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From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis

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Review article

From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis

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Abstract – Brucellosis is not a sustainable disease in humans. The source of human infection always resides in domestic or wild animal reservoirs. The routes of infection are multiple: food-borne, occupational or recreational, linked to travel and even to bioterrorism. New *Brucella* strains or species may emerge and existing *Brucella* species adapt to changing social, cultural, travel and agricultural environment. *Brucella melitensis* is the most important zoonotic agent, followed by *Brucella abortus* and *Brucella suis*. This correlates with the fact that worldwide, the control of bovine brucellosis (due to *B. abortus*) has been achieved to a greater extent than the control of sheep and goat brucellosis (due to *B. melitensis*), these latter species being the most important domestic animals in many developing countries. The long duration and high cost of treatment of human brucellosis reduces the efficacy of the therapy. There is no human vaccine for brucellosis and the occurrence of brucellosis is directly linked to the status of animal brucellosis in a region. In this context, the World Health Organization has defined the development of a human vaccine, besides the implementation of control and eradication programs in animals, as a high priority. The pathogenicity for humans of *B. suis* biovars 1, 3 and 4 is well established, whereas *B. suis* biovar 2 seems to be less pathogenic. Indeed, although hunters and pig farmers have repeatedly experienced infectious contact with *B. suis* biovar 2 (found in wild boar and outdoor-rearing pigs in Europe), isolation of *B. suis* biovar 2 from human samples have only been seldom reported. Marine mammal brucellosis, due to two new proposed *Brucella* species i.e. *B. cetaceae* and *B. pinnipediae*, represents a new zoonotic threat but the pathogenicity for humans of the different *Brucella* species found in cetaceans and pinnipeds still has to be clearly established.

***Brucella* / animal and human brucellosis / zoonoses / domestic and wildlife reservoir**

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Table of contents

1. Introduction	314
2. Brucellae taxonomy	315
3. Prevalence of animal brucellosis	315
4. Zoonoses	316
4.1. Human brucellosis case definition	317
4.1.1. Clinical description	317
4.1.2. Laboratory criteria for diagnosis	317
4.1.3. Case classification	317
4.2. Food-borne, occupational exposure and travel-health	318
4.3. Bioterrorism	319
4.4. Wildlife	320
4.4.1. <i>B. suis</i> biovar 2	321
4.4.2. Marine mammal brucellosis	321
4.5. Conclusions	322

1. INTRODUCTION

We know from written resources that sheep and goats were the primary domestic animals in the Roman Empire. Small ruminants milk was used to make cheese, one of the primary ingredients in Roman cuisine. It was therefore hypothesized that milk and milk products were important sources of an infectious food-borne disease that was later known as the “Maltese fever” (i.e. brucellosis due to *Brucella melitensis*). The Roman town of Herculaneum was destroyed by the tremendous volcanic eruption of Mount Vesuvius in August 79 A.D. Recently, L. Capasso found bone lesions typical of brucellosis in adult skeletal remains of people killed during the first volcanic surge of Mount Vesuvius [16]. He also demonstrated by scanning electron microscopy analysis of a buried carbonized cheese, the presence of cocco-like forms that were morphologically consistent with *Brucella* spp. [16]. Sir David Bruce isolated in 1887 the organism (*Micrococcus melitensis*) responsible for Maltese fever from a British soldier who died from the disease in Malta [15]. This bacterium was renamed *Brucella melitensis* in his honor. In 1905, Zammit demonstrated, again in Malta, the zoonotic nature of *B. melitensis* by isolating it from goat’s milk [78].

In April 2003, the first report of community-acquired human infections with marine

mammal-associated *Brucella* spp. was published. The authors described the identification of these strains in two patients with neurobrucellosis and intracerebral granulomas [66].

These facts highlight that *Brucella* spp. has always been of zoonotic concern throughout History. Human brucellosis has always been associated with an animal (domestic or wild) reservoir of *Brucella* spp. However, the main sources of infection(s) as well as the routes of contamination may differ. Because each *Brucella* species (or even biovar) has distinctive epidemiologic features, the complexity of the interaction between *Brucella* spp., animals and humans has increased. Moreover, new *Brucella* strains or species may emerge and existing *Brucella* spp. adapt to changing social, cultural, travel and agricultural environments. Hence, the global human and animal brucellosis picture will, in essence, always remain incomplete.

