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Review

Human brucellosis caused by raw dairy products: A review on the occurrence, major risk factors and prevention

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ABSTRACT

Despite considerable efforts made to address the issue of brucellosis worldwide, its prevalence in dairy products remains difficult to estimate and represents an important public health issue in many areas of the globe today. This is partly explained by the increasing tendency for consumption of raw dairy products, making the prevention and control of this zoonosis even more critical. This review summarizes reports published since the early 2000s on human brucellosis caused by contaminated dairy products and the systems of *evaluation and assessment* which are used to improve the diagnosis, surveillance, control and prevention of the disease. For this purpose, five comprehensive electronic databases were investigated and relevant studies were identified for systematic review. The design and quality of the studies revealed notable variation, especially in the methods used for the detection and characterization of *Brucella* spp. This report provides helpful information about the health risk associated with the consumption of raw milk and relevant preventive strategies.

1. Introduction

Brucellosis is a widespread zoonosis caused by closely related bacterial species belonging to the genus *Brucella*. This disease is mainly transmitted to humans through the consumption of contaminated dairy products (Kaynak-Onurdag et al., 2016; Oliver et al., 2009; Verraes et al., 2015). Brucellosis has been identified by the World Health Organization (WHO), the Office International des Epizootics (OIE) and the Food and Agriculture Organization of the United Nations (FAO) as one of the most significant neglected zoonotic diseases in the world (Corbel, 1997; Franc et al., 2018; Hosein et al., 2016; McLeod, 2011; Musallam et al., 2016). Brucellosis also generates significant economic impact by causing serious production losses through abortions, infertility, and decreased milk production in cattle, goats, sheep, swine and camels (McLeod, 2011).

Brucella melitensis represents the most virulent species for humans, although *B. abortus*, *B. canis*, *B. suis* (Corbel, 1997; Whatmore, 2009; Young, 1995) and, more rarely, the marine *Brucella strains* such as *B. ceti* and *B. pinnipedialis* (Dawson et al., 2008; McDonald et al., 2006) or *B. inopinata* (De et al., 2008; Scholz et al., 2010), can cause serious infections. Long term clinical signs of human brucellosis include sweats, arthralgia, undulant fever, back pain, hepatomegaly, abdominal pain, headaches, myalgia, and personality changes (Buzgan et al., 2010; Cutler et al., 2005; Valderas and Roop, 2006). Some other brucellosis symptoms are arthritis, leukopenia, anemia, hepatitis, thrombocytopenia, endocarditis, and meningitis (Buzgan et al., 2010). Brucellosis is most commonly associated with the consumption of contaminated dairy products, but has also been reported following close contact with infected animals and among microbiology laboratory workers (Corbel, 1997; Staszkiewicz et al., 1991). Although brucellosis has been eradicated in some developed countries, it still represents one of the most economically important diseases in Latin America, the Middle East, North and East Africa and South and Central Asia (McDermott and Arimi, 2002; Musallam et al., 2016; Oliveira et al., 2017). One of the main issues in brucellosis-endemic regions is possible under-diagnosis and the lack of availability of specific treatment regimens to cure complicated brucellosis in humans in order to achieve universal care and control in susceptible populations (Buzgan et al., 2010).

Thus, *Brucella* contamination of raw dairy products remains one of the main concerns for dairy product consumers in developing countries (McDermott and Arimi, 2002; Oliver et al., 2009), while most developed countries try to maintain brucellosis-free status in livestock (Falenski et al., 2011). Recently, the increasing tendency for consumption of raw dairy products, not only from cows, sheep, and goats, but also camels, llamas, donkeys, horses, buffaloes, reindeer, and yaks (Falenski et al., 2011) constitutes an additional risk of brucellosis transmission. As an effective preventive strategy in the absence of pasteurization facilities, thermal treatment such as boiling or heating

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milk to a minimum temperature of 80–85° for several minutes (Corbel, 2006) can be used to control brucellosis. At farm level, control strategies should be supported by national eradication programs and a preliminary serological screening using a simple, rapid and cost-effective method such as Rose Bengal Test (RBT) appeared to be effective (Mustafa and Nicoletti, 1995). In addition, vaccination campaigns for small ruminants and cattle have been used in some countries and could represent a cost effective and useful control approach for both health and agricultural sectors (Roth et al., 2003).

