



## FOOD-BORNE PATHOGENS AND CONTAMINANTS IN RAW MILK – A REVIEW\*

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### Abstract

The aim of the present review is to highlight the threats to human health posed by consumption of milk and dairy products. The interest in drinking raw milk has been growing in some societies as many people believe it has health benefits. Raw milk is promoted as ‘health food’ despite the fact that it poses a realistic microbiological hazard for the consumers’ health or life. Food-borne disease outbreaks associated with *Campylobacter* spp., *Salmonella* spp., shigatoxin-producing *Escherichia coli*, *Brucella melitensis*, *Mycobacterium bovis* and tick-borne encephalitis virus have been traced to the consumption of raw milk, however, many other microorganisms that can be present in milk are considered as potential food-borne pathogens to humans. The other common causative agents in food-borne disease outbreaks are bacterial toxins produced by *Bacillus*, *Clostridium* and *Staphylococcus* spp. Some of the milk pathogens harbour antimicrobial resistant genes, which can be transferred to commensal bacteria. Most dangerous are methicillin-resistant *Staphylococcus aureus* and extended spectrum beta lactamase/*AmpC* gene-carrying bacteria from the family of *Enterobacteriaceae*, which might negatively affect the treatment of infections in humans. Fungi are not considered as food-borne pathogens for humans, however their secondary metabolites, mycotoxins, constitute a potential threat to public health. Mycotoxins or their metabolites detected so far in milk samples include aflatoxins, ochratoxin A, zearalenone and its metabolites, fumonisins, de-epoxy-deoxynivalenol and cyclopiazonic acid.

**Key words:** public health, milk, food-borne pathogens, enterotoxins, mycotoxins

Milk and dairy products are important in the diet of humans, because they are a source of numerous essential nutrients such as proteins, fats, carbohydrates, vitamins and minerals (van Hooijdonk and Hettinga, 2015). A significant share in worldwide milk production is represented by milk from ruminants such as cattle (85.4%), buffalo (11.1%), goat (2.1%), sheep (1.4%) and camel (0.2%) (Gerosa and Skoet, 2012). Milk production by yak (ruminant), horse and donkey (equids) is estimated as less

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than 0.1% of the global milk production. Other species of ruminants, reindeer and llama, produce milk for human consumption in marginal amount (Faye and Konuspayeva, 2012). Total consumption of milk and dairy products is high and increasing in most parts of the world, especially in developing countries (FAO, 2006; Gerosa and Skoet, 2012; Kearney, 2010). The milk intake in industrial countries has stabilized or declined over the last decades, since milk has been replaced by carbonated beverages and juices. It seems that there will be no appreciable changes in consumption of dairy products (butter and cheese) at the global level in the future (Kearney, 2010). This trend meets growing consumer demands for healthier and more natural animal products (Egger-Danner et al., 2015). One of the products promoted as ‘health food’ is raw milk. Raw milk is defined by European Union legislation as: “milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect” (European Commission, 2004 a). The consumption of raw milk among the general population is rather low, while it seems to be high in case of health-conscious people, who wish to consume natural, unprocessed food and believe that raw unpasteurized milk, which has not been subject to any heating process, is characterized by particular healthy properties, a reduced susceptibility to allergies, enhanced nutritional quality and a better taste (Claeys et al., 2014; Oliver et al., 2009). This approach results in milk consumption by individuals who may have lowered immunity such as the very young, very old, immune-compromised or the people with specific dietary needs.

In Europe, raw milk may be available through numerous distribution channels, including direct sale to consumers at the farm, sale through vending machines and the internet. In Germany, France, the Netherlands, Belgium, Denmark, Italy, Ireland and parts of UK raw cow’s milk can be purchased directly from farms, whereas in Poland, Spain and Norway it is prohibited. Sale through vending machines is not practiced or is prohibited in Denmark, Ireland, Greece, the Netherlands, Spain and the UK (EFSA BIOHAZ Panel, 2015). In the United States direct sale, cow-share or leasing programs and the purchase of raw milk sold as “animal or pet food” have been used as means for consumers to obtain raw milk (Oliver et al., 2009).

