying heat treatment. The contamination of milk powders may also have a external origin. Contamination of pasteurized cheeses can only be done after the pasteurization step. The growth and survival of salmonella during the manufacturing and refining vary according to different parameters, in particular, the acidification of the medium. A low pH variation can reduce (pH 4.55) or contrary to promoting (pH: 4.95) the development of salmonella (68). Lactic acid bacteria like certain Streptococcus or Lactobacillus that produce acids result in the elimination of salmonella. During stages of manufacture and ripening, salmonella can also multiply according to the ambient temperature. They can then persist until the moment of the market for these products. However, for cheeses long ripening period (cooked pressed pasta), elaboration substances with bactericidal action makes it possible to eliminate salmonella; other substances present in cheeses marbling may also have an inhibitory action on the growth of certain strains: this is the case with ferments according to their composition play an important role in the inhibition and elimination of salmonella (30.) Salmonellosis can be classified in two major categories: those due to serovars strictly humans responsible for typhoid syndromes or paratyphoid appearing in our regions as a case isolated sporadic or restricted localized foci, and due to ubiquitous serovars of lower pathogenicity in healthy adults, leading to clinical symptoms of food poisoning of favorable prognosis if the Host resistance is not lowered. After a period incubation period usually from 12 to 36 hours, the table classic clinical is that of a gastroenteritis that can be accompanied by fever, diarrhea, abdominal pain and vomiting. Fragile populations such as subjects immunocompromised, infants and the elderly are more susceptible to infection. The infectious dose needed to the onset of these symptoms is variable depending on the samovar, the nature of the food and the host: it is usually estimated at 10⁶ live bacteria but can be above or below this value (50). During the year 2014 data from the Weekly Epidemiological Bulletin identified 267 reported outbreaks of food-borne illness Collaborative Salmonella (TIAC) for 3,840

%16.7 were hospitalized .

Serovar Enteritidis was found in 65.5% of the outbreaks due to Salmonella. Salmonella TIACs are more likely to occur family catering (60%) in a collective setting (35%). When the offending food could be identified, it was the more often eggs or egg products (47.5%). Milk and dairy products were involved in 5.5% of the cases during the year 2014; no case from these same products has declared during the year 2015.

In France and other European countries, different types products have been involved in recent years in episodes of food poisoning: this is raw milk, goat cheese, mozzarella, soft cow cheese, Cheddar, Vacherin, Milk Powder, Cream and Sauce cream and ice cream. Of the milk powders, those intended for infant feeding have recently been responsible for gastroenteritis in infants in France and in the

United Kingdom. The survey conducted by the National Network public health network and the "Salm-net" European network enabled trace the origin of this contamination and to identify the serovar Anatum. Such contamination by salmonella had already been responsible for TIAC in Austria, there is a ten years and in Australia, twenty years ago. Raw milk cheeses were responsible for two community outbreaks of salmonellosis in the seven occurred in France between 2016 and 2017; the first was due to serovar Paratyphi B; the second, caused by serovar Dublin, covered both France and Switzerland (24, 25). In France, the surveillance of the TIAC is carried out jointly by the National Reference Centers of Pasteur Institute of Paris, by the National Laboratory of (LNS) receiving the reports of diagnosed cases retransmitted by the Departmental Directorates of Affairs health and social services (DDASS). The National Health Network The public carries out epidemiological investigations in the event of declared outbreaks. At the same time, surveillance of zoonoses is under the control of the Directorates of Services veterinarians from each department and the centralization of data is carried out at the level of the Directorate-General Food. Strengthening and better organization monitoring systems have allowed for better census of cases in recent years.

A European network has recently been set up to collect all the data from the centers of national reference of each country. He allowed to observe Isolation frequencies of some identical serovars for each country and also to carry out surveys epidemiological data covering several European countries (34(. / 46/92EEC helped to increase the

