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Human Brucellosis and Adverse Pregnancy Outcomes

Angela M. Arenas-Gamboa[1], **Carlos A. Rossetti**[2], **Sankar P. Chaki**[1], **Daniel G. Garcia-Gonzalez**[1], **Leslie G. Adams**[1], and **Thomas A. Ficht**[1]

[1]Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, 4467 TAMU, College Station, TX 77843, USA

[2]Instituto de Patobiología, CICVyA-CNIA, INTA, Nicolas Repetto y de Los Reseros s/n, Hurlingham, Buenos Aires, Argentina

Abstract

Purpose of Review—Brucellosis is a neglected, zoonotic disease of nearly worldwide distribution. Despite brucellosis being recognized as a reproductive disease in animals, it has been historically known as a flu-like illness in humans with little or no significant role in maternal or newborn health. This review focuses on what is currently known relative to the epidemiology of brucellosis in human pregnancy as well as new insights of placental immunology.

Recent Findings—New evidence suggests that maternal infection poses a significant risk factor for adverse pregnancy outcomes including increased risk for miscarriage during the first and second trimester of gestation, preterm delivery, and vertical transmission to the fetus. Adverse pregnancy outcomes were not associated with any specific clinical sign. However, prompt diagnosis and treatment significantly decreased the risk of miscarriage or any other adverse effect.

Summary—Brucellosis during pregnancy should be considered a significant risk factor for adverse pregnancy outcomes in humans. The identification of the mechanism behind bacterial tropism should prove powerful for the development of new countermeasures to prevent these detrimental effects. Increased awareness concerning brucellosis in pregnant women, its transmission, and prevention measures should be considered as a pressing need.

Keywords

Human; Abortion; Brucellosis; Zoonosis; Placenta

Introduction

Brucellosis is a neglected, under-recognized zoonosis of widespread geographic distribution. Among the different *Brucella* species, *B. melitensis* (goat and sheep), *B. suis* (pig), *B. abortus* (cattle), and *B. canis* (dog) are pathogenic and virulent not only for their target

Correspondence to: Angela M. Arenas-Gamboa.

Compliance with Ethical Standards

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species but also for humans. In most cases, human infections occur through the consumption of unpasteurized milk and dairy products or exposure to infected body fluids and tissues (mainly placenta) from infected animals. Despite the bacterium being recognized as a cause of disease in humans for more than 130 years, little information is available describing the mechanism related to adverse pregnancy outcomes in humans. Brucellosis is still considered an emerging disease with tremendous economic and public health impact under resource-limited settings or within emerging economies [1]. This has led to the classification of brucellosis by the World Health Organization (WHO) as one of the “top 10” neglected zoonoses, a group of diseases that are simultaneously ongoing threats to human health and a source of perpetuation of poverty.

The Disease

In animals, brucellosis is recognized as a reproductive disease often leading to abortion in the middle to last trimester of gestation (sheep, goats, cattle, dogs, and pigs), following bacterial colonization of the placenta (Table 1). Other reproductive symptoms associated with infection include apparent failure to conceive or stillbirths. In males, *Brucella canis* targets the epididymis and prostate, a feature shared by *Brucella ovis* and infrequently by other *Brucella* species [2]. Less commonly reported clinical signs include arthritis, discospondylitis, and carpal hygromas [3–5]. The range of signs of infection can vary from asymptomatic to severe, despite ongoing systemic infection [6, 7]. Although *Brucella*-induced abortion is commonly associated with agricultural species and dogs, it is also been reported among other hosts including dolphins, pinnipeds, camels, and non-human primates [8–12].

Traditionally, clinical signs associated with human infection are different from those described in animals. Human brucellosis is referred to as a “flu-like illness” characterized by a non-specific clinical syndrome with relapsing fever and arthritis being the most commonly reported symptoms [7]. *Brucella* infection can also cause splenomegaly or hepatomegaly, as well as life-threatening conditions including endocarditis and various neuropathies [7, 13]. The lack of pathognomonic signs associated with infection, along with the absence of accurate point of care diagnostic assays, has led to the misassumption that presentation of fever without any other associated condition in endemic countries is indicative of malaria. This in many cases impedes accurate assessment of the overall spectrum of disease manifestation. The lack of accurate diagnostic tools, as well as non-specific symptomatology, has made it extremely challenging to prevent and control the disease, making brucellosis a classic example of an old zoonotic disease that is emerging and re-emerging worldwide [14].