The interest in drinking raw unpasteurized milk or consuming products made from such raw milk is increasing in the European Union (EFSA BIOHAZ Panel, 2015) and in the United States, despite the fact that this practice poses a realistic microbiological hazard for the consumers’ health or life. The presence of food-borne pathogens in bulk tank milk has been demonstrated in many surveys and food-borne outbreaks associated with *Campylobacter*, *Salmonella* spp., *Listeria monocytogenes* and shigatoxin-producing *Escherichia coli* (STEC) have been traced to the consumption of raw milk (Oliver et al., 2009). This review highlights the threats to human health posed by consumption of milk and dairy products.

### **Sources of microbiological contamination in milk**

Milk is thought to be sterile as it is secreted into the alveoli of the udder (Tolle, 1980). However, the high content of a variety of nutrients and water in milk as well as its near neutral pH make milk a good growth medium for some microorganisms (Quigley et al., 2013). Microbial contamination of milk can occur from three main

sources: from within the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment (Murphy and Boor, 2000).

Intrinsic contamination of milk may result from systemic disease in the animal or localized infection such as mammary gland inflammation (mastitis). The milk produced by animals with clinical mastitis typically has an altered appearance (i.e. it may contain flakes, clots or blood, or may have changed colour) and therefore milk is withdrawn from human consumption. On the other hand, milk from cows with sub-clinical mastitis does not differ visibly from that produced by uninfected animals and if mixed into bulk milk on a farm, enters a food chain and poses a threat to human health (EFSA BIOHAZ Panel, 2015). Mastitis can be caused by over 150 different microorganisms which include bacteria, mycoplasmas, yeasts and algae (Kuang et al., 2009). Some of the food-borne pathogens, which are causative agents of mastitis, are directly excreted into milk. However, most food-borne pathogens such as *Salmonella*, *Campylobacter* and STEC are not frequently isolated from mastitic milk, while live in the ruminant intestinal tract. The presence of such bacteria in bulk tank milk seems to result from faecal contamination that occurs primarily during milking (Oliver et al., 2005). For example, study performed by Martin and Beutin (2011) has shown that virulence profiles and serotypes of food-borne STEC were remarkably similar to those of faecal STEC that were isolated from the same animal species. The authors have concluded that the food-producing animals are the most important source of STEC in the food chain. Moreover, microbial contamination of raw milk can also occur through contaminated milking machine or milker's hands and due to poor milk handling and storage conditions before consumption (EFSA BIOHAZ Panel, 2015; Murphy and Boor, 2000). Generally, unless there is no intramammary infection or systemic disease diagnosed in animal, milk in the mammary gland at the site of its production should not contain microorganisms or their toxins. However, in spite of the steps intended to minimize the occurrence of pathogens and spoilage organisms in raw milk, it is not possible to eliminate them entirely (EFSA BIOHAZ Panel, 2015). It has been stated that all the factors, i.e. the health and hygiene of the cow, the farm environment, the procedures used for cleaning and sanitizing the milking equipment, and the temperature and duration of milk storage influence the level of microbial contamination and the types of microorganisms present in raw milk (Murphy and Boor, 2000).

### **Milk as a source of human pathogens**

According to public health and food safety specialists, each year millions of diseases worldwide can be associated with a consumption of food contaminated by pathogens including bacteria, fungi, viruses and parasites. Milk can be an important reservoir of food-borne pathogens, since it can harbour a variety of microorganisms (Oliver et al., 2005). A summary of microbial populations within raw milk was reviewed in Quigley et al. (2013). The bacterial flora of milk-producing animals is usually dominated by the group of lactic acid bacteria (LAB), which include *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Enterococcus* spp. Other predominant microorganisms have been classified to *Enterobacteriaceae*, *Staphylococcus* spp., *Micrococcaceae*, filamentous moulds and yeasts (Quigley et al., 2013).