The aim of this paper is (1) to review the known sources and routes of *Brucella* spp. infections for humans and (2) to assess the new potential zoonotic threat of *Brucella* spp. found in wildlife reservoirs, with particular emphasis on *Brucella suis* biovar 2 infection in the wild boar (*Sus scrofa*) in Europe and marine mammal *Brucella* spp. infections that seem to be present in all the

oceans covering our planet. Animal diagnostic procedures, vaccination protocols, control and eradication programs, human brucellosis diagnosis and medical or surgical management as well as recent advances in the understanding of the biology, virulence and evolution of the genus *Brucella*, through genomic and post-genomic techniques, will not be covered per se and the reader is directed to recent reviews and reference books on all these aspects [3, 6–9, 13, 30, 55, 58, 71, 76, 77].

2. BRUCELLAE TAXONOMY

Brucellae are Gram-negative, facultative intracellular bacteria that can infect many species of animals and man. Six species are recognized within the genus *Brucella*: *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis*, and *Brucella neotomae* [3, 27]. This classification is mainly based on the difference in host preference and in pathogenicity. Distinction between species and biovars is currently performed by differential laboratory tests [3, 27]. Although it has been proposed that the *Brucella* species should be grouped as biovars of a single species based on DNA-DNA hybridization studies [71] and on the comparison of the genome of *B. melitensis* [30] and *B. suis* [58], the current classification of brucellae in species according to differences in host preference and in pathogenicity should be preferred [23, 53].

Worldwide, the main pathogenic species for domestic animals are *B. abortus*, responsible for bovine brucellosis; *B. melitensis*, the main etiologic agent of small ruminant brucellosis; and *B. suis* responsible for swine brucellosis. These three *Brucella* species may cause abortion in their hosts and because of the presence of brucellosis in a herd (or flock), a region or a country, international veterinary regulations impose restrictions on animal movements and trade, which result in huge economic losses [7, 8, 28]. *B. ovis* and *B. canis* are responsible for ram epididymitis and canine brucellosis respec-

tively. For *B. neotomae* only strains isolated from desert rats have been reported. Albeit their respective host preferences, different *Brucella* strains have also been isolated from a great variety of wildlife species such as bison (*Bison bison*), elk (*Cervus elaphus*), feral swine and wild boar (*Sus scrofa*), fox (*Vulpes vulpes*), hare (*Lepus capensis*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandus tarandus*), caribou (*Rangifer tarandus groenlandicus*), chamois (*Rupicapra rubicapra*) and ibex (*Capra ibex*) and wildlife has to be considered as a reservoir for zoonotic brucellosis [29, 39, 60]. The broad spectrum of *Brucella* isolates has recently been enlarged to marine mammals. A number of recent reports describe the isolation and characterization of *Brucella* strains from a wide variety of marine mammals [22, 32, 36]. These strains have been identified as brucellae, however their overall characteristics are not assimilable to those of any of the six recognized *Brucella* species [22, 23, 45].

3. PREVALENCE OF ANIMAL BRUCELLOSIS

Information about animal brucellosis (world wide) is available on the Office International des Epizooties (OIE) website (<http://www.oie.int>), by using the HandiStatus tool (i.e. Help with World Animal Disease Status, version 2) containing information on animal diseases (like brucellosis) that have serious consequences for international trade or public health. This information is regularly updated based on emergency, monthly and annual reports sent to the Central Bureau of the OIE by the veterinary administrations of countries and other official sources. Globally, despite the remarkable results achieved by the majority of industrialized countries, where bovine brucellosis has been eradicated or controlled, small ruminant brucellosis remains a problem in some of these countries as well as in all developing countries [50]. Basically, in developing countries, brucellosis is almost

always present where small ruminants are kept. *B. melitensis* in cattle has emerged as an increasingly serious public health problem in some southern European countries and Israel as a result of the consumption of unpasteurized milk since *B. melitensis* is capable of colonizing the bovine udder [11, 72]. A related problem has been noted in Mexico and some South American countries, where *B. suis* biovar 1 has become established in cattle [12, 47, 64]. Moreover, in some areas of these countries, cattle are now believed to be more important than pigs as a source of *B. suis* biovar 1 infection for humans, because *B. suis* biovar 1 is capable of colonizing the bovine udder as *B. melitensis* does [26]. *B. melitensis* infections occur in cattle almost exclusively when these infections are present in small ruminants [72]. It is therefore likely that *B. melitensis* infections in cattle are not sustainable infections. In this case, the implementation of control measures in the primary host (i.e. small ruminants), without any additional specific measures in cattle (like vaccination, for example) is likely to solve the problem by exhausting the source of infection for cattle [40]. Although it is claimed that *B. suis* biovar 1 has become established in cattle [26], it still remains to be proven that it may do so without a continuous influx of new bacteria from the pig reservoir.

