Hygienic characteristics and microbiological hazard identification in horse and donkey raw milk

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Health,
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Hygiene,
Pathogens,
Risk assessment.

Summary
Today the interest toward horse (*Equus caballus*) and donkey (*Equus asinus*) milk for human consumption is receiving a renewed attention because of its particular composition, hypoallergenicity, and nutraceutical properties. The realistic perspective of global use of this aliment in balanced diets, especially for infancy and geriatrics, poses the need for a more in depth knowledge on milk hygiene and on the health status of dairy animals, as a prerequisite of consumers' safety. The aim of this paper was to review the available literature on the health and hygiene parameters as well as on the potential microbiological hazards in horse and donkey milk and the risks related to their consumption. Both microbial contamination and somatic cell count are reasonably low in equine milk and also the presence of pathogens, like *Escherichia coli* O157, *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Brucella* spp., *Mycobacterium* spp., *Bacillus cereus, Cronobacter sakazakii, Streptococcus equi* subsp. *zooepidemicus*, *Rhodococcus equi, Streptococcus dysgalactiae* subsp. *equisimilis*, *Clostridium difficile* and *Burkholderia mallei* is low. However, in those regions of the world where the prevalence of *Brucella* spp. and *Rhodococcus equi* is high, the alimentary risks could increase. Similarly, in areas with higher incidence of immunocompromised people, the increased risks should be warned not only for pathogens but also for opportunistic microbiota.

Caratteristiche igienico-sanitarie ed identificazione dei rischi microbiologici nel latte di cavalla e di asina

Parole chiave
Igiene,
Latte equino e asinino,
Patogeni,
Pericoli,
Salute,
Valutazione del rischio.

Riassunto
In questi ultimi anni il latte di cavalla (*Equus caballus*) e il latte di asina (*Equus asinus*) hanno fatto registrare un rinnovato interesse sia per la loro particolare composizione sia per le proprietà ipoallergeniche e nutraceutiche. La reale prospettiva per una diffusione globale di questo alimento innovativo, specialmente nell'ambito dell'alimentazione per i lattanti e gli anziani, richiede una più approfondita conoscenza dei pre-requisiti di sicurezza. Lo scopo di questa review è stato quello di esaminare i dati disponibili in letteratura circa i parametri igienico-sanitari e i potenziali pericoli microbiologici che possono riscontrarsi nel latte di cavalla e di asina e dei rischi connessi al consumo. Dai dati disponibili risulta che la contaminazione microbica e il numero delle cellule somatiche del latte sono ragionevolmente bassi e così pure la presenza di microrganismi patogeni, in particolare *Escherichia coli* O157, *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica, Brucella* spp., *Mycobacterium* spp., *Bacillus cereus, Cronobacter sakazakii, Streptococcus equi* subsp. *zooepidemicus, Rhodococcus equi, Streptococcus dysgalactiae* subsp. *equisimilis*, *Clostridium difficile* e *Burkholderia mallei*. Tuttavia, il livello di rischio alimentare potrebbe essere elevato in quelle regioni dove la prevalenza di *Brucella* spp. e di *Rhodococcus equi* è elevata. Inoltre, nelle aree con più alta presenza di persone immunocompromesse, un maggior livello di rischio è riconducibile non solo agli agenti patogeni, ma anche a quelli opportunisti.

Introduction

During the past few years the European scientific community has increasingly devoted more attention to the analysis of horse (*Equus caballus*) and donkey (*Equus asinus*) milk, mainly owing to both palatability and attractive nutrient and functional components that make it suitable to be used as an alternative food in the diet of infants with Ig-E mediated cow's milk protein allergy (Businco *et al.* 2000, Monti *et al.* 2007). Moreover, donkey milk can be considered an valuable food also for elderly consumers, due to its immunonutrient components (Salimei & Fantuz 2012). Equine milk is rich in lactose (61.0-65.8 g/kg milk), ω-3 (8.7-12.0% total fatty acids), and ω-6 polyunsaturated fatty acids (7.0-13.1% total fatty acids) (Pavlik *et al.* 2004). The macro mineral (calcium, potassium, phosphorus, magnesium) content is reported to be higher than in human milk, but lower than in ruminant milk; while sodium as well as zinc and manganese contents, are reported to be similar to human milk (Fantuz *et al.* 2012, Fantuz *et al.* 2013).