Although several yeast species occur in raw milk, degree of contamination is relatively high (ranging from  $10^2$  to  $10^4$  CFU/ml) (Lagneau et al., 1996). Yeasts that have been identified in raw milk from healthy animals or those with mastitis include *Kluyveromyces*, *Rhodotorula*, *Debaryomyces*, *Saccharomyces*, *Geotrichum*, *Pichia*, *Candida*, *Trichosporon* and *Cryptococcus* spp. (Delavenne et al., 2011; Dworecka-Kaszak et al., 2012; Ksouri et al., 2015; Malinowski et al., 2001). Moulds are usually present in raw milk at lower levels than yeasts (Quigley et al., 2013). Moulds detected in normal milk and milk from infected quarters have been classified to genera *Penicillium*, *Aspergillus*, *Mucor*, *Fusarium* and *Cladosporium* (Delavenne et al., 2011; Dworecka-Kaszak et al., 2012; Ksouri et al., 2015; Lavoie et al., 2012).

It has been suggested that some raw milk LAB can contribute to the human health by aiding digestion (Grosu-Tudor et al., 2013) and decreasing the risk of allergies such as asthma, hay fever and atopic sensitisation (Braun-Fahrlander and von Mutius, 2011; Debarry et al., 2007). On the other hand, pathogenic bacteria present in unpasteurized milk can be a cause of severe human illness and death (Oliver et al., 2005). Recently, EFSA Panel on Biological Hazards (EFSA BIOHAZ Panel, 2015) has identified the main microbiological hazards of public health significance that may occur in raw milk from different milk-producing animals in the EU. Clear links between consuming raw milk and human illness were found in the case of *Campylobacter* spp., *Salmonella* spp., STEC, *Brucella melitensis*, *Mycobacterium bovis* and tick-borne encephalitis virus (TBEV). The occurrence of these milk-borne microorganisms reported in milk samples from different animal species is presented in Table 1. Frequencies of isolation of human pathogenic microorganisms in raw milk from cows and milk-producing animal species other than cows were recently summarized in the EFSA document (EFSA BIOHAZ Panel, 2015) and in the review by Verraes et al. (2014). Moreover, according to the EFSA Panel, pathogens potentially transmissible through milk include the bacteria *Bacillus cereus*, *Brucella abortus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Corynebacterium* spp., *Streptococcus suis* subsp. *zooepidemicus* and the parasites *Toxoplasma gondii* and *Cryptosporidium parvum*.

According to the EFSA Panel on Biological Hazards, there were 27 reported outbreaks in the EU involving raw milk between 2007 and 2012. Of these, 21 were associated with *Campylobacter* spp. (primarily *C. jejuni*), one with *Salmonella* Typhimurium, two with STEC and three with TBEV. No case of *L. monocytogenes* transmission to humans from raw milk was reported between 2007 and 2012. According to this report, about 85% of outbreaks were due to raw milk from cows, and the rest of them occurred after consumption of caprine raw milk (EFSA BIOHAZ Panel, 2015). Interestingly, raw donkeys' milk generally seems to be free of food-borne pathogens. A study performed by Šarić et al. (2012) revealed that it can result from its antimicrobial properties (e.g. against *Clostridium perfringens*, coagulase-positive staphylococci, fungi, *Salmonella* spp., *E. coli*).

TBEV causes tick-borne encephalitis (TBE), which is regarded as one of the most common and potentially fatal human infections of the central nervous system. This zoonotic disease is endemic to Central and Eastern Europe and Russia (Cisak et al., 2010; Hudopisk et al., 2013). TBEV is mainly transmitted to humans by bites

from an infected tick and less frequently (but more successfully and with a shorter incubation period) through the consumption of raw milk and dairy products from infected livestock, mainly goat's milk and cheese (Caini et al., 2012; EFSA BIOHAZ Panel, 2015).

Table 1. List of microbiological hazards transmissible to humans through milk and their occurrence in milk samples from different animal species

