

Brucellosis in terrestrial wildlife

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Summary

The epidemiological link between brucellosis in wildlife and brucellosis in livestock and people is widely recognised. When studying brucellosis in wildlife, three questions arise: (i) Is this the result of a spillover from livestock or a sustainable infection in one or more host species of wildlife? (ii) Does wildlife brucellosis represent a reservoir of *Brucella* strains for livestock? (iii) Is it of zoonotic concern? Despite their different host preferences, *B. abortus* and *B. suis* have been isolated from a variety of wildlife species, whereas *B. melitensis* is rarely reported in wildlife. The pathogenesis of *Brucella* spp. in wildlife reservoirs is not yet fully defined. The prevalence of brucellosis in some wildlife species is very low and thus the behaviour of individual animals, and interactions between wildlife and livestock, may be the most important drivers for transmission. Since signs of the disease are non-pathognomonic, definitive diagnosis depends on laboratory testing, including indirect tests that can be applied to blood or milk, as well as direct tests (classical bacteriology and methods based on the polymerase chain reaction [PCR]). However, serological tests cannot determine which *Brucella* species has induced anti-*Brucella* antibodies in the host. Only the isolation of *Brucella* spp. (or specific DNA detection by PCR) allows a definitive diagnosis, using classical or molecular techniques to identify and type specific strains. There is as yet no brucellosis vaccine that demonstrates satisfactory safety and efficacy in wildlife. Therefore, controlling brucellosis in wildlife should be based on good management practices. At present, transmission of *Brucella* spp. from wildlife to humans seems to be linked to the butchering of meat and dressing of infected wild or feral pig carcasses in the developed world, and infected African buffalo in the developing world. In the Arctic, the traditional consumption of raw bone marrow and the internal organs of freshly killed caribou or reindeer is an important risk factor.

Keywords

Bacteriology – *Brucella* spp. – Brucellosis – Epidemiology – Livestock/wildlife interface – Serology – Wildlife.

Introduction

Brucellosis is a major zoonotic bacterial disease, widely distributed in mammals, both humans and animals. The occurrence of the disease in humans depends largely on the occurrence of brucellosis in an animal reservoir, including wildlife. In 1887, Sir David Bruce isolated the organism (*Micrococcus melitensis*) responsible for the 'Malta fever' from a British soldier who died from the disease in Malta. This bacterium was renamed *Brucella melitensis* in his honour. In 1905, Themistocles Zammit demonstrated, again in Malta, the zoonotic nature of *B. melitensis* by isolating it from goats' milk. Today, the disease is mainly occupational in humans (abattoir, animal industry, hunters and health workers) but transmission through the consumption of raw milk and milk products remains important in developing countries. Symptoms such as undulant (rising and falling) fever, tiredness, night sweats, headaches and chills may drag on for as long as three months before the illness becomes so severe and debilitating as to require medical attention (29). Brucellosis in animals is clinically characterised by one or more of the following signs: abortion, retained placenta, orchitis and epididymitis, with excretion of the organisms in semen, uterine discharges and in milk.

The epidemiological link between wildlife and many diseases in livestock is now well recognised. The long-standing conflict between livestock owners and animal health authorities, on the one hand, and wildlife conservationists on the other, is largely based on differing attitudes to controlling livestock diseases which are, or can be, associated with wildlife. The creation of new interfaces between livestock and wildlife due to human activity is the most important factor in disease transmission (7). Translocation or the introduction of infected animals into a non-endemic area and a lack of disease surveillance in wildlife may increase these interfaces (8). Thus, when studying brucellosis in wildlife, three main questions arise:

- Is wildlife brucellosis the result of a spillover from livestock or is it a sustainable infection in one or more host wildlife species?
- Does wildlife brucellosis represent a reservoir of *Brucella* strains for livestock?
- Is wildlife brucellosis of zoonotic concern?

To resolve potential conflicts between ecologists, regulatory veterinarians, the production animal industry, the hunting industry and public health authorities, the approach to wildlife brucellosis has evolved from the general diagnosis of brucellosis to include (early) detection of a pre- or subclinical infection and identification of transmission routes between different host species at the livestock/wildlife interface. This general approach is aimed at taking preventive control and management measures to decrease

the number of interfaces between livestock, wildlife and humans, and reduce the risk of brucellosis in both livestock and free-ranging/captive wildlife, thus also minimising the potential for zoonotic transmission (44).