Lysozyme content in horse and donkey milk results to be high (mean value 600-4,000 mg/l) if compared to bovine (traces), caprine (traces), and human milk (Salimei *et al.* 2004, Guo *et al.* 2007, Uniacke-Lowe *et al.* 2010). Lysozyme, together with other factors, including immunoglobulins, lactoferrin, and lactoperoxidase, may function in the infant's digestive tract by reducing the incidence of gastro-intestinal infections and promoting proper healthy growth (Monti *et al.* 2007). The high lysozyme content, as revealed by previous studies, may contribute to the low bacterial concentration of properly milked equine milk (Salimei & Fantuz 2012). Also lactoferrin (average content 0.60-0.82 g/kg milk) is reported to have antimicrobial activity, among its possible functions, consistent with its digestibility (Uniacke-Lowe *et al.* 2010, Tidona *et al.* 2011).

It is worth noting that worldwide equine populations account for about 112 million heads, including 9% of mules. Horse and donkey populations are differently distributed among continents, ranging from 25-39% reported in Africa, Asia, and Americas to 6.3% in Europe and 0.4% Oceania (FAOSTAT 2013[1]).

Donkey world population is estimated to be of 44 million, of which 40 million (96.4%) in the developing countries. Donkeys represent about 73% of Africa's total equine population, while they account for 49.0%, 15.2%, 8.2%, 2.2% in Asia, Americas, Europe, and Oceania, respectively (FAOSTAT 2013). Equine milk and meat are traditionally consumed in many areas of the World as a dietary source of valuable proteins (Blench 2000, Benítez 2011), although cultural and technological constrains may limit their use. In Europe, the dairy donkey enterprise is developing in relatively few farms. One of the largest herds of dairy donkeys is located in Central Italy, with about 700 heads in a single farm. It is well known that milk for human consumption needs to be characterised by a low microbial contamination and by a low somatic cell count. Milk may in fact represent a microbiological risk especially when consumed raw. On this regard, the recent literature highlights cases of infections in humans as related to the consumption of raw milk from ruminants and caused by *Escherichia coli* O157, *Campylobacter* spp., *Salmonella* spp., and other pathogens or potential pathogen microorganisms (Claeys *et al.* 2013).

The aim of this article is to review the available literature on hygienic and safety characteristics of equine milk. A descriptive analysis of the main and potential microbiological hazards and their risk is therefore reported.

Hygiene and health parameters of horse and donkey milk

The limited data available in literature on horse and donkey milk show fairly variable levels of total microbial count (TMC) (3.40-5.87 log CFU/ml) (Figure 1). According to Šarić and colleagues (Šarić *et al.* 2012), breed and other animal factors, as well as interactivity of lysozyme and lactoferrin, may affect the antimicrobial properties of equine raw milk but more in depth details are lacking at the moment.

Studies carried out in Sicily (Italy) reported values of TMC between 4.15 and 5.63 log CFU/ml. Moreover, *Enterobacteriaceae* (0.68-1.93 log CFU/ml) and pathogens, or potentially pathogenic microorganisms such as *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae* and *Cronobacter sakazakii* (formerly

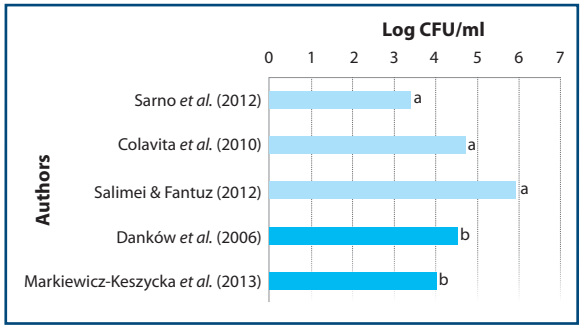

Figure 1. *Total Microbial Count of donkey milk according to literature (mean values).* a = Horse milk; b = Donkey milk.

[1] FAOSTAT, 2013. FAO Statistic Division. Available at http://faostat.fao.org

Enterobacter sakazakii), have been isolated in raw donkey milk (Conte *et al.* 2007).