Microbiological hazards	Animal species	Reference	
<i>Brucella melitensis</i>	goat	Ramos et al. (2008)	
<i>Campylobacter</i> spp. (thermophilic)	cow	Bianchi et al. (2013) Jay-Russell et al. (2013)*	
	goat	Harris et al. (1987)*	
	sheep	FSA (1999)	
<i>Mycobacterium bovis</i>	cow	Doran et al. (2009)* Passchyn et al. (2012)	
	cow	Bianchi et al. (2013) Tackett et al. (1985)*	
<i>Salmonella</i> spp.	sheep	Fotou et al. (2011)	
	camel	Abeer et al. (2012) Al-Tofaily and Al rodhan (2011)	
	Shigatoxin-producing <i>E. coli</i> (STEC)	cow	Martin and Beutin (2011) Solomakos et al. (2009)
		goat	Bielaszewska et al. (1997)* FSA (1999) Martin and Beutin (2011) Solomakos et al. (2009)
		sheep	Martin and Beutin (2011) Solomakos et al. (2009)
Tick-borne encephalitis virus (TBEV)	buffalo	Şeker and Yardimci (2008)	
	camel	Abeer et al. (2012)	
	cow	Caini et al. (2012)* Cisak et al. (2010)	
	goat	Cisak et al. (2010) Hudopisk et al. (2013)* Kohl et al. (1996)* Matuszczyk et al. (1997)*	
	sheep	Cisak et al. (2010)	

\*References that report on confirmed outbreaks of food-borne diseases caused by consumption of raw animal milk.

Other important milk-borne microorganisms with zoonotic potential are algae of the genus *Prototheca*. In a recent study (AbdelHameed, 2015) *P. zopfii*, *P. blaschkeae*, *P. stagnora* and *P. wickerhamii* have been isolated from 29% of the 200 examined raw milk samples and from 55% of the examined 100 cheese samples. It is also worth mentioning that *Prototheca* spp., especially *P. zopfii*, are highly resistant to the most of antifungal and antibacterial drugs (AbdelHameed, 2015).

### **Milk as a source of antimicrobial-resistant bacteria**

The development of bacterial resistance to antimicrobial agents poses a serious threat to human health. The antimicrobial-resistant zoonotic bacteria are of particular concern, as they might negatively affect the treatment of infections in humans (EFSA and ECDC, 2015 b). Intramammary inflammation is the main cause of antimicrobial usage on dairy farms (Sawant et al., 2005) and herd-level associations between the use of antimicrobial agents and antimicrobial resistance in some mastitis pathogens have been demonstrated (Pol and Ruegg, 2007; Saini et al., 2013). The potential public health risks related to milk may result from the presence of pathogens which are resistant to antimicrobials or possess genes encoding resistance to such antibiotics, as well as non-pathogenic bacteria that may transfer their resistance determinants to pathogenic bacteria, which affect the emergence and selection of multi-drug resistant food-borne pathogens. Raw milk may be a source of bacteria that are resistant to antimicrobials, depending on the reservoir of antimicrobial-resistant bacteria in the farm and animal environment (EFSA BIOHAZ Panel, 2015). There is a strong evidence that human consumption of milk carrying antibiotic-resistant bacterium, multidrug-resistant *Salmonella enterica* serovar Typhimurium, has resulted in acquisition of infection resistant to the same antimicrobials (Tacket et al., 1985). Other most significant antimicrobial-resistant human pathogens isolated from raw milk include *Campylobacter* spp., STEC, methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL)/*AmpC* gene-carrying bacteria (EFSA BIOHAZ Panel, 2015). The use of antimicrobial drugs in food-producing animals might also affect human health by increasing the risk of antimicrobial residues in food (WHO, 2001). In the EU, milk is monitored and the maximum residue levels (MRLs) are provided with respect to the concentration of antibiotic residues that are acceptable in milk for sale (European Commission, 2009).

### **Bacterial toxins, enterotoxins and mycotoxins in milk**

In the EU, a total of 5,196 outbreaks of food-borne illness were reported in 2013, affecting 43,183 human cases, causing almost 6,000 hospitalisations and 11 deaths. In the years 2008–2013, the number of outbreaks due to bacterial toxins increased by 58.9%, from 525 to 834 outbreaks. The third most common causative agents in food-borne outbreaks were bacterial toxins produced by *Bacillus*, *Clostridium* and *Staphylococcus* (EFSA and ECDC, 2015 a).