Obstetric Outcomes in Pregnant Women with Brucellosis

In contrast to animal brucellosis, pregnancy-associated complications associated with human brucellosis are thought to be uncommon and are rarely described in the literature. However, early reports of human miscarriages date to 1908, when a case of abortion in a pregnant farmer’s wife was associated with *Brucella* infection [15]. Since this initial observation of human abortion associated with brucellosis more than 100 years ago, less than 40 reports of

adverse pregnancy outcomes have been documented in the literature [16–20, 21•, 22–50]. The majority of these reports consist of individual case reports describing patients residing in low to middle-income countries, where the capacity to conduct appropriate diagnostic examinations is very limited, thus preventing recognition of any association between brucellosis and human pregnancy. More recently, larger retrospective studies investigating the outcomes in pregnant women with a confirmed *Brucella* infection have demonstrated a strong association between infection and an increased incidence of adverse obstetric outcomes [19, 21•, 22, 27, 41]. These larger case studies (Table 2) have been conducted in the Middle East (Turkey, Saudi Arabia), South America (Peru), and Africa (Rwanda), with spontaneous miscarriage rates ranging from 18.6 to 73.3 % [19, 21•, 22, 27, 41]. Interestingly, the majority of the cases are documented to occur during the first and second trimester of gestation (first trimester of pregnancy is defined as a gestational age of <12 weeks, second trimester is considered between 12 and 24 weeks, and third trimester is >24 weeks), and differs from the time of occurrence of abortion in animals, commonly manifested as a late gestational event [51].

The second most commonly documented adverse event occurring in humans is preterm delivery. Preterm birth, defined as the birth of an infant before 37 weeks of pregnancy, is the leading cause of death in children under the age of 5, with as many as 10–11 % of all births estimated to be preterm [52]. However, the incidence of preterm birth in low- to middle-income settings is considerably higher with estimates reaching 15 to 24 % with the highest reported rates occurring in sub Saharan Africa and Asia, in which preterm births associated with brucellosis ranged from 6.9 to 72 % (Table 2) [25, 27, 32, 36, 41–43]. This is a significant finding, not only because the majority of brucellosis occurs in sub Saharan Africa and Asia, but also because of the elevated incidence of child mortality in low-income settings, due to lack of feasible, cost-effective care [52]. In children that survive, preterm delivery is considered to be a major determinant of immediate as well as long-term morbidity and is associated with growth and developmental delay [52]. This clearly suggests the critical need for increased awareness of potential risks associated with brucellosis, especially in endemic countries, where, despite the known-association of preterm delivery with well-identified infectious agents (e.g., *Plasmodium falciparum*, *Listeria monocytogenes*, *herpes simplex virus*, and *influenza*), brucellosis has not been associated with this side-effect. Preterm birth has also been commonly reported for many years in animals, commonly referred to as preterm whelping in bitches and preterm farrowing in sows [53–55]. Another adverse pregnancy outcome reported in the Peruvian study, as well as in single case reports, is “congenital brucellosis.” This is not surprising, since vertical transmission to the fetus is a well-documented effect in animals [2, 3, 51]. In this human case series, Vilchez et al. confirmed that of 86 brucellosis patients, 4.6 % had vertical transmission to the fetus [22].

Clinical signs in pregnant women with brucellosis are nonspecific, ranging from asymptomatic to repeated episodes of excessive sweating and arthralgia, fever, and vaginal bleeding [32]. However, no correlation has been identified between any single clinical sign and pregnancy outcome. Furthermore, 90–95% of all the cases available in the literature have had a history of high-risk factors for *Brucella* infection, including ingestion of non-pasteurized dairy products and close proximity with animals. Interestingly, prompt diagnosis

and treatment significantly decreased the risk of miscarriage and other adverse effects, clearly demonstrating the importance of a rapid and accurate diagnosis to improve pregnancy outcomes in endemic areas [19].