number of It also seems that the implementation of the Directive of Salmonella research in dairy products and either therefore causing the apparent increase in the incidence of Salmonella in these product categories. Apart from the role played by the supervisory bodies and control of food poisoning, the best way to combat the increase in the number of salmonellosis is to set up a number of prevention measures at all levels of production. It is already announced by the World Health Organization. Health (WHO) in 1983 (A. Zuniga Estrada, L. Mota de la Garza and A. Lopez Merino, personal communication), with several recommended control axes since production until consumption. These preventive measures first of all relate to the farms, where they must allow animals to better resist infection with reducing the spread of salmonella and maintaining a good level of hygiene through basic rules. This requires the establishment of awareness-raising actions breeders accompanied by corrective actions in case of animals infected. Procedures on the respect of methods appropriate cleaning and disinfection procedures have been by WHO (70). If the use of vaccination is commonly used in the United States of America, where 45% of cows' dairy farmers in California are probably protected (85), this practice is little used in France. Monitoring milk collected with regular monitoring of milk tank and tank allows a first sort that requires, in case of a positive result, a more thorough investigation the origin of contamination in the production of the milk. Checks are regularly carried out in milk processing workshops, whether at the level of the pasteurization or during the manufacture and refining of cheeses. Control of the manufacturing parameters and ripening (hygrometry, temperature, pH) as well as the place of a hazard analysis critical control system points (HACCP: risk analysis, critical points for their control) can reduce the risks of contamination throughout the processing chain. Finally, the consumer must be informed of the consequences non-compliance with storage or storage instructions some products. In the United States of America, lately, a investigation by the Center for Disease Control and Prevention showed that most toxi-infections were generated by the consumer himself.

Listeria monocytogenes:

The genus *Listeria* belongs to the phylogenetic branch of *Clostridium* as well as *Staphylococcus*, *Streptococcus*, *Lactococcus* and *Bacillus*. On the basis of the DNA / DNA hybridization results and the partial sequencing of 16S ribosomal RNA, the genus *Listeria* is currently divided into six species, divided into two phylogenetic branches (55). The first includes *Listeria (L) monocytogenes*, *L. ivanovii* (subsp.

Ivanovii and subsp. *iondoniensis*), *L. innocua*, *L. welshimeri*, *L. seeligeri*. The second consists of a single species *Listeria* bacteria are in the form of small bacilli of regular shape from 0.5 μm to 2 μm long and 0.4 μm to 0.5 μm in diameter, rounded at the ends and not forming neither capsule nor spore. They are Gram positive, being able to appear in Gram stain, isolated, in V, in clusters and sometimes even in chains.

Their growth is possible between 0 ° C and 45 ° C (temperature optimum temperature: 30 ° C-37 ° C), for pH values between 4.5 and 9.6 up to 10% NaCl and for A w of 0.92. Between 20 ° C and 25 ° C, they are mobile thanks to flagella implantation is peritrich. *Listeria monocytogenes* can be considered as an agent food pathogen "perfect" because it is ubiquitous, very resistant to difficult conditions (temperature, Aw, pH ...) and especially she is able to grow at temperatures of refrigeration of food. The virulence of the strains could besides be exalted by their low development temperature (48).

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Three large reservoirs at *Listeria* are thus identified. All first a "human" reservoir and an "animal" reservoir, since a large frequency of healthy carriers is generally observed (6). Finally, a tank "Environment", namely soil, vegetation, surface, wastewater, silage or food from which the man can be contaminated. However, in the majority of cases, the number of *L. monocytogenes* is low (less than a hundred or a few hundred per gram) (83). This high incidence in food is also at the origin some major *Listeriosis* epidemics, the contaminations being mainly due to of animal origin and in particular to dairy products. Thus, in France, it is estimated that on average between 1% and 9% of raw milk samples are contaminated but the concentration is most often less than one bacterium / ml. Two routes of contamination are usually described

contamination by the cow (mastitis) is infrequent but the level of contamination is often high (1000 to 100,000 *L. monocytogenes* / ml in district milk), associated with an abnormal cell count without clinical signs individuals - ;contamination by the environment is more frequent, but contamination levels are also lower. The poor quality silage are therefore a source significant contamination since the presence of *L. monocytogenes* in these multiplies by twenty the risk of contamination of the tank milk. At the dairy, *L. monocytogenes* is normally destroyed by efficient pasteurization. Contamination possibly highlighted then result usually is a technological problem (insufficient pasteurization) or contamination secondary.

