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Review Article

Laboratory Animal Models for Brucellosis Research

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Brucellosis is a chronic infectious disease caused by *Brucella* spp., a Gram-negative facultative intracellular pathogen that affects humans and animals, leading to significant impact on public health and animal industry. Human brucellosis is considered the most prevalent bacterial zoonosis in the world and is characterized by fever, weight loss, depression, hepato/splenomegaly, osteoarticular, and genital infections. Relevant aspects of *Brucella* pathogenesis have been intensively investigated in culture cells and animal models. The mouse is the animal model more commonly used to study chronic infection caused by *Brucella*. This model is most frequently used to investigate specific pathogenic factors of *Brucella* spp., to characterize the host immune response, and to evaluate therapeutics and vaccines. Other animal species have been used as models for brucellosis including rats, guinea pigs, and monkeys. This paper discusses the murine and other laboratory animal models for human and animal brucellosis.

1. Introduction

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella* that affects humans as well as domestic and wild animals, leading to significant impact on public health and animal industry. *Brucella* spp. is a Gram-negative, facultative intracellular bacterium that is able to survive and replicate in phagocytic and nonphagocytic cells, establishing a chronic infection in both humans and animals [1]. Human brucellosis is considered the most prevalent bacterial zoonosis in the world, with more than 500,000 new reported cases in humans each year, mainly in Mediterranean countries, Central Asia, Arabic Peninsula, India, and Latin America [2]. The disease is characterized by nonspecific symptoms, including undulant fever, weight loss, depression, hepatomegaly, and splenomegaly. Arthritis, spondylitis, osteomyelitis, epididymitis, and orchitis, as well as other more severe complications as neurobrucellosis, liver abscesses, and endocarditis, are also commonly described in patients [1, 2].

There are currently 8 recognized species of *Brucella*, of which six are known to be capable of infecting humans. *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis* are considered important zoonotic agents, and each one has a domestic animal as preferential host: small ruminants, bovines, swine, and dogs, respectively. Humans also can be infected by two *Brucella* species recently isolated from marine mammals, *B. ceti* and *B. pinnipedialis,* and by *B. inopinata,* the new species isolated in breast implant and lung biopsy from human [3–5]. In domestic animals, *Brucella* colonize the reticuloendothelial system and genital organs causing chronic infection and reproductive disease characterized by abortion, stillbirth, orchitis, epididymitis, and infertility, resulting in significant economic losses [3, 6].

Relevant aspects of *Brucella* pathogenesis have been intensively investigated in both cellular and animal models. The mouse is the animal model most extensively used to study chronic infection caused by *Brucella* spp. Moreover, a few other animal species have been used as models for brucellosis. This paper discusses well-characterized murine models of brucellosis as well as other laboratory animal models that have been used to study infection and disease caused by *Brucella* spp.

2. Murine Models for Human and Animal Brucellosis

The mouse is often used as an animal model to investigate the pathogenesis of human and animal brucellosis [7–9]. In addition, the murine models are widely employed to test antimicrobial drugs for treating the disease in humans [10–12]. Availability of new molecular tools allowed the use of murine models for identification of specific pathogenic factors of *Brucella* spp. and the characterization of host immune response. As a result, control methods are being improved and new vaccine candidates are being developed [8, 9, 13].

2.1. Mouse Strain-Specific Differences in Brucella Infection. Several early studies using the mouse model demonstrated that all of the mouse strains tested could be infected by *B. abortus*, suggesting a lack of genetic loci in mice that determine complete resistance to *B. abortus* infection. A comparison of susceptibility of different strains of mice to *B. abortus* strain 19 demonstrated susceptibility of CBA/H, BALB/c, or C57BL/10 to *B. abortus* infection [14]. A subsequent study comparing *B. abortus* infection in CD-1, BALB/cByJ, CBA/NJ (containing the X-linked immunodeficiency trait), C3H/HeJ (deficient in TLR4), and C3H/HeN mice found similar colonization levels between all mouse strains, with a trend for higher colonization of BALB/cByJ mice over a 12-week time course [15]. A higher level of *B. abortus* colonization in the BALB/cByJ strain was demonstrated definitively by comparison with C57BL/10 mice, a mouse strain that is closely related with the commonly used C57BL/6 strain [16, 17]. More detailed studies have demonstrated that an increased Th1 polarization of the immune response in the C57BL strains is responsible for their more resistant phenotype (see below for a more detailed discussion). However, it should be kept in mind that most of these comparative studies were performed using *B. abortus*, so that it is possible that the susceptibility toward infection or the infection kinetics may differ for other *Brucella* species.