Pregnancy and the Immune System

Pregnancy poses a unique challenge for the maternal immune system [56, 57]. Infection during pregnancy differs from infection in non-pregnant individuals as the presence of the fetus and placenta alter maternal immunity and physiology in order to sustain pregnancy. Successful pregnancy requires the maternal host to effectively balance the opposing processes of maternal immune reactivity to the infectious agents while maintaining tolerance to the fetus [56]. For many years, the uterus and amniotic cavity were considered sterile environments. However, this concept has been reviewed in recent studies demonstrating the presence of a “placental microbiome” during healthy pregnancy [58]. These findings further suggest that mechanisms preventing or limiting invading microbial proliferation and pathological consequences are in place to sustain pregnancy [59]. For many years, it was assumed, that pregnancy was associated with a state of cell-mediated immune suppression that subsequently increased susceptibility to intracellular pathogens. As a result, a T helper 2 (Th2)-biased immune response was considered the main reason pregnant women were capable of controlling extracellular pathogens more efficiently [60–62]. This model was considered substantiated by the clinical observation that cell-mediated inflammatory disorders including the clinical signs associated with rheumatoid arthritis were ameliorated during pregnancy, whereas antibody-mediated disorders, such as those observed in systemic lupus erythematosus symptomatology, were exacerbated [62]. Today, increasing evidence suggests that the immune system during pregnancy is fully functional and that the placenta and the decidua (uterine lining during pregnancy) represent important immune modulators affecting the global immunological response [57]. Nevertheless, some pathogens are capable of breaching the maternal-fetal barrier which can lead to adverse obstetric outcomes such as abortion, preterm delivery, or congenital infections. Just how some pathogens are capable of evading immune mechanisms in place is only partially understood at this time.

Pregnancy and Placenta

In an effort to understand the mechanisms leading to placental and fetal infection, it is necessary to consider the different components of the placenta and decidua. The placenta, composed of maternal and fetal tissues, performs a number of important functions throughout gestation including (i) anchoring of the developing fetus to the uterine wall, (ii) oxygen/carbon dioxide exchange, (iii) fetal nutrition, (iv) waste product removal, and (v) maternal immune tolerance [63]. Placentas of different mammals exhibit great differences at the maternal-fetal interface. This is an extremely important characteristic to consider when extrapolating physiological, immunological, or any other observation across species. In general, placentas are classified based on the histological structure of the maternal-fetal interface (epitheliochorial, endotheliochorial, hemochorial; Fig. 1a–c), the type of maternal-fetal interdigitation, and the gross aspect (diffuse, cotyledonary, discoid, or zonary) [63]. In humans, the placenta is composed of individual units termed chorionic villi. Each villus has a connective tissue core that contains (1) mesenchymal cells, (2) macrophages, termed

Hofbauer cells, (3) fetal vascular cells, and (4) trophoblasts. Trophoblasts are fetal-derived cells that, depending on their differentiation, pose different roles. The trophoblast population within the placental villous surface is characterized by an inner layer of cytotrophoblasts that either fuse to form the overlying multinucleated syncytiotrophoblasts or assume invasive capabilities in anchoring villi (extravillous cytotrophoblast, EVT) that attaches the placenta to the uterus. Syncytiotrophoblast cover the entire surface of villous trees and is in direct contact with maternal blood, providing an abundant surface area for gas and nutrient exchange for the mother and fetus [64]. EVTs migrate through the decidua and enters the fetal spiral arterial walls, providing an anchoring capability. Previous studies have demonstrated that trophoblasts express pattern recognition receptors that function as "sensors" capable of recognizing the presence of bacteria, viruses, and parasites present in the surrounding environment, and are capable of secreting cytokines and chemokines able to act on cells of the innate immune system present in the decidua guiding them to work together in support of the growing fetus [65]. This supports the theory that the placenta plays an active role during immune regulation. Within the decidua, there are unique immune cell populations that actively contribute to the fetal tolerance and immunity of the placenta. This cell population consists of uterine natural killer (uNK) cells that in humans represent approximately 70 % of the total leukocyte population and are critical for the development of the placenta [66, 67]. uNK cells are also believed to play a role in facilitating invasion by fetal HLA-G+ extravillous trophoblasts (EVT) into maternal tissues for the establishment of healthy pregnancies. In addition, uNK contain cytotoxic granules, functioning in immunity to viral infections. Interaction of these cells with EVT leads to the acquisition of HLA-G. Thus, uNK cells provide tolerance as wells as anti-viral immunity [68]. Macrophages are the predominant subset of antigen-presenting cells (APC) and compromise about 20–25 % of the total decidua leukocytes, and are necessary for a wide range of gestational processes including implantation, ovarian function, placental development, and immunity [69].