*Staphylococcus aureus* food poisoning is associated frequently with milk and other dairy products and therefore milk from infected animals is considered as the main source of enterotoxigenic *S. aureus* of animal origin (Srinivasan et al., 2006). *S. aureus* can produce a variety of toxins including staphylococcal enterotoxins (SEs), SE-like toxins (SEI) and toxic shock syndrome toxin (TSST-1) (Hu and Nakane, 2014). SEs are short, extracellular proteins responsible for staphylococcal food poisoning syndrome (nausea, vomiting and abdominal cramps) in humans who consumed contaminated food (Hameed et al., 2006; Schelin et al., 2011). Approximately 95% of staphylococcal food-poisoning outbreaks are caused by classic SE types SEA–SEE, whilst the remaining 5% of outbreaks may be associated with other newly described SEs (SEG–SEI, SER–SET) and SEIs (SEIJ–SEIQ, SEIU, SEIV,



SEIX). These toxins and TSST-1 are superantigens of T cells and have the ability to stimulate these cells (Heidinger et al., 2009; Hu and Nakane, 2014; Srinivasan et al. 2006). SEs can be produced in milk that is not cooled quickly and/or is not efficiently pasteurized. In comparison with pasteurized milk, consumption of raw milk may pose a greater risk of SE intoxication due to the lack of microbiological 'kill step' that eliminates SE-producing bacteria (Heidinger et al., 2009). Furthermore, SEs are considered as heat- and protease-stable proteins that cannot be destroyed during pasteurization (Heidinger et al., 2009; Hu and Nakane, 2014; Schelin et al., 2011).

Other toxin-producing bacteria important in milk contamination include spore-forming pathogens from the genus of *Bacillus* and *Clostridium*, which are able to survive heat treatment and processing in spore form (Doyle et al., 2015). These bacteria may shorten the shelf life of pasteurized milk and dairy products as well as cause food poisoning (Te Giffel et al., 2002). *Bacillus cereus* can produce two major enterotoxins, haemolysin BL (HBL) and a nonhaemolytic enterotoxin Nhe, which both cause the diarrhoeal type of food poisoning (Moravek et al., 2006). *Clostridium perfringens* has been detected in milk of cows (McAuley et al., 2014) and other ruminants (Ribeiro et al., 2007). Food poisoning is associated with *C. perfringens* enterotoxin (CPE) which causes severe abdominal cramps and diarrhoea (Doyle et al., 2015). Clostridia species have been frequently isolated from powdered infant formulas (Barash et al., 2010), however, infant botulism (IB) has been associated with the presence of only *Clostridium botulinum* spores in contaminated powder (Johnson et al., 2005). The outbreaks of botulism have been reported as a result of consumption of contaminated cheeses and yoghurts containing type B toxin. Some strains of *Clostridium butyricum*, which can occur in milk, may also be able to produce botulinum toxin. Spores of *Bacillus* and *Clostridium* bacteria are heat-stable and may persist in raw milk and its products during heat treatments such as thermization and pasteurization. Thermization may adversely affect the milk quality due to activating spore germination, whereas ultra-high temperature (UHT) treatment and commercial sterilisation eliminate up to 99.99% of spores (Doyle et al., 2015).

Shiga toxins (cytotoxins) produced by *E. coli* strains may be a cause of severe tissue damage. Especially STX2 is associated with a variety of human illnesses such as diarrhoea, haemorrhagic colitis (HC), thrombotic thrombocytopenia purpurea (TTP), and hemolytic uremic syndrome (HUS) with possible fatal consequences. Ruminants, especially cattle, are the main reservoir of STEC and outbreaks associated with consumption of ground beef, pasteurized milk and dairy products (yoghurt and cheese) have been reported worldwide (Vendramin et al., 2014).

Although fungi present in milk are not considered as food-borne pathogens for humans (EFSA and ECDC, 2015 a; EFSA BIOHAZ Panel, 2015), their secondary metabolites, mycotoxins, constitute a potential threat to public health (Assem et al., 2011; EFSA and ECDC, 2015 a). Mycotoxins are natural contaminants produced by a range of fungal species (mainly *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* sp.) during plant growth in the field, harvesting, storage or food processing (Bilandžić et al., 2014 a; Marin et al., 2013). Human exposure to mycotoxins can result either from consumption of contaminated foods of plant origin or ingestion of mycotoxins carried over from feed into animal tissues, meat, eggs or milk.

