Chapter 1

Risk Factors for *Brucella* spp. in Domestic and Wild Animals

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Additional information is available at the end of the chapter

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Abstract

Brucella spp. is the aetiological agent of brucellosis, a serious contagious disease that results in reproductive failure and that has profound public health significance because of its zoonotic characteristics. This disease also is responsible for a high economic impact associated with the application of prevention, surveillance and test-and-slaughter programmes in animals by national authorities. *Brucella* spp. infects a large variety of animals and their prevalence is variable worldwide, mainly associated with the presence or absence of control programmes and also with the vaccination of animals against brucellosis. To achieve the control and eradication of brucellosis, the identification of the risk factors of brucellosis that maintain the infection in animals and/or the environment is fundamental. Although several risks have been identified, the most important have been associated with the biology of the bacteria, animal management (age, sex, species or breed), herd management (herd/flock size, number of species, contact with wild animals or type of animal production), farm management (facilities, cleaning and disinfection or veterinary support) and farmers' knowledge about the disease. Thus, to benefit from proper risk identification of brucellosis, it is essential to put a cost-effective and efficient brucellosis control programme into place.

Keywords: Brucellosis, risk factors, animals, prevalence

1. Introduction

Brucellosis is a serious contagious disease that results in reproductive failure and has profound public health significance because of its zoonotic characteristics [1]. In animals, brucellosis can be considered as one of the most economically important zoonosis worldwide, resulting in clinical disease, abortion, neonatal losses, increased calving intervals, reduced fertility, decreased milk production, increased culling rates because of metritis and the emergency slaughtering of infected animals and also an impediment to free animal movement and trade [2-4]. However, a high economic impact is associated with the human disease and also by the application of prevention, surveillance and test-and-slaughter programmes in animals by national authorities [4,5].

Brucella spp. infects a large variety of animals as described in Table 1. Classically, the genus *Brucella* includes six recognized species based on antigenic/biochemical characteristics and primary host species [6]. *Brucella abortus* (cattle), *Brucella melitensis* (sheep and goats), *Brucella suis* (swine, cattle, rodents, wild ungulates), *Brucella ovis* (sheep), *Brucella canis* (dogs) and *Brucella neotomae* (rodents). More recently, other species have been recognized such as *B. ceti* (cetaceans), *Brucella pinnipedialis* (seals), *Brucella microti* (voles) and *Brucella inopinata.* The last was isolated from a breast implant in a human with clinical signs of brucellosis [7].

Animals	*Brucella spp. Hosts*	Reference
Domestic/Farm animals	Alpacas, Cattle, Dogs, Goats, Horses, Llamas, Pigs, Sheep	[8,9,10-14]
Wild animals	Bears, Bison, Buffalo, Camelids, Caribou, Deer, Elk, Ferrets, Foxes, Rabbits, Rodents, Wolves	[15,16,17-22]
Birds	Partridges, Quails	[23,24]
Marine mammals	Dolphins, Dugongs, Manatees, Otters, Sea porpoise	[15,23-29]

Table 1. Hosts of *Brucella* spp. among the literature

The epidemiology of brucellosis is variable worldwide. In developed countries, brucellosis has been eradicated or presents low individual prevalence due to control programmes and vaccination of animals [30,31]. Currently, the brucellosis status of a country is based on the epidemiology in domestic animals. However, to consider a country free of brucellosis, it may also include epidemiological data regarding brucellosis in both wild animals and in marine animals [15,25].

According to the data available at the World Organisation for Animal Health (OIE) (2009), brucellosis (including *B. abortus* and *B. melitensis*) was not reported in several countries such as the USA, Australia and several European countries. Among the latter, Mediterranean countries such as Spain, Portugal, Italy and Greece are not brucellosis free today [32-35]. On the other hand, the picture of the prevalence of brucellosis has changed in South America, Africa, Middle East and Asia, where brucellosis is endemic because control programmes are insufficient or they basically do not have a great impact in animal and human health [5,26]. Since official data about prevalence of brucellosis in these countries is scarce, reports carried out in these areas show a large variability in the prevalence (Table 2).

The aim of brucellosis control is both to decrease the impact on human health and avoid economic consequences through reducing exposure to *Brucella* spp. and increase resistance to the infection among animal populations. To achieve this objective, several measures such as test-and-slaughter programmes and/or biosecurity measures (hygiene, control of animal movements, vaccination or reproductive management, etc.) should be performed [30].

However, to achieve success against brucellosis, the elaboration of control and eradication programmes must first identify all the potential risks that maintain the infection in animals and/or the environment.

