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MINIREVIEW

Cell and Tissue Tropism of *Brucella* spp.

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ABSTRACT *Brucella* spp. are facultatively intracellular bacteria that can infect, survive, and multiply in various host cell types *in vivo* and/or *in vitro*. The genus *Brucella* has markedly expanded in recent years with the identification of novel species and hosts, which has revealed additional information about the cell and tissue tropism of these pathogens. Classically, *Brucella* spp. are considered to have tropism for organs that contain large populations of phagocytes such as lymph nodes, spleen, and liver, as well as for organs of the genital system, including the uterus, epididymis, testis, and placenta. However, experimental infections of several different cultured cell types indicate that *Brucella* may actually have a broader cell tropism than previously thought. Indeed, recent studies indicate that certain *Brucella* species in particular hosts may display a pantropic distribution *in vivo*. This review discusses the available knowledge on cell and tissue tropism of *Brucella* spp. in natural infections of various host species, as well as in experimental animal models and cultured cells.

KEYWORDS macrophage, trophoblast, *Brucella*, tropism

Brucella spp. are Gram-negative facultatively intracellular coccobacilli (1) that belong to subclass alpha-2 of the class Proteobacteria (2, 3). *Brucella* spp. can infect several host species (1). Historically, six species have been recognized as "classical" *Brucella* species that were named according to their host preferences, including *Brucella abortus* (cattle), *Brucella melitensis* (goats and sheep), *Brucella ovis* (sheep), *Brucella suis* (pigs), *Brucella canis* (dogs), and *Brucella neotomae* (desert rats) (4, 5). The terminology "classical" refers to *Brucella* species that were originally identified in terrestrial mammalian hosts, particularly domestic animal species, but this classification may be confusing since in the recent past decades, the genus has expanded with the recognition of novel species (6), including *Brucella ceti* (cetaceans), *Brucella pinnipedialis* (pinnipeds) (7), *Brucella microti* (common vole) (8), *Brucella inopinata* (breast implant infection) (9), *Brucella papionis* (baboon) (10, 11), *Brucella vulpis* (red fox) (12), and *Brucella*-like (amphibians) (13, 14). However, in spite of this expanding number of species in this genus (15), *Brucella* species have a low degree of genetic diversity, with approximately 97% similarity between species (16, 17).

Brucellosis is a neglected and a re-emerging zoonosis with worldwide distribution (18). The organism was first described in 1887 by David Bruce, when the microorganism was isolated from military personnel that died after developing a disease that was known as Malta fever (19). *B. melitensis* is the most pathogenic for humans, while *B. abortus* and *B. suis* show intermediate zoonotic potential, and *B. canis* has a lower zoonotic potential (20, 21). However, the number of new human cases is still unclear, and a lack of accurate epidemiologic data suggests that published studies on the prevalence of brucellosis

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underestimate the actual case numbers (22). Transmission occurs through contact with contaminated animal secretions, by ingestion, or via aerosols. For humans, both ingestion of contaminated raw milk or nonpasteurized dairy products and occupational exposure are important risk factors (1, 23, 24). Venereal transmission may occur in animals, although it is considered to be an uncommon route of infection (25). However, infected semen used in artificial insemination in cattle may be a source of transmission (26).

Brucellosis results in highly relevant economic losses for the animal industry as a result of abortions, reduced fertility or infertility, and reduction in milk production (27, 28). Clinical signs and lesions include placentitis and retained placenta in females, and orchitis and epididymitis in males, and, less commonly, arthritis (1, 23) or bursitis (29). In human patients, brucellosis is associated with a chronic and debilitating disease, although its mortality rate is low. Common clinical symptoms are intermittent or undulating fever and fatigue. The initial symptoms may progress to other localized clinical manifestations such as osteomyelitis, arthritis, spondylitis, neurobrucellosis, and endocarditis (1, 30–33).

Brucella is considered a facultative intracellular bacterium, and its ability to survive in the environment is considered to be low (34). Therefore, *Brucella* spp. must interact with various host cell types in order to establish infection (35–37). Our goal is to provide a comprehensive review of the literature on cell and tissue tropism of *Brucella* spp. in various host species, in the context of both experimental conditions and natural disease.

BRUCELLA SPP. TROPISM IN THEIR NATURAL PREFERENTIAL HOSTS

Over the past several decades, scientific data accumulated demonstrating the tissue distribution of *Brucella* spp. by using various laboratory techniques such as bacterial isolation, immunohistochemistry, electron microscopic, PCR, and *in vivo* imaging systems, among others. Early studies demonstrated a tropism of *B. abortus* to the pregnant uterus of cows (38), resulting in very high numbers of *B. abortus* CFU in cotyledons of infected pregnant cows, up to 1.4×10^{13} CFU/gram of tissue (39). In the placental cotelydons, an abundant cell type that is unique to pregnancy and key for *Brucella* infection is the trophoblast. The ability of *Brucella* spp. to infect and multiply in trophoblasts often results in necrosis and abundant extracellular bacterial aggregates (Fig. 1) and is a key factor for transmission to other animals and contamination of the environment (38). Ultrastructural examination of tissues from experimentally infected pregnant goats, used as models for *B. abortus*-induced placentitis in ruminants, revealed large numbers of intracellular bacteria in trophoblasts, as well as within organelles having ultrastructural features of rough endoplasmic reticulum (40). In this caprine model of infection, there is fetal death as observed in cattle. *B. abortus* is also isolated from infected goats' milk, uterine secretions, and several different fetal tissues, particularly lymph nodes (41). These findings in experimentally infected goats (40, 41) are quite similar to experimental infections of pregnant heifers with *B. abortus*, in which the pathogen is frequently present in uterine and placental tissues, lymph nodes, and mammary gland, (23). Indeed, in experimentally infected cows shedding *B. abortus* in milk, the mammary gland was shown by immunostaining of *B. abortus* to be a target organ, with *B. abortus* mostly associated with mammary gland macrophages (23). In pregnant cattle, *B. abortus* infections usually are not associated with gross lesions in mammary glands. However, in spite of the absence of grossly recognizable lesions, Xavier et al. (23) demonstrated that 21 of 42 *B. abortus*-infected cows (50%) had a multifocal interstitial lymphohistiocytic infiltrate, with neutrophils in the acinar lumen of mammary gland. In addition, immunolabeling demonstrated *B. abortus* intracellularly in macrophages, as well as in the acinar lumen, and these findings were associated with bacterial isolation from mammary tissues. These findings are in good agreement with those described by Meador et al. (42), in which tissues from goats experimentally infected with *B. abortus* had abundant immunolabeling of *B. abortus*, especially in macrophages and neutrophils within the mammary alveoli and ducts, as well as in the adjacent interstitium.