Several mouse strains have been used to characterize suitable murine models to study *Brucella* sp. infection. Four- to 9-week-old BALB/c female mice are often used to evaluate systemic distribution of *Brucella* sp. during the course of infection [7, 17, 18]. Additionally, this animal model has been used to study gene expression during *Brucella* sp. infection, leading to the identification of several host genes associated with innate and adaptive immune responses that are activated during the course of infection [8, 19, 20]. Previous studies have shown that a high production of interferon-gamma (IFN-γ) and interleukin 12 (IL-12) may lead to efficient control of *Brucella* spp. infection in the mouse model, due to activation of macrophages and induction of natural killer cells and Th1 cellular response [8, 21, 22]. High serum levels of both IFN-γ and IL-12 are also described in humans during *Brucella* sp. infection, which is associated with the induction of a Th1 response at early stages of infection [23–25]. Additionally, natural killer (NK) cells play an important role in controlling intracellular bacterial infections, due to their ability to kill infected cells and secrete IFN-γ. NK cells have a deficient cytotoxic activity in patients with acute brucellosis, although these cells show normal activity in treated patients [26]. However, it seems that NK cells are not required to control *B. abortus* early infection in the mouse, since mice with nonfunctional NK cells have similar bacterial load when compared to immunocompetent mice [27]. Moreover, previous studies have shown that CD8+ T cells may also play a role against *Brucella* sp. persistent infection [28–30]. In BALB/c mice, i*n vivo* depletion of CD8+ T cells leads to increased bacterial load in the spleen [30]. In humans, peripheral blood CD8+ T cells that are stimulated with heat-killed *B. abortus* or lipopolysaccharide produce IFN-γ, which elicit a Th1 immune response [29].

C57BL/6 and C57BL/10 mice strains have also been used to evaluate *Brucella* sp. infection, since they are considered more resistant to *Brucella* sp. infection than BALB/c mice [16, 17, 22]. Comparative studies among these mice strains helped detecting specific mechanisms of C57BL mice immune response that are defective in BALB/c mice. These mechanisms are likely important for controlling *Brucella* sp. infection [8, 17, 22]. Additionally, various knockout mice were developed by using C57BL/6 or 129/Sv as background mice. The results obtained in this model seem to have high similarity to host-pathogen interaction mechanisms that were previously described in humans and domestic animals [8, 31, 32]. Moreover, knockout mice with defective production of cytokines related to innate immune response illustrate the crucial role of specific cytokines against *Brucella* sp. infection in hosts [22, 33, 34]. Interestingly, interferon regulatory factor-1-deficient (IRF-$1^{-/-}$) mice infected with *B. abortus* developed an acute hepatitis similar to humans but, unlike the natural hosts, IRF-$1^{-/-}$ mice are unable to control the infection and die within a short period of time. While uncontrolled infection and death are not typical endpoints of *Brucella* infection, the IRF-$1^{-/-}$ knockout mouse has been useful for identifying and comparing residual virulence of highly attenuated *Brucella* vaccine candidates [33].