Species	**Country**	**Individual prevalence**	**Lab method**	**Reference**
Cattle	Brazil	2.9%	RBT+2ME	[43]
	Libya	42%	RBT	[44]
	Bangladesh	2.66%	RBT + I-ELISA	[45]
	Nigeria	24.0%	RBT+ELISA	[46]
	India	5,00%	RBT + ELISA	[47]
	Uganda	14%	RBT	[48]
	Uganda	5%	ELISA	[49]
Sheep	Libya	24%	RBT	[44]
	Bangladesh	2.31%	RBT + I-ELISA	[45]
	Nigeria	14.5%	RBT + SAT	[49]
	Kyrgyzstan	8.9%	RBT	[50]
Goat	Libya	31%	RBT	[44]
	Bangladesh	3.15%	RBT + I-ELISA	[45]
	Uganda	17%	RBT	[48]
	Nigeria	16.1%	RBT+SAT	[49]
	Kyrgyzstan	2.5%	RBT	[50]
Horses	Iran	2.5%	RBT	[51]
	Nigeria	14.7%	RBT	[52]
	Pakistan	20.7%	RBT	[53]
Dog	Iran	4.90%	IA	[54]
	Argentina	14.7%	RBT	[55]
	Nigeria	5.46%	RBT	[56]
	Nigeria	28.6%	RBT	[57]
	Iran	10.62%	IA	[58]
Swine	Croatia	1%	RBT	[11]
Coyote	USA	18%	Card test	[59]
Camels	Egypt	5.7%	RBT	[18]
Wild boars	Switzerland	1.5%	RBT	[60]
	USA	23.4%	CT+STT+RT+CFT	[61]
Marine mammals	USA	0.03%	C-ELISA	[62]
	USA	38%	RBT+CFT+ELISA	[63]

IA: Immunochromatography assay; RT: rivanol test; C-ELISA: competitive ELISA; CT: card test; STT: standard tube test; LAB method: laboratory method for brucellosis diagnostic; ELISA: Enzyme-Linked Immunosorbent Assay; RBT: Rose Bengal Test; 2ME: 2-mercaptoetanol test; CFT: Complement Fixation Test; I-ELISA: indirect ELISA; SAT: serum agglutination test

Table 2. Seroprevalence of brucellosis among the different species

Identification of risk factors of brucellosis has been reported in epidemiological studies [36-41]. Although several risks have been identified, the most important are related to farm management, animal management and knowledge about the disease [42]. Thus, to benefit from proper risk identification of brucellosis, it is essential to put a cost-effective and efficient brucellosis control programme into place.

2. Risk factors of brucellosis in animals

The risk factors can be categorized into those associated with characteristics of animal populations, management and the parasite biology.

2.1. Risk factors associated with the biology of *Brucella* spp.

Brucella abortus is the aetiological agent of bovine brucellosis and responsible for an economically important cause of abortions in cattle [31]. *B. abortus* also affects other species such as bison, buffalo or elks representing an important risk for the maintenance of the agent in the animal population with special importance in areas where wildlife and cattle rearing occur together [15]. Moreover, infections in wildlife can hinder eradication efforts in cattle. *B. abortus* is still a human pathogen and outbreaks associated from infected cattle and also from ingesting contaminated dairy products represent an important risk of infection [4].

Brucella melitensis can affect most domestic animals, but dairy sheep and goats are especially susceptible. Sheep have different receptivity according to breed, while in goats this association has not been reported [64]. *B. melitensis* is the main etiological agent of brucellosis in small ruminants, although sheep can be also infected by *B. ovis*. Sporadic cases of brucellosis have been described in sheep and goats as *B. abortus* and *B. suis* [65,66]. The dogs that guard the herds and flocks can also be infected [67].

Dogs, cats and other wild carnivores such as foxes or wolves present an important role in the epidemiology of brucellosis, because they act as mechanical disseminators due to the transportation of infected foetuses or placentas from abortions in infected herds and flocks. Since pigs are susceptible to infection by *B. melitensis*, pig farms present some epidemiological importance where both species are reared [68]. In addition, wild ruminants with potential contact with infected sheep and/or goats could be infected with *B. melitensis*, maintaining the infection in natural environment [15].

Porcine brucellosis is caused by *Brucella suis* biovars 1, 2 or 3. The disease caused by biovars 1 and 3 is similar, while that caused by biovar 2 differs from 1 and 3 in its host range, its limited geographical distribution and its pathology [66]. In domestic pigs, risk factors associated with infection are ingestion of aborted foetuses, foetal membranes, abortion products and uterine discharges, or contaminated foodstuffs. Transmission during copulation is very common [66, 69,70]. Artificial insemination with contaminated semen or conjunctival mucosal should also be considered a risk [66,71].

The infection of a pig herd by brucellosis could be associated with the purchase/entrance of infected animals, contact with wildlife reservoirs, use of contaminated semen or feed [72] or the use of a lend boar. Other risk factors could be attributed to transmission of the disease by mechanical vectors due to contamination of vehicles, holding equipment or utensils and also to the introduction of infected offal (e.g. placenta and afterbirths) [70]. Serological screening and purchase from brucellosis-free herds should reduce this risk [70].

The likelihood of the introduction of the infection from potentially infected wild boar, free-range pigs or hares and its establishment in outdoor and backyard pig populations depends on housing management such as the type of housing (outdoor vs indoor), low levels of biosecurity, direct or indirect contact with infected wild boar, free-ranging pigs or hares, feeding practices (*i.e.* home prepared food vs commercial food), purchasing animals or semen without testing, no testing of live pigs, husbandry systems, lack of detection of unapparent infections, contamination of semen collection centres and equipment, contamination of transport vehicles, transport of pigs from different holdings or mixing of pigs [70].