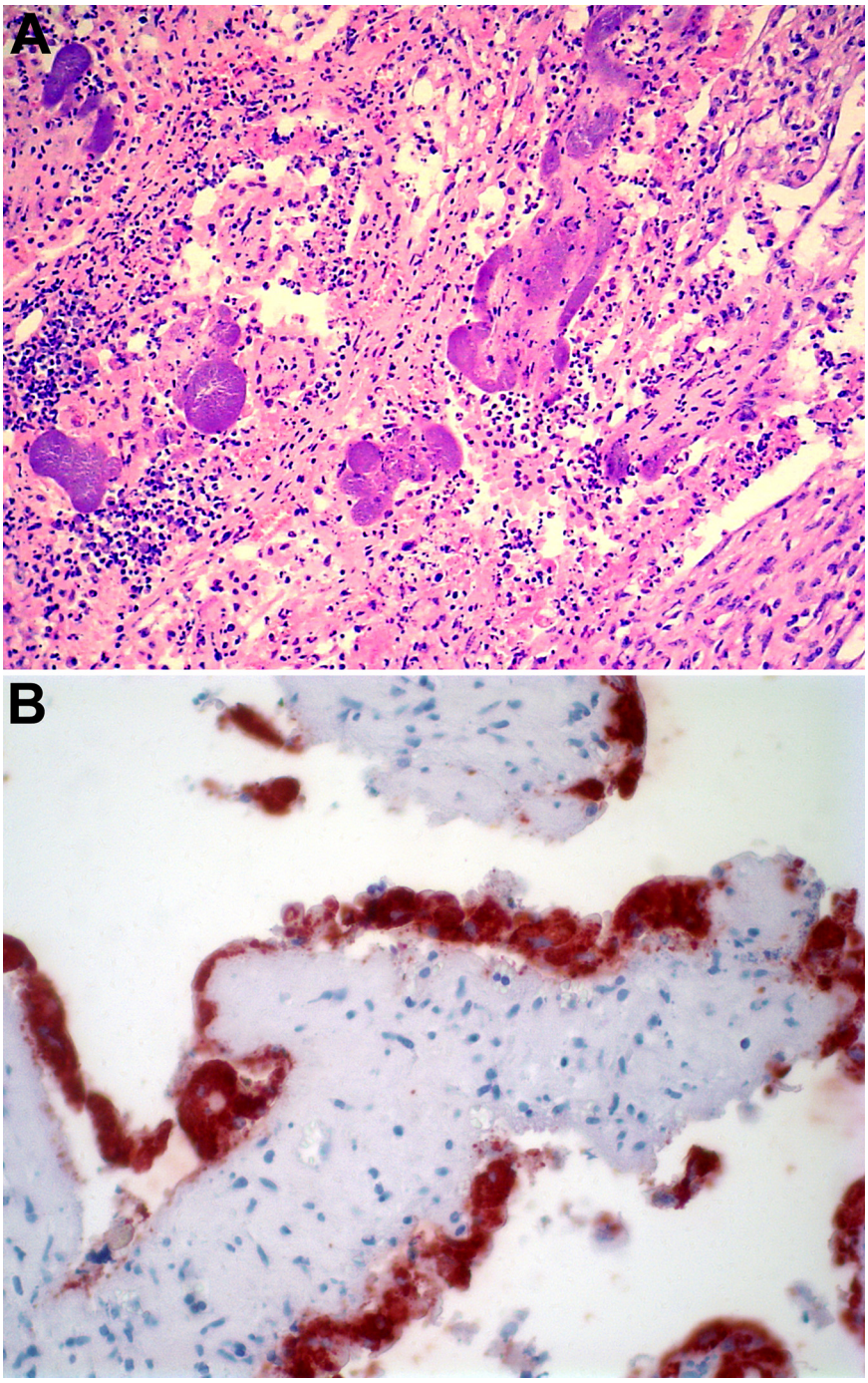

FIG 1 Placentitis in a cow with intralesional *Brucella abortus*. (A) Neutrophilic and necrotizing placentitis with large colonies of *B. abortus* in a placentome from a cow experimentally infected as previously described (23). (B) Immunolabeling of *Brucella* sp. with a polyclonal primary antibody anti-*Brucella* sp. in trophoblasts of a bovine placenta from a naturally infected cow.

Clinical manifestations of *B. canis* infection in dogs were also originally characterized by abortion (43), which is common when a given group or population of naive dogs are first exposed to this pathogen, although neonatal lethality is more common under enzootic conditions (44). Therefore, *B. canis* has a tropism for the pregnant uterus, causing placentitis and fetal and/or neonatal lethality (44), which are clinical and pathological features similar to those resulting from infection with *B. abortus* or *B. melitensis* in cattle or in small ruminants, respectively (45). In contrast, pregnant sows infected with *B. suis* tend to have higher rates of early embryonal or fetal losses, as well as stillbirths and

neonatal mortality. Remarkably, infected pigs have a prolonged bacteremia, which favors shedding of the pathogen in the urine and mucosal surfaces, thereby promoting transmission in the absence of abortion, including venereal transmission, which is much less common in bovine and canine brucellosis (46). Interestingly, although *B. ovis* may cause abortion in pregnant ewes, in contrast to *B. abortus* and *B. melitensis* that also infect ruminants, *B. ovis* induces lesions in males more often than in females. Although *Brucella* spp. may cause lesions in the male genital system of various host species, *B. ovis* primarily causes epididymitis and vesicular seminitis in rams (47).

The reproductive tract provides an environment where the abundance of bacteria creates the conditions for key aspects of the transmission cycle of *Brucella* spp. (36). As pointed out, early studies indicated that the bovine placenta was a privileged site for *B. abortus* multiplication and hypothesized that erythritol was responsible for favoring *B. abortus* multiplication in the placenta (38, 48, 49). *Brucella* spp. can colonize trophoblasts in naturally infected cows, does, ewes, sows, and bitches (40). Erythritol may serve as a carbon source during the multiplication of *Brucella* spp. in the placenta and genital organs of natural hosts. Erythritol is found in high concentrations in fetal fluids, placenta, epididymis, and semen (48, 50, 51). *In vitro* experiments indicate that *Brucella* spp. utilize erythritol as a source of carbon preferentially over glucose (49). However, some strains and/or species of *Brucella* spp. do not metabolize erythritol but are still capable of colonizing the placenta, testis, and uterus. For instance, *B. ovis* has tropism for genital tissues of sheep and may eventually cause abortion; yet, it is defective for the oxidative metabolism of arabinose, galactose, ribose, xylose, glucose, and erythritol (49). This defect is due to a stop codon in *eryA* (*BOV_A0811*) and a frameshift in *eryD* (*BOV_A0814*) that render them pseudogenes in *B. ovis*, although erythritol does not inhibit *B. ovis* growth (52). In contrast, the vaccine strain S19, which may induce abortion in cattle (53), is inhibited by erythritol *in vitro* (54). Importantly, other studies indicate that not just erythritol but other suitable energy sources available in the genital organs may have a relevant role in defining organ and tissue tropism of *Brucella* spp. (36, 55).

The tropism of *Brucella* spp. for the maternal-fetal barrier favors fetal infection. The mechanism of fetal infection is still unclear. However, the presence of *Brucella* spp. in amniotic liquid and placenta certainly exposes fetuses to infection (41). Therefore, abortion is not just due to impairment of placental functions but also to fetal lesions (23, 56). *Brucella* spp. can be found in the lungs, liver, spleen, and kidney of bovine and caprine fetuses (45).

Animals may be infected through various routes of infection, although natural infections are commonly a consequence of exposure through ingestion (1). Experimental inoculation of bovine ligated ileal loops with *B. abortus* demonstrated that the pathogen invades primarily through M cells at the domed villi, which are associated with lymphoid nodules of the Peyer's patches. Once in the lamina propria, *B. abortus* is found within macrophages and neutrophils (57). In contrast to most domestic animal species, venereal transmission is a relevant route of natural infection for *B. suis* in pigs (46).

Although much less common, osteoarticular lesions are also observed in some preferential animal host species in the context of natural infection with *Brucella* spp. (1). For instance, *B. abortus* is often associated with bursitis in cattle (29).

Organs of the reticuloendothelial system have been recognized as target for *Brucella* infection since brucellosis was first described (19). In a recent review, González-Espinoza et al. (58) indicated that organs comprising the reticuloendothelial system or monocyte phagocyte system, particularly spleen, liver, bone marrow, and lymph nodes, which contain abundant populations of monocytes, macrophages, dendritic cells, and/or myeloid cells, provides a favorable environment for *Brucella* spp. multiplication. In addition, organs of the genital system, particularly the placenta in pregnant females and the epididymis in males, also provide optimal conditions for multiplication of *Brucella* spp. In contrast, lymph nodes, spleen, liver, and bone marrow may act as reservoir organs for *Brucella* spp., providing an environment that is suitable for persistence and long-term survival within the host (58). In fact, the bone marrow is commonly affected by *Brucella* sp. infection in humans.