Areas currently considered to have high brucellosis prevalence areas are the Middle East, the Mediterranean Basin (Portugal, Spain, Italy, Greece, Turkey, Near East, North Africa), South and Central America, South-Eastern Europe, Asia, Africa, and the Caribbean. In some regions, camel brucellosis due to *B. melitensis* or *B. abortus* infections is another important source of human infections [1, 2, 10, 25, 59]. As a consequence, the importance of zoonotic brucellosis reservoirs is such that the World Health Organization (WHO) stated: "We regard brucellosis as the world's most widespread of all zoonoses and apart from its toll on people, it has an enormous impact on the animal industry". As the international trade in ani-

mals and animal products and movement of people increase, the risk of introduction and re-introduction of brucellosis is growing.

4. ZONOOSES

Brucellosis is an established zoonosis, infection having been attributed to at least four of the six recognized *Brucella* species in terrestrial mammals, *B. ovis* and *B. neotomae* being the exceptions. *B. melitensis* is by far, the most important zoonotic agent, but *B. abortus* and *B. suis* also constitute a significant threat [6]. *B. ovis* has not been demonstrated to cause overt disease in humans, although it is widespread in sheep. *B. canis* can cause disease in humans, although this is rare even in countries where the infection is common in dogs [17]. Recently, human brucellosis has also been attributed to some marine mammal strains [14, 66]. Human-to-human transmission by tissue transplantation or sexual contact have occasionally been reported but are, in epidemiological terms, insignificant [48].

Control and eradication of brucellosis in domestic animals have important public health implications. Test-and-slaughter programs, in conjunction with vaccination and, in a later stage, whole herd depopulation, are the major methods of control and eventually eradication of animal brucellosis [6–8, 26, 28]. As a general rule, prevention of human brucellosis depends predominantly on the control of the disease in animals [6, 7, 68]. For example, the rise of human brucellosis cases reported in Greece in 1995 is assumed to be due to the discontinuation of the small ruminant vaccination program against brucellosis that was followed by an increase in the prevalence of the disease in small ruminants [52]. Once an emergency mass-vaccination program of young and adult animals was reinforced, the number of human cases again decreased [52]. Specific regulations have been implemented in the European Union (EU) concerning measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order

to prevent outbreaks of food-borne infections and intoxications (Council Directive 92/117/EEC of 17 December 1992).

4.1. Human brucellosis case definition

The following case definitions are taken from the US Center of Disease Control (CDC) [18].

4.1.1. Clinical description

An illness characterized by acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache, and arthralgia.

4.1.2. Laboratory criteria for diagnosis

- Isolation of *Brucella* spp. from a clinical specimen, or
- Fourfold or greater rise in *Brucella* agglutination titer between acute- and convalescent-phase serum specimens obtained greater than or equal to two weeks apart and studied at the same laboratory, or
- Demonstration by immunofluorescence of *Brucella* spp. in a clinical specimen.

4.1.3. Case classification

- Probable: a clinically compatible case that is epidemiologically linked to a confirmed case or that has supportive serology (i.e. *Brucella* agglutination titer of greater than or equal to 160 in one or more serum specimens obtained after the onset of symptoms).
- Confirmed: a clinically compatible case that is laboratory confirmed.