During a 2 year study, 39 samples of bulk milk produced by 10 Martina Franca donkeys were evaluated for hygiene and health parameters. The value of the TMC at 30°C was 4.7 (± 0.73 s.d.) log CFU/ml and *Escherichia coli* was isolated in only 2 samples (2 log CFU/ml), while total coliforms showed counts ranging between less than 1.00 and 6.57 log CFU/ml. These data were strongly influenced by the values of 2 samples collected from a refrigerated tank, which had not been cleaned and sanitized properly, as verified later. This case underlined that, when hygiene is neglected, the level of microbial contamination can reach very high values in donkey milk. Pathogens, such as *Listeria monocytogenes* and *Salmonella* spp., have never been detected, yet; *Listeria* spp. were detected by polymerase chain reaction (PCR). This highlighted the importance of hygiene of both equipments and environment (Colavita *et al.* 2010a).

The wholesomeness of milk strictly depends on the mammary gland health. The values of Somatic Count Cells (SCC) ranged from 3.86 to 4.66 log/ml in one study (Conte *et al.* 2007), and 4.37 (± 0.32 s.d.) log/ml in another study (Colavita *et al.* 2010a) (Figure 2).

In a study on milk parameters over an entire lactation of jennies raised as companion animals, Pilla et colleagues (Pilla *et al.* 2010) noted a SCC content consistent with values observed in dairy jennies (Beghelli *et al.* 2009, Colavita *et al.* 2010 a, b). However, the number of SCC can be influenced by the milking intervals, as it remains almost unchanged for milking intervals of 3 and 5 hours but it increases at longer interval (D'Alessandro *et al.* 2009). It is important to note that horse and donkey need to be separated from the foal at least 3 hours before mechanical or manual milking. For this reason, data on donkey milk production are frequently expressed per 'milking session', *i.e.* from foal separation until donkeys leave the milking parlour (Salimei and Fantuz 2012). The low levels of SCC indicate an overall healthy status of mammary gland in the dairy equine where mastitis is less frequent than in the dairy cow (Svendsen 1997). This phenomenon is partly due to both the small volume of the mammary gland (max. 2-2.5 l) and its protected position so that teats are less exposed to traumas and infections. In addition, the mammary gland cistern has a limited capacity so that its emptying is guaranteed by foal suckling, when dams are not milked. Nonetheless, management of milking can affect the udder health. Clinical forms are often the result of traumatic lesions of teats caused by suckling or milking.

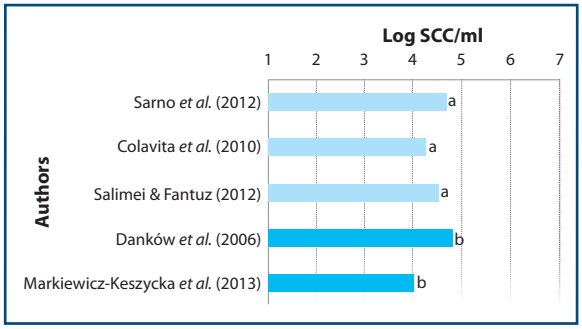

Figure 2. *Somatic cell counts (SCC) of donkey milk (mean values) reported in literature.* a = Horse milk; b = Donkey milk.

The prevalence of mastitis in donkeys is not known, with available data referring to mares: out of 100 lactating horses examined by Bartmann and colleagues (Bartmann *et al.* 1996), mastitis was diagnosed in 21 subjects. Although there are no data on the incidence of subclinical mastitis, these are considered more common than clinical mastitis. Perkins and Threlfall (Perkins and Threlfall 2002) reported *Streptococcus equi* subsp. *zooepidemicus* (32%), *Staphylococcus* spp. (15%), and *Actinobacillus* spp. (8.1%) as the main agents of mastitis in horses.

Cases of purulent mastitis caused primarily by streptococci and staphylococci have been reported in mares, glanders mastitis has rarely been reported, as well as mastites caused by *Mycobacterium avium* (Pavlik *et al.* 2004, Ryhner *et al.* 2009). In general, values of SCC/ml milk greater than 100,000 are considered pathological and index of mastitis. It is frequently possible to isolate *Streptococcus equi* subsp. *zooepidemicus* from mare udders, but pathogens may also be present in milk from healthy udders (Bohem *et al.* 2009). As Kocabiyik and colleagues (Kocabiyik *et al.* 2008) reported, out of 28 mares affected by mastitis, *S. equi* subsp. *zooepidemicus* was isolated in 37% of cases.

