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Brucellosis: Evolution and expected comeback

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ABSTRACT

Brucellosis is a serious infectious disease which causes great direct and indirect economic loses for animal holders worldwide such as the reduction of milk and meat production through abortions/culling of positive reactors, the expense of disease control/eradication and farmers compensation. Although the disease was eradicated from most of the industrial countries, it remains one of the most common zoonotic diseases in developing countries being responsible for more than 500,000 new cases yearly. Brucella is considered to be a bioterrorism organism due to its low infectious doses (10–100 bacteria), capability of persistence in the environment, rapid transmission via different routes including aerosols, and finally due to its difficult treatment by antibiotics.There are many reasons to believe that a new comeback of brucellosis may occur in near future. This expectation is supported by the recent discovery of new atypical Brucella species with new genetic properties and the recent reports of (man to man) disease transmission as will be discussed later. The development of new concepts and measurements for disease control is urgently required. In the present review, the evolution of Brucella and the different factors favoring its comeback are discussed.

1. Introduction

Brucellosis is a serious infectious disease affecting different mammalian species including man. Natural infection of farm animals occurs mainly through ingestion of food or water contaminated by uterine discharges, aborted feti or fetal membranes and even through licking the genitalia of diseased animals. In addition, infected males can also spread the infection among females through natural mating and artificial insemination. Brucellae can pass through intact or injured skin and through all mucous membranes [1].

Direct and indirect contact with diseased animals or foodstuffs of animal origin represents the major source of infection to humans. It was thought that the infected human are the dead end of the infection, however, human to human transmission was recorded recently [2]. Ice cream and homemade cheese play an important role in the spread of the disease among human as they are prepared in a way which does not eliminate viable Brucella bacilli [3].

Investigation of burned cheese rests found in the old Roman city (Herculaneum) which was suddenly destroyed in August 79 AD by the volcanic eruption (Vesuvius) revealed the presence of bacterial colonies morphologically resemble Brucella, which may be the first sign of brucellosis in the old ages [4]. In 1884, Dr. Bruce was able to differentiate between brucellosis (Malta fever) and typhoid outbreaks affected Malta. Three years later, he isolated the causative agent of Malta fever and named the bacterium *Micrococcus melitensis*. In 1897, Dr. Bang studied the disease in Denmark and could isolate *Brucella abortus* strains from aborted cattle. He noticed that the pathogen can also infect sheep, goat and horses, the disease became known as (Bang's disease). Later on, in 1918, Evans could detect the connection between animal and human cases after he isolated an organism from human aborted foetus which was closely related to Bruces's organism. In the year 1938, it was possible to differentiate among the caprine, bovine and swine forms of Undulant fever caused by *B. melitensis*, *B. abortus* and *B. suis,* respectively. Since 1884 till now, brucellosis represents a continuous re-emerging zoonoses worldwide [4–6].

Brucella is a Gram-negative, non-motile coccobacilli. It belongs to alpha-Proteobacteria, which include in addition to Brucella other members such as Agrobacterium, Rickettsia, Rhodobacterium, and Rhizobium. However, recently atypical motile Brucella isolates were isolated from diseased frogs [7].

Brucella was considered to be a facultative intracellular pathogen in most references; however, they were re-designated as facultative extracellular intracellular pathogens due to their evolutionary relationship to other alpha-Proteobacteria. Brucellae are stealth microbes which prefer induction of chronic rather than acute infections [8].

Due to the high genomic homology among the typical Brucella species, it was supposed in the 1980 s that Brucella is a monospecific genus (*Brucella melitensis*) which has 6 biovars distinguished according

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to their host prevalence, the different Brucella species were renamed e.g. *Brucella abortus* was called *Brucella melitensis* biovar *abortus*. However, this classification did not survive the new data delivered by molecular biological genotyping tools [9,10].

Through the modern molecular tools it was possible to prove that *B. melitensis, B. abortus, B. ovis* and *B. neotomae* represent 4 related clones of one organism while *B. suis* (including *B. suis* biovar 5) forms a distinct cluster from them but closely related to the marine mammals Brucella species isolated from dolphin, seal and porpoise. Meanwhile, *B. suis* biovars 3 and 4 seem to be evolved from *B. suis* biovar 1 and *B. canis*. These relationships were confirmed by the data delivered by whole genome sequencing [9,10].

However, after the discovery of the new Brucella species, the old debate arose again. Positioning of the recently detected atypical Brucella species (specially *B. microti* and *B. inopinata*) was problematic due to their clear distinction from the classical ones on phenotypic and genetic levels. Both *B. microti* and *B. inopinata* are fast growers and highly active metabolically. They have a unique *16S rRNA* gene with 5 different nucleotide sequences when blasted with the highly conserved corresponding gene of the other Brucella species. The genetic diversity among the different species of Brucella is clearer than the diversity between the closely related genera Brucella and Ochrobactrum. Trials to group both Brucella and Ochobactrum spp together were carried out through the fusion of current Brucella species in one species with subspecies and biovars (e.g. *B. melitensis* subsp. *abortus* biovar 1) and in the same time to translocate all species of Ochrobactrum into the genus Brucella. However, these trials failed as the Brucella spp. are obligatory pathogens while the Ochobactrum spp. are opportunistic pathogens. This close phenotypic relationship is best seen when blasting the genomes of both *B. microti* and Ochrobactrum. This closeness lead to the false identification of *B. microti* in the past as a new member in genus Ochrobactrum [9–11].