The purpose of the current review is to summarize current data about virulent *Brucella* spp. contaminating different dairy products and different methods/conditions used for their detection. The *Brucella* hazards reported in raw milk of different animal species such as cows, sheep, goats, and camels are also highlighted.

2. Material and methods

2.1. Searching approach

A systematic search was done according to the databases PubMed, Web of Science, Science Direct, Scopus, CABI Direct databases on relevant studies performed from 2000 to 2018. The MESH-terms applied were “milk”, “dairy products”, “and brucellosis”, “detection methods”. After a first screening, all potentially acceptable articles were extracted from the data bases and their data were downloaded and checked. Data from each article included the geographical regions of study; methods of brucellosis detection; the kind of dairy samples; total sample size; year of study and year of publication.

Inclusion criteria were: (1) Full-text availability; (2) English language for published full text; (3) Evaluation of brucellosis incidence in small and large ruminants; (4) Original epidemiological studies and case reports on brucellosis incidence after confirmed consumption of raw dairy products; (5) Research reporting the presence, growth and survival of *Brucella* spp. in dairy products using different methods. Articles were excluded when any of the above criteria were not met. We did not consider redundant articles or data, conference abstracts and letters.

3. Results

From a total of 1331 papers identified in PubMed (214), Web of Science (75), Science Direct (878), Scopus (146), CABI Direct (18) databases, 409 were excluded in the first literature review. According to titles and abstracts, 922 papers were identified as probably suitable. Eight hundred and ten papers were excluded according the criteria mentioned in the “searching strategy” section and 112 papers have been used for this review.

After identifying eligible articles, 28 papers containing 14,225 samples of contaminated dairy products, published between 2000 and June 2018, were included to evaluate the occurrence of *Brucella* contaminations in raw dairy products and major risk factors. Overall, the incidence rate of brucellosis through the consumption of raw dairy products in different geographic area was extracted from 16 papers containing 2955 samples. The methods used to define the rate of brucellosis in small and large ruminants and humans including enzyme-linked immunosorbent assay (ELISA), indirect enzyme-linked immunosorbent assay (iELISA), milk ring test (MRT), rose bengal test (RBT), polymerase chain reaction (PCR), real time- PCR and loop-mediated isothermal amplification (LAMP) were extracted from 68 papers.

3.1. Occurrence of Brucella spp. in raw dairy products and major risk factors

Generally, raw milk can be contaminated by *Brucella* spp. via two ways i.e. by endogenous contamination from the blood of infected animals to the milk or by exogenous contamination during or after milking through environmental agents (Verraes et al., 2015). Brucellosis in livestock populations has been successfully eradicated in many developed countries such as the majority of northern European countries, Canada, Australia, and New Zealand (McDermott et al., 2013; Pappas et al., 2006). In North America, *Brucella* reservoirs persist in wildlife such as bison, elk and wild boar (Abe et al., 2017; Cross et al., 2010; McDermott and Arimi, 2002). Similarly in Europe brucellosis cases in humans have resulted from consumption of raw dairy produce with the likely reservoir of infection identified as chamois (Mick et al., 2014). Among high-income countries, Saudi Arabia and Kuwait are highly impacted by this zoonosis and are still considered as brucellosis-endemic countries (Alenazi et al., 2018; El-Gohary et al., 2016; Elmoslemany et al., 2016; Mousa et al., 1988; Paul et al., 2017).

The prevalence of brucellosis in middle income countries such as Bangladesh, Iran, India, South Africa, Nigeria, Brazil, Turkey, Egypt, China and Pakistan is highly variable, because of different management systems, various implicated livestock species, and specific national or regional medical and veterinary programs (McDermott et al., 2013). Inappropriate management systems for animal transportation, unprotected borders, poor cross-border veterinary collaboration and lack of veterinary staff are known as the main constraints for controlling *Brucella* spread (Godfroid et al., 2013).

In some low-income countries, such as Uganda and Tanzania, brucellosis represents a neglected endemic disease without any effective control in animals, and high risk populations including farmers, butchers, shepherds, veterinary personnel, cattle handlers/transporters and cattle businessmen (Agasthya et al., 2007; Hoffman et al., 2016; Kamwine et al., 2017). The elimination of an infected livestock population and an adequate surveillance and diagnostic system for a better evaluation of the distribution and prevalence of brucellosis are required for effective control of the disease in these regions (McDermott et al., 2013).