2.2. Routes of Infection. *Brucella* infection may occur by digestive route, inhalation or through nasal mucosa or conjunctiva [6, 9]. After crossing the mucosal barrier, the organisms reach regional lymph nodes, replicate in macrophages, and establish a systemic and persistent infection. A bacteremic phase of infection results in colonization of the spleen, liver, and osteoarticular tissues, and depending on the *Brucella* species and host, it may also colonize the mammary gland and the reproductive system [6, 9, 35]. In murine models of *Brucella* sp. infection, experimental inoculation is performed mostly through three routes: intraperitoneal, digestive, or nasal (aerosol).

The intraperitoneal route of infection is frequently used to establish a persistent infection in the mouse, as it results in a rapid systemic distribution of *Brucella* sp. and high bacterial loads in the spleen and liver [7, 8, 18]. Initially, *Brucella* multiplies during the first week, progressing to a slow decrease in bacterial numbers at systemic sites of infection. During the first 5 to 6 weeks after inoculation, C57BL/6 or BALB/c mice infected with 10^6 CFU of *B. abortus* strain 19 remain with stable numbers of organisms at systemic sites of infection, and bacteria can be isolated

up to two months after infection. However, *B. abortus* strain 2308 infection in BALB/c mice may persist over 6 months [7, 36]. Murine models of intraperitoneal infection with *Brucella* sp. allow the identification of pathogenic factors that are required for establishment of chronic infection [37, 38]. For instance, comparison between input and output loads of wild-type and mutant strains of *Brucella* in mouse models resulted in the identification of pathogenic factors, including the role of the type IV secretion system encoded by the *virB* operon during *Brucella* sp. persistent infection *in vivo* [31, 32, 38, 39]. *virB* mutant strains of *Brucella* sp. are not capable of surviving and replicating intracellularly in macrophages and, therefore, are attenuated in mouse models *in vivo* [37–39].

The digestive tract is the main route of *Brucella* infection in humans, which is associated with the ingestion of unpasteurized milk and dairy products from infected animals [2, 40]. Murine models of intestinal infection allow the identification of bacterial pathogenic factors that are required to establish infection through the digestive tract [41–44]. Recently, Paixão and colleagues described a murine model for intestinal infection of *B. melitensis*, in which a high intragastric dose ($\sim 10^{10}$ CFU per animal) leads to a systemic infection in BALB/cByJ mice, probably due to bacterial translocation through the intestinal mucosa via M cells [44]. Interestingly, this high infectious dose did not result in intestinal inflammation in the mouse. Previous studies have shown that mice can control intestinal *Brucella* infection when they are previously vaccinated through the same route [13, 45, 46]. Pasquali and colleagues demonstrated that BALB/c mice previously treated with sodium bicarbonate to neutralize gastric acid are more susceptible to *B. abortus* systemic infection by digestive route than untreated mice [46]. This result suggests that gastric acidity may interfere with *Brucella* sp. However, a previous work has shown that *Brucella* sp. challenge through the digestive tract is an inadequate method to produce a uniform and consistent infection in mice [47]. Additionally, it is important to consider experimental issues in murine models, including artificial inoculation using intragastric gavages and gastric acid neutralization, which may significantly differ from *Brucella* sp. natural infection in humans and animals. Bacterial factors mediating intestinal infection by *Brucella* sp. and their target molecules at mucosal surfaces of the digestive tract are still poorly understood; so additional studies evaluating carefully this route are required.