In humans, chronic consumption of mycotoxins may be a cause of many severe diseases (liver cancer, nephropathies, reproductive disorders, immunosuppression) due to their carcinogenic, genotoxic, immunotoxic, hepatotoxic, nephrotoxic, oestrogenic and teratogenic potency (Marin et al., 2013). Ruminants are generally less susceptible as compared to other animal species, since the forestomach flora has an important function in the metabolism of some mycotoxins. Ochratoxin A (OTA), deoxynivalenol (DON), aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and zearalenone (ZEN) are converted to less toxic compounds, whereas others, like patulin (PAT) and fumonisin (FUM), pass the rumen barrier intact (Fink-Gremmels, 2008). Nevertheless, some studies demonstrated that carry-over of mycotoxins into milk can occur. The mycotoxins that were detected in milk and dairy products include aflatoxins, OTA, ZEN and its metabolites, fumonisins, cyclopiazonic acid (CPA), sterigmatocystin (STC) and PAT.

Aflatoxins are mainly produced by the moulds *Aspergillus flavus* and *Aspergillus parasiticus* (EFSA, 2004). The most toxic AFB<sub>1</sub> consumed with contaminated feed is partly degraded by ruminal flora and does not enter systemic circulation. The absorbed portion of AFB<sub>1</sub> is metabolized in the liver to equally toxic but less carcinogenic aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) and subsequently excreted in milk (EFSA, 2004; Marin et al., 2013). In high yielding dairy cows, the carry-over of aflatoxins from feed to milk has been estimated to be up to 8% of the total amount of ingested AFB<sub>1</sub> (Britzi et al., 2013).

The U.S. Food and Drug Administration has established the maximum concentration of aflatoxin permitted in milk consumed by humans at the level 0.5 µg/kg (FDA, 2005), whereas the European Commission (2010) has indicated that the MRL of AFM<sub>1</sub> in raw milk, heat-treated milk and milk for the manufacturer of milk-based products must not exceed 0.05 µg/kg (0.05 ppb). Additionally, the concentration of AFM<sub>1</sub> in infant milk and follow-on milk should not exceed 0.025 µg/kg. Other dairy products must be produced from milk complying with these AFM<sub>1</sub> limits. The presence of AFM<sub>1</sub> concentration exceeding the legislations mentioned above has been detected in raw, pasteurized, powder, organic, concentrated and ultra-high temperature treated (UHT) milk samples originated from different species of milk-producing animals (Assem et al., 2011; Bilandžić et al., 2014 a; Bilandžić et al., 2014 b; Ghannem and Orfi, 2009; Ghidini et al., 2005; Heshmati and Milani, 2010; Scaglioni et al., 2014). The occurrence of AFM<sub>1</sub> in animal milk samples in Europe has been recently summarized in detail in a review by Flores-Flores et al. (2015). AFM<sub>1</sub> has affinity for casein (protein) fraction of milk, therefore, cream and butter contain lower concentrations of AFM<sub>1</sub> than the milk these products are made of (Grant and Carlson, 1971). AFM<sub>1</sub> concentration in cheese is four times higher on average in comparison to the original milk (Cavallarin et al., 2014; Rubio et al., 2011). AFM<sub>1</sub> is frequently detected in powder milk. In a study carried out by Londoño et al. (2013), AFM<sub>1</sub> was found in all 30 investigated samples of powdered milk in the range of 0.1–0.92 µg/kg. A study carried out by Meucci et al. (2010) revealed that AFM<sub>1</sub> was present in 1% out of 185 analysed infant formula milk samples at concentration (range 11.8 to 15.3 ng/l) below maximum tolerance limit accepted by the EU. Recent studies performed in Poland (Kosicki et al., 2015) have reported the lack of AFM<sub>1</sub> in examined samples of infant and UHT milk. AFM<sub>1</sub> has been detected in breast milk



of nursing mothers who consumed AFB<sub>1</sub>-contaminated food. El-Sayed et al. (2002) found AFM<sub>1</sub> in 55% of milk samples (in the range of 0.02 and 2.09 ng/ml). Navas et al. (2005) reported the presence of AFM<sub>1</sub> in one out of 50 human breast milk samples at a concentration of 24 ng/l.