During processing, it is estimated that 0.5% to 10% cheeses are contaminated with *L. monocytogenes*; it's about mainly soft cheeses, however 75% contaminated cheeses have low levels of 1 to 100 *L. monocytogenes* / g. However, some cheeses pasteurized soft can hold up to 10⁶ / g. Contamination may be limited to crust or extended to the dough. Indeed, *Listeria* generally follows the evolution of the pH which is not homogeneous in cheese (the pH gradient can reach one or two units between the heart and the crust). The development of *Listeria* is therefore more favorable under crust, thanks to the flores of surfaces that alkalize the matrix. We can globally distinguish several types of behavior of *L. monocytogenes* according to the matrix cheese (48). For some cheeses such as cheeses fresh, acidic soft pasta (goat cheese,) Hard cheese ripening very long (parmesan, mozzarella), the matrix itself has destructive properties. Other cheeses have inhibiting properties: pressed pastes some blues. Still others allow growth of *Listeria*; these can reach levels of contamination sometimes very high in pasta soft pasteurized (10⁶ / g). Levels of contamination in soft pasta with raw milk are very variable, depending on the contamination of the raw material and following the flora associated, since some flora have an inhibitory action it can be added that *L. monocytogenes* has been isolated in other dairy products such as unsweetened condensed milk, concentrated milk by ultra filtration, ice cream, or certain dried milks (drying by the "spray" destroys *L. monocytogenes*, which survives but does not develop not).

Although listeriosis is a rare infection caused by virulent strains of *L. monocytogenes*, it has done much talk about her in recent years because of the seriousness of the disease. This is, indeed, food-borne infection most lethal (20% to 30% of cases). Thus, even diagnosed early, listeriosis high mortality rates, on the one hand because the diagnosis is difficult to achieve, given the very long incubation times variables (2-

70 days) (6), on the other hand because treatments are quite random (development of antibiotic resistance).

The infection can evolve into a mild form (episode febrile influenza type with sometimes meningeal syndrome) but it can evolve into a more serious form like a meningitis or sepsis, leaving in 5% to 10% of cases neurological sequelae.

For example, 60% of patients during the Swiss epidemic do not have any predisposing condition). The infectious dose is still unknown but no case human listeriosis has been linked to food containing less than 100 *L. monocytogenes* / g or ml. This dose will be difficult to evaluate because it depends, among other factors, the immune status of the host and the virulence of the strain. Since the early 1980s, the number of listeriosis has tendency to increase, mainly in the industrialized. Thus, if we list fifty cases per year in France until 1970, then about 150 cases per year until 1975, the 1980s were marked by significant increase in the number of listeriosis cases 824(cases in 2014, 522 in 2017). This situation is mainly due to an increase in food chains and a restoration development collective, but also to more epidemiological surveillance organized as well as an increase in life expectancy and number of immunocompromised individuals. Establishment of listeriosis surveillance systems in many industrialized countries has thus made it possible to estimate the incidence of this disease. This one is also variable from one country to another because it probably depends on everything less in part, eating habits. So, for the year 2013, the incidence is close to ten cases / 106 inhabitants in France, while it is estimated at around two cases / 106 inhabitants in Switzerland or Canada and seven to eight cases / 106 inhabitants in the United States of America. In France, surveillance is carried out by the National Center reference for phage typing and molecular typing *Listeria* (Institut Pasteur, Paris) and by the LNS which receives the reports of cases diagnosed by a network of biologists in the field of regulation.

Staphylococcus aureus:

Staphylococcus and Micrococcus are two genera bacteria that have long been grouped together in the family of Micrococcaceae. This family is going to be soon overhauled, because of the remoteness phylogenetic of these two genera. The micrococci represent a heterogeneous group of the branch of Actinomycetes, while staphylococci form a homogeneous group connected to the branch of Clostridium (20). The bacteria of the genus *Staphylococcus* are Gram cocci positive, non-sporulating, grouped into clusters,