In countries where brucellosis is enzootic (i.e. present in animal reservoirs), human confirmed cases are based on clinical symptoms associated with positive serology without attempts to isolate *Brucella* spp., in the vast majority of suspected or confirmed cases. In countries where animal brucellosis has virtually been eradicated, for every single suspected case of human brucellosis,

particularly in the presence of positive serology, the emphasis is put on the epidemiological inquiry in order to confirm the case. This inquiry has to trace back the potential source of contamination. The importance of the epidemiological investigation can be exemplified by the situation in Belgium. In this country which has been free of brucellosis in cattle since 2000 and in sheep, goats and domestic pigs for decades (and thus where no domestic animal reservoir exists), more than a hundred positive human brucellosis serology cases are registered every year in the national reference centre for animal and human brucellosis. Beforehand, we know that the number of false positive serological reactions will increase with the decrease of the actual prevalence of the disease. Therefore, in a country virtually free of animal brucellosis, confirmation by isolation of *Brucella* spp. from a clinical specimen is required. In Belgium, for the 1993–2003 period, from zero to seven human cases/year were confirmed by the isolation of *Brucella* spp. in clinical specimens. For all these confirmed cases, an epidemiological link was clearly identified. Indeed, besides one occupational case of *B. abortus* infection (in the nineties some foci of infections remained in the Belgian cattle population) and one occupational case of *B. suis* infection (this latter case occurred in a butcher preparing imported feral pig meat [41]), all other human brucellosis cases were food-borne infections due to *B. melitensis* and were “imported” cases, from Mediterranean countries. Thus, in countries virtually free of animal brucellosis and in the absence of an established epidemiological link, human brucellosis can virtually be ruled out and positive serological results have to be classified as false positive serological results [43]. False positive serological reactions in animal brucellosis are well documented in EU Member States virtually free of animal brucellosis and they represent currently one of the most important problems the veterinary authorities have to deal with [42].

4.2. Food-borne, occupational exposure and travel-health

Worldwide, the true incidence of human brucellosis is unknown. Reported incidence in endemic-disease areas varies widely, from <0.01 to >200 per 100 000 population [26]. For example, Egypt, the Islamic Republic of Iran, Jordan, Oman, Saudi Arabia and Syrian Arab Republic reported a combined annual total of more than 90 000 cases of human brucellosis in 1990 [10]. The low incidence reported in known brucellosis-endemic areas may reflect the absence or the low levels of surveillance and reporting [26, 50, 68]. In 2000, altogether 2 857 human cases (in 14 Member States) were reported in the EU compared to 3 899 cases (in 13 Member States) in 1999 [40]. As in previous years, only very few human cases occurred in those Member States that had acquired the "Officially Brucellosis Free" status in cattle and small ruminants [40].

Human brucellosis is not considered a contagious disease [6, 21, 35]. Therefore, clustering could result from common-source outbreaks or time-space clustering of factors that increase the risk of infection [21, 35]. In California, human brucellosis evolved between 1973 and 1992 from an occupational (slaughterhouse cases) to a food-borne illness with a higher incidence in Hispanics, especially in children and teenagers, compared with non-Hispanic whites and African Americans [21]. Therefore, public health programs in California should focus on educating Hispanic populations about the risk of consuming dairy products, such as soft cheeses, made from unpasteurized milk [35]. The importance of screening household members of acute brucellosis cases in endemic areas, like Saudi Arabia, has recently been emphasized: screening family members of an index case of acute brucellosis detected additional cases [4]. Humans are often the first to be reported as infected in an area into which the disease has recently been introduced and epidemic human infections by *B. melitensis* have developed among

people frequently in contact with infected goat herds or goat manure, as recently reported in Argentina [73].

Consumption of contaminated foods and occupational contact are the major risks of infection. Consumption of unpasteurized cow, small ruminants or camel milk and milk products (cheese and sour milk, for example) is considered to be the main route of infection [4]. Meat of animals with brucellosis may also be a source of infection if eaten when insufficiently cooked. Some particular food habits, such as eating aborted foetuses as seen in Ecuador, may be responsible for human brucellosis.

Crushing the umbilical cord of newborn lambs and kids with the teeth is another risky habit. Skinning stillborn lambs and kids and aborted foetuses, which may be heavily contaminated with *Brucella* spp., also presents a high risk of brucellosis [10].

Infection may also result from the entry of the bacteria from diseased animals through skin lesions or mucous membranes or from inhalation of contaminated dust or aerosols. Inhalation is often responsible for a significant percentage of cases in abattoir employees [62]. Contamination of skin wounds may be a problem for persons working in slaughterhouses or meat packing plants or for veterinarians. Hunters may be infected through skin wounds or by accidentally ingesting the bacteria after cleaning deer, elk, moose, or wild pigs that they have killed. It is worthy to re-emphasize that in the USA and Australia, human brucellosis caused by *B. suis* biovars 1 or 3 is considered to be almost entirely occupational [62, 74], whereas in the Americas, brucellosis in humans caused by *B. suis* biovar 1 is emerging as an increasingly serious public health problem as a result of the consumption of unpasteurized cow milk since *B. suis* biovar 1 is capable of colonizing the bovine udder [26].