Ochratoxin A (OTA) produced by *Penicillium* and *Aspergillus* moulds has been detected in ruminants' milk, however its concentration was rather low (ranging from 5 to 110 ng/l) (Boudra et al., 2007; Huang et al., 2014; Pattono et al., 2011; Skaug, 1999). This mycotoxin is rapidly metabolised into the less toxic ochratoxin  $\alpha$  (OT $\alpha$ ) by rumen microorganisms and only very small amounts of intact ochratoxin A are absorbed (Fink-Gremmels, 2008 a). These small amounts may be important to consumers of large quantities of this product, mainly children. It was found that small children who consume large quantities of milk may have a total daily intake of OTA which exceeds the Tolerable Daily Intake (TDI) (Skaug, 1999). Particularly dangerous for public health is exposure of newborn infants to ochratoxin A. In contrast to ruminants, human and monogastric animals excrete ochratoxin A in milk. Micco et al. (1991) detected OTA in 18% human milk samples at a concentration ranging between 1.7 and 6.6 ng/l. Other studies reported the occurrence of OTA in 21% (in the range of 10 to 182 ng/l) (Skaug et al., 2001), 35.8% (range 5.07 to 45.01 ng/ml) (El-Sayed et al., 2002), 85.7% (average value of 6.01 ng/l) (Turconi et al., 2004), 4% (in the range of 11 and 24 ng/l) (Navas et al., 2005) and in 72% of samples (range 35.1 to 689.5 ng/l) of breast milk samples (Meucci et al., 2010). Postupolski et al. (2006) detected OTA in 5 out of 13 examined maternal milk (in the range of 5.3 and 17 ng/l) and confirmed the correlation between OTA concentration in maternal and foetal blood serum and between OTA concentration in maternal serum and milk. Gürbay et al. (2010) found both AFM<sub>1</sub> and AFB<sub>1</sub> in all 75 studied breast milk samples at the levels in the range of 60.90–299.99 ng/l and 94.50–4123.80 ng/l, respectively. According to Adejumo et al. (2013), 82% of human milk in Nigeria was contaminated with AFM<sub>1</sub> (range 3.49–35 ng/l) and 16% exceeded the EU limit of 25 ng/l. These AFM<sub>1</sub> concentration data were significantly correlated with the mothers' dietary exposure to AFB<sub>1</sub> and socioeconomic status. Polychronaki et al. (2006) detected AFM<sub>1</sub> in about 36% of milk samples from Egyptian nursing mothers. Non-working status, obesity, high corn oil consumption, number of children and early lactation stage contributed to the occurrence of AFM<sub>1</sub> in breast milk. A study performed by Muñoz et al. (2010) revealed that OTA was found in all nine examined breast milk samples from Chilean nursing mothers, with an average concentration of 106 ng/l (range 44–184 ng/l). In a more recent study (Muñoz et al., 2014), the authors stated that a greater fraction of circulating OTA (derived from maternal blood plasma) is excreted in colostrum than with mature milk. OTA exposure of Chilean infants was calculated as an average intake of 12.7 ng/kg body weight at early stage of nursing (first 6 days after delivery), while the intake with mature milk had average values close to 5.0 ng/kg body weight/day (Muñoz et al., 2014). In Germany, infant exposure to OTA from breast milk is considered to be low compared to several other countries, however, a study has shown that more than 50% of the 90 examined milk samples contained detectable OTA levels and in the case of 29% of samples, the TDI of 3 ng/kg body weight/day was exceeded (Muñoz et al., 2013).

A few studies reported natural contamination of milk with mycoestrogen ZEN and its metabolites formed by moulds from the *Fusarium* genera. ZEN, zearalanone (ZAN),  $\alpha$ -zearalanol ( $\alpha$ -ZAL),  $\alpha$ -zearalenol ( $\alpha$ -ZEL) were detected in 0.97–30% milk samples (maximum 12.5  $\mu\text{g}/\text{kg}$  of ZEN) (El-Hoshy, 1999; Huang et al., 2014; Sándor, 1984; SCOOP, 2003; Xia et al., 2009), whereas the presence of ZEN,  $\alpha$ -ZEL;  $\beta$ -zearalenol ( $\beta$ -ZEL) was reported in 9–28% of the analysed infant formula milk samples (maximum 73.24  $\mu\text{g}/\text{l}$  for  $\beta$ -ZEL) (Meucci et al., 2011). Human exposure to ZEN from milk is not considered to be a health risk, since an adult (50–70 kg) should drink 2–2.8 l of milk daily to reach the provisional maximum tolerable daily intake (PMTDI).