Causative agents

Brucellae are Gram-negative, facultative intracellular bacteria that can infect many mammalian species, including humans. Ten species are recognised within the genus *Brucella*:

- a group composed of six 'classical' *Brucella* species, some of which include different biovars: *B. abortus* (biovars 1, 2, 3, 4, 5, 6, 7, 9), *B. melitensis* (biovars 1, 2, 3), *B. suis* (biovars 1, 2, 3, 4, 5), *B. ovis*, *B. canis* and *B. neotomae* (3, 14)
- a group represented by the 'new' recently described species: *B. ceti* and *B. pinnipedialis* (32), *B. microti* (88) and *B. inopinata* (89). The distinction between species and biovars is currently made by several differential laboratory tests (3, 14).

Classification of the classical species was based mainly on differences in microbiological and molecular characteristics, host preference(s) and pathogenicity. In 1985, it was proposed that the *Brucella* species should be grouped as biovars of a single nomen species, based on DNA-DNA hybridisation studies (94). However, this change has never been accepted by the international community of *Brucella* researchers, and a return to the pre-1986 taxonomy was advocated and eventually adopted by the *Brucella* Taxonomic Subcommittee (68).

Worldwide, the main pathogenic species for livestock are:

- *B. abortus* (all biovars), responsible for bovine brucellosis
- *B. melitensis* (all biovars), the main causative agent of brucellosis in both small ruminants and humans
- *B. suis* (biovars 1, 2 and 3), responsible for swine brucellosis
- *B. ovis*, which causes brucellosis in sheep.

If brucellosis occurs in a herd or flock, national and international veterinary regulations impose restrictions on animal movements and trade, which can result in huge economic losses (29, 97).

Despite their respective host preferences, *B. abortus* and *B. suis* have also been isolated from a great variety of wildlife species, such as bison (*Bison bison*), elk/red deer (*Cervus elaphus*), feral swine and wild boar (*Sus scrofa*), the red fox (*Vulpes vulpes*), the European brown hare (*Lepus europaeus*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandus tarandus*) and caribou (*Rangifer tarandus groenlandicus*). For

this reason, wildlife must be considered a potential reservoir for brucellosis in livestock (16, 40, 78). *Brucella melitensis* is rarely reported in wildlife. However, sporadic cases have been reported in Europe in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in the Alps (28, 37).

Brucella ovis and *B. canis* (responsible for ovine epididymitis and canine brucellosis, respectively) have never been reported in wildlife in Europe. However, *B. ovis* infection has been reported in red deer in New Zealand (79). For *B. neotomae*, only strains isolated from desert rats in Utah in the United States (USA) have been reported (29).

Since the first description of an abortion due to *Brucella* spp. in a captive dolphin in California in 1994 (26), several reports have described the isolation and characterisation of *Brucella* spp. from a wide variety of marine mammals, such as seals, porpoises, dolphins and whales, in almost all the waters covering the globe. Although brucellosis seropositivity has been documented in Antarctic seals, there has been no isolation of *Brucella* spp. from this animal so far (for a review, see 65). The overall characteristics of marine mammal strains are different from those of any of the six 'classical' *Brucella* species and, since 2007, *B. ceti* and *B. pinnipedialis* (preferentially infecting cetaceans and pinnipeds, respectively) have been recognised as new *Brucella* species (32).

During the years 1999 to 2003, an acute disease was reported in the common vole (*Microtus arvalis*) in South Moravia (Czech Republic). A new *Brucella* species (*B. microti*) was isolated and characterised from clinical samples of diseased voles (51, 88). *Brucella inopinata* was recently isolated from a breast implant infection in an elderly woman with clinical signs of brucellosis (89). At this time, *B. inopinata* has not been isolated from any animal reservoir.

Experimental studies and epidemiological evidence suggest that birds are very resistant to *Brucella* spp. infection. On the other hand, it has been shown that *B. melitensis* biovar 3 can be cultured from both skin swabs and the visceral organs of Nile catfish (23, 83). These findings suggest that fish may also be susceptible to *B. ceti* and *B. pinnipedialis* infection, which would have veterinary public health implications, given the potential zoonotic concern of these *Brucella* species, and this warrants further investigation. Brucellosis in marine mammals will be addressed elsewhere and will not be discussed further in this review.