Brucellosis vaccines for animal use (*B. abortus* B19 and RB51, for cattle; *B. melitensis* Rev. 1 for small ruminants) are live attenuated vaccines. These vaccines

show a residual virulence for humans and are known to cause disease in humans [6, 7, 11, 19]. Adverse effects of strain persistence and secretion in milk have been encountered with the *B. melitensis* vaccine strain Rev. 1 [11]. Moreover, in Israel, field isolates obtained from vaccinated animals and from a human were classified as Rev.1-like field isolates and it was demonstrated that they originated from the Rev. 1 vaccine strain [11]. In 1997, nine persons (a farmer, four veterinary clinicians, and four veterinary students) in Manhattan, Kansas, were infected when participating in an attempted vaginal delivery, a caesarean delivery, and a necropsy on a stillborn calf that died because of *Brucella abortus* RB51 infection [19].

Laboratory-acquired infections are occasionally reported and the inhalation of infective aerosols produced accidentally by microbiological techniques is the most frequent source of infection. Hence, in the laboratory, handling *Brucella* spp. in biosafety level 3 cabinets should always take place [3, 49].

Although many travellers are willing to experience new and exotic food and food preparations, they need to be educated with regards to the modes of disease transmission, so that they can take care when eating abroad, especially food from street vendors or traditional shops. Otherwise, they may expose themselves to *Brucella* spp. infections. *Brucella* spp. persist for several days in milk (even when it turns sour) and is known to flourish in soft fresh small ruminant cheese [6, 51]. It may also persist for weeks in ice cream and months in butter. Therefore, these products always have to be made from pasteurized milk [51]. For those travellers who may have contact with live or dead animals, infection may be transmitted via direct contact or aerosol. It is also worthy remembering that *Brucella* spp. can survive in the soil as well as in tap water for several weeks [51].

Despite many studies on the medical management of brucellosis over the past two decades, no major changes in the known therapeutic modalities have taken place [9,

76, 77]. The treatment recommended by the WHO for acute brucellosis in adults is rifampicin 600 to 900 mg and doxycycline 200 mg daily for a minimum of six weeks [6]. However, although the most appropriate antibiotic combination is not known, other reports suggest that the combination of doxycycline and an aminoglycoside is probably best and the addition of rifampicin may be a reasonable option [9, 55]. There is no consensus with regards to the best therapeutic regimen for chronic brucellosis in adults, simple or complicated brucellosis in children under 7 years of age and surgical management is only indicated in special cases [9, 51, 55, 68, 76, 77]. Moreover, the long duration and high cost of treatment of human brucellosis reduce the efficacy of the therapy [68, 76, 77].

No human brucellosis vaccine exists and it has been suggested that the development of such a vaccine should be treated as a priority area under the WHO global program on vaccination and immunization [7].

4.3. Bioterrorism

Bioterrorism and its potential for mass destruction have been subjects of increasing international concern. Production costs of biological weapons are low, and aerosol dispersal equipment from commercial sources can be adapted for biological weapon dissemination. As far as brucellosis is concerned, inhalation of only a few organisms is sufficient to cause a significant likelihood of infection. In a theoretical model of a bioterrorist attack and in the absence of an intervention program for 100 000 persons exposed, a *B. melitensis* cloud would result in 82 500 cases of brucellosis requiring extended therapy, with 413 deaths [46]. The economic impact of such a brucellosis bioterrorist attack would cost \$ 477.7 million per 100 000 persons exposed [46].