The detection rates of *Brucella* species in dairy products widely differ according to the geographical areas (Table 1). It should be noted that several methods, both direct and indirect, with different specificity and sensitivity have been used. In this respect, Uganda and Iran have reported the highest detection rates in dairy products using ELISA and Real time PCR methods, respectively (Kamwine et al., 2017; Moslemi et al., 2018).

The detection rate of *Brucella* spp. in contaminated milk provides key information for risk evaluation in high risk populations given the fact that *B. abortus* and *B. melitensis* infections usually affect humans through the consumption of cattle, camel, goat or sheep milk (Table 1). In numerous middle income countries, such as Iran (Moosazadeh et al., 2016), India (Proch et al., 2018), Nigeria (Salisu et al., 2017), Brazil (Oliveira et al., 2017), Turkey (Kaynak-Onurdag et al., 2016), Egypt (Hosein et al., 2016; Wareth et al., 2014), China (Ning et al., 2013; Song et al., 2018) and Pakistan (Shafee et al., 2011), the detection rate of *Brucella* spp. remains high (Rubach et al., 2013). The incidence of *Brucella* spp. contamination has been reported in the milk of different animals, in various parts of the world. Moreover, it has been shown that brucellosis prevalence could be influenced by differences in hygienic conditions, animal age and species along with other experimental conditions such as the examined sample size, animal pregnancy conditions, breed, study area, herd size, breeding approaches, reproductive disorders, and diagnostic tests (Akbarmehr, 2011; Ayoola et al., 2017; El-Diasty et al., 2016; El-Gohary et al., 2016; Gafirita et al., 2017; Hosein et al., 2016; Kamwine et al., 2017; Kumar et al., 2017; Rahman et al., 2016; Salisu et al., 2017; Shafee et al., 2011). Regarding the diagnostic methods used in *Brucella* identification, it has been reported that some buffalo milk that was positive using PCR and serological tests, could show negative results with milk culture (Marianelli et al., 2008). However, bacterial isolation is still considered as the “gold standard” for the diagnosis of brucellosis.

On the other hand, this method presents limitations such as the need

Table 1
The detection rate of *Brucella* spp. in contaminated dairy food. Methods used for prevalence determination: Enzyme-linked immunosorbent assay (ELISA), Indirect Enzyme-Linked Immunosorbent Assay (iELISA), milk ring test (MRT), Rose Bengal test (RBT), Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP).