Human patients diagnosed with brucellosis may develop granulomatous inflammation in the bone marrow associated with hematological changes, including anemia, leukopenia, and thrombocytopenia. The bone marrow has been considered a more sensitive site for bacterial isolation compared to blood in patients with previous antibiotic treatment in chronic cases of brucellosis (59).

TISSUE TROPISM OF *BRUCELLA* SPP. IN LABORATORY ANIMAL MODELS

In addition to infections in natural hosts, as well as experimental infection of small ruminants (40, 41), some of the knowledge on tropism of *Brucella* spp. emerged from experimental models such as the mouse (60–62) and guinea pigs (63, 64). Particularly the mouse has been extensively used as an animal model for *Brucella* spp., providing a significant contribution to several aspects of brucellosis, including immunology, host-pathogen interactions, pathogenesis, and vaccinology (65–67). The mouse model became even more important due to technical difficulties such as biocontainment needs, for experimental infections in natural hosts. Mice may be challenged through various routes such as oral, intragastric, intraperitoneal, intravenous, or intratracheal, and bacteria may be recovered from the spleen and liver after 1 to 7 days of infection (35, 66, 68, 69). Interestingly, *B. melitensis* can cause systemic infection in mice after intragastric inoculation without causing any intestinal lesion or inflammation in the intestinal mucosa, and there is evidence that M cells are specifically targeted by *B. melitensis* to cross the intestinal epithelial layer (35). These findings in the mouse model seem to parallel what has been described in cattle infected with *B. abortus* (57).

The hepatic tissue of *Brucella*-infected mice presents areas with inflammatory infiltrate composed of neutrophils during the early stages of infection (first 3 to 4 days postinfection) followed by an inflammatory infiltrate composed predominantly of macrophages and epithelioid macrophages, forming microgranulomas at the chronic stages of infection (Fig. 2). *Brucella* can be detected in phagocytic cells, especially macrophages (65, 66, 70). In the spleen of *Brucella*-infected mice, there is an increase in the organ size and weight that is associated with an inflammatory reaction (splenitis). At approximately 7 to 10 days postinfection, the number of intracellular *Brucella* spp. in macrophages reaches its peak (65, 66).

Another tissue that has been investigated and considered important for *Brucella* persistence is the bone marrow. Persistence of *B. abortus* or *B. canis* has been demonstrated in the bone marrow of mice, coinciding with the chronicity of the disease in this animal model (71, 72). In mice infected with *B. abortus*, histopathological changes in the bone marrow include granulomas, as well as an increase of multipotent progenitor and active hematopoietic stem cells, neutrophils, and $CD4^+$ T lymphocytes. The three types of cells infected in bone marrow by *B. abortus* 2308 expressing red fluorescent protein were monocytes, neutrophils, and granulocyte-macrophage progenitors (GMPs). At 8 days postinfection, the proportion of neutrophils containing bacteria was greater than other cells but significantly decreased after 30 days. Interestingly, *B. abortus* resists killing by neutrophils and induces premature death of these cells, and *B. abortus*-infected neutrophils display phosphatidylserine on their cell membrane, which favors phagocytosis of infected neutrophils by macrophages. Therefore, apparently *B. abortus* adopts a "Trojan horse" strategy by using infected neutrophils as vehicles for dispersion throughout the host mononuclear phagocytic system (72, 73).

Human patients with brucellosis frequently display symptoms arising from osteoarticular complications (1, 30), which as pointed out above may also occur in naturally infected animals (29). The use of bioluminescent *B. melitensis* in the mouse model of infection enabled the evaluation of bacterial dissemination to osteoarticular tissues of BALB/c mice, followed by histological characterization of the infection sites (74). *In vivo* imaging demonstrated the rapid dispersion of bacterium to multiple sites in the skeletal structure. Mice infected for a period of 26 weeks exhibited multiple skeletal complications, including massive infiltration of inflammatory cells in synovial joints of the hind paws, with pannus and bone lesions (74). A marked reduction of bacterial infection

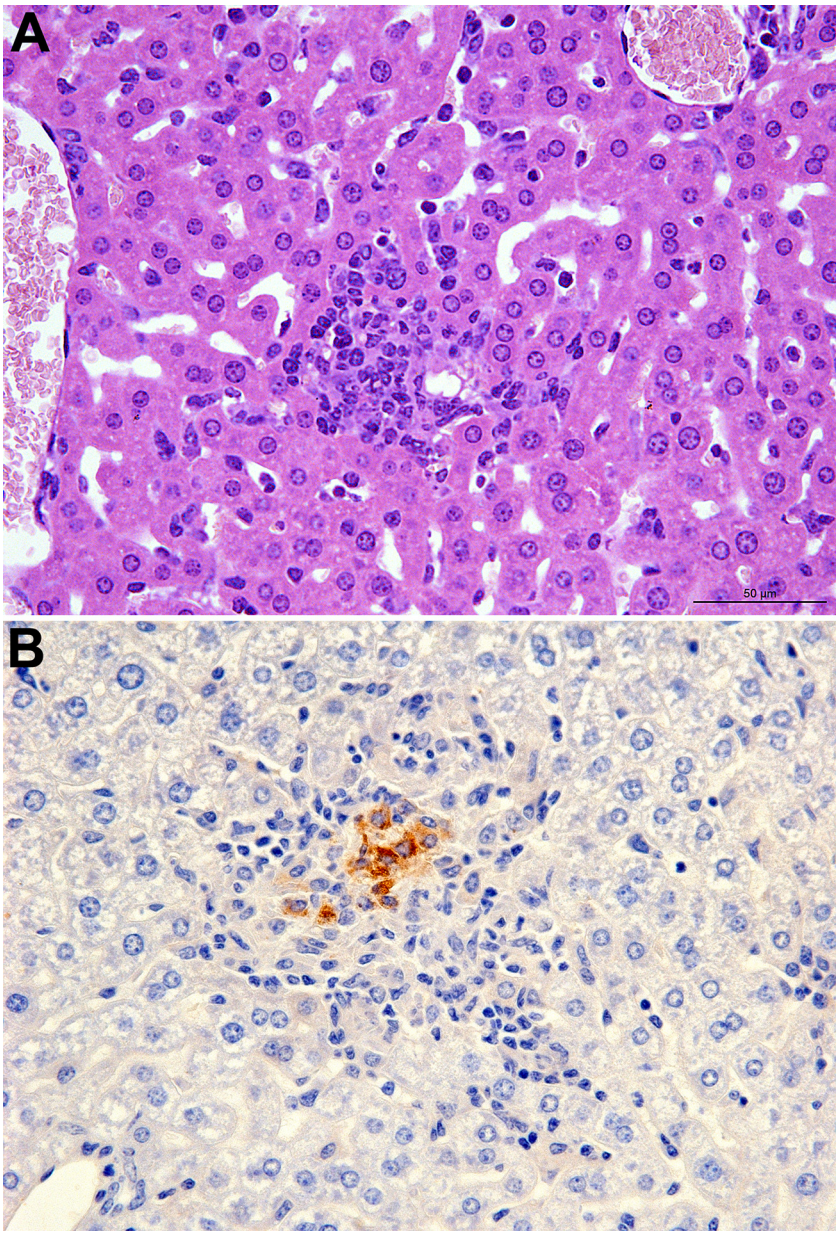

FIG 2 Mouse model of *Brucella* spp. infection. (A) Microgranuloma in the liver of a mouse experimentally infected with *Brucella ovis*. (B) Immunolabeling of intralesional *Brucella* sp. (brown) in a hepatic granuloma of an experimentally infected mouse. The mice in panels A and B were inoculated with 10^6 CFU/mouse immunoprecipitate and sampled at 7 days postinfection.

in joints was observed after 28 days of infection in mice deficient for CXCR2, which plays an important role in neutrophil recruitment. The mechanism for arthritis in mice is dependent on CXCR2, supporting the hypothesis that blocking CXCR2 ligands may be useful as a complementary treatment for human osteoarthritis provoked by *Brucella* (75).