In 1999, in Massachusetts, USA, a suspected case of brucellosis in a woman in which the atypical clinical presentation and suspicious circumstances surrounding the

case raised the possibility of a bioterrorist attack [20]. The patient showed clinical signs consistent with brucellosis and positive serological results in brucellosis tests. The patient died of a cause that was unrelated to brucellosis. However, this episode highlighted the difficulties linked to the implementation of an early bioterrorism warning system. Because agents high on the list of possible biological terrorism have a very low incidence of natural infection in the United States and other industrialized countries, the risk for a false-positive result is high. Therefore, diagnostic laboratory testing should be integrated with epidemiological investigation when assessing potential covert biological terrorism events to rule out false-positive laboratory findings. In addition, support systems should be established to facilitate early recognition of rare and unusual emerging infectious diseases [20]. In order to re-emphasize the importance of epidemiological investigations, it is worth mentioning that in France, human cases of food-borne *Yersinia enterocolitica* serogroup O:9 (YO9, a known cross-reactive bacterium in brucellosis serology) infections were reported in the 1990s. Patients had positive serological results for brucellosis but no history of contact with *Brucella*-infected animals. Gastrointestinal symptoms suggested yersiniosis [43].

4.4. Wildlife

Wildlife brucellosis is a political issue: the livestock, hunting and game farming industries, and those involved in wildlife conservation and welfare, have conflicting interests [31, 75]. Moreover, wildlife brucellosis represents a potential zoonotic threat [60]. When addressing wildlife brucellosis, it is very important to distinguish between a spillover of infection contracted from domestic animals and a sustainable infection in order to implement right decisions [39]. In the USA, the only known focus of *B. abortus* infection left is in bison (50% of the animals tested positive) and elk in the Greater Yellowstone Area [60]. These last

foci of infections have a very strong impact on the cattle industry in the region. Indeed, spill over from wildlife to cattle is regularly reported around these natural parks [60]. In zoonotic terms, due to the behavior of bison and elk, there is more risk of disease transmission from bison to man than from elk to man. But this pattern is changing due to the introduction of artificial elk feed grounds. Elk are more concentrated and less likely to calve in seclusion. Infected elk may also abort during the time they are congregated in the feed grounds. Under these conditions, the risk of disease spread from elk to elk and from elk to man (through skin wounds or by accidentally ingesting the bacteria after cleaning elk) is increased [60]. It is also worthy to note that to date, there is no vaccine that has proven to be safe and to provide a significative degree of protection in elk and bison, as well as in other wildlife species [39, 54, 65].

All *Brucella* species may infect different wildlife species, but *B. suis* deserves particular attention because, although there is a general host restriction pattern among the different species of *Brucella*, within the *B. suis* species, the different biovars preferentially infect different animal host species. Indeed, *B. suis* biovar 1 and 3 infect *suidae*, *B. suis* biovar 2 infects *suidae* and hares, *B. suis* biovar 4 infects reindeer and caribou and *B. suis* biovar 5 has been isolated from rodents in the former USSR.

B. suis biovars 1 or 3 infections in feral pigs are regularly reported in the southeastern states of the USA and in Queensland, Australia [60, 62, 70, 79]. In the USA, an extension of the geographical range of *B. suis* has occurred these last decades by release of *B. suis* infected feral pigs [70]. Human brucellosis is re-emerging in Queensland because of the recreational and occupational exposure to feral pigs infected with *B. suis* [62]. Moreover, the number of humans at risk is increasing because of the expansion of an export industry based on feral pig meat [38, 41]. For example, in 1994, *B. suis* biovar 1 was isolated from a butcher manipulating imported feral pig meat in Belgium

where the last *B. suis* biovar 1 infection had been reported in a pig farmer in 1983 [41]. Although few recent outbreaks of human disease caused by *B. suis* biovar 4 have been reported [34], foci of the infection persist in the Arctic regions of North America and Russia and constitute a potential hazard for the local population. Surprisingly, the isolation of *B. suis* biovar 2 in humans has only been exceptionally reported in the international literature [57, 67]. Furthermore, the characterization of *B. suis* biovar 2 is missing in some of these reports, raising questions on the accuracy of the typing method used [57, 67].

4.4.1. *B. suis* biovar 2

Recently, brucellosis in wild boar due to *B. suis* biovar 2 was described in Belgium [41] and seems to be widely distributed among wild boar throughout Europe. Frequent contact between wild boar and cattle have been reported and the risk of cattle being exposed to *B. suis* biovar 2 has been assessed under field [5] and experimental conditions [42]. *B. suis* biovar 2 does not persist in cattle (self-cure mechanism) [42]. Hares have also been suspected to be the source of contamination of a cow in Denmark, a country where there are no free-ranging wild boar populations [5]. So, there is a huge reservoir of *B. suis* biovar 2 in wildlife within the EU and wild boar (and possibly hares) have been reported to be widely and sustainably infected. A significant characteristic of *B. suis* biovar 2 is that this particular biovar is not an important pathogen for humans in contrast to *B. suis* biovars 1, 3 and 4. This is certainly one of the main reasons why no specific control measures have been taken so far to control *B. suis* biovar 2 infections in wild boar. There is a huge wild boar hunting industry in Europe. According to the very high prevalence rate of *B. suis* biovar 2 infections, recreational and occupational exposures of people to *B. suis* biovar 2 must occur frequently [41]. Albeit this important infection pressure, there is, to date, no documented

