Epidemiology of brucellosis in domestic animals caused by *Brucella melitensis*, *Brucella suis* and *Brucella abortus*

E. Díaz Aparicio

Centro Nacional de Investigación Disciplinaria en Microbiología Animal (CENID Microbiología), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), Km 15.5 Carretera Federal México–Toluca, C.P. 05110, Mexico, DF, Mexico
E-mail: efredia@yahoo.com

Summary
Brucellosis is a disease that causes severe economic losses for livestock farms worldwide. *Brucella melitensis, B. abortus* and *B. suis*, which are transmitted between animals both vertically and horizontally, cause abortion and infertility in their primary natural hosts – goats and sheep (*B. melitensis*), cows (*B. abortus*) and sows (*B. suis*). *Brucella* spp. infect not only their preferred hosts but also other domestic and wild animal species, which in turn can act as reservoirs of the disease for other animal species and humans. Brucellosis is therefore considered to be a major zoonosis transmitted by direct contact with animals and/or their secretions, or by consuming milk and dairy products.

Keywords
*Brucella abortus* – *Brucella melitensis* – *Brucella suis* – Brucellosis – Epidemiology.

Introduction

The main characteristic of the *Brucella* genus is its ability to survive within phagocytic and non-phagocytic cells. While a wide variety of factors explain the capacity of the *Brucella* genus to multiply and spread to new cells, so far no single factor has been shown to be responsible for its virulence (19).

*Brucella* usually enter the body via the oral route and lodge in the mucosa, where the bacteria are ingested by professional phagocytes beneath the sub-mucosa. Once internalised, *Brucella* is localised in a vacuole that matures from an early to a late endosome and, unless destroyed, goes on to multiply in the endoplasmic reticulum of macrophages. However, not all *brucellae* survive: where bacteria are not sufficiently numerous and the animal has a competent immune system, they are directed towards the lysosomes where they are destroyed and the major histocompatibility complex on the cell surface presents the peptides to Th1 and Th2 lymphocytes to elicit an immune response (6, 43).

These bacteria multiply abundantly in the placental cotyledons, chorion and fetal fluids, where they cause lesions in the organ wall, inducing endometriosis ulcerosa in the intercotyledonary spaces and destruction of the villi, leading to death and expulsion of the fetus (75). Three species of *Brucella* affect humans: *B. melitensis, B. abortus* and *B. suis* (other species can cause infection in humans, but only rarely). Of these three species, infections by *B. melitensis* are the most common in humans and are also the most serious (55).

Epidemiology of *Brucella abortus*

Bovine brucellosis is usually caused by *B. abortus*. Non-bovine animals, including humans, can also contract the disease and play a role in its persistence and transmission. *Brucella abortus* has seven recognised biovars, the most reported of which are biovars 1, 2, 3, 4 and 9, with biovar 1 being the most prevalent in Latin America. The distribution of biovars could be important in ascertaining the source of some infections (22, 31, 33, 46, 76). Bovine brucellosis is reported in virtually all countries where cattle are farmed, with some northern and central European countries, Australia, Canada, Japan and New Zealand considered free. In 2008, 12 European Union countries were considered officially free from bovine brucellosis, as well as caprine and ovine brucellosis. In 2008, 15 countries
that were not free reported cases of bovine brucellosis (herd prevalence of 0.12%). The situation is less favourable in southern Europe but, even there, prevalence is less than 1% (28). Although all states of the United States (USA) are ranked free from B. abortus in cattle, the infection remains in wildlife in and around the Yellowstone area, with occasional spread to cattle. Brucellosis-infected cattle herds have been detected in the states of Montana, Wyoming and Idaho since 2007. The primary source of infection for cattle is believed to be elk (Cervus elaphus) (67, 73).