Brucella has an intense tropism for the reproductive tract of domestic animals. Considering that the male genital tract is also affected in brucellosis, Izadjoo et al. (76) developed a male mouse model to study the genitourinary pathogenesis of *Brucella*. Testis, epididymis, or both were infected in 13 of 57 animals (22.8%) inoculated with *B. melitensis* strain 16M, with a histiocytic inflammation in the testicular periarterial tissue and the superficial lymph nodes. *B. ovis*, which causes lesions primarily in the male genital system of sheep (47), is capable of infecting mice, in which it induces systemic lesions that are similar to those induced by other *Brucella* species in the mouse, but it

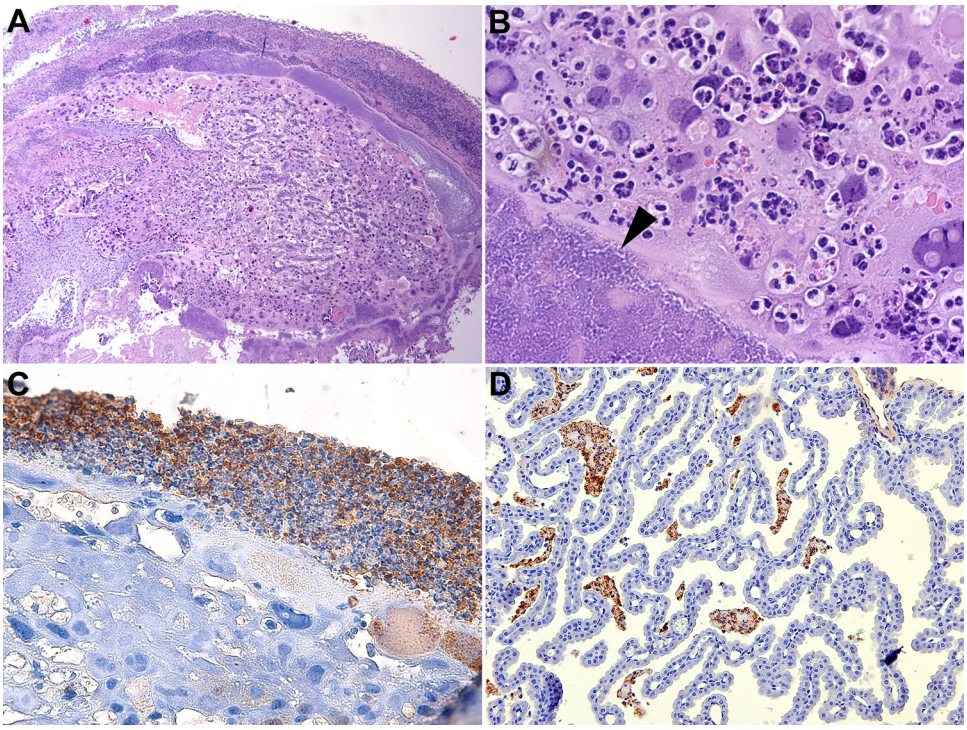

FIG 3 Placentitis in a mouse with intralesional *B. abortus*. (A) Neutrophilic and necrotizing placentitis with large colonies of *B. abortus*. (B) Necrosis with neutrophilic infiltration in the junctional zone of the placenta with large extracellular aggregates of *B. abortus* (arrowhead). The pregnant mice in panels A and B were inoculated with10^5 CFU/mice immunoprecipitate at the fifth day of gestation and sampled at 13 days after infection. (C) Immunolabeling of *Brucella* sp. (brown) in the decidua and junctional zone of the placenta from experimentally infected mice as previously described (81). (D) Immunolabeling of *Brucella* sp. (brown) in the yolk sac from experimentally infected mice as previously described (81).

does not consistently result in lesions in the genital system (68), indicating that in this particular case, the mouse is a suitable model for infection but not necessarily a model for the disease or tissue tropism.

The placenta is a complex and transient structure in the pregnant uterus of mammals. It might be morphologically classified into various categories. The mouse placenta is hemochorial and discoid, which is quite distinct from the placenta of domestic ruminants, which are highly susceptible to *Brucella*-induced abortion and have synepitheliochorial and cotyledonary placentas (45, 77). Despite these morphologic differences, pregnant mice have been employed as a model for *Brucella*-induced placentitis and fetal loss (62, 78–82). *B. abortus* colonizes and persists in the placenta and uterine tissues of pregnant mice. In the mouse placenta, *B. abortus* infects trophoblastic giant cells localized mostly in periphery of the placenta, but it may also be found extracellularly, particularly in association with necrosis (Fig. 3). Histologically, infected placentas develop marked necrosis and mild infiltration of neutrophils in spongiotrophoblastic zone extending into the decidua basalis (62). At the junctional zone of infected placenta, trophoblastic giant cells may have a dark blue and granular cytoplasm because of the presence of intracellular bacteria. *Brucella* may also present in the spongiotrophoblast, endodermal cells of the visceral yolk sac adjacent to the remnants of Reichert's membrane. Considering the importance of placentitis and abortion in natural hosts (43, 56, 83) and the lack of mechanistic studies in these species, the mouse model significantly contributed to the identification of pathogen and host mechanisms involved in *B. abortus*-induced placentitis. Indeed, in the mouse, the effector *Brucella* protein VceC triggers endoplasmic reticulum (ER) stress response (79), which is linked to inflammation in *B. abortus*-infected placenta (84). This results in trophoblast cell death, inflammation, and fetal loss (79). VceC-triggered ER stress response is also associated with induction of tumor necrosis factor α

(TNF-α), which is associated with placentitis and fetal loss (80). Interestingly, smooth *Brucella* species may have variable pathogenic potential for causing placentitis in pregnant mice, with *B. melitensis* displaying a more intense placental tropism compared to *B. suis* biovar 2 (82). However, *B. ovis* that is primarily associated with epididymitis in rams (47) is also capable of inducing placentitis and fetal loss in pregnant mice (81). Interestingly, nonpregnant uteruses from experimentally infected mice yield markedly lower numbers of *B. abortus* per gram of tissue (85) compared with placentas (86), demonstrating the tropism for the placenta in the pregnant uterus in this model (62).

The mouse model may also be useful for studying the pathogenesis of *Brucella* spp. in the mammary gland. In contrast to the placenta, the mammary gland is morphologically similar among all mammals (87). *Brucella* can survive and colonize the mammary gland of lactating mice (81, 88). Macrophages and neutrophils are target cells for colonization in the mammary gland and may carry the pathogen through the excreted milk of mice, which may be a source of infection for the litter (88). These findings suggest the mouse as a potential model for future studies to understand mechanisms of vertical transmission in natural hosts since infection parallels what has been described in the bovine mammary gland (23).