immobile, facultative anaerobes and possessing a catalase. Thirty three species have already been identified by DNA / DNA hybridization and new species or subspecies are regularly described (44). The basic criterion of their classification is the coagulase production. There are three species to positive coagulase: *Staphylococcus aureus*, *S. intermedius*, *S. hyicus*, and thirty coagulase negative species. The presence of staphylococci in food represents a risk to human health, because some strains mainly belonging to the species *S. aureus* produce enterotoxins whose ingestion causes toxoinfection Staphylococcal food. Milk and dairy products only become toxic if they are contaminated with enterotoxin producing strains and if favorable conditions for multiplication important bacterial and toxinogenesis can be found met. *Staphylococcus aureus* is part of the flora of the skin and mucous membranes of man and animal. Parasite usually harmless, it can cause infections (skin abscess, mastitis). Contamination of milk may occur by through healthy or infected carriers, or by the environment. In cattle, *S. aureus* is isolated in nostrils. It is found in small skin lesions and in the sleeves of milking machines. Colonization of teats can lead to infection of the udder. It can be estimated that 25% of dairy cows can be have breast infections. During investigations carried out in France and the United Kingdom from 2006 to 2016, the frequency of isolation of *S. aureus* in infected quarters has been 20% to 40% for unseen infections, and %15to 30% in clinical mastitis (76). *S. aureus* is the most common bacterium involved in latent infections and chronic subclinical mastitis. Mastitis being difficult to eradicate, they represent the main source of contamination of raw milks by *S. aureus*. Excretion of *S. aureus* in district milk varies from 0 to 1 0 4 - 1 0 5 bacteria / ml in case of subclinical mastitis and up to 1 0 8 bacteria / ml in case of clinical mastitis. In the mixing milk, there are on average 102 to 103 *S. aureus* / ml. In humans, nasal carriage concerns 20% to 50% of people. *S. aureus* is disseminated on the skin and hands of temporarily or permanently. The man is considered as the main vector of contamination during the manipulations intervening throughout the chain food. However in raw milk and milk cheeses raw, human biotype strains remain a minority compared to strains of bovine biotype . The *S. aureus* strains are not all toxinogenic. According to the numerous surveys carried out on this subject, and optimal conditions of culture, in the laboratory, the percentage of toxigenic strains would be 30% to 60 sheep and goat strains, compared to only %10in cattle strains (21). The temperature of milk storage and manufacturing of cheeses plays a vital role. *Staphylococcus aureus* grows at temperatures included between 6 ° C and 46 ° C (optimum temperature: 37 ° C) at a pH between 4 and 9.8

(optimum pH: 6 to 7). This species tolerate a high concentration of NaCl (up to 20%) and a A_w very reduced (0.83). Toxinogenesis is involved in conditions a little more restrictive than those required for the growth (20).

Once formed, the enterotoxins are remarkably stable. They resist the irradiation, the enzymes proteolytic and especially to heat. While the bacteria is destroyed during pasteurization of milk, enterotoxins are only partially inactivated. They are not completely inactivated after 20 to 40 min at 120 ° C, according to a study with unpurified toxins at 100 ng / ml in phosphate buffer (90). More importantly, they could form complexes with each other or with the food, preventing their detection after heat treatment while their biological activity persists (82, 90).

Pasteurized milk is more favorable for the growth of *S. aureus* than raw milk because this microorganism is a bad competitor in the presence of other bacterial flora. In the raw milk, the initial number of *S. aureus* must be equal or greater than that of the concomitant flora to be able to multiply sufficiently and produce enterotoxins .

The technological parameters of cheese making are usually favorable for the growth of *S. aureus*. This occurs in the tank, then, if the pH does not drop Normally, it continues during pressing but usually not beyond. The first twenty-four hours manufacturing therefore seem decisive. However, in case of lactic flora, staphylococci can continue to multiply during the first weeks refining. Their number then decreases gradually . Enterotoxins can be detected when the number of *S. aureus* reaches about 10⁶ to 10⁷ / g. In milk cheeses believed, this is exceptional because even when the level bacterial exceeds this value, the surrounding conditions are not usually favorable for toxinogenesis. The type of cheese also matters (temperature and pH gradient during manufacture, salt concentration, activity of the antagonistic flora) (5, 21).