Mechanism of *Brucella*-Induced Abortion in Animals and Humans

Studies conducted over the last 30 years have demonstrated a correlation between an increased rate of adverse pregnancy outcomes with infection by certain microbial agents [70]. Historical, experimental, and epidemiological evidence supports the concept that *Brucella* infection in animals is a significant risk factor for abortion [19, 21•, 22]. Despite this evidence, very little is known about the mechanism of infection-induced abortion. Placental tissue tropism for *Brucella* was originally defined in ruminants based on (i) preference for erythritol as a carbon source, (ii) elevated erythritol levels in the ruminant placenta, and (iii) growth inhibition exhibited by erythritol sensitive *B. abortus* vaccine strain S19 [71]. However, genetic experimentation, in which restoration of the erythritol locus (*ery*) did not restore S19 to virulence and deletion of the *ery* locus from virulent *B. abortus* S2308 did not attenuate virulence, disproved this hypothesis [72]. Although genetic experimentation has disproved any relationship between virulence and erythritol utilization, placental tissue tropism remains a well-documented phenomenon in ruminants [73]. In an effort to identify cellular tropism and the mechanism of *Brucella*-induced abortion, the uterus and placenta from pregnant goats inoculated intravenously with a single dose of highly virulent *B. abortus* were evaluated via light and electron microscopy [74]. Placental

infection was observed as early as 5 days post inoculation with the subsequent occurrence of abortion within 11 days. Interestingly, *Brucella* were observed within trophoblasts and chorionic villi [75]. More recently, Salcedo et al. investigated the ability of *Brucella* spp. to infect human trophoblasts using different cell lines and primary cultures representing the different trophoblast populations present in the placenta. Trophoblast colonization and replication was observed in the JEG-3 (EVT-like cells) when cells were infected using *B. abortus* or *B. suis*; however, replication was unusual since it was not completely dependent on *virB* type IV secretion system and replication was observed in large acidic LAMP-1 and CD63 positive compartments. *B. melitensis*, however, was able to replicate in a typical *Brucella* containing vacuole BCV compartment in a *virB* type IV-dependent manner. When other cell lines reflecting a syncytiotrophoblast phenotype were used, *B. abortus* was not found in acidic, LAMP-1 positive inclusions, but in ER-derived BCV [76•]. More recently, Fernandez et al. investigated the ability of *B. abortus* to infect a cytotrophoblast cell line Swan-71, demonstrating that *B. abortus* was capable of replicating inside these cells but survival was *virB*-type IV dependent. Infection elicited secretion of IL-8, MCP-1, and IL-6, and the authors suggested that trophoblasts may provide a local inflammatory environment that could potentially contribute to abortion [77•] (Fig. 1d).

Conclusions: Comment on Future Work

It is obvious that in order to address the important questions raised in this review pertaining to the precise details and/or mechanism of *Brucella*-induced abortion, there is the need to develop model systems capable of appropriate investigation. From an agricultural perspective, small ruminants represent one potential model. Reduction in human disease closely parallels reduction in animal disease. Thus, the ability to eliminate transmission may be expected to have a significant impact on public health in a relatively short period of time. Direct intervention to reduce human disease may follow the development of primate models based on similarities in placental structure. In either case, support for the development of in vivo systems is warranted based on the variability observed in tissue culture systems related to differences in experimental outcomes including novel trafficking, reduced virulence, and variable readouts associated with the use of different cell lines. Although work has focused on the capacity of *Brucella* to replicate in trophoblasts, it is unclear whether such replication reflects the prime function of these cells, i.e., to protect the placenta and intercept pathogens or the precipitating event leading to pathology. In contrast, examination of organism distribution in the infected placenta over time may be expected to provide improved insight with regard to abortion and intracellular invasion/replication.