report of *B. suis* biovar 2 infection in hunters in Europe, although *B. suis* biovar 2 was recently isolated in an immunocompromized hunter in France (B. Garin-Bastuji et al., manuscript in preparation). Moreover, for the period 1993–2003, *B. suis* biovar 2 infections have been reported in more than forty out-door rearing pig farms in France [37]. Although a huge percentage of pigs were infected, farmers did not complain of any clinical symptom consistent with brucellosis and all attempts to isolate *B. suis* biovar 2 from human clinical samples failed, despite some of the farmers showing transient serological titers [37]. Our preliminary observations indicate that *B. suis* biovar 2 is attenuated in the THP-1 human macrophage cell line compared to the fully virulent *B. suis* biovar 1, although replication is similar in the J774 mouse macrophage cell line for both biovars (J. Godfroid and S. Kohler, unpublished results). Now that the genome of *B. suis* biovar 1 is sequenced [58], it would be interesting to identify the genomic sequences that are absent or differentially expressed in the *B. suis* biovar 2 genome as compared to the *B. suis* biovar 1 genome in order to identify sequences that could possibly be implicated in the pathogenicity of *Brucella* spp. in human macrophages. Once identified, these sequences could be further studied in the context of the development of new attenuated vaccine-candidates.

4.4.2. Marine mammal brucellosis

Prior to the first reports in 1994 of the isolation of *Brucella* spp. from stranded harbor seals (*Phoca vitulina*), harbor porpoises (*Phocoena phocoena*) and common dolphins (*Delphinus delphis*) on the coast of Scotland [63], and from an aborted foetus of a captive bottlenose dolphin (*Tursiops truncatus*) in California [32], brucellosis was not only unrecognized, but was not even suspected in marine mammals. The recent isolation of marine mammal *Brucella* strains extends the ecologic range of the genus and, potentially, its scope as a

zoonosis [26]. Currently, two reports indicate that marine mammal *Brucella* strains have a zoonotic potential. In England, a laboratory worker got infected and developed clinical brucellosis while handling cultures of a *Brucella* isolate from a seal [14]. In April 2003, the first report of community-acquired human infections with marine mammal-associated *Brucella* spp. was published. The authors described the identification of these strains in two patients with neurobrucellosis and intracerebral granulomas [66]. Both patients were young men from Peru and the route of infection was not discovered. One possible route of infection was the consumption of infected marine mammalian products; another was that marine mammal *Brucella* strains have been carried by other animal species. Seroconversion and abortion in cattle experimentally infected with *Brucella* spp. isolated from the Pacific harbor seal (*Phoca vitulina richardsi*) has been reported [61].

In Norway, there is a long tradition of harp seal (*Phoca groenlandica*), hooded seal (*Cystophora cristata*) and minke whale (*Balaenoptera acutorostrata*) meat consumption. Individuals from all these species have been found to be infected with *Brucella* spp. However, brucellosis has not been reported in humans at risk in Norway (e.g. whale- and seal-hunters, veterinary meat inspectors, other meat handlers and consumers) [69]. Brucellosis was recently identified in two species of baleen whales in the western North Pacific [56]. Although these animals are hunted for human consumption in Japan, there is to date no report of foodborne brucellosis due to the consumption of raw meat of these animals.

It has been proposed, based on host preference and molecular classification methods, that brucellae isolated from such diverse marine mammal species such as seals and dolphins could actually comprise at least two new species, *Brucella cetaceae* (preferentially infecting cetaceans) and *Brucella pinnipediae* (preferentially infecting pinnipeds) [23]. The respective pathogenicity for

humans of *B. cetaceae* and *B. pinnipediae* needs to be further studied and documented.