2.2. Risk factors associated with the host

2.2.1. Age

Age has been referred to as one of the intrinsic factors associated with brucellosis. Higher seroprevalence of brucellosis has been observed in older animals, both in cattle and small ruminants with a prevalence odds ratio (POR) of about 2.0 in cattle over 5 years old and a POR of about 1.7 in small ruminants over 2 years old [43,73-77]. Similar results have been observed in wild boars and camels [78,79]. Brucellosis has traditionally been considered a disease of adult animals since susceptibility increases after sexual maturity and pregnancy [80]. However, variations in the age of sexual maturity among breeds could present differences between age and brucellosis positivity [81]. *Brucella spp*. presented a tropism to the reproductive tract due to the production of erythritol, a 4-carbon sugar produced in the foetal tissues of ruminants that stimulates the growth of *Brucella* [82]. Thus, it may also explain the higher prevalence in adult animals than in young [83]. On the other hand, a higher prevalence of brucellosis in adults has also been associated with longer contact with infected animals or with the environment. This potential risk may be significant in those herds without culling of positive animals [84]. It must be kept in mind though that this low prevalence might be faulty because young animals can be infected without clinical symptoms presenting serologic response for only one week [83,84].

2.2.2. Sex

The influence of sex in the prevalence of brucellosis has been studied in cattle, small ruminants and wild animals [74,77,79,80,84]. Female ruminants presented a higher odds of brucellosis infection, the same has been observed in female dogs compared to male dogs [85]. Although this is difficult to explain, it could be associated with the intrinsic biology of the microorganisms and its tropism to the foetal tissues as previously described. Since brucellosis infection in males presented clinical signs such as epididymitis and orchitis, the prevalence in males could be lower than females because they may be culled faster [86]. On the other hand, the absence of clinical signs such as abortion or metritis in non-pregnant infected females or the absence of farmers' observation/identification of abortions in extensive herds may also explain the higher prevalence in females. In addition, in non-pregnant females, brucellosis becomes chronic. This fact has important epidemiological implications since, after an initial immune response, animals may be asymptomatic carriers, the antibodies disappear from circulation and are difficult to detect with traditional serological techniques [87]. Since brucellosis in pigs may affect both males and females equally, sex susceptibility has not been fully demonstrated [72]. Regarding wild boars, the behavior of females living in matriarchal groups could explain the higher prevalence [79].

2.2.3. Species and breed

The prevalence of brucellosis is variable among species and region as described in Table 2. However, prevalence in farm animals seems to be lower in small ruminants than large ruminants [44,84] and lower in sheep than in goats [45,88,89]. Transmission of brucellosis occurs in ruminants through the excretion of contaminated materials from the female genital tract, which constitutes the main form of transmission to other animals and humans. In most of the circumstances, the main route of spread is the placenta, foetal fluids and vaginal discharges expelled after delivery or abortion. At that time, large numbers of *Brucella* are released [90]. The vaginal excretion of *Brucella* spp. in goats is greater and more prolonged than sheep, lasting for 2-3 months. In sheep, it is generally lower and normally ceases within 3 weeks after birth or abortion. It is also common that excretion occurs through milk or semen [91]. The excretion of *Brucella* in milk is generally intermittent and usually only appears 6 to 12 days after the abortion. In goats, the excretion is more abundant and more prolonged, so there is an increased risk of infection via the consumption of milk from this species [92,93].

The phenomenon of latent brucellosis in sheep was observed in lambs born from infected mothers that breast-feed with milk contaminated with *Brucella*. These lambs are seronegative to adulthood, while in females, the latency of brucellosis is maintained until the first pregnancy, a period in which the disease process develops [94].

Infected females thus present a high number of abortions with special importance in primiparous females [87].

In game animals, seroprevalence in wild boars seems to be higher than wild ruminants [8,95-98]. To the best of our knowledge, there is no evidence of higher susceptibility to brucellosis within specific species. In the case of horses, they have usually been considered more resistant to brucellosis than ruminants [51], but the variation of prevalence reported in endemic areas of brucellosis [99,100] seems to be discussible. The information available about differences of brucellosis infection by species is scarce. In sheep, pregnant dams do not present *Brucella* spp. in vaginal discharges, contrary to that observed in goats [101], where excretion may extend over two or three months [102]. Thus, the higher prevalence in specific species could be achievable through the intrinsic characteristics of the etiological agent [103].

Regarding the breed, a higher prevalence of brucellosis has been reported [104] in cross-breed cattle than local breeds, although other reports indicated no statistical differences among cattle breeds [46,105]. In small ruminants, Maltese and South American sheep breeds seem to present a greater resistance to brucellosis compared to the sheep breeds of Southwest Asia and the Mediterranean, such as the Awassi breed [13,106,107]. Although Husky and Chihuahua dog breeds appeared to be more prone to *Brucella* infection than other breeds, their infection seems most likely influenced by other factors such as the local dog population or owners than by dog breed [107]. In swine, some breeds such as Duroc and Jersey Red crosses may be less susceptible to experimental challenge with *B. suis*, suggesting some genetic resistance [108]. Previous studies showed that stray dogs demonstrated a greater than three-fold rate of infection versus non-stray dogs [109].

Keypoint: Risk factors associated with *Brucella* spp. and the host

Brucella abortus is the aetiological agent of bovine brucellosis in cattle although also infects other species such as bison, buffalo or elks. It represents an important risk to the maintenance of the agent in the animal population with special importance in areas where wildlife and cattle commingled. *B. melitensis* is the main etiological agent of brucellosis in small ruminants, although sheep can be also infected by *B. ovis*. Sporadic cases of brucellosis have been described in sheep and goats as *B. abortus* and *B. suis.* Porcine brucellosis is caused by *Brucella suis* biovars 1, 2 or 3. The disease caused by biovars 1 and 3 is similar, while that caused by biovar 2 differs from 1 and 3 in its host range, its limited geographical distribution and its pathology.