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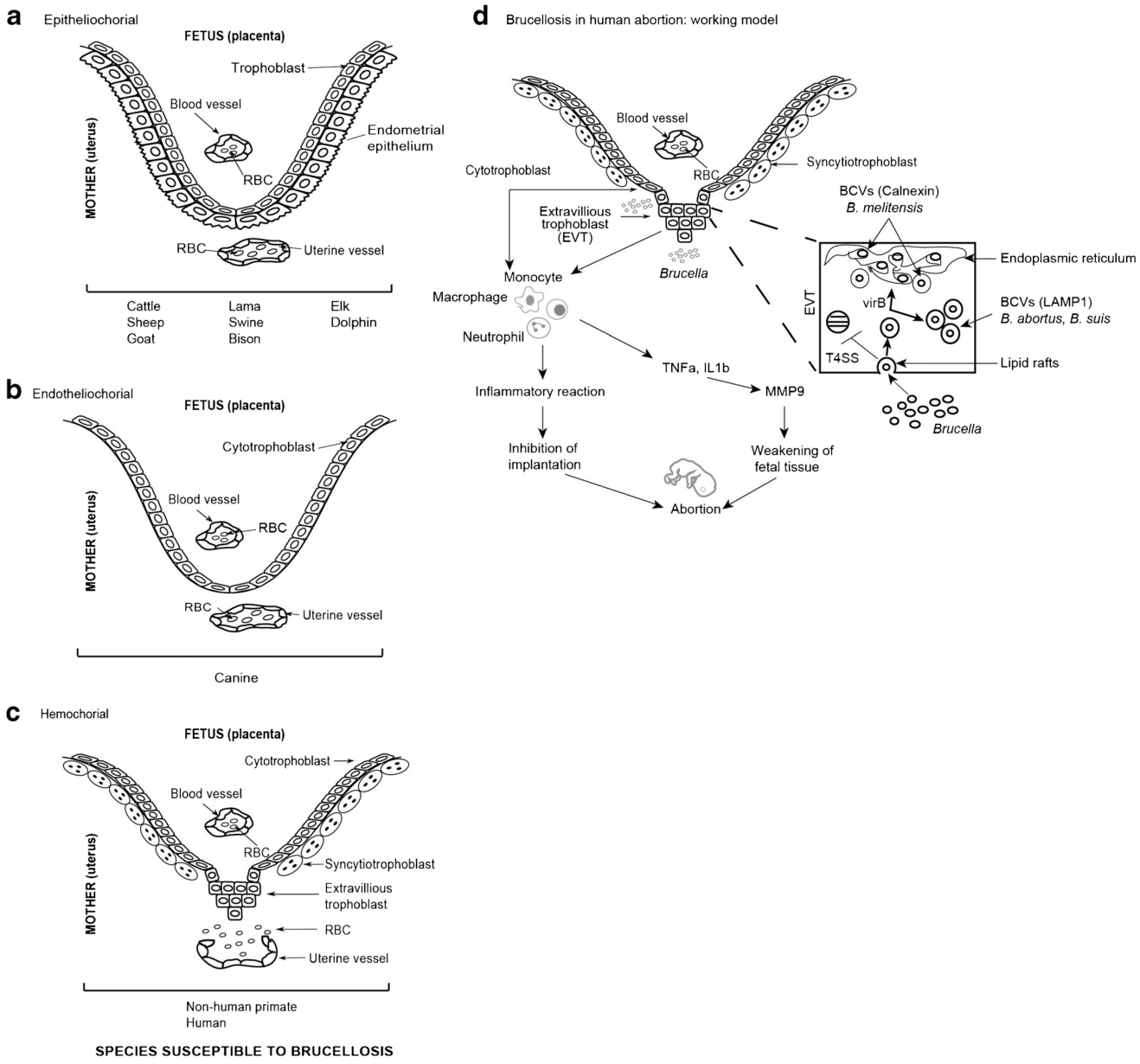

Fig. 1.

Schematic representation illustrating the relationship between the fetal trophoblast cells and maternal blood of the three main types of placentation, susceptible to *Brucella* infection (**a**–**c**) and the working model regarding the pathogenesis of brucellosis in human abortion (**d**). **a** Epitheliochorial: Trophoblast cells are in direct apposition with the surface of the uterine epithelial cells with no trophoblast invasion beyond this layer. **b** Endotheliochorial: The uterine epithelium is breached and trophoblasts are in direct contact with endothelial cells of maternal uterine vessels. **c** Hemochorial: Maternal blood directly bathes the chorionic villi. **d** *Brucella* in human pregnancy target trophoblasts to survive and replicate. *B. abortus* and *B. suis* replicate inside LAMP1 positive acidic vesicles, whereas *B. melitensis* replicate inside vesicles positive for endoplasmic reticulum marker calnexin and inhibits implantation of trophoblasts. *Brucella*-infected cytotrophoblasts secrete cytokines and interleukins like

IL8, IL6, MCP1 that cause infiltration of neutrophils, macrophages, and monocytes in the placenta. The inflammatory reaction might lead to implantation inhibition and abortion of the fetus. At the same time, macrophages and neutrophils secrete TNFα and IL1β that cause increase in MMP9 secretion potentially leading to weakening of fetal tissue and abortion