4.5. Conclusions

Brucellosis is not a sustainable disease in humans. The source of human infection always resides in domestic or wild animal reservoirs and the routes of infection are multiple: food-borne, occupational or recreational, linked to travel and even to bioterrorism. *B. melitensis* is the most important zoonotic agent, followed by *B. abortus* and *B. suis*. In regions where human brucellosis is endemic, there is a great need for high-level recognition that animal and human health are inextricably linked and that the veterinary and public health sectors share the common goal of protecting, promoting and improving the health and well being of human populations. To date, no human vaccine exists and the long duration and high cost of treatment of human brucellosis reduce the efficacy of the therapy. Therefore, the development of a human vaccine should be treated as a priority.

Due to the lack of vaccine and to the burden associated with the disease management in man, the actual challenge remains to eradicate animal brucellosis. Besides the implementation of sound proficient eradication, surveillance and vigilance programs, the changing nature of the disease due to the changing animal husbandry and farming systems also has to be taken into account.

There is a huge reservoir of *B. suis* biovar 2 in wildlife in Europe: hares and wild boars have been reported to be widely and sustainably infected and spill over to outdoor reared domestic pigs and cattle has occurred. *B. suis* biovar 2 is peculiar in many aspects. A significant characteristic is that this particular biovar is not an important pathogen for humans, in contrast to *B. suis* biovars 1, 3 and 4. In the USA, there is a *B. abortus* reservoir in bison and elk that causes a threat to bovine brucellosis eradication programs [60]. Thus, wildlife should always be carefully monitored in order to prevent the

re-emergence of brucellosis from the wild-life reservoir [39, 60].

B. melitensis has regularly been isolated from cattle in contact with infected sheep and goats in Mediterranean countries and represent a major zoonotic threat [8, 72]. This challenges the preferred animal host speciation of brucellae. Indeed, *B. abortus* abortions in sheep, goats and pigs have been seldom reported in the past and not further documented in recent decades. Although *B. suis* biovars 1 and 3 isolations from cattle have been reported in the Americas and Australia, *B. suis* biovars 1 and 3 abortions have never been documented in cattle [24, 33]. *B. suis* biovar 2 is a self limited infection in cattle [42]. Although *B. melitensis* abortions in cattle have been reported [72], the knowledge in the pathobiology of *B. melitensis* in cattle is surprisingly very scarce. In this perspective, the most important issue is whether *B. melitensis* is a sustainable infection in cattle, i.e. able to maintain itself in cattle without spill over from a reservoir in sheep and goats.

In the context of bioterrorism, it is of crucial importance to be able to discriminate quickly between true brucellosis and other diseases appearing in the differential diagnosis of brucellosis, among which are infections due to cross-reactive bacteria in brucellosis serology, like YO9 [43]. It is worthy to note that it is only since the early nineteen-nineties that YO9 has been regularly isolated in cattle in Europe [42], but also in other parts of the world including New Zealand [44]. Although YO9 infections do not seem to be associated with clinical signs in cattle, they induce what has been called "False Positive Serological Reactions" (FPSR) in brucellosis tests. Up to now, no single test is able to discriminate beyond any doubt a YO9 infection from brucellosis in cattle [42]. This causes a tremendous burden to trade of living animals and eradication programs. A very crucial question is to understand how and why YO9 was able to establish itself as an infective agent in cattle in the early nineteen-nineties. If the infec-

tion becomes widely distributed and sustainable among cattle, we may face the same problems as observed in pigs. Essentially all brucellosis serological tests have so many limitations that the OIE questions the validity of brucellosis serological testing in this animal species [8].

Experimentally, a marine mammal *Brucella* spp. isolate from a Pacific harbor seal induced seroconversion and abortion in cattle [61]. Another important aspect of marine mammals brucellosis is the zoonotic potential of marine mammal brucellae [14, 66]. The clinical symptoms recorded during the accidental human laboratory infection were rather non-specific and the cause of such symptoms may not be easy to verify unless *Brucella* spp. infection is suspected [14]. Recently, marine mammal brucellae were identified in two patients from Peru with neurobrucellosis and intracerebral granulomas, which were the first reported cases on community-acquired human infections with marine mammal brucellae [66]. These last reported cases might raise new awareness and interest for such infections in humans.

New *Brucella* strains or species may emerge and existing *Brucella* spp. adapt to changing social, cultural, travel and agricultural environments. Hence, the global animal and human brucellosis picture will, in essence, always remain incomplete and regular updates are required.

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