Human brucellosis may also be acquired by inhalation. The number of organisms required to establish the infection in humans by this route is low, with an estimated infectious dose of 10 to 100 organisms for humans by aerosol [48]. Therefore, *Brucella* sp. is considered a potential biological warfare agent [49]. Characterization of murine models for *Brucella* sp. infection by the nasal route (aerosol) may be used to evaluate vaccines candidates and therapeutics for human brucellosis [19, 50, 51]. A recent study demonstrated that BALB/c female mice immunized with *B. melitensis* attenuated strain Rev1 followed by aerosol infection with 10^4 CFU of *B. melitensis* 16M had a decreased bacterial load in the spleen, suggesting that this animal is a suitable model to evaluate protection during *Brucella* sp. aerosol infection [51]. Mice infection by aerosol with 10^6 CFU of *B. melitensis* or 10^7 CFU of *B. abortus* resulted in high bacterial load in the spleen, liver, and lungs. However, infection doses as low as 10^2 and 10^3 CFU per animal are also sufficient to establish a systemic infection in the mouse [19, 52, 53]. Apparently the lung is only affected in the mouse during aerosol infection with pathogenic species of *Brucella*. No histopathological lesions have been described in the lung, but high bacterial loads are recovered from the lungs at early time points during infection, which indicates that *Brucella* sp. is able to replicate in this organ without eliciting innate immune responses [19, 52]. Although aerosol chambers have been effectively used to study bacterial infections in mouse models [19], it is important to consider that the infection dose that reaches the lung of a mouse may be significantly lower than expected [53]. Additionally, *Brucella* sp. infection in these models may be established due to coinfection through the conjunctiva or oral mucosa, since it was previously shown that bacteria can be detected also in the fur of infected mice [19]. Therefore, it is essential to critically evaluate the results of *Brucella* sp. aerosol infection during vaccine studies in murine models. An open question in the field is the identity of the *Brucella* factors that are important for its efficient infection of the respiratory tract.

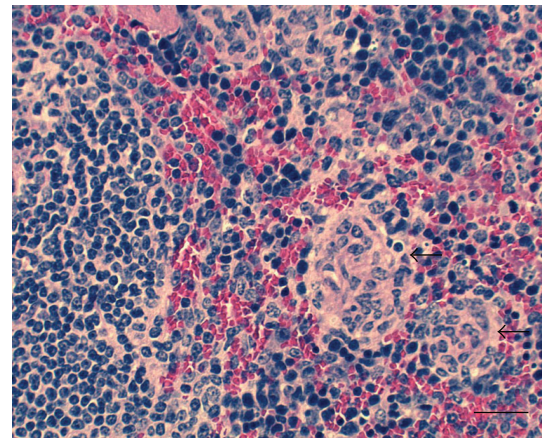

Figure 1: Spleen of BALB/c mouse at 21 days of infection by *Brucella melitensis*. The mouse was intragastrically infected with 10^5 CFU of *B. melitensis* 16 M. Microgranulomas in red pulp (arrows). HE. Bar: 100 μm.

2.3. Histopathological Changes during Brucella Infection. During *Brucella* sp. infection in the mouse, the spleen is the most heavily colonized organ, and it develops histiocytic infiltrates and multifocal microgranulomas (Figure 1) [7, 18, 54]. BALB/c mice intraperitoneally (i.p.) infected with *B. abortus* or *B. melitensis* develop significant splenomegaly, which is more prominent than in mice infected by aerosol (Figure 2) [19, 54]. The liver is also an important site for colonization and replication of *Brucella* sp. in the mouse [7, 13, 19]. Usually, mice infected with virulent strains of *Brucella* sp. have mild to moderate hepatitis, which is characterized by neutrophilic infiltrate at early

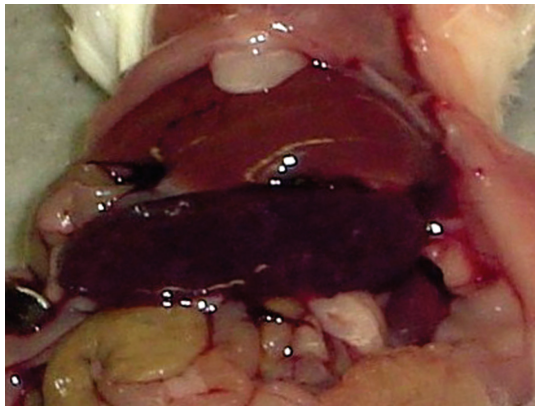

Figure 2: BALB/c mouse i.p. infected with 10^6 CFU of *Brucella ovis* ATCC25840 with severe splenomegaly at 30 days of infection.