Ruminants are generally susceptible to B. abortus, which is of particular relevance in areas where eradication programmes are in operation. Buffaloes, camels, deer, goats and sheep are highly susceptible to infection (20, 23). Brucellosis has been reported in the one-humped camel (Camelus dromedarius) and two-humped camel (C. bactrianus), as well as in a number of South American camelids – llama (Lama glama), alpaca (Lama pacos), guanaco (Lama guanicoe) and vicuña (Vicugne vicugne) – following contact with ruminants infected with B. abortus or B. melitensis. It has also been observed in the water buffalo (Bubalus bubalis), American bison (Bison bison), European bison (Bison bonasus), yak (Bos grunniens) and elk (Cervus elaphus), as well as in the African buffalo (Syncerus caffer) and several species of African antelope. The manifestations of brucellosis in these animals are similar to those of bovine brucellosis and can become epidemiologically important in sustaining the infection in cattle where they share pasture and water holes (20, 23, 30, 35, 71). The infection is prevalent in horses cohabiting with cattle, it presents with characteristic swelling of the supraspinous bursa, known as fistulous withers. The infection is usually transmitted to pigs by feeding them whey as a by-product from cheese-making (52, 54). Brucella abortus infection of dogs has been demonstrated orally, by the ingestion of weak calves, but infected cows suffer repeated uterine infections, with microorganisms evident in birth products and milk. This means that such cows will continue shedding large numbers of brucellae at delivery that will go on to become sources of infection for other animals. Adult males can develop orchitis, and brucellosis can cause sterility in both sexes. Neutered males used for artificial insemination could be important. Infected calves of infected cows. While the venereal route is not generally considered to be epidemiologically important in transmitting brucellosis in cattle, infected semen used in artificial insemination could be important. Infected cows shed Brucella in their milk and this is key in its transmission to calves. Infected semen used in artificial insemination could be important. Infected cows shed Brucella in their milk and this is key in its transmission to calves. In dairies, milking is another mode of transmission that must be taken into account because the bacteria are highly likely to be transmitted from cow to cow if the same teat cups are used for milking. For this reason, it is recommended that healthy cows be milked first and infected cows last (61, 62, 71).

The disease is usually asymptomatic in non-pregnant females, but pregnant adult females infected with B. abortus develop placentitis, which normally causes abortion between the fifth and ninth month of pregnancy. Even in the absence of abortion, there is heavy shedding of bacteria through the placenta, fetal fluids and vaginal exudates. The mammary gland and regional lymph nodes can also be infected and bacteria can be excreted in milk (56, 71).

Vertical transmission was proved by Plommet, who states that between 60% and 70% of the fetuses born to infected mothers carry the infection. Female calves can also be infected during birth when passing through the birth canal, or by suckling colostrum or milk from infected cows. While most of these calves rid themselves of infection by feeding on aborted fetuses, dragging them along and spreading the bacteria (8, 15).

A herd becomes infected with Brucella when animals that are infected but not yet diagnosed are introduced into it. Livestock fairs and shows are a risk, since animals can become infected and infect other animals when they return to their herd of origin. It is therefore advisable not to purchase animals that are infected or do not come from brucellosis-free herds (61, 62).

Cows aborting in stables and farmyards are the main factor for the spread of brucellosis. It has been determined that cows infected with Brucella are three to four times more likely to abort than unexposed cows (51, 63).

The main route of entry for Brucella is oral, by the ingestion of food or water contaminated with secretions or aborted fetal remains from infected cows, or by licking the vaginal secretions, genitals, aborted fetuses or newborn calves of infected cows. While the venereal route is not generally considered to be epidemiologically important in transmitting brucellosis in cattle, infected semen used in artificial insemination could be important. Infected cows shed Brucella in their milk and this is key in its transmission to calves. In dairies, milking is another mode of transmission that must be taken into account because the bacteria are highly likely to be transmitted from cow to cow if the same teat cups are used for milking. For this reason, it is recommended that healthy cows be milked first and infected cows last (61, 62, 71).

After contracting the infection, cows commonly abort during their next pregnancy, but 80% of infected cows abort only this once. In most cases, subsequent pregnancies will come to term with no apparent signs, or with the birth of weak calves, but infected cows suffer repeated uterine and mammary infections, with microorganisms evident in birth products and milk. This means that such cows will continue shedding large numbers of brucellae at delivery that will go on to become sources of infection for other animals. Adult males can develop orchitis, and brucellosis can cause sterility in both sexes. Neutered males used for artificial insemination could be important. Infected calves of infected cows. While the venereal route is not generally considered to be epidemiologically important in transmitting brucellosis in cattle, infected semen used in artificial insemination could be important. Infected cows shed Brucella in their milk and this is key in its transmission to calves. In dairies, milking is another mode of transmission that must be taken into account because the bacteria are highly likely to be transmitted from cow to cow if the same teat cups are used for milking. For this reason, it is recommended that healthy cows be milked first and infected cows last (61, 62, 71).

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There have been reports of cows infected with B. abortus that test seronegative. Brucella abortus was isolated in 119 milk samples from seronegative cows and the isolates were biovars 2, 3 and 9 (76). In Mexico, the field strain of B. abortus with a smooth phenotype is reported to have been isolated from the vaginal exudate of two primiparous cows, vaccinated with reduced-dose RB51, that had calved normally and were seronegative (7).
In stables with a high prevalence of brucellosis, a major risk factor for spread is when infected cows contaminate the herd's pasture, where Brucella can remain viable for long periods assisted by adequate moisture levels. The microorganism becomes even more resistant in the presence of organic material (1).