Table 1

Abortions in animals

Susceptible species	Type of placenta	Average length of gestation (days)	Most susceptible stage for abortion	*Brucella* species	Reference
Baboon (*Papio* spp.)	Hemochorial	180	117 days	*Brucella* spp.	[8][a]
Bison	Epitheliochorial	283	195–225 days	*Brucella abortus*	[78, 79]
Camel	Epitheliochorial	396	–	*Brucella melitensis*	[80][b]
Canine	Endotheliochorial	65	45–55 days	*Brucella canis*	[81, 82]
Cattle	Epitheliochorial	283	226–242 days	*Brucella abortus*	[78]
			Mid to late gestation	*Brucella* spp.	[83][c]
			180–283 days	*Brucella abortus*	[84, 85]
Dolphin (*Tursiops truncates*)	Epitheliochorial	365	270 days	*Brucella delphini* (ceti)	[86][d]
Elk	Epitheliochorial	250	35–84 days post challenge	*Brucella abortus*	[87, 88]
Goats	Epitheliochorial	150	117–135 days	*Brucella melitensis*	[89–92]
Lama	Epitheliochorial	345	240 days	*Brucella abortus*	[93]
Sheep	Epitheliochorial	148	107–146 days	*Brucella melitensis*	[92, 94–96]
Swine	Epitheliochorial	114	May occur at anytime	*Brucella suis*	[51, 81, 97]

[a] Reported two cases of stillbirths in baboons with positive culture of a novel *Brucella* spp. isolate confirmed by PCR

[b] Based on aborted camels tested by RBT and confirmed by CFT. *Brucella melitensis* was isolated from two fetuses

[c] Based on 150 abortion cases positives to RBT and confirmed by PCR

[d] *Brucella* spp. were isolated from two specimens of *Tursiops truncates* that aborted

Table 2

Reported case series of adverse pregnancy-associated complications in pregnant women diagnosed with brucellosis

Study year and country	Pregnant women[a]	Diagnostic criteria	Spontaneous abortion rate	Preterm delivery rate	Intrauterine fetal death rate	Associated clinical signs	Reference
2015, Peru	86	SAT (>1/160) and blood culture	16 (18.6 %)	12 (13.9 %)	7 (8.14 %)	Osteoarticular, haematological and congenital disorders	[22]
2014, Africa	15	RBPT	11 (73.3 %)	–	4 (26.7 %)	–	[41]
2011, Turkey	39	Coombs test (>1/160) and blood culture	1 (2.56 %)	7 (17.95 %)	–	Fever, arthralgia, myalgia, hepatospenomegaly	[32]
2011, Iran	19	SAT (>1/160), 2ME titer >1/80	10 (53 %)	–	–	Fever, arthralgia, back pain, sweating	[31]
2010, Turkey	29	SAT (>1/160) and blood culture	7 (24.14 %)	2 (6.9 %)	1 (3.45 %)	Sacroiliitis with Joint pain, hepatosplenomegaly	[27]
2008, Saudi Arabia and Kuwait	55	SAT (>1/160)	19 (34.5 %)	13 (23.6 %)	11 (20 %)	Common symptoms and signs of brucellosis in 35 women	[25]
2001, Saudi Arabia	92	SAT (1:2560) and blood culture	40 (43.5 %)	–	2 (2.17 %)	Vaginal bleeding, febrile illness, 24 patients with recurrent abortion	[21•]
2001, Saudi Arabia	30	SAT and blood culture	12 (41 %)	9 (30 %)	1 (3.4 %)	Fever, arthritis, headache, sweating,	[42]
1998, Kuwait	25	SAT (1/160), ELISA and tissue culture	2 (8 %)	18 (72 %)	5 (20 %)	Fever, arthralgia, myalgia backache, headache, anorexia	[43]
1991, Lebanon	6	SAT (>1/160, direct >1/80)	1 (17 %)	1 (17 %)	–	Fever, back pain, sweating, fatigue and malaise, chills	[36]
1988, Kuwait	35	SAT (>1/160) and ELISA: (IgG>1/1600, IgM>1/400, IgA≫1/200)	11 (31.4 %)	–	–	Fever, arthralgia, back pain, hepatosplenomegaly, headache, sweating	[44]
1974, Iran	51	SAT, urine, uterine and blood culture	6 (11.6 %)	–	–	Undulant fever, arthralgia, sweating and muscle pain	[45]
1954, Argentina	200	SAT	52 (26 %)	–	–	Febrile and non-febrile	[38]

[a]Positive for *Brucella*. Fetal death before 24 weeks of gestation is considered spontaneous abortion and fetal death after 24 weeks of gestation is considered intrauterine fetal death. Delivery before 37 weeks is considered preterm delivery. *SAT* serum agglutination test, *RBPT* Rose Bengal plate test