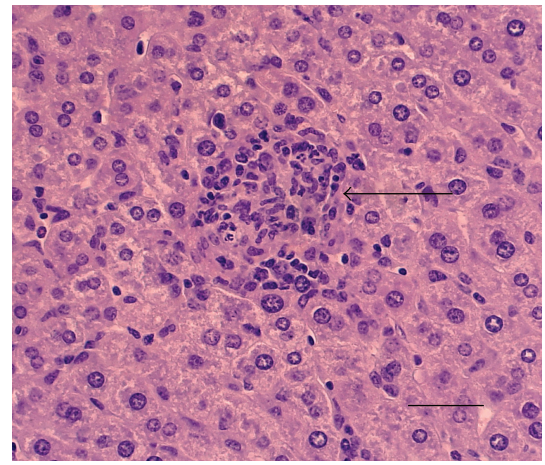

Figure 3: Liver of BALB/c mouse at 30 days of infection by *Brucella ovis*. The mouse was i.p. infected with 10^6 CFU of *Brucella ovis* ATCC25840. Microgranuloma containing predominantly macrophages and neutrophils (arrow). HE. Bar: 100 μm.

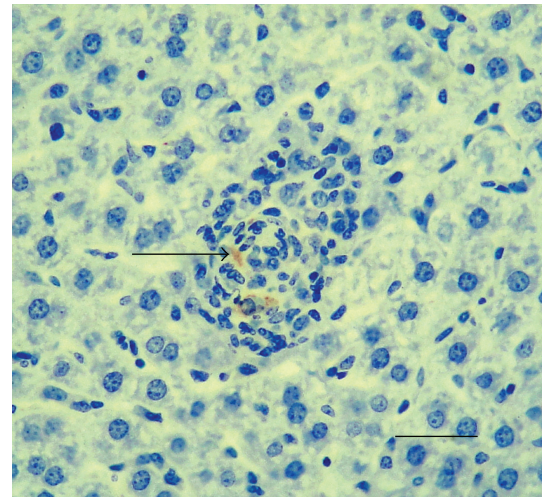

Figure 4: Liver of BALB/c mouse at 30 days of infection by *Brucella ovis*. The mouse was i.p. infected with 10^6 CFU of *Brucella ovis* ATCC25840. Microgranuloma with immunolabelled *B. ovis* in macrophages (arrow). IHC. Bar: 100 μm.

stages of infection, followed by histiocytic infiltrate with epithelioid cells and microgranulomas at chronic stages of infection (Figure 3) with bacteria localizing intracellularly in macrophages within microgranulomatous lesions (Figure 4) [7, 54]. It is noteworthy that *Brucella* infection in mice results in lesions that mimic those described in chronic infections in humans. Patients with chronic brucellosis may develop splenomegaly and hepatomegaly. Additionally, multifocal granulomas with epithelioid macrophages are observed in the parenchyma of the liver and spleen in biopsy samples from infected patients [55, 56]. However, hepatic and splenic abscess were described as uncommon complication in some patients during the acute phase of *Brucella* sp. infection [57]. *Brucella* sp. chronic infection in humans may also lead to osteoarticular disease, including osteoarthritis, spondliytis, and osteomyelitis [1, 2]. A previous study [58] reported that mice may develop bacterial colonization in osteoarticular tissues during chronic stages of *B. melitensis* infection. In IRF-$1^{-/-}$ mice that survived more than 45 days after i.p. infection with 10^7 CFU of *B. melitensis*, a high number ($\sim 10^5$ CFU) of bioluminescent *B. melitensis* were detected in vertebral joints in the tail, suggesting that these mice might be a useful model for the study of human osteoarticular disease. However, a comparison of actual osteoarticular lesions in mice and humans would help to assess the potential utility of this model to study a common clinical presentation of brucellosis in man.