Even though cattle have been shown to exhibit considerable variation in their immune response to disease challenges, and much of this difference is attributable to genetics, cattle breeds are not genetically susceptible or resistant to brucellosis. The higher incidence of brucellosis in dairy cattle breeds are not genetically susceptible or resistant to Brucella suis. The higher incidence of brucellosis in dairy cows is the result of the close cohabitation that results from this form of farming (50, 69).

Epidemiology of Brucella suis

While domestic pigs are infected mainly by B. suis, less frequently they may become infected with B. abortus or B. melitensis in regions where brucellosis is endemic in cattle or small ruminants. There are five biovars of B. suis, with 1, 2 and 3 being responsible for porcine brucellosis worldwide (27).

Brucella suis biovars 1 and 3 are distributed worldwide in most areas where there are pigs. They affect both sexes, causing infertility, abortion, orchitis and bone and joint lesions. Biovar 1 is present in the Americas and Asia, while biovar 3 has been reported in China, the USA, and Europe (72). Prevalence is generally low, except in parts of South America and South-East Asia. Within the European Union, the epidemiological status of porcine brucellosis varies. Some countries are free from the disease, while others report sporadic outbreaks and some report infections as an emerging problem. Available epidemiological evidence shows that, in Europe, B. suis biovar 2 is the most common source of infection in pigs but biovars 1 and 3 are also present (27, 34).

Brucella suis is moderately influenced by environmental factors, with the microorganism's survival time in the environment decreasing as temperatures rise. However, the bacteria often survive dessication and can survive freezing temperatures for over two years (58). Facilities and pasture can remain contaminated for long periods but direct sunlight reduces the bacteria's survival significantly (38).

The B. suis entry sites are similar to those identified for other types of Brucella infection, being essentially the oral, nasopharyngeal, conjunctival and vaginal mucosa. There is generally a relatively long incubation period before clinical signs appear. These are not usually visible in young animals, and their occurrence will depend mainly on the age, sex and physiological state of animals at the time they are infected.

As an example, animals infected during critical periods of the pregnancy (the first third to half of pregnancy) will abort approximately 30 to 45 days after infection. However, animals infected at full term will not abort, and animals infected out of the pregnancy period will not abort during their next pregnancies (26).

In a primary infection with B. suis in pig farms, the bacteria can spread within a few months from one infected pig to more than 50% of animals on the farm. The infection can often reach 70% to 80% of infected animals at the start of the outbreak (11, 56). As there tend to be few or mild clinical signs, the disease can go unnoticed in infected herds. However, recently infected herds may manifest major signs of infection, such as a high percentage of abortions, increased neonatal mortality and infertility, causing adverse economic consequences. Although the minimum infective dose of the various B. suis biovars has yet to be established, it has been demonstrated that doses as low as 10^4 to 10^5 colony-forming units are sufficient to infect most pigs challenged by the conjunctival route, but the severity of the infection is not correlated with the dose nor with the route of challenge (18).

Porcine brucellosis is believed to affect both sexes equally and age is no determinant of susceptibility, although this is not proven (4). It has also been reported that some pig breeds, such as Duroc and Jersey Red crosses, may be less susceptible to experimental challenge with B. suis, which suggests the existence of genetic resistance to infection (16). The main risks associated with the entry of porcine brucellosis into pig farms are: the introduction of infected animals, contact with wildlife reservoirs, and artificial insemination with semen from infected boars. Infected boars can shed 10^3 to 10^5 colony-forming units of B. suis per millilitre of semen (45), with semen being one of the routes of spread in artificial insemination. Many combinations of antibiotics are used to preserve semen in the mistaken belief that this inactivates the pathogens; in fact, no combination of commercial antibiotics is capable of completely inactivating B. suis in semen (27).

While there is no hard evidence to prove it, as with other animal species, infected sows can also transmit infection to their piglets, either transplacentally (resulting in the birth of seronegative latent carriers) or when piglets ingest B. suis in their mother's milk, as the bacterium is shed in the milk of infected sows (4).

Other domestic species apart from pigs may be susceptible to B. suis infection. Horses kept in close contact with infected pigs can become infected, as evidenced by fistulous withers (23). The infection has also been reported in dogs, causing lameness and granulomatous lesions in genital organs (59). While all states in the USA, with the exception of Texas, are classified as free from porcine brucellosis (20), B. suis was
isolated from the testicles of two dogs on small farms in the state of Georgia (54). Similarly, B. suis biovars 1 and 3 have been isolated as the cause of brucellosis cases in cattle (29).

Brucella suis biovar 4 causes fever, depression, abortion, retained placentas, mastitis, bursitis and orchitis in reindeer and caribou (Rangifer tarandus and its various subspecies) throughout the Arctic region, including Alaska, Canada and Siberia (72). Arctic foxes and wolves may contract B. suis biovar 4 from reindeer and dogs, while rodents, such as rats and mice, may acquire other B. suis biovars by cohabitation with infected hosts. In Australia, Kenya and the former Union of Soviet Socialist Republics there have been reports of small rodents becoming infected with B. suis biovar 5 (72).