Several risk factors of brucellosis have been associated with the host such as age, sex, species or breed. Regarding age, higher seroprevalence of brucellosis is observed in older animals since susceptibility increases after sexual maturity and pregnancy. It could be associated with the tropism of *Brucella* spp. to erythritol, a 4-carbon sugar produced in the foetal tissues of ruminants that stimulates the growth of *Brucella.* This fact may explain the higher prevalence in adult animals than in young ones. With regard to sex, the odds of infection by brucellosis in ruminants are higher in female than male probably associated with the tropism to the foetal tissues as previously described. Species and breed have also been described as risk factors. In farm animals, the prevalence seems to be lower in small ruminants than large ruminants and lower in sheep than in goats. In this last case, the vaginal excretion of *Brucella* spp. in goats is greater and more prolonged than sheep, lasting for 2-3 months whereas excretion in sheep is generally lower and normally ceases within 3 weeks after birth or abortion. Regarding the breed, there is not consensus among the studies. Thus, some of them reported higher prevalence of brucellosis in cross-breed cattle than local breeds. In small ruminant, Maltese and South American sheep breeds seem to present a greater resistance to brucellosis compared to the sheep breeds of Southwest Asia and the Mediterranean, such as the Awassi breed.

2.3. Risk factors associated with herds

2.3.1. Herd/flock size

An important risk factor for brucellosis seropositivity is herd size, being higher in large herds and/or flocks. An increased odds ratio for seropositivity has been largely reported in cattle

[82,84,104,110] as well as in small ruminants [77,86,111]. In contrast, no statistical differences among goat flocks were observed in the literature [112,113].

The higher prevalence of brucellosis in large herds and/or flocks has been associated with several factors, such as a higher number of animals tested in larger herds means the probability of detecting at least one seropositive animal is greater [77] or the higher number of animals increases the likelihood of transmission of the disease by contact among them [114]. The low prevalence of brucellosis in small-sized herds could also be associated with the herd and/or farm management [86]. Thus, small-sized flocks usually graze at pastures near or contiguous to the farm, avoiding contact with other flocks or utilization of common paths and/or roads. Because premises for small herds or flocks are smaller, cleaning, disinfection and manure removal procedures are easier and less time consuming to the farmer. Disinfection is also facilitated by the low resistance of *Brucella* spp. to most disinfectant agents [115] and by the low cost of this operation. Farmers of small-sized herds may easily control the partum period and usually keep dams away from the flock during parturition. This measure is very important in case of abortions, to avoid pasture contamination. In these small sized herds, replacement is usually made by reposition and economic trade is not frequent. Thus, the absence of an elevated rate of animal movement decreases the likelihood of infection.

The health status of a flock may influence the predisposition to brucellosis infection. Thus, in small-sized herds, farmers can easily identify sick animals and veterinary and preventive treatments are usually carried out at low financial cost. Regarding the official control of brucellosis by the official veterinary authority, small-sized flocks are easily controlled and in the case of a positive finding, most farmers agree to cull the whole flock to maintain the brucellosis-free status and also to avoid a zoonotic infection [116,117]. In addition, the vaccination coverage of young animals with RB-51 or Rev-1 is more easily achievable in these herds.

On the other hand, the higher prevalence of brucellosis observed in large flocks may be also associated with the utilization of communal pasture areas, utilization of common paths and/or roads and due to contact with others flocks [114]. Cleaning and disinfection procedures of premises and manure removal in large-sized flocks is more difficult than in medium or small flocks because it requires the availability of mechanical equipment and consequently a higher financial cost. An increased prevalence of brucellosis associated with decreased of proper manure removal, cleaning and disinfection procedures has been described [118]. The control of reproductive management is difficult in large flocks, where parturitions on grazing areas are frequent and abortions are a source of pasture contamination. In addition, animal movement in large herds is frequent, both for replacement and/or trade, thereby increasing the risk of infection by brucellosis. Due to the higher cost of veterinary treatments and/or application of preventive programmes, animals in large flocks may be more susceptible to brucellosis infection. Moreover, associated with high numbers of animals unvaccinated and/or non-blood sampled animals may occur and remained unprotected and susceptible in case of infection. In addition, these animals act as a source of brucellosis contamination to the rest of the herds [74,118] and in the case of positive animals, farmers hesitate to slaughter the entire flock.

In dogs, the risk of transmission increases in kennel environments due to the high interaction among the animals and reduced space, which infected dogs share with other healthy ones to play, defecate or urinate [119]. Kennels with a history of abortion are 13 times more likely to be seropositive than kennels without this record [120].

Transmission studies have demonstrated that the exposure of healthy dogs to abortion products is an easy way for *B. canis* transmission [119]. The aborting bitch presents a high risk for the spread of infection to healthy dogs. *B. canis* is also found in the milk of infected lactating bitches, which might lead to the potential infection of nursing pups [121]. The high POR of seropositivity in kennels with a history of abortions could be associated with the presence of *Brucella* over long periods of time, caused by the absence of good reproductive practices and exposure to body fluids in the environment [120].