Epidemiology

Spillover versus sustainable infection

One very important issue related to brucellosis in terrestrial wildlife is how to distinguish between a spillover of infection from livestock and a sustainable infection in wildlife. If the

latter does exist, the concern of the livestock industry is to prevent the re-introduction of the infection into livestock, particularly in regions or states that are 'officially free from brucellosis' because of the costs linked to pre-movement testing.

It should be emphasised that the introduction of infected individuals into a healthy population is not, on its own, sufficient to cause the effective transmission of *Brucella* spp. within a given wildlife host. The probability of brucellosis becoming established and being sustainable in a wild species will be less than the probability of infection, and close to zero in some cases. This is because sustainability depends on a combination of factors, including: host susceptibility (or resistance), infectious dose, (repeated) contacts with infected animals, and management and environmental factors (36). In North America, the highest probability of transmission of infectious agents between wildlife and cattle occurs in the extensive management systems used to raise beef cattle. Wildlife and cattle ranges overlap, and feed, water and mineral sources are shared between species on many farms and ranches (93). The development of the game-farming industry has contributed to the re-emergence of brucellosis as an international concern for both livestock and wildlife, by increasing the density of potentially infected game species and the introduction of artificial feeding (78, 93). *Brucella* spp. cannot multiply outside their mammalian hosts but may remain viable within the environment for a period of time (several years for *B. microti*). In general, the viability of *Brucella* spp. outside the mammalian host is enhanced by cool temperatures and moisture and limited by high temperatures, dryness and direct exposure to sunlight. For example, *B. abortus* survives a couple of hours under direct sunlight but up to 185 days in the cold and shade. *Brucella abortus* also survives in aborted fetuses, manure and water for periods of up to 150 to 240 days (81). The epidemiological importance of environmental contamination as a source of *Brucella* spp. for wildlife, scavengers and non-target species has to be assessed on a case-by-case basis, but the possibility of direct transmission from an infected host must be excluded first. The following sections will review the epidemiology of different *Brucella* species in terrestrial wildlife.

Brucella abortus

Since brucellosis eradication efforts in the European Union (EU) and in the USA focused on bovine brucellosis, the emphasis in these countries was put on the identification of a possible reservoir of *B. abortus* in wildlife. When brucellosis was prevalent in cattle, numerous surveys identified occasional seropositive results in wild ungulates, particularly cervids (78, 93), and under free-ranging conditions, the infection was considered self-limiting or the consequence of spillover from infection in cattle. For example, in 1995, *B. abortus* was isolated from 7/112 culled

chamois but brucellosis did not seem to be present in larger areas of the Western Italian Alps, where bovine brucellosis is absent (27).

Nowadays, in countries where bovine brucellosis eradication programmes are close to their end, there are no known sustainable reservoirs of *B. abortus* in wild species, other than bison and elk in and adjacent to the American National Parks of the Greater Yellowstone Area (GYA) and in the Canadian Wood Buffalo National Park. However, recent studies in feral pigs on the Atlantic coast of South Carolina, USA, may challenge these thoughts. Indeed, *B. abortus* wild-type and the *B. abortus* S19 and RB51 vaccine strains have been isolated from feral pigs on a property where no cattle had been kept since at least 1970 (90). Although these findings need further confirmation, this is the first report of a wildlife reservoir of *B. abortus* outside the GYA in the USA. In Europe, the isolation of *B. abortus* in wild boar has, so far, not been reported.

In Africa, the African buffalo is considered a reservoir of *B. abortus* (9). In Zimbabwe, antibodies against *Brucella* spp. have been detected mainly in buffalo, which were considered a possible source of infection for domestic stock (59). However, in a recent study in Zimbabwe, no anti-*Brucella* antibodies were detected in buffalo in an area where 9.9% of cattle tested positive (45). A recent report from Botswana suggests that household practices for processing buffalo bushmeat represent a significant *Brucella* spp. exposure risk to family members and the community (2). In areas of livestock and wildlife interaction, brucellosis seropositivity has been reported in Kafue lechwe antelope (*Kobus leche kafuensis*) (60) and in unspecified wildlife in Uganda (63).