Staphylococcal food poisoning is characterized by violent and repetitive vomiting occurring 30 minutes to 8 hours after ingestion. Disease is short lived but very challenging and spectacular complications are sometimes observed in ingested toxin dose and sensitivity individual. Hospitalization is reported in 14% of case on average, but mortality is exceptional (22). In France, from 1988 to 1995, staphylococci were incriminated in more than 300 households, or 10% of TIAC reported to the health authorities. They occupy the second row for the number of outbreaks after salmonella and reached during this period more than 7,000 patients of which 55% in schools (2 2, 4 3). Foods responsible for food poisoning Staphylococci are varied. Milk and dairy

products are suspected in 25% of the 300 households mentioned above (22). On the one hand, Generally, dairy products are infrequently incriminated in original food-borne illness bacteria: they accounted for 4% of total outbreaks, France, from 1988 to 1995 (22) and, in other countries, from 0.9% to 8.3% of households by country (32). In contrast, staphylococci are the most common bacteria more often implicated in the food-borne infections due to dairy products in France as United Kingdom (22, 56). European Directive 92/46 / EEC of 16 June 1992 fixes the health rules for the production and placing on the market of raw milk, heat-treated milk and products based on milk (15). Staphylococci are presented as germs evidence of lack of hygiene, but it must be remembered that in the case of raw milk products, the main source of contamination is bovine mastitis (5). *S. aureus* must be enumerated in raw cow's milk ($m = 500$), cheeses fresh ($m = 10$), soft cheeses and marbled pasteurized milk ($m = 100$), raw milk and milk cheeses thermised ($m = 1000$). If they contain more than 10,000 colony forming units (CFU) per gram of *S. aureus*, an enterotoxin test should be performed (culture toxins isolated strains from cheeses or toxins extracted from cheeses). However, the implementation evidence of enterotoxins remains technically difficult despite the existence of many detection kits. Prevention of food-borne illness Staphylococci involves setting up a program action against bovine mastitis, the maintenance of milk refrigeration temperature and strict compliance with the rules hygiene during handling on the farm and at the dairy, in order to limit the number of *S. aureus* present in the milk. She also requires know-how, a monitoring of parameters technologies and a choice of lactic ferments to inhibit the growth of *S. aureus* as much as possible during the manufacture of raw milk cheeses (5, 20, 61). Finally, improvements are needed at the level of declaration and investigation of food-borne infections. Indeed, many homes are not reported and when they are, it is not always possible to evidence of the causative bacteria or the food in question (43, 56). In addition, most food poisoning reported are very poorly documented. Lack of information epidemiological data, the risk due to the presence of Staphylococci in dairy products will remain difficult to assess.

Escherichia coli:

Escherichia (E) coli form a group of motile bacilli gold still, Gram-negative, of the family of Enterobacteriaceae. They can multiply at temperatures between 4 ° C and 46 ° C, with an optimum growth at 37 ° C and pH between 4.6 and 9.5. *E. coli* that

causes diarrhea, acute gastritis or colitis of man are referred to as *E. coli* pathogens. Differentiation criteria based on their serotype, their virulence and their clinical consequences have classified these pathogenic strains into four groups: Enteropathogenic *E. coli* distinguishes (EPEC), *E. coli* enterotoxigenic agents (ETEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC).

EPECs are associated with epidemics of childhood diarrhea. They may, depending on the strain, produce toxins or invade epithelial or intestinal cells. Clinically, the disease is characterized by fever, vomiting, abdominal pain and severe diarrhea, accompanied by a large amount of mucus in the stool and a little blood (49). ETECs are characterized by the production of one or two toxins, one thermolabile (LT), the other thermostable (ST). Tea disease is characterized by watery diarrhea by abdominal pain, discomfort and nausea. ETECs are also recognized agents of the traveler's diarrhea (49). The infectious dose for ETEC is high: $10^8 - 10^{10}$ (58). EIECs are characterized by signs of toxemias with discomfort and fever. EIECs proliferate in tissues epithelial cells until necrosis occurs (49). The infectious dose for this pathovar is also important: $10^6 - 10^8$ (58). EHEC is responsible for hemorrhagic colitis, uremic hemolytic syndromes (HUS) and purpura thrombocytopenia. *E. coli* 0157: H7 are the most often responsible for this hemorrhagic colitis. These *E. coli* can produce two potent cytotoxins (VT toxins). The infectious dose is not known with certainty purpose it is weak ($<10^3$ / g). *Escherichia coli* are normal commensal of the intestine of man and animals. It represents 80% of the flora aerobic intestinal. It is found in very large numbers in the faecal faeces. From there, it spreads in nature: soil, waters. Its presence in the environment always signifies a faecal contamination. A commensal bacterium, whatever its species, can acquire certain pathogenicity factors through the contribution a new generic support (plasmid, bacteriophage, transposons) or by the expression of genes previously silent, and thus become pathogenic (39). Of the pathogenic bacteria that can end up in raw milk, some are usually at a very low level and are unlikely to develop. Others are at appreciable levels and can multiply. It's the case, among others, from *E. coli* that usually comes from the skin udders (77). This bacterium of fecal origin can survive on soiled soil. Its implantation in the material milking is unusual. Some strains, fortunately rarely present, when they are at a high level in the raw milk or in cheese, can produce gastroenteritis due to the production of toxins. The transmission of enterohemorrhagic *E. coli* is essentially related to product contamination food. Food contamination can occur during factory manufacture (followed by multiplication possible during transport and storage), ie when meal