2.3.2. Number of species

Farming several species in the same herd has been described as a risk factor [78,80,84], although there is no evidence of higher susceptibility of brucellosis in specific species. Thus, an increase in prevalence where several species intermingle is difficult to explain but could be associated with higher chances of being *Brucella* seropositive because of multiple sources of infection.

It has been suggested that brucellosis is transmitted only rarely from sheep and goats to cattle, or among cattle [88]. However, the higher risk for cattle on farms which also had sheep or goats suggests that some of the cattle infections may have originated from small ruminants since *B. melitensis* biovar 3 was isolated from cow's milk.

Because *B. melitensis* is considered the most virulent of *Brucella*, it may explain the increased POR in cattle rearing with small ruminants [103]. In addition, the susceptibility of all ruminants to infection by *B. abortus* may explain the higher prevalence of brucellosis in cattle herds in contact with buffaloes or wild ruminants [37,122]. Horses seem to be resistant to brucellosis, although the risk of infection increases when they intermingle with cattle [46].

In regions where *B. melitensis* has been confirmed in sheep and goats, cattle can become infected with this bacterium [74]. It has not yet been determined whether *B. melitensis* can be kept alone in a population of cattle in the absence of small ruminants. *B. melitensis* causes abortion in cattle similarly to *B. abortus.*

As previously described, horses present a certain resistance against brucellosis, however, seropositivity has been associated with horses in areas without brucellosis control programmes for large and small ruminants. In addition, *B. suis* infection in horses has been reported in those commingling with swine [123].

The presence of swine could be a risk for brucellosis transmission to cattle [123] and is a public health concern. However, recent studies showed that cattle intermingling with pigs in the same area do not seem to be infected by *Brucella* spp. and do not contribute to its maintenance [125]. In contrast, the risk of cattle infection by *B. suis* from wild boar has been recently described [126].

The practice of mixing cattle, either through grazing or sharing watering points, is a significant risk factor for brucellosis [104,127,128]. Community pastures should be treated as livestock unit and control measures must be applied to all animals [129].

Other researchers [84] found that the disease is easily transmitted in areas where extensive production systems predominate, based on grazing and the high mobility of herds, the mixture of species in the same herd and where sharing pastures, roads and water sources occurs. Mobility increases the likelihood of contact with other potentially infected herds or wild animals that are reservoirs of disease.

The presence of dogs has been described as a risk for brucellosis infection in farm animals [125] and represents a potential epidemiological threat in endemic and/or brucellosis areas without brucellosis control programmes. However, dogs are a potential risk in the diffusion of brucellosis, acting as mechanical disseminators by feeding on aborted foetuses, dragging them along and spreading the bacteria [107].

Canine brucellosis is usually caused by *B. canis*, although infection by *B. abortus*, *B. suis* and *B. melitensis* have been reported [129]. Previous studies showed that dogs have been identified as a link in the brucellosis transmission chain. *B. abortus* and *B. melitensis* can be transmitted from cattle to farm dogs playing the role as vector. *Brucella* can produce disease in dogs via ingestion of infected reproductive tissues [9]. Infected dogs with *B. abortus* can spread organisms into the environment through urine, vaginal secretions, aborted foetuses or faeces. If a pregnant dog is infected with *B. abortus*, it may abort, and the tissues and vaginal discharges have a great potential for transmitting *Brucella* to susceptible cattle [9,129]. Dogs can also be infected with *B. suis* via ingesting aborted swine foetuses [71]. Thus, the elimination of infected cattle may not necessarily eradicate the disease [9].

2.4. Risk factors associated with farm management and environment

Several risk factors of brucellosis associated with farm management and environment have been referred to in the literature as presented in Table 3.

Regarding the main seroprevalence, dairy animals have a much greater chance of not only contracting brucellosis but also of spreading it faster than beef animals. The reason is not a genetic or physiological factor but due to husbandry. Animals that live in concentrated smaller areas come into close contact when they are grazing and when they are milked [129]. The zoonotic transmission of brucellosis by improper milking procedures was observed [128] associated with skin lesions in hands. Thus, transmission through skin lesions of the udder is not a neglectable source of infection. In addition, it is considered that dairy animals are subjected to more stress conditions on farms, leading to a higher susceptibility to brucellosis infection [135]. The persistence of the infection of the udder and supramammary lymph nodes leads to a constant or intermittent excretion in milk in successive lactations. This fact constitutes an important source of infection for humans and for the young animals [136].

Animal purchase has been considered as a risk for brucellosis. Purchasing in larger herds has usually been associated with more animal movements on and off the farm, and this practice

Factors described	Reference
Absence of calving paddock	[14,43]
Age	[75,77]
Breed	[39,77]
Cleaning and disinfection	[40,112,114,130]
Climatology	[79]
Commingling with other animals	[14,88,114,131]
Communal pastures	[36,43,112,130]
Contact with wildlife	[36,74,104]
Education	[40,42,80]
Handling of aborted material	[43,80]
Intensive management	[104,132,133]
Herd size	[36,40,75,77,88]
Lending males	[112]
Main animal production (beef /dairy)	[76]
Milking procedures	[80]
Purchase/entrance of new animals	[39,112]
Sex	[77]
Specie	[114]
Stocking rate	[14,77,125]
Transhumance	[104]
Veterinary services	[43,104,112,117,131]
Water sources	[40,125,130]
Handling of aborted material	[43,80,134]

Table 3. Risk factors of brucellosis infection in animals

increases the risk of introducing an infected animal into a herd [81]. Introduction of animals from market fairs also presents a higher risk of infection. The majority of infections or reinfection in disease-free herds starts through buying infected animals of unknown status [128]. This has a higher importance in those endemic areas or countries where there is an absence of control programmes. However, in countries with test-and-slaughter control programmes, the movement of cattle are subjected to a compulsory pre-movement test that consists in the serological brucellosis diagnostics before an animal leaves the farm [42]. Moreover, animal movement restriction measures are applied in brucellosis positive herds to avoid spreading the disease [34].