Brucella spp.	Dairy products (n)	Method	Detection rate (%)/product/method	Geographical area	References
Not determined	Cow milk (360)	RBT, culture, PCR	6.6/cow milk/PCR	Bangladesh	(Islam et al., 2018)
Not determined	Sheep milk (33) Goat milk (33) Cow milk (57) Pasteurized milk (34) Pasteurized cheese (28) Unpasteurized cheese (23)	Real-time PCR	27.3/sheep milk 45.5/goats milk 26.3/cow milk 14.7/pasteurized milk 25/pasteurized cheese 39.1/unpasteurized cheese	Iran	(Moslemi et al., 2018)
B. abortus, B. melitensis, B. abortus vaccine strain S19	Sheep milk (555) Goat milk (520) Cow milk (880)	PCR	2.3/sheep/PCR 0/goats/PCR 13.8/cow/PCR	South Africa	(Caine et al., 2017)
B. melitensis biovar 1 *Brucella ovis*	Sheep milk (300) Goat milk (400)	Culture, PCR	1/sheep/PCR 1.5/goats/PCR	Iran	(Ashrafganjooyi et al., 2017)
Not determined	Goat milk (470) Sheep milk (330)	PCR	10.9/goats/PCR 5.5/sheep/PCR	Iran	(Shirazi et al., 2017)
Not determined	Cow milk (185)	MRT, iELISA	33.5/cow milk/MRT, 49.5/cow milk/iELISA	Uganda	(Kamwine et al., 2017)
B. abortus	Cow milk (174)	ELISA, RBT	3.4/cow milk/ELISA	Nigeria	(Ayinmode et al., 2017)
B. melitensis *B. abortus*	Cow milk (564)	Real-time PCR	10.3/cow milk/Real-time PCR	Tajikistan	(Lindahl-Rajala et al., 2017)
Not determined	Cow milk (57)	MRT, iELISA	33.3/cow milk/MRT 3.5/cow milk/ELISA	Nigeria	(Ayoola et al., 2017)
Not determined	Cow milk (15) Fresh cheese (38)	Culture, PCR	0/cow milk/PCR 13.2/cow cheese/PCR	Brazil	(de Freitas Kobayashi et al., 2017)
B. melitensis	Camel milk (80)	RBT, ELISA and PCR	22/camel milk/RBT 15/camel milk/ELISA 11.3/camel milk/PCR	Saudi Arabia	(Khan et al., 2017)
Not determined	Cow milk (483)	MRT, ELISA	4.4/cow milk/MRT 5.8/cow milk/ELISA	India	(Kumar et al., 2017)
Not determined	Cow milk (57)	RBT, iELISA	33.3/cow milk/MRT 3.5/cow milk/ELISA	Nigeria	(Ayoola et al., 2017)
B. abortus strains and *B. abortus* S19 vaccine strain.	Cow milk (99)	Real-time PCR, culture	2/cow milk/Real-time PCR	Turkey	(Kaynak-Onurdag et al., 2016)
B. abortus	Cow milk (215) Cow cheese (50) Cow butter (50)	Culture, PCR	1.9/cow milk/culture and PCR	Turkey	(Gulbaz and Kamber, 2016)
B. abortus	Cow milk (324)	Real-time PCR	6.5/cow milk/Real-time PCR	Uganda	(Hoffman et al., 2016)
B. abortus	Cow milk (185)	MRT, i-ELISA	26.5/cow milk/MRT, i-ELISA	Uganda	(Kamwine et al., 2017)
B. melitensis biovar 2	Cow milk (60)	MRT	61.7/cow milk/MRT	Kuwait	(El-Gohary et al., 2016)
B. abortus biovar 1	Cow milk (205)	PCR, culture	6.3/cow milk/PCR	Egypt	(El-Diasty et al., 2016)
B. abortus biovar 3	Cow milk (63)	RBT, ELISA	6.3/cow milk/ELISA	Tanzania	(Mathew et al., 2015)
B. melitensis *B. abortus*	Cow and buffalo milk (215)	iELISA, Real-time PCR	16/cow and buffalo milk/iELISA 7.9 cow and buffalo milk/Real-time PCR	Egypt	(Wareth et al., 2014)
Not determined	Cow milk (5211)	PCR	1.1/cow milk/PCR	China	(Ning et al., 2013)
Not determined	Sheep milk (110)	LAMP	6.4/sheep milk/LAMP	China	(Song et al., 2012)
B. melitensis *B. abortus*	Cow cheese (1000)	Culture	2.2/cow cheese/culture	Iran	(Akbarmehr, 2011)
Not determined	Cow milk (86) Buffalo milk (114)	MRT, i-ELISA	4.6/cow milk/MRT 1.7/buffalo milk/MRT 20/cow milk/ELISA 0/buffalo milk/ELISA	Pakistan	(Shafee et al., 2011)
B. abortus *B. melitensis* *B. ovis*	Cow milk (120) Buffalo milk (120) Sheep milk (180)	MRT	24.2/raw milk/MRT	Iraq	(Abbas and Aldeewan, 2009)
B. abortus	Goat milk (54)	PCR, MRT	88.8/goat milk/PCR 59/goat milk/MRT	India	(Gupta et al., 2006)
B. melitensis	Cow milk (52) Sheep milk (21) Goat milk (18) Camel milk (12)	Culture, PCR	46.1/cow milk/culture 55.7/cow milk/PCR 57.2/sheep milk/culture 47.6/sheep milk/PCR 55.6/goat milk/culture 72.3/goat milk/PCR 0/camel milk/culture 0/camel milk/PCR	Qatar	(Hamdy and Amin, 2002)

for viable bacteria in tested samples, rich media for the culture of *Brucella* spp. and biosafety level-3 protocols and facilities to prevent laboratory-acquired infections. In addition, appropriate storage conditions and rapid delivery of biological samples to diagnostic laboratories are crucial requirements for the use of this approach (Brucellosis, OIE, 2016). In order to overcome these issues, molecular methods have been broadly used for a rapid, simple, sensitive and specific diagnosis and epidemiological surveys and been suggested to improve the sensitivity