preparation (kitchen staff). The interpersonal contamination also exists by intermediate healthy carriers with a low dose infective. Infectious diarrhea is a case sporadic or anademic episodes (TIAC, for example), even epidemic. Specificities exist, on the one hand according to the regions and seasons of infection, but also according to the age and socio-economic level of concerned and their way of life (diets, consumption of imported foods, fast and collective catering, travel) (39).

Seven biovars are currently identified in *B. abortus*, three in *B. melitensis* and five in *B. suis* (Table 2). The studies hybrid DNA / DNA and DNA / RNA compatible with the existence of a single species of *Brucella ovis* and *B. canis* are the only two species naturally in rough phase. Bacteria of the genus *Brucella* are small sticks, very frequently coccobacillary, aerobic and low glucidolytic. Their length varies from 0.6 μm to 1.5 μm and their thickness 0.5 μm to 0.7 μm . Their morphology is very constant except sometimes in older cultures. The *Brucella* are immobile and do not form spores. No flagella, pili or capsule. Gram-negative bacteria, *Brucella* do not show bipolar staining and are resistant to discolouration by weak acids (2, 16). Many species animals (especially ruminants, but also suidae, carnivores, equidae and rodents) can be naturally infected by various species of *Brucella* (Table 2). Because of the frequency and severity of contracted human cases directly or indirectly from the animal, the brucellosis is considered a major zoonosis.

Table 2. *Brucella* (species and biovars) (2,69)

Species	biovar	Morphology colonies	Host Preferential
<i>Brucella abortus</i>	1.2.3.4.5.6.9	s	Cattle
<i>B. melitensis</i>	1.2.3	s	Sheep, goats
<i>B. am</i>	1	s	Pigs
	2	s	Pigs, hares
	3	s	pigs
	4	s	Rennes
	5	s	Wild rodents
<i>B. neotomae</i>	-	s	Neotomes *
<i>B. ovis</i>	-	R	Sheep
<i>B. canis</i>	-	R	Dogs

S: naturally in smooth phase R: naturally in rough phase * *Neotoma lepida* (desert wood rat)

In the South, the prevalence of bovine infection is now very low (for example, in

France prevalence of livestock was 0.28% in 1995) (4). In these regions of southern Europe, ovine and caprine considerably diminished over the last ten years because of strengthening of control measures, but the prevalence remains significant (livestock prevalence rate sheep infected in France in 1995: 2.27%) (4), in particular in mountain areas and around the Mediterranean. Brucellosis in these species largely explains the human cases still observed in these areas. The most common vehicle of human infection by ingestion is raw milk, or one of its drifts, especially the cream and, in many countries, fresh cheeses, which sometimes the cheapest source of protein and no longer available (69). Milk and dairy products of origin cattle, sheep or goats are most frequently incriminated, but the infection can also be contracted atfrom dairy products derived from buffaloes, camelids or yaks. Brucellosis of small ruminants is considered nevertheless as being responsible for the majority of the cases food-borne humans. In France, for example, the consumption of goat cheese is the first cause of unproductive human brucellosis (54%), absorption cow's milk accounted for only 16% of these cases (80, The generalization of the pasteurization of the milk of origin intended for consumption or processing and level of sanitary requirements allowing the marketing raw milk products explain this situation. The eating habits, and especially the predilection of certain populations for raw milk and its derivatives concur still largely to the maintenance of human brucellosis. The survival of *Brucella* in milk and dairy products is related to many factors, including the type of product, the water, temperature, pH changes, action the other bacteria present, and the duration and storage conditions of the product (38). The results of some studies (11, 19, 65, 75, A. Zuniga Estrada, L. Mota Garza and A. Lopez Merino, personal communication) are shown in Table 3. Low concentration in medium liquid, *Brucella* is easily destroyed by heat. So, the classic pasteurization, the ultra-high method temperature or a simple prolonged boil (10 min) kill the *Brucella* contained in the milk (19).