The proximity to other infected herds or flocks has also been described an infection risk, although, small ruminant contact with other flocks was reported to have no impact on *Brucella* seropositivity in Spain [137].

The influence of the agro-ecological zone has been also referred to as a brucellosis risk factor, having a higher prevalence in dry zones [132]. Since pasture areas are scarce in dry zones, animals must seek pastures over large areas implying an unrestricted animal-to-animal contact with potential transmission as previously described. In addition, transmission due to aerosol inhalation of contaminated dust from foetal discharges or abortions is possible [138]. In contrast, a lower prevalence of brucellosis in these areas has been proposed by other authors [139] due to lower survival of *Brucella* spp. in aborted material in dry-zones.

Larger herds might be expected to be associated with intensive management practices that are typically more difficult to control and allow for closer contact between animals and their environment, which increases the potential for exposure to infectious excretions [130]. In addition, the stressful conditions of animals subjected to intensive production may make them more susceptible to the infection, as previously described. On the other hand, extensive management may also imply a risk of brucellosis and higher prevalence has been reported in small ruminants. Although difficult to explain, it could be associated with controlling abortions, observation of sick animals or contact with animals, among others [86]. Since extensive management implies rearing a large number of animals in large areas and/or sharing communal pastures, the contamination of pastures with placentas or abortions is a source of infection to other animals in the herds, as we described previously in the risk factors of brucellosis by the herd size.

Animal handling and environmental conditions are risk factors which influence the transmission of infection, such as births and breeding in semi-dark settings, confinement in closed spaces and high animal densities [130]. Another risk of intensive systems could be associated with airborne dust transmission indoors [138].

The season also has an impact on herd management and animal nutrition, mainly in production systems involving transhumance or nomadic practices [114]. Rainfall affects the development and the nutritional state of the pasture. These factors influence the reproduction of animals kept in extensive systems and thus the time of delivery/miscarriages. In intensive systems, isolation of post-parturient animals in maternity facilities reduces the spread of infection to the rest of the herd or flock [128].

Cleaning and disinfection of farm facilities and proper manure removal have been described as a protective factor against brucellosis infection [114,118,130]. This fact is associated with the low resistance of *Brucella* spp. to most disinfectant agents [115] although their effectiveness is based on the previous elimination of organic material since it decreases the bactericidal effect of the disinfectant [140]. A similar risk of brucellosis was reported in kennels [119,120]. Kennels with improper management of excrement and built with materials such as tile, wood and earthern floors were considered to have a higher risk for infection since they maintain exposure to urine, faeces, or reproductive secretions [120].

Insect rodents on dogs could act as a mechanical vector of brucellosis. Blood-sucking insects have been reported as disseminators of brucellosis. *Brucella* was isolated from the stomach contents of *Stomoxys calcitrans*, *Ornithodoros* and *Musca autumnalis* (stable fly). The stable fly is dipterous in contact with ruminants. The female lays eggs in the faeces of these animals and feeds on their blood, tears and placental secretions. It is thought that these insects and ticks contribute to disease transmission [92,138]. As mentioned earlier, dogs intermingling with large and small ruminants in farms have presented an important role in the epidemiology of brucellosis. However, stray dogs which remain free on the streets and travel long distances also act as disseminators of the agent and provide chances for infection of other animals and humans through environmental contamination [141].

Environmental factors that affect the ability of *Brucella* to survive outside mammalian hosts need to be considered in the epidemiology of brucellosis. High humidity, low temperatures and absence of direct sun light may favour the survival of *Brucella* for several months in water, aborted foetuses, placental membranes, liquid manure, hay, buildings, equipment and clothes [129].

The survival of *Brucella* outside a mammalian host is relatively persistent compared to other non-sporulating pathogenic bacteria in similar circumstances [142]. Favourable conditions are pH>4, low temperatures, absence of direct sunlight and high humidity. *Brucella* can persist for several months in water, aborted placentas, faeces, manure, wool, facilities, equipment and clothes [143]. *Brucella* can survive for 40 days in dry soil and 60 days in moist soils, 144 days at 20 °C and 40% relative humidity, for several months in drinking water at 4 °C to 8 °C and two and a half years at 0 °C, 30 days in urine, 75 days in aborted foetuses, more than 200 days in uterine secretions and several years in frozen tissues or culture media. *Brucella* resistance to different environmental conditions increases in the presence of abundant organic matter. The spread of the disease via waterways is not frequent and can only be effective over short distances [129].