and specificity in the detection of *Brucella* infections (Boeri et al., 2018; Das et al., 2018; Kaden et al., 2018). A recent study, evaluating different methods for *Brucella* detection in several matrices, showed that the limit of detection (LOD) for cultivation and direct real-time PCR techniques was in a range of 10^3–10^4 CFU/g and 10^4–10^5 CFU/g, respectively (Kaden et al., 2018). Through their comparative experiments and by determining the lowest possible limit of detection (LLD), the authors concluded that real-time PCR was the most sensitive method to detect *B. abortus* in concentrations near the infection dose (Kaden et al., 2018). In milk samples, although the LLD of both cultivation and PCR methods was almost the same (approximately 100 CFU/ml), cultivation method led to significantly lower LOD when compared to those obtained using real-time PCR preformed directly (without enrichment) on milk samples (825 vs 8667 CFU/ml). The authors suggested that the 10-fold increase in LOD using real-time PCR could be due to the presence of interfering substances in milk, such as casein micelles, which are able to bind DNA. Hence, PCR-based methods are appropriate for a first screening and in case of negative results, bacterial culture experiments are further recommended.

3.2. Reported human cases associated with the consumption of contaminated dairy products

Human brucellosis is described as an acute or sub-acute zoonotic disease usually characterized by an undulant fever with prostration, sweating, anorexia, malaise and muscle pain (Rahman et al., 2016; Smits and Kadri, 2005). Certain occupations, including veterinarians, abattoir workers, butchers, farmers, meat inspectors, and laboratory workers, are recognized to be at high risk from human brucellosis. The consumption of contaminated milk, butter and cheese could also transmit this disease (Kunda et al., 2007; Makita et al., 2010). Informally marketed raw milk as well as the consumption of dairy products made from raw milk represent the main risk factor (Buzgan et al., 2010; Makita et al., 2010). In addition, several factors like strain pathogenicity, the amount of food-ingested *Brucella* and the health condition of the consumer could influence the disease development following the consumption of contaminated dairy products. A few systematic studies were available on the prevalence of human brucellosis linked to the consumption of raw milk (Baron-Epel et al., 2018; El-Amin et al., 2001; Gur et al., 2003; Moosazadeh et al., 2016; Roushan et al., 2004). These studies highlighted the fact that the occurrence of the disease was mainly reported after consumption of raw milk and cheeses derived from the processing of raw milk (Table 2). In some countries, such as France, after milk pasteurization and application of strict surveillance and control policies throughout the production process of raw dairy products, the incidence of brucellosis significantly declined in humans (Mailles et al., 2016). As depicted in Table 2, the Middle Eastern countries are highly impacted by this disease and infected dairy products were responsible for several brucellosis outbreaks over the last two decades. In Turkey, a survey on 1028 brucellosis patients, admitted over a ten-year period, revealed that 63.6% had a history of raw dairy products and/or raw milk consumption (Buzgan et al., 2010). In Turkish studies, the level of reported human *Brucella* contaminations resulting from infected dairy products consumption varied from 62.6% (Gur et al., 2003) to 94.6% (Buzgan et al., 2010). Infected raw milk consumption was also responsible for 69% of brucellosis cases in Kuwait (Mousa et al., 1988), 57.1% in Iran (Moosazadeh et al., 2016) and 63% in Oman (El-Amin et al., 2001). Recently in Qatar, an outbreak of *B. melitensis* and *B. abortus* infections has been associated with camel milk consumption (Garcell et al., 2016). The ingestion of unpasteurized raw milk and cheese has also been reported as an important source of human brucellosis in other Middle Eastern areas such as Israel (Baron-Epel et al., 2018; Shimol et al., 2012) and Saudi Arabia (Somily et al., 2017).

In Africa, most of the human brucellosis reports come from Uganda where 5.8 per 10,000 people living in urban areas are annually contaminated by *B. abortus* strains due to the consumption of informally marketed raw milk (Makita et al., 2010). A recent study has also reported several cases of human brucellosis among travelers to and immigrants coming from the Horn of Africa due to the consumption of infected camel milk (Rhodes et al., 2016).

The ingestion of infected dairy products has also been at the origin of human brucellosis outbreaks in Southern Europe (Colmenero et al., 2011; Méndez et al., 2003; Ramos et al., 2008), South Eastern Asia (Leong et al., 2015) and Northern America (Serpa et al., 2018).

These data clearly show that the consumption of raw milk is the main risk factor for human brucellosis and is strongly conditioned by the geographical situation which impact the number of infected animals and the occurrence of *Brucella* contamination in dairy products (Franc et al., 2018; Seleem et al., 2010; Shirazi et al., 2017). However, some of these prevalence data should be interpreted with caution considering the fact that many people who are infected by raw milk are also in direct contact with animal or animal products.