Wirth *Brucella*, it is usually reduced in milk that has been kept for some days. Survival in ripened fermented cheeses seems pretty short. We do not know the time of minimal fermentation necessary for their total destruction, but it is conventionally estimated that three months are sufficient in fresh cheeses, the survival of *Brucella* can be much longer, strictly lactic fermentation and of short duration and desiccation favoring their survival. Alone past pasteurization of the milk or cream allows to prevent the health hazard posed by these products human. *Brucella* are also sensitive to radiation ionizing agents at commonly used doses, especially in colostrum.

Prevention of food-borne human brucellosis therefore goes first and foremost by the heat treatment of milk intended for direct consumption or processing. The sale of raw milk and dairy products untreated heat must be, for its part, closely monitored and limited to free holdings. These measures, even when accompanied by campaigns information, are insufficient, however, to reduce significantly the incidence of human brucellosis, numerous cases originating from direct contact with infected animals. Only countries that have implemented animal brucellosis control programs were able to observe a consequent reduction in the number of cases humans. The control and prevention of animal brucellosis requires the respect of a general hygiene in the farms and the setting in place at regional level a restorative on sanitary and / or medical measures. All of these measures cannot be really effective without a health education, training and mobilization of professionals concerned. Finally, no measure of prophylaxis cannot be considered without identification sustainable use of animals and flocks and strict control of their movements (trade, transhumance).

Various general hygiene measures make it possible to limit the extension of the infection. At the farm level, isolation animals at the time of parturition and the destruction of non-living products and foeto-maternal appendages as well as disinfection of livestock premises are two measures essential in this respect. Staff must also submit to a systematic disinfectant treatment of body parts and clothing that may have been in contact with contaminated products. Given the relative inefficiency and the cost of treatment antibiotic in livestock, elimination by slaughter of seropositive animals is one of the solutions used in the fight against animal brucellosis. This solution implies, when setting up the program of fight, a relatively low disease prevalence rate so that this plan is economically bearable. Such plans are usually based on the systematic control of flocks (individual serology or regular ring-test on milk tanker) and animal health measures positive or in clinical outbreaks, with slaughter positive animals or total slaughter of herds infected. Bacteria of the genus *Mycobacterium* belong to the family of *Mycobacteriaceae* which consists of *Actinomycetales* with rudimentary pseudomyelium usually presents as small bacilli, immobile, sometimes having bulging, cuneiform or branched (0.2-0.6 μm over 1.0-10 μm). They are characterized by their ability to maintain color despite action combined with alcohol and diluted acids: they are acid-resistant. The optimal temperature of mycobacteria ranges from approximately 28 ° C to 45 ° C. Within the genus *Mycobacterium*, there are more than 40 species recognized, including *M. tuberculosis*, *M. leprae* and several mycobacteria called "atypical". The classification taxonomy

within the genus is based on the form of colonies, pigmentation, growth characteristics bacterial and biochemical reactivity (Table 4). The bacteria are divided into two main groups depending on whether their growth is slow or fast. In practice, we consider that growth is slow when it takes more than seven days incubation to obtain visible growth.

Table 3. Cultural characteristics of the main bacteria of the genus *Mycobacterium*

Mycobacteria	Pigmentation	37 C°	42 C°	> 7 day s	Mycobactin	Pyr .	Pn b	Tc h	pz a	Tb l	em b
M. tuberculosis	-	+	-	+	-	-	-	+	-	-	-
M. bovis	-	+	-	+	-	+	-	-	+	-	-
M. africanum	-	+	-	+	-	+	-	V	-	V	-
M. paratuberculosis	-	+	-	+	+	+	V	+	v	Nf	+
M. avium	-	+	+	+	v	+	+	+	nf	v	+