2.5. Other factors associated with brucellosis

The role of farmers' knowledge about brucellosis has been discussed in the literature. It was noted that knowledge ages equal to or older than 55 years was a protective factor for brucellosis prevention [40,42]. This observation is difficult to explain and may be due to younger farmers' lack of experience. Older farmers have more familiarity with recognizing the clinical signs of the disease or the main route of transmission and can be more aware of the importance of preventive measures [67,144,145]. Farmers who had previously experienced brucellosis in their herds had a higher probability of having greater knowledge of bovine brucellosis, which is consistent with having experience with the disease. Producer's associations, education and veterinary support have been recognized as protective factors [42,118]. Farmer's lack of awareness about brucellosis, improper handling of aborted materials and the habit of consuming raw milk, among other factors, might contribute to further spread of brucellosis in their livestock and expose the community to a public health hazard [80].

Keypoint - Risk factors associated with farm management and environment

The risk factors of brucellosis associated with the herd are size and the number of animal species. The higher prevalence of brucellosis in large herds could be explained by the higher odds of detecting at least one seropositive animal, the increase of the transmission of the disease by contact among them, utilization of communal pasture areas or improper cleaning and disinfection procedures in large farms. Farming several species in the same herd has been described as a risk of infection due to multiple sources of infection. Thus, presence of dogs in large herds may spread *Brucella* spp. by both mechanical carriers or by the spread of the organisms into the environment through urine, vaginal secretions, aborted foetuses or faeces.

Dairy animals have a much greater chance of not only contracting brucellosis but also of spreading it faster than beef animals. Because animals that live in concentrated smaller areas come into close contact when they are grazing and when they are milked. In addition, it is considered that dairy animals (intensive production) are subjected to more stress conditions on farms, leading to a higher susceptibility to brucellosis infection. Purchasing in larger herds has usually been associated with more animal movements on and off the farm, and this practice increases the risk of introducing an infected animal of unknown status with special importance in areas with absence of control programmes.

Also the influence of the agro-ecological zone has been also referred as a brucellosis risk factor. High humidity, low temperatures and absence of direct sun light may favour the survival of *Brucella* for several months in the environment. In addition, cleaning and disinfection of farm facilities and proper manure removal have been described as a protective factor against brucellosis infection. This fact could be explained to the low resistance of *Brucella* spp. to the disinfectant agents.

2.6. Brucellosis in wild animals — A threat to farm animals

Brucella abortus and *B. suis* have been isolated worldwide from a great variety of wildlife species [15]. Some general risk factors, which can be identified in most of the wildlife diseases are wildlife overabundance, movements of wild and domestic animals and fomites [146]. Artificial management of wild species, including fencing, feeding and translocation, can also increase the risk of transmission of infectious brucellosis. [147] The risk of infection increases dramatically with increasing wildlife density and their exposure to *Bucella abortus* around feeding grounds [148]. Wild ruminants have been suggested as brucellosis carriers, but they are probably not true reservoirs [146,148]. Other works showed that wild ruminants do not play a relevant role in the maintenance of *B. abortus* and *B. melitensis* infections since limited cases of brucellosis have been reported in wild ruminants [79,149,150]. Only weak evidence for a direct relationship between brucellosis and size/density of the population of wild animals has been reported [151]. However, a potential risk for brucellosis infection of livestock by wild animals could be associated when artificial management such as winter feeding increases aggregation [146,151]. Thus, wild animals are often at risk as a consequence of contact with infected livestock, particularly in extensive breeding systems [79].

With regards to elk and bison, artificial feeding management during winter results in significant congregations in the feeding grounds and increases the risk of elk being exposed to *B.*

abortus [15]. A possible risk factor for bison in the USA is environmental contamination by the RB51 vaccine strain, which is a rifampicin resistant strain released in the environment [15].

Rangiferine brucellosis (brucellosis in reindeer and caribou) is caused by *B. suis* biovar 4 in the Arctic regions of Siberia, Canada and Alaska, constituting a serious zoonosis. *B. suis* may also infect moose (*Alces alces*) and occasionally various carnivores [15]. In European wild boar *B. suis* biovar 2 was observed in all age categories [152,153].

2.7. Brucellosis in marine mammals — New threat?

Brucella was detected in free-ranging pinnipeds and cetaceans from America, Europe, Japan, New Zealand, the Solomon Islands and the Antarctic, as well as in captive bottlenose dolphin (*Tursiops truncates)* [154-157].

Brucella ceti and *B. pinnipedialis* prefer cetaceans and seal hosts respectively [157, 158]. Epidemiological studies of risk factors for *Brucella* infection in cetaceans and pinnipeds have not yet been performed, and the role of environmental factors in the emergence of marine mammal brucellosis is still unknown [157]. It seems unlikely that *B. ceti* could survive for long periods outside marine mammals.