Analysis of microbiological hazards in horse and donkey milk

Since the data are currently scarce and fragmentary, it is not possible to approach properly the assessment of microbiological hazards associated with the consumption of equine milk. For this reason, in this context only a descriptive analysis of the actual and potential microbiological hazards and their risk is provided by considering both common risks with raw cow milk and specific ones for equids.

Escherichia coli *O157 group and* Salmonella *spp.*

With regard to microorganisms of the *E. coli* O157 group, the cow is considered the main reservoir, while

other species may serve as a secondary reservoir to humans, especially after contact with ruminants.

The few data available in literature show that the prevalence of *E. coli* O157 in horses is low. During a study conducted on 242 faecal samples of horses, the germ was isolated only in 1 subject (0.4%), which was farmed in promiscuity with cattle (Lengacher *et al.* 2010).

In some epidemiological cases, mixed farming can represent a risk factor, as evidenced by a survey in England and Wales, in which *E. coli* O157 was sought in the faeces of animals in some free stalls. The germ was found in 29% of the faeces samples of 365 cattle, in the 24% of the faeces samples from 426 sheep, in the 14.6% and 12.3% of the faeces samples of 26 donkeys and 85 horses, respectively (Pritchard *et al.* 2009).

In Germany, out of 400 samples of horse faeces, only in 1 case a strain of *E. coli* STEC belonging to serotype O113:H21 was identified by PCR, whereas all samples were negative for *Salmonella enterica* (Pichner *et al.* 2005).

Like other enteric pathogens, *Salmonella* spp. may contaminate the milk from faecal material during and after milking, although there are no reported cases of salmonellosis from consumption of equine raw milk so far (de Jager 2009). In studies carried out at slaughter on samples of faeces from horses coming from different European countries, *Salmonella* spp. was isolated in only 1.43% cases. However, this result may be misrepresented by the intermittent elimination of germs in the faeces (Colavita *et al.* 2010b).

Campylobacter *spp. and* Yersinia enterocolitica

Campylobacter spp. and *Yersinia enterocolitica* were found with low frequency in horse faeces (Browning *et al.* 1991). However, out of 100 samples of horse muscles from animals slaughtered in Piedmont (Italy), thermo-tolerant *Campylobacter* spp. were isolated in 3% cases. Outbreaks of campylobacteriosis caused by dairy products are most commonly associated with drinking unpasteurized milk. The infective dose of these bacteria is generally low. In European Union, the occurrence of *Campylobacter* ranges between 0% and 2.7% in raw milk and from 2.4% to 4.1% in cheese (EFSA 2012).

Moreover, *Yersinia enterocolitica* serotype O:5 was isolated in 9% of cases (Ercolini *et al.* 1997). Regarding the presence of these pathogens in milk, only data on cow's milk are available. In the same region of Italy on 30 samples of raw cow's milk, *Y. enterocolitica* was isolated in 4 cases (Franzin *et al.* 1984), while in Sicily it was detected in 8 samples of raw cow's milk out of 316 tested (Aiello *et al.* 1985). *Yersinia enterocolitica* was found in 5% samples of bovine raw milk on 111 collected samples in Argentina (Mercado and Ibañez 1986). It is estimated that *Y. enterocolitica* is responsible for 2% of all infectious enteritis affecting children (Bottone 1999).

Brucellosis

In Europe, equine brucellosis is rare. Although there have been cases of infections by *Brucella suis* and *Brucella abortus* with rare episodes of abortion in the mare, and 2 cases of equine brucellosis caused by *B. suis* biovar 3 have been recently reported (Cvetnic *et al.* 2005).

The infection can cause mainly suppurative spinous bursitis and fistulizing. Brucellosis in horses has a prevalence of 5.8% in Egypt (donkey 7.3%), of 12.8% in India, and of 5.8% in Pakistan (Gul and Khan 2007). On a population of 346 horses and 28 donkeys, examined in Africa, 4.9% horses (17 subjects) and 3.6% donkeys (1 animal) were positive for brucellosis (Musa 2004). Moreover, on about 412 donkeys examined with seropositivity, a prevalence of 2.1%. *B. abortus* was reported (Elsalam Abdalla *et al.* 2010). Another sero-epidemiological survey carried out in Middle East reported that sero-prevalence for *Brucella* spp. was of 1% and 8.5% in horses and donkeys, respectively (Abo-Shehada 2009).