Carry-over of fumonisins produced by *Fusarium verticillioides* from feed to milk (estimated rate 0–0.05%) has been considered to be limited and insignificant for total human exposure (Fink-Gremmels, 2008; Scott et al., 1994). However, some studies showed that fumonisins can be transferred to bovine and human milk. Maragos and Richard (1994) have reported fumonisin B<sub>1</sub> (FB<sub>1</sub>) in 1 out of 155 analysed samples, at a concentration of 1290 ng/l. In another study, Gazzotti et al. (2009) have reported FB<sub>1</sub> in 80% of tested milk samples, with a maximum level of 430 ng/kg. The study performed by Magoha et al. (2014) has revealed that 44.3% of analysed breast milk samples were contaminated with FB<sub>1</sub> (at a maximum level of 471.05 ng/ml), and about 10% of the positive samples did not meet the EU standards for total fumonisins in infant foods (above the limit of 200 ppb).

Other mycotoxins have been occasionally detected in milk and dairy products. These mycotoxins include cyclopiazonic acid produced by *Penicillium* and *Aspergillus*, sterigmatocystin formed mainly by *Aspergillus* moulds and less toxic metabolite of deoxynivalenol, de-epoxy-deoxynivalenol (DOM-1) produced by *Fusarium* species. CPA has been found in 15% (Losito et al., 2002) and 4.2% of analysed cow milk samples (with a maximum level of 9.7  $\mu\text{g}/\text{l}$ ) (Oliveira et al., 2006). STC was present in 9.5% of analysed cheese samples at maximum level of 1.23  $\mu\text{g}/\text{kg}$  (Veršilovskis et al., 2009). DOM-1 has been reported in 25% of raw bovine milk samples (with a maximum value of 300 ng/l) (Sørensen and Elbæk, 2005). The occurrence of patulin produced by some *Aspergillus* and *Penicillium* species in milk has been reported in 28% of cheese samples (with a range 15.4 to 460.8  $\mu\text{g}/\text{kg}$ ) (Pattono et al., 2013).

Mycotoxins are commonly resistant to the normal heat treatments of milk. Studies have shown that pasteurization and heating to higher temperatures (90–95°C) does not affect considerably AFM<sub>1</sub> and fumonisins concentration in milk (Jasutiene et al., 2007; Maragos and Richard, 1994; Scott et al., 1994). Better results have been obtained after milk fermentation, since concentration decreased by 25% on average as compared to unpasteurized milk (Jasutiene et al., 2007). Therefore, avoiding contamination seems to be the only rational mean to ensure safety of milk for human consumption.

## Conclusion

Milk production is a process susceptible to contamination with microorganisms and their toxins at each stage of animal milk production: during feeding with mycotoxins-contaminated feed (especially AFB<sub>1</sub>, OTA, ZEN and its metabolites,

FB1), mammary gland infection with human food-borne pathogens, during milking, poor milk handling and improper milk storage. Data gathered by EFSA confirm that some of the food-borne disease outbreaks were associated with the consumption of raw milk contaminated with *Campylobacter* spp., *Salmonella* spp., STEC, *Brucella melitensis*, *Mycobacterium bovis* and tick-borne encephalitis virus (TBEV). Generally, bacteria are killed or inactivated during heat treatments, whilst toxins produced by these microorganisms are often heat-stable and may persist in milk as unaffected. Food-borne pathogens and their toxins as well as chronic exposure to mycotoxins can cause severe diseases in humans such as diarrhoea, liver cancer, nephropathies, reproductive disorders and immunosuppression. The other threat to human health related to milk is the presence of antibiotic-resistant bacteria, as they might negatively affect the treatment of infections in humans. Newborns and children seem to be more exposed to milk contaminants than adults, since they consume larger quantities of milk and are more susceptible. Therefore, it is very important to control the level of contaminants in milk. The health risks to consumers associated with the consumption of milk and products made from such milk should also be repeatedly assessed.

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