The transmission of brucellosis in marine mammals is not totally understood [158]. The dilution of the agent in sea water may make transmission difficult due to a low infecting dose. It is likely that the mode of transmission is through close contact between hosts, such as sexual intercourse or maternal feeding, contact with aborted foetuses and placental tissues or through fish or helminth reservoirs [159]. A second alternative corresponds to vertical transmission from mother to foetus, which is feasible since foetuses and placenta from infected animals have been found to contain large quantities of *Brucella* [156]. In addition, the behaviour of assisting the births observed in several cetaceans could be a risk due to the close contact with foetal tissues and discharges [27]. This hypothesis should be considered since *B. ceti* have been found in aborted foetuses and the reproductive organs of captive bottlenose dolphins [156] and in the uterus of a stranded striped dolphin with placentitis [160]. *B. ceti* has been also associated with mastitis and endometritis in cetaceans [161]. Both *B. ceti* and *B. pinnipedialis* have also been isolated from the testes, uterus and mammary glands of cetaceans and pinnipeds without any apparent pathology [162-164]. A potential risk factor could be the infection through ingestion of *Brucella* contaminated fish or helminth vectors [165]. *B. ceti* and *B. pinnipedialis* have been isolated from lungworms (*Pseudalius inflexus*) in the lungs of cetaceans and pinnipeds and these parasites can be a reservoir and vector for *Brucella* in these animals [165].

2.8. Animal brucellosis and zoonotic risk

In endemic regions without brucellosis eradication programmes, zoonotic risk still represents an important public health threat [166]. Infection happens due to contact with infected animals or consumption of their products, mostly unpasteurized milk and milk products of sheep and goats [167]. It presents special importance in those regions where trading of raw milk and raw milk products is a common practice among farmers [168]. The survival of *Brucella* in milk and dairy products is related with curing methods, humidity, temperature and/or changes in pH.

For milk, *Brucella* survival is inversely proportional to the pH [169]. *Brucella* can be responsible for milk-borne diseases, particularly since the appearance and taste of the milk are rarely affected by the presence of the bacteria [170]. Boiling or heating of milk at 80–85 ºC [176–185 ºF] for several minutes [approximately 10 minutes] will destroy the bacteria [30]. Bacteria cannot survive if the cheese is cured longer than 3 months [171]. In acidified soft cheeses and dry cheese, their survival is greater. Thus, European legislation requires that all cheeses made from raw milk be submitted to a cure period of not less than 60 days [172]. Survival time in meat is lower, except in frozen meat where the microorganism can survive for several years [173].

Although zoonotic brucellosis is mainly associated with farmers in high prevalence areas, even in low prevalence countries brucellosis represents an important threat as a work-acquired infection among dairy farmers, butchers, veterinary practitioners, meat inspectors, slaughterhouse personnel or artificial inseminators who do not take adequate biosafety precautions while performing their jobs [174-176]. In addition, brucellosis vaccines such as Rev-1 and RB51 are live dried living vaccines. Thus, needlestick accidents during their preparation or administration could also be a risk factor for human infection. Close contact with animals may occur when farmers or veterinarians assist animals during parturition or abortion or handling of stillbirth. In some parts of the world it is also common practice for farmers to separate the placenta manually, thereby increasing their exposition to tissues infected with *Brucella* [168].

Dairy farmers who milk with bare hands have a greater chance of becoming infected from *Brucella* infected animals [177] as do farmers or slaughterhouse workers who have skin lesions which provide an entry point for the bacteria [128]. Also, inhalation of *Brucella* has been previously reported in slaughterhouse workers where the concentration of *Brucella* can be high due to aerosol generation [129].

Zoonotic brucellosis from marine mammal includes individuals in traditional communities where products from whales and seals are still an important part of their diet [16]. In addition, occupational acquired infection in people handling stranded marine mammals, whale and seal hunters, marine researchers and other people handling raw products from the ocean could be exposed [25,178]. Also, it is suggested that marine avian species may harbour *Brucella* by eating infected fish and thus become vectors of zoonotic infections [158]. Tourists who swim and interact closely with captive dolphins can be at risk when *Brucella* spp. could be circulating in these colonies [163].

Keypoint: Emerging risk factors for brucellosis

Wild animals have been referred as reservoir of brucellosis and represent an important risk of infection to farm animals, particularly in extensive breeding systems.

The prevalence of brucellosis in wildlife varies worldwide and several species such as bison, reindeer, caribou or wild boar have been described as potential source of infection of livestock. However, their role as risk factors of infection is still discussed since the microbiological isolation of *Brucella* spp. has been reported in wild ruminants. The zoonotical potential of *Brucella* spp. still represents an important public health threat not only in areas without eradication control programmes but as a work-acquired infection among dairy farmers, veterinarians or meat inspectors among others while performing their jobs. The discovery of brucellosis in marine mammals also represents a public health threat with special interest in occupational acquired infection in people handling stranded marine mammals.

Foodborne brucellosis is an important biological hazard associated with dairy products. However, the presence of *Brucella* spp. in marine animals indicates that fish-borne brucellosis could be a future hazard to be considered.

3. Conclusions

Brucella spp. is responsible for a contagious disease that results in reproductive failure and has an important economic impact, not only in animal health but also in public health because of its zoonotic characteristics. To achieve the control and eradication of brucellosis, the identification of all potential risks is necessary. Given the important role of domestic and wild animals as potential sources of *Brucella* infection, further risk assessment will require more complete and reliable data on the infection prevalence. Several risk factors have been described for brucellosis infection, although the herd or flock size, species and age have been cited as the most important. Brucellosis has traditionally been associated with farm animals, however, risks of brucellosis associated with wildlife and marine mammals could be a new threat and further epidemiological studies are necessary. In addition to animal sanitary measures, complementary measures such as good farm practices, biosecurity, training and education are necessary to control this old disease that is still of concern today.

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