Brucellosis infection caused by B. suis biovar 2 differs from that caused by biovars 1 and 3 in terms of geographical distribution, host and virulence, and is considered less pathogenic for humans than the highly infectious biovars 1 and 3; humans must be immunocompromised to become infected with biovar 2 (33, 44, 47).

Although the epidemiological cycle of B. suis biovar 2 has not been described fully, there is evidence of a close relationship between infection in domestic animals and infection in wildlife. Biovar 2 has been isolated in wild boar (Sus scrofa) in Belgium, Croatia, France, Germany, Spain and Switzerland (23, 36, 42, 52, 74), as well as in the European hare (Lepus europaeus) (65). Brucella suis biovar 2 can be transmitted to cattle (29, 32), dogs (10) and horses (23).

Epidemiology of Brucella melitensis

Brucella melitensis is the most virulent species of the Brucella genus and has three biovars, with biovars 1 and 3 being the ones isolated most frequently in small ruminants in the Mediterranean, the Middle East and Latin America (14, 46). Brucellosis is a barrier to trade in animals and animal products and causes significant losses from abortion, as well as being a serious zoonosis (9, 12, 64).

Goats are the classic and natural host of B. melitensis and, together with sheep, are its preferred hosts. In pathological and epidemiological terms, B. melitensis infection in small ruminants is similar to B. abortus infection in cattle: the main clinical manifestations of brucellosis in ruminants are abortion and stillbirths, which usually occur in the last third of the pregnancy following infection and usually only once in the animal’s lifetime (14, 25).

Healthy animals can be exposed to Brucella infection in many ways, as a large number of bacteria are shed in the birth fluids or fetus, placenta and abortion secretions of infected females. The bacteria have the ability to survive several months outdoors, especially in cold, wet conditions, where they remain infectious to other animals, mainly through ingestion. Brucellae also colonise the udder and contaminate milk (9, 13). Although females calve apparently normally in pregnancies following the first abortion, they continue to shed large numbers of bacteria into the environment.

As with B. abortus infection in cows, B. melitensis can be transmitted congenitally in utero but only a small proportion of lambs and kids are infected in this way and most latent infections of B. melitensis are probably acquired by ingesting colostrum or milk (37). Despite the low transmission rate, the existence of such latent infections makes it even more difficult to eradicate the disease because, as the bacteria persist without inducing detectable immune response, infected animals are silent carriers of the disease. It is therefore recommended that infected females and their offspring be culled as part of an eradication programme in infected herds (9). The exact mechanism enabling latent Brucella infection to develop is unknown (14).

Some female hoggets testing seropositive to brucellosis have been found to shed B. melitensis in milk postpartum, whereas others do not shed brucellae despite being infected. While lambs sampled for seven months showed seropositivity, some tested seronegative for brucellosis in routine tests even though a post-mortem study later revealed them to have been infected with B. melitensis. This was also observed in lambs from the same herd that had been born from mothers seronegative for brucellosis (34). A previously unreported fact is that B. melitensis was successfully isolated from the vaginal discharge of a goat that had aborted but tested seronegative for brucellosis, making the animal a potential risk for spread undetectable by serological diagnosis (39).

While orchitis and epididymitis are uncommon in rams and billy goats, they do occur (21). Brucella melitensis biovar 3 has been isolated from a testicular hygroma of a ram (53). Brucella melitensis can infect not only cattle but also calves, through the ingestion of infected milk (5, 41, 68). The isolation of B. melitensis in dogs has been demonstrated and this has been observed to favour incidence of the disease, as dogs can drag placenta or aborted fetuses to uninfect ed areas (40, 48). There is one report of B. melitensis biovar 3 having been isolated from a black bullhead catfish (Amia calva) (24), but clarification is needed as to whether such animals can act as disease carriers or whether the finding was a result of water pollution.

In extensive goat and sheep farms, it is common practice for herds to share pasture and water holes before returning to their pens. Such mixing of animals is a factor of risk for spreading the disease from infected to free herds and makes it harder to control. In this case, all goats sharing such sites
must be considered as a single, large herd, and all goat farmers must carry out control activities, e.g. vaccination and the separation of positive and negative animals. Failure by goat farmers to act will negate the efforts of those that do take action to reduce the incidence of brucellosis (2, 3, 60).

Any strategy for the control or eradication of brucellosis should begin by establishing the different epidemiological contexts within a country or even a region or district, and must have the support and collaboration of farmers. Above all, the effectiveness of any such strategy will rely heavily on the quality of the Veterinary Services and administrative organisations involved, because the requisite diagnostic and prophylactic tools are already fully validated and standardised (14, 49).

References