IN VITRO STUDIES OF CELL SUSCEPTIBILITY TO *BRUCELLA* SPP. INFECTION

Informed by information gleaned from animal infection studies, early studies evaluated the ability of *B. abortus* to infect various host cell types in culture (89). These studies provided a foundation for studying the interaction of *Brucella* spp. with the cell types identified during *in vivo* infection. In addition, immortalized cell lines of various origins, such as HeLa and Vero cells, were then used as models for investigating features of *Brucella*-host cell interaction, such as its intracellular trafficking and cellular adhesion (90). Subsequent *in vitro* studies have verified a large spectrum of susceptible cells to *Brucella* spp. infection and survival, enhancing our knowledge about intracellular pathways and pathogenesis.

It is well known that *Brucella* spp. target professional phagocytes such as macrophages (91), dendritic cells (92), monocytes, and neutrophils (93, 94). Macrophages are considered one of the most important target cells for *Brucella* infection (Fig. 4) (91). *Brucella* spp. is capable of modulating intracellular trafficking, excluding lysosomal markers from the *Brucella*-containing vacuole, which progressively accumulates markers of the rough endoplasmic reticulum, in which *Brucella* resides in its intracellular replicative niche that favors intracellular growth, and finally the *Brucella*-containing vacuole acquires features of an unconventional autophagosome, which eventually promotes a controlled exit of the pathogen from the cell. This pattern of intracellular trafficking is dependent on bacterial effector proteins that are secreted through the *virB* operon-encoded type IV secretion system (95). Neutrophils are also susceptible to *Brucella* spp. infection. *Brucella* resists the hostile intracellular environment of neutrophils, but in contrast to macrophages, it does not multiply well in these host cells, although the pathogen induces a neutrophil cell death. Importantly, dying neutrophils generate signs for other phagocytic cells, such as macrophages, to remove the dying infected neutrophils, via a process known as efferocytosis (96). This mechanism is thought to be important for dispersing *Brucella* to other organs while avoiding innate immune system (93, 97). Further, *in vitro*, dying infected macrophages can be taken up by neutrophils, which in the host may be a mechanism to promote cell-to-cell spread while avoiding extracellular antibody-mediated responses (98). Monocytes may also be infected with *Brucella*. In fact, *B. abortus* establish platelet-leukocyte complexes mainly involving monocytes and neutrophils (99), which enhances infection. These interactions may allow infected monocytes to cross the human brain microvascular endothelial cells, making infected cells act as a "Trojan horse," since under *in vitro* conditions, *B. abortus* is not capable of crossing a brain endothelial monolayer, whereas previously infected monocytes can traverse the endothelial layer carrying intracellular *B. abortus* (100).

During the course of infection, *Brucella* also targets nonphagocytic cells. A nonphagocytic cell line that has been extensively used for *Brucella* research is the HeLa (85, 101–103).

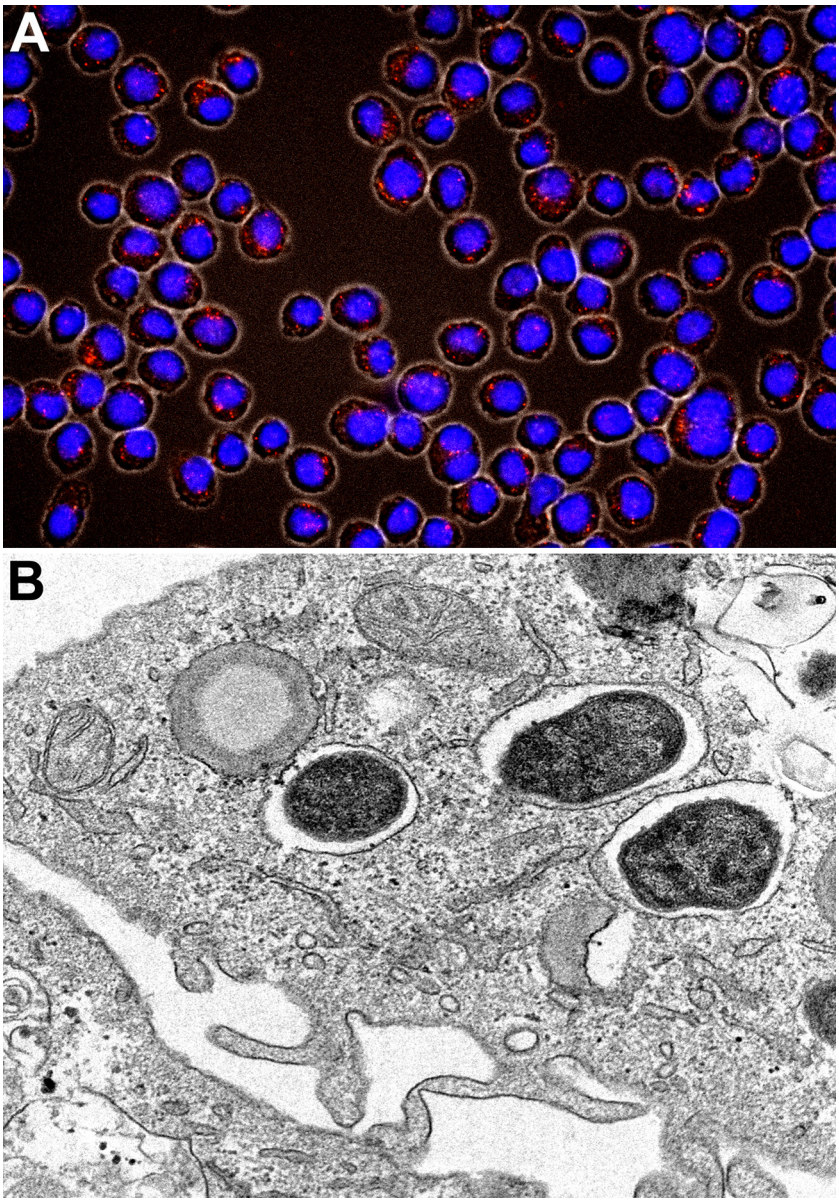

FIG 4 Infection of macrophages with *Brucella* sp. (A) Mouse macrophage cell line (J774) infected with *B. ovis* expressing mCherry (red) at 24 h postinfection with a multiplicity of infection of 1:100. (B) Transmission electron micrograph demonstrating the intracellular localization of *B. abortus* in membrane-bound vacuoles in cultured bovine macrophages experimentally infected as previously described (142).

In addition to assessment of invasion and intracellular survival, HeLa cells have been often used as a model for *Brucella* spp. adhesion to epithelial cells (104–106). As mentioned above, M cells serve as a port of entry for *Brucella* to infect through the gastrointestinal route (35, 57). Cell culture experiments also provided valuable knowledge on the interaction of *Brucella* with trophoblasts, which are highly relevant target cells *in vivo*. *Brucella* spp. invades, survives, and multiplies in human trophoblasts (86, 107). Importantly, the interaction of *B. abortus* with bovine trophoblasts has been investigated by using cultured placental explants, demonstrating that *B. abortus* invades and multiplies within bovine trophoblasts in cultured placental explants (108, 109).