At the time, at least 12 Brucella species are known (Table 1). Due to its great economic and zoonotic importance, it is important to identify field isolates of Brucella not only at their species level but also their genotypes. This enables the detection of hidden foci of Brucella and to tract the sources of infection in the population. As an example, genotypic analysis of different *B. abortus* field strains isolated from cattle, bison and elk showed that the cattle isolates are closely related to elk isolates but completely divergent from those of bison [12]. Genotyping of the field isolates enables also the differentiation between infected animals/veterinarians due to accidental exposure to vaccinal strains (*B. abortus* S19 and RB51) from those infected with field strains although the *B. abortus* genome is highly conserved among various *B. abortus* biovars including S19 *B. abortus* smooth vaccinal strain which is closely related to strain 2308 [10,13]. Proper genotyping differentiates among vaccinal strains from other field genotypes using specific primers targeting the *ery* locus (for S19) or the *wbo*A gene (for RB51) [14].

Similarly, genotypic investigation of the field isolates in Germany enabled the detection of the source of human infections there. It was long believed that the human infections in Germany are related to tourisms in the Middle East countries, however, the genotypes of *B. melitensis* isolated from German patients were more related to the clades present in Southeast Europe, Turkey, Afghanistan, Turkmenistan, Far East and Southeast Asia with a clear genetic diversity from those originating from Middle East [15]. Genotyping of animal field isolates is also important for public health issues. As an example, *B. suis*, the etiological agent of swine brucellosis, consists of 5 biovars [1–5], while biovar 2 is rarely zoonotic, biovars 1 and 3 are extremely pathogenic to humans [16]. The close relationship between *B. canis* and *B. suis* enabled *B. suis* to reemerge recently among dogs causing sever reproductive problems in dogs and health hazards to humans in contact with diseased dogs. Even cattle, horses, sheep and deer in contact can catch the infection with *B. suis* also [17,18].

2. Evolution of brucellosis

Blasting the genomes of *B. melitensis, B. abortus, B. suis, B. neotomae* and *B. canis* against that of *B. ovis* reveals an overall DNA homologies of 95% indicating that they all were diverged from a common ancestor very close to the *B. ovis* 86,000–296,000 years ago[10]. This occurred as a result of the infection of wild mammals with the *B. ovis* ancestor

Table 1
List of different Brucella species and their natural hosts.*

Brucella species	Colony type	Natural host**	Zoonoses	Year of first isolation
B. melitensis (bv1-3)	Smooth	Goat and sheep	+ + +	Bruce (1893)
B. abortus (bv 1–6, 7, 9)	Smooth	Cattle	+ +	Schmidt (1901)
B. suis biovar***				Huddleson (1929)
1–3	Smooth	Pig	+ +	
2	Smooth	Wild boar, Hare	+	
4	Smooth	Reindeer, Caribou	+ +	
5	Smooth	Rodent	–	
B. ovis	Rough	Sheep	–***	Buddle (1956)
B. neotomae	Smooth	Desert rat	+	Stoenner and Lackman (1957)
B. canis	Rough	Dog	+	Carmichael and Bruner (1968)
B. ceti (B. delphini)	Smooth	Dolphins	+	Foster et al. (2007)
B. pinnipedialis (B. phocae)	Smooth	Seals	+	Foster et al. (2007)
B. microti	Smooth	Wild voles	(?)	Scholz et al. (2008)
B. inopinata	Smooth	Human	+ +	Scholz et al. (2009)
B. papionis	(?)	Baboons (*Papio* spp.)	(?)	Whatmore et al. (2014)
B. vulpis	(?)	Red foxes (*Vulpes vulpes*)	(?)	Scholz et al. (2016)
N.N.****	Smooth	Frog	(?)	Soler-Lloréns et al. (2016)

* Different Brucella species and their natural hosts according to [4,5,7,39,41–46].

** The host susceptibility range of Brucella species is not extremely narrow. Nearly all Brucella species can infect other mammals beside their primary host with the exception of *B. ovis*. In such cases, the infection is mostly mild and even self-limiting.

*** Different *B. suis* biovars vary in their zoonotic potential, while biovars 1, 3 and 4 are more pathogenic to human than *B. abortus* but less than *B. melitensis*, other *B. suis* biovars have obviously limited potential to infect humans. The reason why the *B. ovis* is not zoonotic in opposite to the rest of Brucella species is attributed to the fact that the genome of *B. ovis* contains a high percentage of pseudogenes and other mobile genetic elements compared to the rest Brucella species due to genome degradation in parallel with narrowing of the host susceptibility scope of *B. ovis*. This genomic degradation and re-arrangement lead to the deletion of the genomic island 2, which is responsible for lipopolysaccharide biosynthesis in addition to the inactivation of essential genes regulating nutrient uptake and utilization. All of these factors, beside the inactivation of genes responsible for the synthesis of the envelop outer membrane proteins, lead to the loss of the ability of *B. ovis* to invade humans and many other mammalian species [25].

**** An intermediate trait between the soil associated ancestor of Brucella species and the known host adapted Brucella species. No data are yet available about its zoonotic capability.