Mycobacteriosis

Tuberculosis in horses is extremely rare, but when it occurs it is often caused by *Mycobacterium avium* subsp. *avium* (agent of avian tuberculosis). Very sporadically both *Mycobacterium tuberculosis* and *Mycobacterium bovis* are the etiological cause of equine tuberculosis (Keck *et al.* 2010, Monreal *et al.* 2011). As also observed for *Rhodococcus equi*, the risk of transmission of tuberculosis infection from horses to humans can be considered very low, except for immunocompromised patients who are particularly susceptible to mycobacterial infection (Pavlik *et al.* 2004).

Bacillus cereus

Bacillus cereus causes 2 types of gastrointestinal disorders (diarrheic and emetic). The organism is ubiquitous in nature, and as a consequence, it is present in feedstuffs and contaminates raw milk from faeces, soil, and during processing. Its heat-stable spores survive to the pasteurization process. Several reports have shown that it can proliferate rapidly in milk, depending on storage temperature, and some authors indicated the release of enterotoxin in milk (Notermans *et al.* 2009). The contamination

of bovine milk by *B. cereus* is very common. Scatassa and colleagues (Scatassa *et al.* 2009) have isolated *B. cereus* at 3 log CFU/ml in samples of donkey milk. In the Netherlands, de Jager (de Jager 2009) analysed milk from 12 horse farms and found the organism in 2 samples, with counts ranging from 40 to 120 CFU/ml.

Cronobacter sakazakii

About 120-150 neonatal *Cronobacter* infections have been described as frequently associated with immunodeficiency syndrome in babies mainly fed with contaminated infant formula milk. Moreover, FAO/WHO cited cases of colonisation and infection with *Cronobacter* spp. in the elderly population. Microbiological criteria for food and process hygiene, relevant for the European Union, consider *Cronobacter* spp. as a possible risk factor in powdered infant formula and dried dietary foods for special medical purposes for infants of less than 6 months of age (Friedemann 2009). The pathogen was found in 2 samples of donkey milk (Conte 2007). However, the ingestion of low numbers of this micro-organism by healthy infants and children does not lead to illness (EFSA 2004).

Streptococcus equi *subsp.* zooepidemicus

Human infection by *Streptococcus equi* subsp. *zooepidemicus* is a known zoonosis, primarily in patients who consume milk or raw milk products (Barnham *et al.* 1989). There are numerous reported cases of illness, often severe (septicaemia, meningitis, abscess, fever), from the consumption of cow or goat raw milk. An episode caused by the consumption of unpasteurized cow's milk has been described in England, in which 11 people were affected and 7 died (Edwards *et al.* 1998). A case of streptococcal toxic shock syndrome (STSS) involving a woman suffering from meningitis caused by *S. equi* subsp. *zooepidemicus* and referable to the consumption of raw milk from mare has been reported in Japan (Hashikawa *et al.* 2004).

Rhodococcus equi

Rhodococcus equi causes pneumonia and enteritis with a high mortality rate in foals of 1-3 months. Isolated for the first time in 1923, it was named *Corynebacterium equi*. *Rhodococcus equi* is considered an important emerging pathogen in immunocompromised individuals, particularly if affected by Acquired Immune Deficiency Syndrome (AIDS), in which it plays the role of opportunistic pathogen, with a high mortality rate. Eradication is difficult since it is transmitted by inhalation, through skin and tissues lesions as well as by food (Kedlaya *et al.* 2001). It lives and proliferates in soils rich in organic matter and it has been isolated from faeces of cattle, sheep, goats, pigs, and wild birds but, most frequently, from horse faeces (Takai *et al.* 1991). In foals it can be found at 3-4 log CFU/g, whereas in adults the counts are much lower. Thus, the foals are the true responsible for the maintenance of contamination at the farm level. In pigs and cattle, *R. equi* can cause injury, particularly at the level of the submaxillary lymph nodes, with some difficulty in the differential diagnosis with tuberculosis (Marcato 2008). In other species, albeit rarely, *R. equi* can give pyometra, mastitis, and purulent injuries (Cattabiani 2003); *R. equi* was also isolated from mammary quarters (13.7%) of dairy buffaloes (Bonini *et al.* 2007). The contamination of milk may be caused by environmental contamination or during suckling of the foal. While being a danger frequently present in some epidemiological conditions, the risk should be considered higher for immunocompromised consumers.