During recent years, several studies addressed the interaction of *Brucella* spp. with several different cultured cell types (Fig. 5). Table 1 has a comprehensive list of cultured cells that have been experimentally infected with *Brucella* spp. However, in some

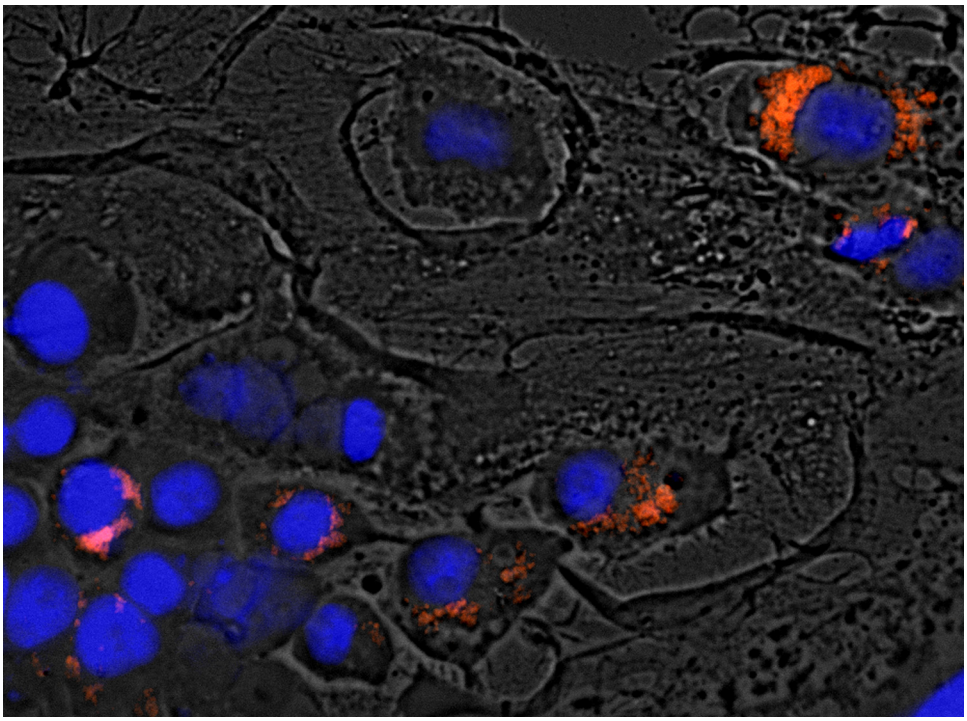

FIG 5 Mouse endothelial cells experimentally infected with *B. ovis* expressing mCherry (red) at 24 h postinfection with a multiplicity of infection of 1:1,000.

cases, how the interaction of *Brucella* spp. with cultured cells correlates with cell tropism *in vivo* remains to be elucidated.

EVIDENCE OF A BROADER TROPISM OF *BRUCELLA* SPP. IN NATURAL HOSTS

In natural hosts, *Brucella* has tropism and ability to replicate in hemolymphopoietic organs including spleen, liver, bone marrow, and lymph nodes, as well as reproductive organs such as the placenta, testis, and epididymis (58). In these target organs, *Brucella* resides especially within phagocytes but can invade and replicate in a range of nonphagocytic cells. A previous study (110) demonstrated *B. melitensis* in erythrocytes of intraperitoneally inoculated mice. Under those experimental conditions, *B. melitensis* could persist for at least 2 weeks in the blood, being initially located extracellularly but rapidly transitioning to an intracellular location in erythrocytes. While there was no evidence that erythrocytes provide a suitable niche for *Brucella* replication (since each infected cell contained only a single bacterium), and the role of these cells as a niche for pathogen reservoir may be limited by the short life span of erythrocytes, targeting these cells may be relevant for pathogen dissemination in the host, considering the uptake of senescent erythrocytes by splenic macrophages. Although this study in the mouse model could initially be seen as disconnected with the available knowledge on tissue and cell distribution of *Brucella* spp., a more recent study demonstrated large numbers of *B. canis* within intravascular erythrocytes of naturally infected newborn dogs (37). Indeed, the study by Souza et al. (37) demonstrated a pantropic distribution of *B. canis* in fetal and neonatal canine tissues (Fig. 6). In naturally infected fetuses or neonatal dogs, as expected, *B. canis* was isolated from the placenta, spleen, liver, and lung, but the pathogen was also identified in all organs subjected to analysis, including the gastrointestinal tract, heart, kidney, urinary bladder, lymph nodes, adipose tissue, blood, genital organs, umbilical cord, central nervous system, and the eye. Importantly, although often located in cells morphologically compatible with macrophages, *B. canis* was detected in several different cell types in neonatal dogs (Fig. 6), including cardiomyocytes, adipocytes, enterocytes, renal tubular epithelia, and erythrocytes, among

TABLE 1 Cultured cell lines or primary cells experimentally infected with *Brucella* spp.[a]

Cell type	Origin	*Brucella* species	MOI	Outcome of infection	References
Macrophages	Primary mouse BMDMs	*B. abortus*, *B. melitensis*	1 to 200	Cell infection and intracellular multiplication without inducing cytotoxicity	97, 142–144
Macrophages	Primary from bovine or ovine peripheral blood	*B. abortus*	100	Cell infection and intracellular multiplication	145, 146
Macrophages	Cell lines DH82, THP-1, RAW 264.7, and J774.A1	*B. abortus*, *B. suis*, *B. melitensis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. pinnipedialis*, *Brucella* sp. frog isolate	1 to 1,000	Cell infection and intracellular multiplication	13, 68, 71, 147–156
DCs or macrophages	Primary human, canine, ovine, and bovine PBMCs; mouse peritoneal and alveolar cells	*B. abortus*, *B. suis*, *B. canis*, *B. ovis*	1 to 300	Cell infection and intracellular multiplication; *Brucella*-induced DC maturation	149, 157–164
Neutrophils	Primary human, caprine, and bovine from peripheral blood or primary mouse from bone marrow	*B. abortus*, *B. melitensis*, *B. canis*, *B. suis*	50 to 1,000	Cell infection; *Brucella* can survive in intracellular environment in the absence of proinflammatory stimulus; neutrophil could be a Trojan horse for macrophage infection with *Brucella*; cell death	73, 93, 99, 149, 165–168
Platelets	Primary culture from human peripheral blood	*B. abortus*	1 to 30	*Brucella* internalization	169
HeLa cells	Human epithelial cell line	*B. canis*, *B. melitensis*, *B. abortus*, *B. ovis*, *B. pinnipedialis*, *Brucella* sp. frog isolate, *B. neotomae*	200 to 500	Cell infection and intracellular multiplication without inducing cytotoxicity; model of attachment to mammalian cells	13, 71, 97, 104–106, 148, 150, 170, 171
Trophoblasts	Primary cultures from placenta of dogs or mice or immortalized from caprine placenta	*B. canis*, *B. suis*, *B. melitensis*	100 to 250	Cell infection and intracellular multiplication without inducing cytotoxicity; decrease of cell multiplication	172–174
Trophoblasts	Human cell lines (derived from choriocarcinoma) Bewo, JeG-3, and SW.71	*B. melitensis*, *B. papionis*, *B. abortus*, *B. suis*, *B. ovis*	100 to 250	Cell infection and intracellular multiplication; *B. melitensis* caused changes in hormonal production; *Brucella* spp. can be localized in "*Brucella* inclusions"; *B. papionis* induced a decrease of cell fusion	86, 107, 170, 175, 176
Chorioallatoic membrane explants	Explants from bovine placenta	*B. abortus*	1,000	Internalization of bacteria in trophoblasts and intracellular multiplication	108, 109, 177
Enterocytes	Human cell lines (derived from colorectal epithelia) HT-29 and Caco-2	*B. suis*, *B. abortus*, *B. melitensis*, *B. canis*	100 to 200	Cell infection and intracellular multiplication with cytotoxicity and low inflammatory response	175, 178
M cells	Derived from human cell line Caco-2 cocultured with B lymphocytes	*B. melitensis*	10 to 100	Invasion and epithelial transmigration	35
Alveolar epithelia	Commercial line of human type II alveolar epithelial	*B. abortus*, *B. suis*, *B. canis*	200 to 500	Cell infection and intracellular multiplication with low cytotoxicity	179, 180
Esophageal epithelia	Primary cells of hooded seal (*Cystophora cristata*)	*B. pinnipedialis*	500	Cell infection without intracellular multiplication, with a significant reduction of CFU at 24 hpi	181
Testicular epithelial cells	Ovine cell line OA3.Ts	*B. ovis*	200	Cell infection and intracellular multiplication	170
Endometrial epithelial cells	Human or caprine cell lines	*B. suis* *B. abortus*	100 to 250	Cell infection and intracellular multiplication with low cytotoxicity	182, 183