2.4. Evaluation of Therapeutic Interventions and Vaccines. The efficiency of different chemotherapies for human brucellosis has also been evaluated in the mouse model [10, 11, 59]. The recommended treatment for human brucellosis is a combination of rifampicin and doxycycline daily for at least six weeks [60]. However, other antibiotic combinations have been tested in animal models and infected patients. Previous studies showed that mice infected with *B. melitensis* and treated with ciprofloxacin, by subcutaneous (40 mg/kg), digestive (200 mg/kg), or intraperitoneal (20 mg/kg) route, are not able to control the infection [10, 12], whereas mice treated with doxycycline (40 mg/kg) at 24 hours after infection efficiently clear the infection [12]. Additionally, Shasha and coworkers [10] reported that mice treated with rifampin (25 mg/kg) or doxycycline (40 mg/kg) by intraperitoneal route had high levels of antibiotics in the blood (rifampin: 18 μg/ml; doxycycline: 5.4 μg/ml) and were able to clear the infection. Moreover, new antibiotic carriers, like microspheres, have been tested against *Brucella* sp. infection. Microspheres are phagocytized by monocytes, allowing direct access of the antibiotic to the intracellular site of bacterial replication. However, a previous study showed that mice infected with *B. abortus* and treated with gentamicin microspheres (100 μg/animal) for three days were not able to reduce bacterial load in the spleen after 1 and 3 weeks after treatment [11].

Additionally, the quality of live vaccines that are commercially used for preventing animal brucellosis is evaluated in murine models [61]. Live *B. abortus* S19 strain, which is the most widely used vaccine in cattle, has been tested in female CD1 mice 5 to 7 weeks old. Mice are previously treated with 10^5 CFU of *B. abortus* reference vaccine (strain S19), a commercial vaccine sample or PBS. After 30 days of vaccination, all mice are i.p. infected with 10^5 CFU of *B. abortus* virulent strain. Then, bacterial loads in the spleen are evaluated in each group at 15 days after infection. A commercial vaccine is considered efficient when mice have significantly lower bacterial load than the unvaccinated control group and when the vaccinated group has similar immunogenicity value to mice group vaccinated with S19 reference strain [61].

2.5. Pathogenesis of the Reproductive Tract. Furthermore, murine models were developed to study reproductive changes described in human and animal brucellosis. Previous studies evaluated the occurrence of abortion and placental colonization in female pregnant mice during *B. abortus* infection [3, 35]. Although *B. abortus* infection is not characterized by abortion in women [2], it is extremely relevant to study *Brucella* pathogenesis in pregnant female models, due to its significant economic impact in cattle production as well as in other domestic animal species. Moreover, uterine secretion and products from abortion are the most important source of infection within a herd maintaining the disease and may also represent an occupational source of infection to humans [6, 35].

Previous studies from Bosseray characterized the infection in pregnant CD1 mice with *B. abortus* strain 544. The infection did not lead to abortion or fetus death at early stages of pregnancy, although high colonization of placenta was described when mice were infected at 7 and 11 days of pregnancy. Additionally, placental and splenic colonization increased with higher challenge accordance to the infection dose and each placenta was considered an independent unit, as some placentas were colonized and others were not in the same uterus [62]. Another study demonstrated the congenital infection of *B. abortus* in the mouse at 7 days of pregnancy, which resulted in the colonization of 60% of newborns. In this study, newborns remained infected until 30 days and no significant difference of *Brucella* sp. infection was observed between male and female newborns [63]. Moreover, Bosseray described the kinetics of placental colonization in mice that were intravenously infected with *B. abortus* at 15 days of pregnancy. Although low bacterial loads were recovered from the placenta at early stages of infection (4–6 hours), apparently local bacterial replication resulted in higher colonization in the placenta at 72 hours after infection [64].