A recent survey in the Iberian Peninsula highlighted that wild ruminants were not a significant brucellosis reservoir for livestock (61). *Brucella abortus* biovar 1 was only isolated from a single red deer, probably as a spillover from infected cattle. Thus, these results suggest that, in Europe, wild ruminants are occasional victims of brucellosis transmitted from infected livestock, rather than true reservoirs of the infection for livestock (61). The available epidemiological information suggests that wild ruminants are not able to sustain *B. abortus* infection without introductions from infected cattle. Given the progress in the eradication programme of bovine brucellosis in the EU Member States, it is unlikely that *B. abortus* will become a threat to wildlife in the EU in the future.

Brucella melitensis

The known ecological range of *B. melitensis* in wildlife is more restricted than those of *B. abortus* and *B. suis*, although it is still a major problem in small ruminants in southern European states. Spillover from infected small ruminants has been documented in a few wildlife species, such as

chamois (*R. rupicapra*) and ibex (*C. ibex*) in the French and Italian Alps (28, 37), and the Iberian wild goat in Spain (61), suggesting that these wild species are unable to sustain the infection and thus cannot act as reservoirs for domestic animals and human beings. These reports highlight the fact that *B. melitensis* infection in European wildlife is nowadays anecdotic and always linked to the small ruminant reservoir. Given the effective eradication of small ruminant brucellosis in Northern and Central Europe a long time ago, and the important progress made by *B. melitensis* eradication programmes in some Mediterranean EU Member Countries, this infection will probably not become a relevant issue in the future. However, important foci of *B. melitensis* persist in small ruminants in the Balkans, possibly threatening the wild ruminants of this region.

In the Middle East, *B. melitensis* has been isolated from an Arabian oryx (*Oryx leucoryx*) in Saudi Arabia (69). However, most *B. melitensis* infection cases occur in nomadic one-humped camels (*Camelus dromedarius*) that have been in contact with sheep and goats (1). The same situation occurs in the two-humped camel (*C. bactrianus*) and yak (*Bos grunniens*) in Central Asia (80). *Brucella melitensis* has been isolated from camel milk, indicating that camels may also constitute a serious public health problem (48). Brucellosis also occurs in llamas and other small camelids in some South American countries (29), but its epidemiological relevance is unknown. There is no documented report of the isolation of *B. melitensis* in wildlife in North America. In Africa, *B. melitensis* has been isolated once, from a sable antelope (*Hippotragus niger*) in 2005 (M. McFarlane, personal communication).

Brucella suis

Brucella suis (biovars 1, 2, 3) is still widely distributed in the world, although information is scarce in Africa (58), where no isolation of *B. suis* has been documented in the international scientific literature. *Brucella suis* biovar 3 has only been isolated in South-East Asia and America. Generally, the prevalence in domestic pigs is low, with the exceptions of those in South-East Asia and Central and South America. *Brucella suis* infection transmitted from the pig reservoir has been reported in cattle (13) and dogs (77), but the epidemiological importance of these findings is unknown. A recent report described the isolation of atypical *B. suis* biovar 3 in horses in Croatia (15). Further molecular studies grouped these Croatian strains with *B. suis* biovar 1 isolates (33). The taxonomical relevance of *B. suis* biovar 3 or, at least, the representativeness of its reference strain is questionable, since there is no single field strain of *B. suis* that matched both microbiological and molecular profiles of the biovar 3 reference strain (33).

Brucella suis infection due to biovar 1 is restricted to feral pigs in the USA and Australia, with human infections

linked to butchering swine being regularly reported (20, 84). *Brucella suis* biovar 1 has been isolated from people, domestic pigs (57) and collared peccaries (*Tayassu tajacu*) in South America (56). In Croatia, during the period 1980–2003, *B. suis* biovar 1 was isolated from wild boar, hares, domestic pigs and horses (35). However, to date, no human *B. suis* biovar 1 infection has been reported in Croatia (72). In 1993, *B. suis* biovar 1 was isolated in Belgium from a butcher handling imported feral pig meat. The most recent *B. suis* biovar 1 infection before that had been reported in a pig farmer in 1983 (43).