(Continued on next page)

TABLE 1 (Continued)

Cell type	Origin	*Brucella* species	MOI	Outcome of infection	References
Osteoblasts	Primary from newborn-mouse calvaria	*B. suis*	100	*Brucella* adherence	175
Osteoblasts	Human or mouse cell lines SaOS-2, MG-63, and MC3T3-E1	*B. abortus*, *B. suis*, *B. melitensis*, *B. canis*	10 to 1,000	Cell infection and intracellular multiplication; *Brucella* invasion inhibits bone formation; cortisol levels increased *Brucella* intracellular multiplication; no recovery of *B. canis* at 50 hpi; induction of osteoblast autophagy	147, 184, 185
Osteoclasts	Differentiation from primary BMDMs	*B. abortus*	50 to 500	Cell infection and intracellular multiplication	186
Osteocytes	Cell line MLO-Y4 (with high expression of osteocalcin)	*B. abortus*	100 to 1,000	Cell infection and intracellular multiplication *Brucella* infection decreased the expression of cx-43.	187, 188
Synoviocytes	Human cell line or primary culture	*B. abortus*	100 to 1,000	Cell infection and intracellular multiplication	149, 189, 190
Fibroblasts	Human fibroblast-like cell line SW982 or primary synovial fibroblasts	*B. abortus*, *B. suis*	100 to 1,000	Cell infection and intracellular multiplication	175, 189
Endothelial cells	Human cell lines HBMECs and HUVECs	*B. abortus*	25 to 1,000	Cell infection and intracellular multiplication	100, 167, 191
Hepatocytes	Human hepatoma cell line HepG2	*B. abortus*	10 to 1,000	Cell infection and intracellular multiplication with cytotoxic effect	166
Hepatic stellate cells	Human cell line LX-2	*B. abortus*	100 to 1,000	Cell infection; *Brucella*-induced increased of MHC-II in coculture with macrophages	154, 192, 193
B Lymphocytes	Primary culture from mouse splenocytes	*B. abortus*	100 to 1,000	Cell infection and intracellular multiplication	194, 195
T Lymphocytes	Primary culture from human PBMCs	*B. abortus*	25 to 1,000	Low cell infection, with low CFU counts; infection induced apoptosis	196
Microglia	Human or mouse cell lines BV-2, C13NJ, and HMC3	*B. suis*, *B. melitensis*, *B. abortus*	10 to 1,000	Cell infection and intracellular multiplication with low cytotoxicity	197–200
Astrocytes	Primary culture from mice	*B. abortus*, *B. melitensis*, *B. canis*	50 to 1,000	Cell infection and intracellular multiplication	200, 201
Adipocyte	Differentiated from fibroblast cell line 3T3-L1	*B. abortus*	100 to 1,000	Cell infection and intracellular multiplication	202

[a]BMDM, bone marrow-derived macrophage; DC, dendritic cell; hpi, hours postinfection; HBMEC, human brain microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; MOI, multiplicity of infection; PBMC, peripheral blood mononuclear cell.

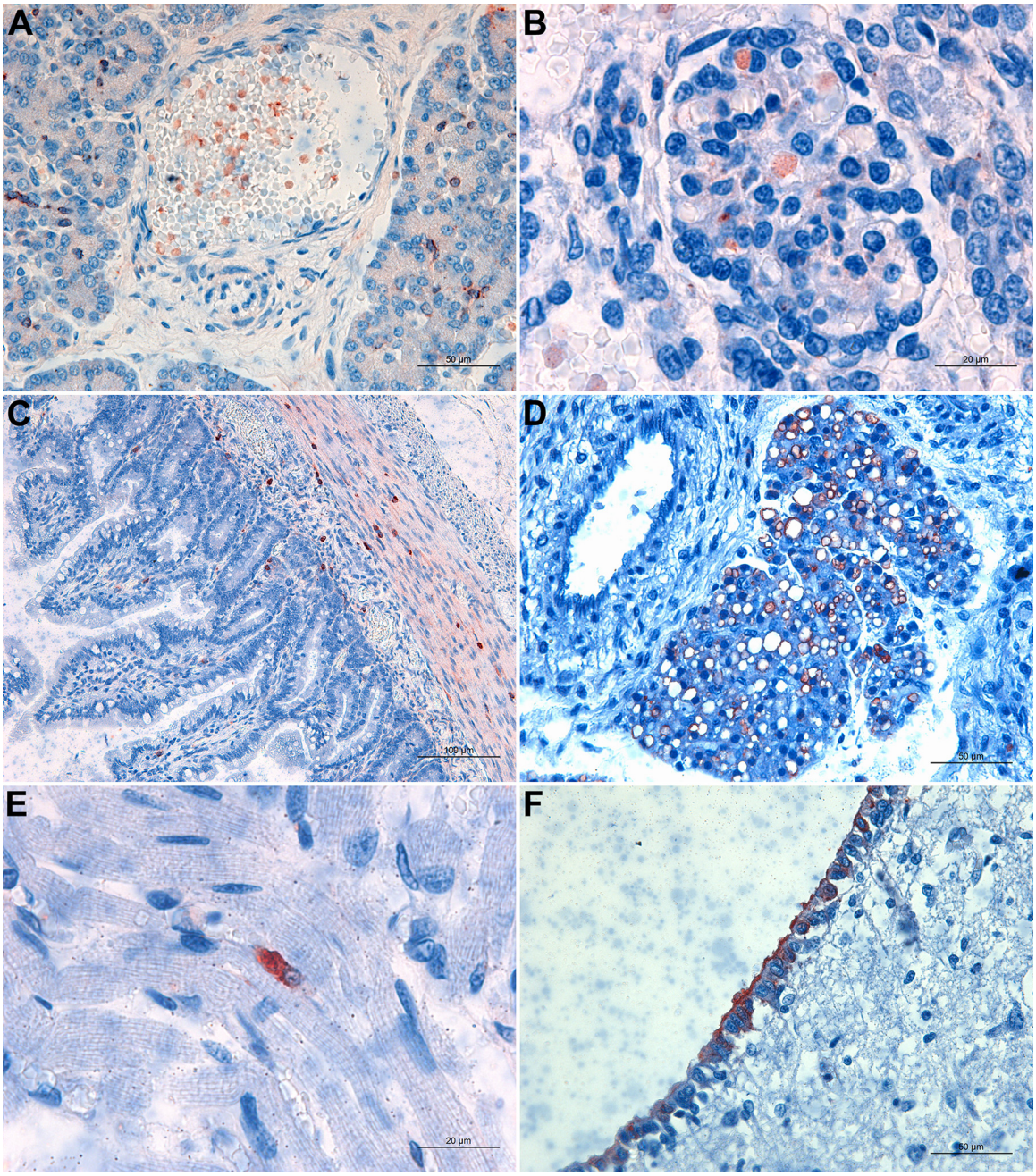

FIG 6 Distribution of *Brucella canis* in tissues of naturally infected neonatal dogs demonstrated by immunohistochemistry (3-amino-9-ethylcarbazole [AEC]; red chromogen) as previously described (37). (A) In intravascular erythrocytes. (B) In erythrocytes in a glomerulus. (C) In the small intestine. (D) In adipose tissue (adipocytes). (E) In the myocardium. (F) In the ependyma in the central nervous system.

other cells (37). *B. abortus*-infected bovine fetuses seem to develop a systemic infection, often with severe lesions in the lungs, among other organs (23, 56). These few studies discussed above suggest that the tropism of *B. abortus* and *B. canis* in fetuses and neonates is broader than previously appreciated; however, additional studies such as the one with *B. canis* (37) will be needed to determine whether these observations will hold true in *B. abortus* and in other *Brucella*-host combinations.