3.3. Growth and survival of Brucella spp. in dairy products made from raw milk

The survival, growth and virulence of *Brucella* spp. in dairy products are highly influenced by production methods and storage conditions. (Falenski et al., 2011; Verraes et al., 2015). *Brucella* survival is highly dependent on the NaCl concentration, temperature, time of storage, pH, water activity, competing microbiota, and the dry matter content of contaminated dairy products. Salting with NaCl reduces the growth and survival of *Brucella* strains by decreasing the water activity. The level of fermentation is another important factor as the presence of fermenting bacteria in cheese and yoghurt decreases the growth of *Brucella* spp. by nutrient competition and by decreasing the pH (Verraes et al., 2015). The pH value plays a significant role in the survival of *Brucella* with optimal pH conditions ranging from 6.6 to 7.4 at 37 °C and a survival capacity between pH 8.4 and pH 4.1–4.5 (Falenski et al., 2011). It has been shown that *B. melitensis* could not survive at pH less than 4 (El-Daher et al., 1990), whereas *B. abortus* strains appeared to be more tolerant to acidic pH and could even survive in a pH below 4.0 in fermented milk for 10 days. This indicates that non-pasteurized fermented milk, contaminated with *B. abortus*, could be a route of bacterial transmission (Estrada et al., 2005).

Throughout cheese production processes and during the storage and ripening, *Brucella* spp. can also recur (El-Daher et al., 1990; Falenski et al., 2011; Reu et al., 2002). Several studies revealed long survival periods for *Brucella* in different types of ripened goat and cow cheeses produced from contaminated milk (Méndez-González et al., 2011; Plommet et al., 1988; Santiago-Rodríguez et al., 2015). Interestingly, in raw milk-derived cheeses, *Brucella* spp. can grow during the soft cheese production, but cannot spread in hard cheeses and semi-hard cheeses (Reu et al., 2002; Tantillo et al., 2003; Verraes et al., 2015). Ice creams produced from raw milk may also represent an important threat in endemic regions and have been reported as a way to spread *Brucella* infection in Turkey (Kuplulu and Sarimehmetoglu, 2004).

Milk and other dairy products could also be contaminated by *Brucella* spp. through exogenous environmental agents during or after milking (Verraes et al., 2015). Deliberate contamination of UHT-milk with B. abortus led to multiplication from 4.7×10^7 cfu/ml to 1.5×10^8 cfu/ml within two days and the bacteria could reach 6.7×10^8 cfu/ml at the end of the product's shelf-life (day 87) (Falenski et al., 2011). The same study revealed that *B. abortus* could survive in sterilized milk for 10 months at room temperature (Falenski et al., 2011).

3.4. The consumption of raw dairy products: a major risk factor for brucellosis

The determination of main risk factors associated with human

Table 2
Reported human cases of brucellosis due to contaminated dairy products. Methods used for reported human cases of brucellosis are comprised of Enzyme-Linked Immunosorbent Assay (ELISA), Rose Bengal test (RBT), Polymerase chain reaction (PCR), Standard tube agglutination test (STAT), blood culture, real time PCR and immunocapture–agglutination test.