+: positive v: variable Pyr. : pyruvate TCH: hydrazide of thiophene carboxylic acid, 2 µg /
Tb1:thiosemicarbazone, 10 µg / ml ,, - : negative nf: not done PNB:
Paranitrobenzoate, 500 µg / pza : pyrazinamide EMB: Ethambutol, 2 µg / ml

The bacteria belong to the tuberculosis complex (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*) are mandatory pathogens. Apart from *M. tuberculosis* and *M. leprae*, several species of mycobacteria are pathogenic for humans under certain conditions. Some species like the *M. avium* complex, *M. kansasii*, *M. malmoense* and *M. xenopi* are more likely to cause diseases than others. The majority of potentially pathogenic atypical mycobacteria have a slow growth. From an economic point of view, the main diseases Mycobacterial infections in animals are due to *M. bovis* and *M. paratuberculosis*. *Mycobacterium bovis* can be transmitted from animals to humans and to provoke in the latter a disease identical to tuberculosis due to *M. tuberculosis*. Other mycobacteria, such as *M. avium*, *M. marinum*, *M. farcinogenes* and *M. silvaticum* may be responsible infections in animals of very different species. *M. bovis*, agent of bovine tuberculosis, is able to infect humans and certain animal species, especially goat, pork, dog and cat. From the point of view of veterinary medicine and public health, this germ is the most important cause of diseases mycobacteria in animals. The symptoms of the disease in cattle vary according to the organ reached. In case of pulmonary infection, a dry cough appears and worsens as the disease develops. This cough is accompanied by slimming. Infection of the uterus and / or mammary glands is responsible for a sterility and decreased milk production.

The disease tends to slow worsening. Cattle with tuberculosis are the main source of *M. bovis* which is transmitted from cattle to humans mainly in two ways: by air (aerosols) and digestive (consumption of raw milk infected). Man with pulmonary tuberculosis *M. bovis* is also a source of infection for other subjects and possibly for cattle.

Table 4. Biochemical characters of the main bacteria of the genus *Mycobacterium*

Mycobacteria	Nia.	Nit.	Cat. 20°	Cat. 68 °	Urea	1	2	3	4	5	6
M. tuberculosis	+	+	+	-	+	+		+	+		
M. bovis	-	-	+	-	+	+		+	+		
M. africanum	V	V	+	-	V	+		+	+		
M. paratuberculosis	-	-	+	+	-	+			+		+
M. avium	-	-	+	+	-	+			+		+

Nia. : niacin Cat. : catalase v: variable +: positive -negative Nit. : nitrate reductase

Conclusion:

Other pathogenic microorganisms may be encountered in milk and milk products, among which *Yersinia enterocolitica*, *Campylobacter jejuni*, *Coxiella burnetii*, *Streptococcus agalactiae*, *Clostridium botulinum*, *Bacillus cereus*, toxin-producing molds and viruses. The presence and persistence of these germs in milks and dairy products depend on their resistance to treatments raw milk (pasteurization, acidification, curd heating, ripening conditions) and the initial level of contamination in raw milk. Pasteurization treatments (72 ° C for 15 s) eliminate pathogenic bacteria in vegetative form, but those in sporulated form resist) *B. cereus*, *C. botulinum*). *Campylobacter*, very fragile germs, are found only in raw milks and insufficiently pasteurized milks. The number increased by 4-5 log instead of 3 log the foot-and-mouth disease virus is also susceptible to pH drop. It is inactivated in 17 s at pH 6.7 at 72 ° C and in 55 s at pH 7.6 at 72 ° C . The survival of these pathogenic microorganisms in the dairy products is also more or less dependent on manufacturing process. So *C. burnetii* can be put in evident in butter and cooked pressed cheeses after several weeks, while in the cheeses soft and acidic dairy

products, germs are very fast inactivated (33). The FMD virus also survives longer in cooked pressed cheeses than in soft cheeses. Studies from milk milks cow naturally infected with fever virus foot-and-mouth disease products for the production of cheddar and camembert cheese revealed that the foot-and-mouth disease virus survives in the cheddar after 60 days of storage but not after 120 days .In the pie chart, the virus survives 21 days at 2 ° C but not 35days. As for poisoning due to *C. botulinum*, in cheese technology, the parameters (acidification, Aw, salt content) do not allow cell growth vegetative growth of *C. botulinum* or spore germination (12).

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