Most of the studies on cell or tissue tropism were based on "classical" *Brucella* species, i.e., the ones that were originally identified in terrestrial mammalian species, particularly domestic animals. However, the genus *Brucella* had a marked expansion in recent years (15). Brucellosis has emerged as an important disease in marine mammals

since the first report of a *Brucella* species in an aborted fetus of a bottlenose dolphin (111). *B. ceti* and *B. pinnipedialis* had been detected in various marine coastal regions affecting cetaceans and pinnipeds, respectively (112). *B. ceti* was detected in various organs and tissues, including blubber abscesses, subcutaneous lesion, skin lesions, pleura (pleuritis), lymph node, meninges, brain, kidney, liver, and lung. In all these organs and tissues, the bacteria was found inside macrophage, neutrophils and trophoblasts (113–121). Interestingly, *B. ceti* infection in many species of cetaceans, including dolphins and whales, appears to result in neurologic disease much more often than other *Brucella* spp. (118, 122–126). Although *B. ceti* may also cause genital or osteoarticular lesions (118, 122, 123, 125, 127) similar to other *Brucella* spp., neurologic disease attributable to *Brucella* is rare in domestic animals and occurs in only 5% of human patients infected with *B. melitensis*, so although not common, neurobrucellosis is a serious complication of brucellosis (1). The mechanisms for this presumed neurotropism of *B. ceti* remain to be investigated. In contrast, the pathogenic potential of *B. pinnipedialis* is still unclear (128), although the organism has been isolated from various organs of stranded or healthy pinnipeds (129, 130). Interestingly, *B. pinnipedialis* is often associated with parasitic pneumonia (129), whereas lungworms are susceptible to infection with this pathogen (131–133), so it is thought to be a potential vector for transmission of the organism among hosts. Alternatively, considering the pathogenic potential of *B. pinnipedialis* to the lungworm *Parafilaroides* sp., including tropism for the male and female genital organs of the parasite and evidence of sexual transmission between these nematodes, the lungworm not the pinniped may hypothetically be considered the preferential host for *B. pinnipedialis* (133).

Not much is known about tissue and cell tropism of other *Brucella* spp. identified in recent years. *B. microti*, originally isolated from the common vole *Microtus arvalis* (8), or *B. microti*-like organisms have ever since been isolated from many other host species, including red foxes, wild boars, and frogs (134–136), so its pathogenic potential and tissue tropism are still to be investigated. Similarly, *B. inopinata*, originally isolated from an infected breast implant in a woman (9), had an expansion of its host range with the identification of *B. inopinata*-like organisms in frogs (13, 137–140), from which the organism was detected in various organs and tissues (137, 141). *B. papionis* was isolated from baboons that had delivered stillborn offspring (10), which supports the hypothesis of a genital tropism for this particular species. Finally, *B. vulpis* was isolated from mandibular lymph nodes of two red foxes (*Vulpes vulpes*) with insufficient data to indicate any particular tissue tropism (12).

FUTURE PERSPECTIVES

Original studies focusing on "classical" *Brucella* species established a dogma about the tissue and cell tropism of *Brucella* spp. Considering the various *Brucella* and host species (i.e., brucellosis is not a single disease), solid knowledge in this field indicates that *Brucella* spp. stealthily shelter in phagocytic cells in organs of the reticuloendothelial system or monocyte phagocyte system, particularly spleen, liver, bone marrow, and lymph nodes, with a particular tropism for genital organs, specially the placenta in females and the epididymis and sexual glands in males (Fig. 7). However, recent studies have demonstrated the ability of *Brucella* to infect and multiply in a large variety of cultured cell types (Table 1). Although the correlation between these findings *in vitro* and actual clinical manifestations or *Brucella*-induced lesions is often not clear, *in vivo* studies have challenged this dogma. As detailed in this review, *B. canis* has a pantropic distribution in neonatal dogs (37). Furthermore, as-yet-unknown host factors may play key roles in tissue tropism. For instance, although domestic animals usually do not develop neurobrucellosis, approximately 5% of *Brucella*-infected human patients may develop neurologic disease (1), whereas neurobrucellosis seems to be common in dolphins naturally infected with *B. ceti* (118). Obviously, bacterial factors may also play important roles in tissue tropism, which may be illustrated by the fact that *B. ovis* frequently causes lesions in males (47), whereas other *Brucella* spp. affects predominantly

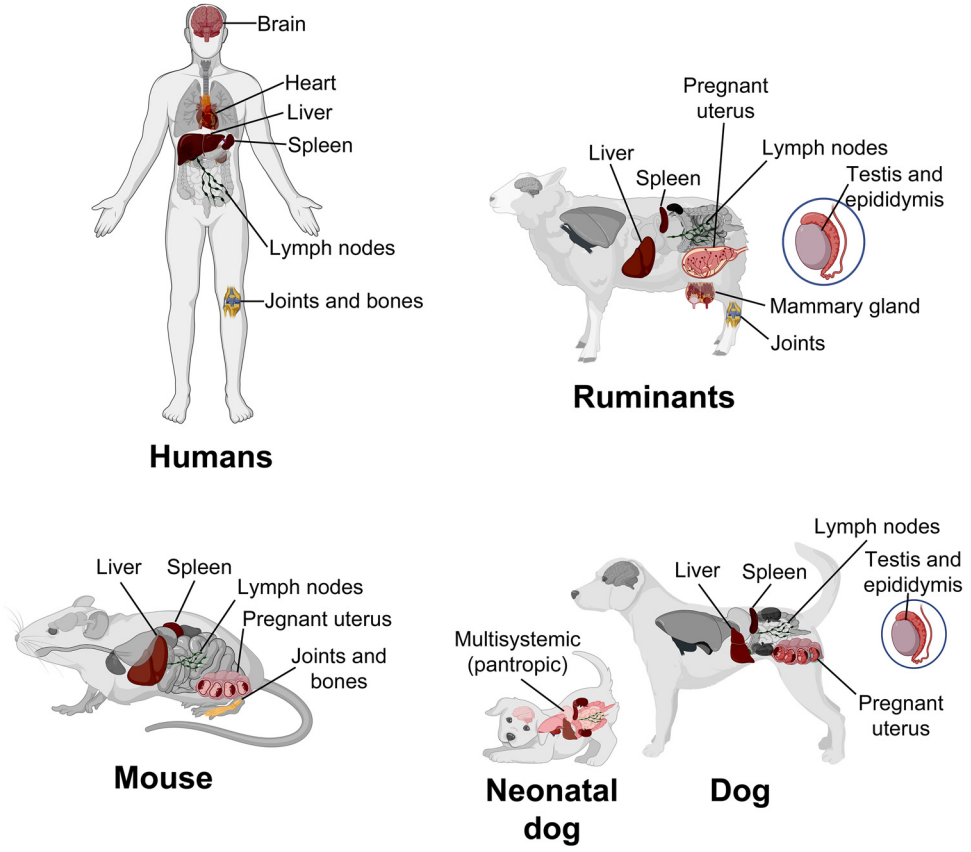

FIG 7 Most important target organs in various host species for *Brucella* spp. The figure was created with BioRender.com.

pregnant females (45). Furthermore, the expansion of the genus with the emergence of novel *Brucella* species will require significant research effort to accurately establish tissue tropism. Therefore, significant advances in this particular field of study are expected in the foreseeable future.

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