Brucella spp.	Dairy product	Country	Year	The number of human cases	Diagnostic test	Positive cases of human brucellosis (%)	References
Not determined	Raw milk	Oman	2001	375	ELISA, blood culture	63	(El-Amin et al., 2001)
B. melitensis biovar 3	Unpasteurized raw goat cheese	Spain	2002	11	Blood culture	100	(Méndez et al., 2003)
B. melitensis	Raw milk and fresh cheese	Turkey	1992–2000	283	STAT, blood culture	Not determined	(Gur et al., 2003)
Not determined	Fresh cheese	Iran	1992–2002	469		100	(Roushan et al., 2004)
B. melitensis biovar 3	Raw milk and unpasteurized fresh cheese	Spain	2008	3	RBT, blood culture	100	(Ramos et al., 2008)
Not determined	Herbaceous cheese produced from raw milk	Turkey	1998 to 2007	1028	STAT	63.6	(Buzgan et al., 2010)
B. melitensis	Unpasteurized cheese	Spain	2011	7	RBT, blood culture, STAT, real time PCR, immunocapture–agglutination test	100	(Colmenero et al., 2011)
B. melitensis	Raw milk	Israel		15	RBT, culture	100	(Shimol et al., 2012)
Not determined	Raw milk	Malaysia	2011 to 2012	79	Blood culture, PCR, tissue culture tests, ELISA	69.8 (blood culture) 17.5 (PCR) 1.6 (tissue culture tests) 87.3 (ELISA)	(Leong et al., 2015)
B. abortus, *B. melitensis*	Raw milk	Qatar	2015	14	Blood culture, STAT	100	(Garcell et al., 2016)
Not determined	Raw milk	Iran	Up to 2015	Meta-analysis on 47 articles	Meta-analysis	57.1	(Moosazadeh et al., 2016)
B. melitensis	Raw milk	Horn of Africa	2007 to 2013	3	Blood culture	100	(Rhodes et al., 2016)
B. melitensis *B. abortus*	Raw milk	Saudi Arabia	2003 to 2013	163	Blood culture, STAT	45.4	(Somily et al., 2017)
Not determined	Raw milk	Rwanda	2017	198	RBT, blood culture	6.1	(Gafirita et al., 2017)
Not determined	Raw milk and fresh cheese	Israel	2014	306	Questionnaire	100	(Baron-Epel et al., 2018)
B. abortus vaccine strain RB51	(Unpasteurized) cow's milk	United States of America	2017	A human case	PCR, blood culture	A human case	(Cossaboom et al., 2018)

brucellosis is crucial to set up efficient preventive and control strategies (Kumar et al., 2017). Unfortunately, the lack of in depth investigations for identifying risk factors is the most important cause of the brucellosis persistence in some counties (Ning et al., 2013). *Brucella* may be shed into the milk frequently, thereby presenting a serious risk for the consumers of unpasteurized dairy products. Although pasteurization could completely kill *Brucella* strains, the elevated consumption of contaminated dairy products without pasteurization is still responsible for the highest proportion of brucellosis cases in different outbreaks (Table 2). Besides, living around and in close contact with infected livestock is another important risk factor (Al-Shamahy et al., 2000). Unpasteurized cheeses produced from infected raw goat milk have been responsible for brucellosis outbreaks in Spain (Castell et al., 1996; Méndez et al., 2003). In addition, the illegal sale of contaminated raw milk has been reported as another important factor influencing the brucellosis incidence rate in some Spanish and Moroccan cases (Méndez et al., 2003; Ramos et al., 2008). It has been shown that developing policies and preventive strategies to reduce informally marketed milk, as one of the most important risk factors for human brucellosis, could effectively prevent the spread of this infection (Hoffman et al., 2016). Another study pointed the importance of screening the family members of contaminated patients in order to prevent and control the spread and severity of this zoonosis (Sofian et al., 2008). For this purpose, public health education programs, as well as improved veterinary services, should be developed in high risk populations (Asakura et al., 2018; Earhart et al., 2009). The reduction of contamination in livestock by controlling abortion rate, avoiding mixing small ruminants and cattle, and culling after testing could considerably reduce the *Brucella* content in milk, thereby preventing human infection (Ning et al., 2013). However, in urban areas with lower animal density and limited contact of human populations with livestock, the systematic pasteurization of dairy products, along with appropriate control and education programs regarding the use of fresh dairy products, represent efficient preventive strategies to reduce the incidence of human brucellosis.

3.5. Detection methods of Brucella spp. in raw milk

As mentioned above, bacterial isolation is considered as the “gold-standard” for specific diagnosis of *Brucella* spp. allowing the biotyping of the isolates (Alton et al., 1988; Leyla et al., 2003). However, the culture of *Brucella* spp. can be challenging and requires optimal biosafety, handling and culture media conditions (Brucellosis, OIE, 2016). Thus, the detection of specific antibodies against *Brucella* antigens in serum or milk using immunological methods such as indirect Enzyme-Linked Immunosorbent Assay (i-ELISA), Enzyme-Linked Immunosorbent Assay (ELISA), Milk Ring Test (MRT) and the Rose Bengal Test (RBT) represent the conventional and most available approaches to indirectly assess the presence of *Brucella* contaminations (Table 1). These techniques are commonly applied for the control and surveillance of brucellosis infections, although serological methods are not a direct measure of the presence of this microorganism in the milk. Despite their numerous advantages, serological tests can generate false-positive results (Munoz et al., 2005) and antibiotic therapy significantly reduces the presence of *Brucella*-specific IgM antibodies, even if specific IgG antibodies remain detectable (Elfaki et al., 2005). Moreover, in the case of seronegative dairy cattle, when the disease goes into chronic stage with declined antibody titers, or in the first 14 days of infection, when the humoral response show undetectable antibody levels, culture of specimens are required to identify contaminated milk samples (Islam et al., 2018).