Streptococcus dysgalactiae *subsp.* equisimilis

Streptococcus dysgalactiae subsp. *equisimilis* comprises β-haemolytic streptococci belonging to the Lancefield group C. In humans, these colonize the pharynx, skin, gastrointestinal tract, and female genitourinary tract. This species causes severe pneumonia, septicemia, endocarditis, and toxic shock syndrome. In horses, *S. equisimilis* is generally considered infrequent or opportunistic, but it has recently been isolated from cases of strangles-like disease from aborted placentas and abscessed lymph nodes, suggesting a possible pathogenic role (Preziuso *et al.* 2010, Rantala *et al.* 2010).

Clostridium difficile

Clostridium difficile is a spore-forming Gram-positive, it produces toxins A and B, which cause gastroenteritis in humans with severe complications. It frequently develops resistance to antibiotics and causes outbreaks of nosocomial and community infections. It is assumed that aliments are a possible source of contamination, even though data are still inconsistent. *Clostridium difficile* has been isolated from foods commonly marketed in the United States, Canada, and Europe. The strains were often indistinguishable from those in humans, so that a common source or a zoonotic transmission is presumed. In 2005, *C. difficile* was isolated in 20% of beef samples in Canada and in 42% of beef and turkey

samples in the United States. In a survey carried out in Europe, *C. difficile* was isolated in 7.5% of samples of ready-to-eat foods. These data show that the germ is common in food and normal cooking of food will not idle the relatively rare spores. Although the issue is still controversial, some researchers believe that aliments of animal origin, including milk, may play a role in the transmission of *C. difficile* in humans, so that further studies are needed to design appropriate arrangements to control such transmission (Gould and Limbago 2010).

In 10% of horses *C. difficile* lives in the gut. Its presence may reach 45% in foals and in horses treated with antibiotics, but this germ rarely causes the disease, although in some cases colitis and diarrheal may arise. While the infection in humans is more common in hospitals, in equines it is found more frequently in the herd (Båverud *et al.* 2003).

Glanders

Burkholderia mallei is the causative agent of this disease that severely affects horses, mules, donkeys, and humans, with ulcerous lesions of the skin and lung that can even lead to death. Formerly widespread, the disease has been eradicated from many countries. Eliminated in Western Europe, glanders remains endemic in several countries in Asia, Africa, and South America (Wittig *et al.* 2006). In the last years, glanders outbreaks have been reported in Iraq, Iran, India, Pakistan, Turkey, and United Arab Emirates (Scholz *et al.* 2006). In Brazil, recent cases of glanders have been reported in some donkeys in the state of Pernambuco. The milk may be contaminated with material derived from pathological skin lesions (Mota *et al.* 2010).

Conclusions

Domestic equines are considered a dairy species worldwide since ancient times, but the recent scientific interest toward their possible role in human nutrition, mainly focused on nutritive, nutraceutical, and hypoallergenic properties, is not yet accompanied by sufficient data on microbiological hygiene and health characteristics. If during milking the hygiene procedures are strictly observed, microbial contaminations of horse and donkey milk are generally low thanks to their anatomical and behavioural peculiarities. Both horses and donkeys exhibit high capacity of adaptation to severe environmental conditions: resistance to mammary gland infections is attested by the low somatic cell count of milk and rare presence of mastitis agents compared to cow milk.

According to the reviewed literature, hazard exposure associated to the consumption of raw horse and donkey milk has to be considered lower than for cow milk, especially for microorganisms like enterotoxigenic *E. coli* and thermo-tolerant *Campylobacter*. However, the hazards related to milk consumption vary depending on whether one considers risks for human or domestic animals health.

Even if the alimentary risks associated to equine milk consumption is reasonably low in Europe compared to bovine milk, thermal treatment before consumption is strongly recommended. The alimentary risks increase in those regions of the world where horse, donkey, and mules are still crucial components of micro-economies and prevalence of *Brucella* spp. and *R. equi* is higher.

For example, in those regions of the world where HIV/AIDS represent a serious health problem, the high incidence of the immunocompromised population warns on the increased risk for pathogens as well as for opportunistic microbiota possibly present in horse and donkey milk.

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