BALB/c female pregnant mice infected with 10^6 CFU of *B. abortus* virulent strain 2308 develop a moderate multifocal necrotic placentitis associated with severe neutrophilic infiltrate and intralesional bacteria in trophoblastic cells [54]. The bacterial load and lesions described in the placenta increase throughout the pregnancy, whereas the bacterial load recovered from the spleen was stable during the course of infection in the mouse [54, 65]. The lesions described in female pregnant mice were similar to those observed in cows, which suggests that this model may be useful to study *Brucella*-induced placental disease, although mice and cattle have different morphological types of placenta [54]. Additionally, Kim and colleagues [65] demonstrated that *B. abortus* infection may lead to 98% of abortion in female mice at 4.5 days of pregnancy. However, intraperitoneal inoculation of the pathogen at any other time point during the pregnancy does not result in a high abortion rate although placentas from both aborted and live fetuses have intracellular *Brucella* sp. in trophoblast giant cells. In natural hosts, *B. abortus* infection leads to abortion in cows at late stages of pregnancy due to placental lesions, which are related to bacterial invasion and intracellular replication in trophoblastic cells [66, 67].

Considering that male genital tract may also be affected during *Brucella* sp. infection, male mice were characterized to study specific bacterial mechanisms that lead to orchitis and epididymitis in men and animals [3, 6]. Previous studies reported that *Brucella* sp. may colonize the male genital tract in the mouse [13, 58]. Izadjoo and colleagues demonstrated that *B. melitensis* infection (10^{10} CFU) through the digestive tract in sexually mature BALB/c male mice leads to perivascular inflammation of the testes and histiocytosis in inguinal lymph nodes [13]. In addition, use of male mice may be important for testing residual pathogenicity of candidates for vaccine strains, by evaluating histopathologic lesions in the genital tract and the immune response against *Brucella* sp. [13]. Recently, our laboratory developed a male mouse model for *Brucella ovis* infection (Silva et al., unpublished data). Although *B. ovis* is one of the few classical *Brucella* species that do not have zoonotic potential, this organism is considered a major cause of reproductive failure in sheep, which leads to significant economic losses in the sheep industry [68]. The characterization of a murine model has allowed the study of pathogenic mechanisms used by *B. ovis* that may determine the bacterial genital tropism in sexually mature rams, causing epididymitis and orchitis exclusively in this animal. Interestingly, *B. ovis* infection in male mice resulted in early colonization of testes, epididymides, and seminal vesicle. However, colonization of these organs quickly decreased at later time points, and the inflammatory lesions were restricted to peripheral tissues of the genital tract. Therefore, male mice were not considered a good model for *B. ovis* genital disease in rams, although it may be used as a suitable infection model (Silva et al., unpublished data).

3. Other Laboratory Animal Models for Brucellosis

Although the mouse is by far the most often used animal model for brucellosis, it is a good animal model for chronic infection of the reticulo-endothelial system but fails to replicate some features of the clinical disease caused by *Brucella* in humans, such as fever. Therefore, there are several reports of experimental work employing other laboratory animals, including rats, guinea pigs, and monkeys that are susceptible to experimental infection with *Brucella* spp.

3.1. Rodent Models Other Than Mice. The rat has been used as a model for human brucellosis due to some peculiarities of this species. Despite the fact that rats do not develop physical signs of infection and are considered more resistant to infection than mice, they develop persistent bacteremia and do not have spontaneous cure after one month of infection [69, 70]. Therefore, rats have been selected as an experimental model for evaluation of increased susceptibility to infection (including *Brucella* infection) in patients with chronic disorders. Wistar Albino rats with diabetes were used to evaluate the course of infection by *B. melitensis*. In this case, diabetes is induced by streptozotocin before challenge with *B. melitensis.* Diabetic rats have higher numbers of bacteria in the liver and spleen when compared to control rats [69]. Other studies investigated the effect of chronic ethanol consumption on the course of *B. melitensis* infection in a rat model [71–73]. Rats chronically treated with ethanol have an increased susceptibility to *B. melitensis* due to a decrease in protective cellular immunity [72]. The rat model has also been used to study the efficacy of various antibiotics for treating *Brucella* infection [74–76]. Sprague Dawley rats were used to evaluate the efficacy of spiramycin, a macrolide antibiotic, for treating brucellosis, since this drug has no teratogenic effect, and therefore it is safe for pregnant women [74]. Similarly to mice, rats are also used to study clinical and pathological effects of *B. abortus* infection during pregnancy. *B. abortus* did not affect pregnancy in Sprague Dawley rats, paralleling what happens in women, although necrosis in the periplacentomal chorionic epithelium and metritis were observed in this model [77]. Although rats do not abort even with placentitis, a previous study demonstrated significant protection against systemic *B. abortus* infection in rats vaccinated with RB51, a vaccine strain against bovine brucellosis [78].