Molecular approaches using PCR-based methods have shown high sensitivity for the diagnosis of *Brucella* spp. in milk samples (Kaden et al., 2018). Recently, real-time quantitative PCR (qPCR) appeared to be highly reliable to assess the occurrence of *Brucella* contamination in milk, and may enable the discrimination of virulent strains from those resulting from vaccination (Awwad et al., 2016; Kaynak-Onurdag et al., 2016). The use of qPCR is also advantageous because of its rapidity and higher sensitivity when compared to conventional PCR. It also reduces the risk of laboratory contamination and false-positive results as it does not require post amplification handling of PCR products. For this purpose, efficient DNA preparation protocols intended to improve the PCR-based identification of *Brucella* in raw milk samples are of paramount importance. Recently, a modified approach for the amplification of the 16*S* rRNA, *omp*2, and *IS*711 targets from *Brucella* was developed for the screening of camel milk samples (Khan et al., 2017). Another approach, using IS711-based qPCR, proved to be a very sensitive method for detecting *Brucella* in milk samples (Hinić et al., 2008). Recently, the presence of the *ery*C gene, involved in the erythritol metabolism in virulent strains and absent from vaccine *Brucella* strains, was also introduced as an effective diagnostic biomarker for the detection of *Brucella* infection through qPCR (Rodríguez-Lázaro et al., 2017).

Considering the practical limitations of culture methods, it has been proposed that both bacterial isolation and molecular tests should be applied for reliable epidemiological screening of herds in surveillance programs (Kattar et al., 2007; Sarker et al., 2017). Some other investigations recommend the combination of qPCR with serological tests for a better control of brucellosis outbreaks in dairy products (Gwida et al., 2015). Despite the importance of these molecular approaches for better brucellosis prevention, a special effort is needed to improve their implementation in farm environments in order to provide accurate and fast tests for dairy products and livestock in endemic regions.

4. Conclusion

With the current industrial norms and regulations for thermal processing of milk, the risk of *Brucella* infection has considerably decreased among urban population in most developed and developing countries. However, the incidence of human brucellosis remains high in some endemic regions, especially among rural populations and small communities of cattle owners where the hygiene rules and industrial standards in milk processing and food preparation are not appropriately applied. Besides, the increasing tendency for consumption of raw dairy products due to their purported health benefits, stresses the need for proactive public information strategies and campaigns to promote individual measures for preventing food-borne *Brucella* infections. Avoiding the consumption of home-made dairy products derived from raw milk as well as unpasteurized cheeses, yoghurts and creams considerably limit the risk of infections. Despite this fact, very few systematic reports on risk assessment of brucellosis resulting from milk consumption are available from endemic areas. Taken together, the data gathered in this review reflects the fact that the consumption of dairy products from raw milk remained the main route of human infection in endemic regions and the higher incidence of human brucellosis in rural areas is mainly due to the consumption of contaminated dairy products (Wernery, 2014). Small and large ruminants are common vectors for these virulent species (Abbas and Aldeewan, 2009; Akbarmehr, 2011; Khan et al., 2017) and the two most prevalent species inducing brucellosis are *B. abortus* and *B. melitensis* (Tables 1 and 2). The high incidence of human brucellosis in Middle Eastern regions stresses the urgent need to control the disease in endemic areas, particularly through the detection and monitoring of pathogen sources. The development of molecular approaches allowing simple, cost-effective, and accurate identification of *Brucella* infections in cow, camel and goat milk samples may lead to a better monitoring and aid eradication of this disease. To achieve this aim, regular and strict control measures alongside production and supply processes of dairy products should be implemented by regional and national authorities in order to ensure *Brucella* free and safe dairy products. In urban areas, given the limited contact of urban populations with cattle, goat and camel reservoirs, managing the dairy processing chain appeared to be a highly efficient strategy to reduce brucellosis incidence. Brucellosis is still a major challenge for public health policies given its wide distribution around

the world, with the potential of damaging livestock and human health worldwide (Valderas and Roop, 2006). Educational programs targeting at risk populations, along with stringently enforced hygiene measures, regulations and inspections, should be implemented to reduce the incidence of this disease in brucellosis endemic regions.

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