Guinea pigs are probably the most susceptible laboratory animal species to *Brucella* infection. Early comparative studies of susceptibility in guinea pigs, mice, rats, and sheep demonstrated that guinea pigs developed granulomatous lesions when inoculated with 10 CFU of *B. melitensis* or *B. suis* [79]. Lesions were consistently observed in the liver, spleen, lungs, and lymph nodes, resembling those described in humans [80]. Guinea pigs inoculated subcutaneously with infectious doses of *B. abortus*, *B. suis*, or *B. melitensis* develop a persistent bacteremia for 6 weeks after infection, whereas the attenuated *B. abortus* S19 is cleared from the blood at one week after infection [81]. Therefore, the guinea pig model may be considered valuable for the evaluation of candidate vaccine strains [82, 83]. All classic *Brucella* species were pathogenic for guinea pigs [70]. Furthermore, the guinea pig has been employed as an animal model for evaluating the efficacy of antibiotics and chemotherapeutic agents for treatment of brucellosis [84, 85].

Although the rabbit is a laboratory animal frequently used as an experimental model, it is not considered a model of choice for *Brucella* infection. Rabbits are partially susceptible to *Brucella* infection [70], and only about 20% of infected animals developed a very short and sporadic bacteremia with *B. abortus* or *B. suis* [86]. The pregnancy increases systemic susceptibility of rabbits to *B. abortus* infection but nevertheless the infecting organism was not recovered from the uterus of pregnant female rabbits [70]. Hamsters (Syrian or Golden Hamster) do not appear to be a good animal model for *B. abortus* infection, due the vast individual differences in susceptibility [70].

3.2. Nonhuman Primate Models. Nonhuman primate models of *Brucella* infection have been reported in *Macaca arctoides* and rhesus macaque (*Macaca mulatta*) infected with *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* [87–90]. These animals are susceptible to *Brucella* organisms administered by digestive, subcutaneous, or respiratory routes and develop persistent bacteremia up to eight weeks after inoculation [89]. The primate infection leads to a multiple-organ disease causing focal granulomatous hepatitis, splenitis, and lymphadenitis, similar to human brucellosis [91]. In a few cases, there is an involvement of the reproductive tract causing granulomatous orchitis, epididymitis, or acute endometritis [89]. Aerosol infection of nonhuman primates has been reported [89, 90], resulting in a number of pathologic changes similar to human brucellosis, suggesting that nonhuman primate model is a suitable model for human brucellosis [90]. It is noteworthy that the aerosol exposure might possibly occur as a result of a bioterrorism event, and studies of dose-dependent infection by this route and animal model are important. Mense and colleagues [89] reported that uninfected macaques, not inoculated with *Brucella* organisms, became infected when housed in the same room with inoculated macaques, suggesting that the macaque is a good model to study *Brucella* infection by aerosol route. Moreover, studies on the efficacy of diagnostic methods for *Brucella* detection after aerosol exposure has been performed, and therefore nonhuman primates could provide an excellent model for testing of diagnostics [90].

4. Conclusions

Human brucellosis results in highly variable clinical manifestations that are not quite paralleled by experimental infections in laboratory animals. However, animal models, particularly the mouse, have been extensively used and allowed for accumulation of valuable information mostly in the past recent years regarding the pathogenesis, immunity, and antibiotic susceptibility of *Brucella* spp. *in vivo*. New technologies in mouse genetics will likely bring about even greater insights into the interaction of *Brucella* spp. with the immune system that lead to disease in humans and in the natural zoonotic reservoir hosts.

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