Brucella suis biovar 2 infection remains restricted to wild boar and European brown hares in Europe, where *B. suis* has been eradicated in the intensive domestic pig industry for decades. The available evidence suggests that wild boar seem to be the main source of infection for domestic pigs, since several outbreaks of *B. suis* biovar 2 have occurred in outdoor rearing systems, even on fenced premises, with the source of infection being traced back to contacts with wild boar. Transmission from wild boar to pigs is thought to be through the venereal route, as crossed (striped) piglets have been reported, at least in France and Portugal; however, other routes might also be possible (24). The role of wild boar hunting (migration pressure, offal remaining in the field, hunters working on the premises, etc.) has not been fully investigated (24).

The geographical distribution of biovar 2 has historically occurred in a broad range between Scandinavia and the Balkans. In recent years, several reports of *B. suis* biovar 2 infections in wild boar and the European hare have been published from the Iberian peninsula (61) to central Europe (73, 91).

The role of hares in the dissemination of brucellosis should be of lesser importance than that played by wild boar, due to the particular migratory behaviour of the latter. Solitary wild boar adult males may range very long distances if disturbed or in the case of food shortage, whereas hares have a non-migratory way of life and occupy small home ranges, reducing the interface with other wildlife. However, ten clinical outbreaks of *B. suis* biovar 2 infection were recorded in domestic pigs in Denmark between 1929 and 1999, and epidemiological evidence linked these outbreaks to hares. *Brucella suis* biovar 2 infections in cattle have also been reported in Denmark (4) and the source of contamination is believed to have been hares, since there is no established population of free-ranging wild boar in Denmark. Hares have been considered as a possible source of *B. suis* biovar 2 outbreaks in domestic pigs via swill feeding with offal from hunted infected hares. Some reported outbreaks have also been traced to the introduction of infected live animals, originating from holdings where the disease had not been detected (24). There is no information available on the presence of *B. suis* biovar 2 in the mountain hare

(*Lepus timidus*) in Northern Europe (24). In contrast to *B. suis* biovars 1 and 3, which can cause severe disease in humans, *B. suis* biovar 2 has only been isolated in a very small number of patients (38).

Brucellosis in reindeer and caribou (also named rangiferine brucellosis) is caused by *B. suis* biovar 4 throughout the Arctic region (Siberia, Canada and Alaska, with the exception of Norway) and constitutes a serious zoonotic problem (30). In Canada and Alaska, human cases were restricted to herders of diseased reindeer or caribou. All the patients were clinically ill native people who ate caribou as part of their diet. Part of a freshly killed caribou is often eaten raw, including bone marrow and some internal organs, known to contain high numbers of *B. suis* biovar 4 (30). Recently, the Alaska Native Tribal Health Consortium published bulletins on brucellosis in Alaska. (These bulletins can be downloaded at: www.anthctoday.org/community/climate_health.html.) It has been experimentally demonstrated that cattle exposed to *B. suis* biovar 4-infected reindeer can become infected (31). *Brucella suis* biovar 4 may also infect moose and musk ox (*Ovibos moschatus*) and, occasionally, carnivores (30). A recent serological study concluded that brucellosis was not present in reindeer in Finnmark, northern Norway (5). *Brucella suis* biovar 5, isolated in rodents in Eastern Europe and of which very few strains are known, is most probably misidentified and will not be discussed further.

Brucella microti

As mentioned previously, a systemic disease occurred in a wild population of the common vole in South Moravia (Czech Republic) during the years 1999–2003. From the clinical samples, small Gram-negative coccobacilli were isolated and identified as *Ochrobactrum intermedium* (51). However, subsequent analysis identified the strains as *Brucella* spp., representing a novel species within the genus *Brucella*, to which the name *B. microti* was given (88). Long-term survival of *B. microti* in soil was described and, thus, soil might act as a reservoir of infection (87). *Brucella microti* was also isolated from the mandibular lymph nodes of red foxes hunted in the region of Gmünd, Lower Austria. *Brucella microti* is thus prevalent in a larger geographical area, covering the region of South Moravia and parts of Lower Austria, and foxes could have become infected by ingesting infected common voles (86). No human or livestock infection with *B. microti* has been reported to date.

Pathogenesis

The sequence of events following the introduction of *Brucella* infection in wildlife is believed to be quite similar to that described during brucellosis infections in domestic animal

species. There is generally a relatively long latency period before clinical signs appear, mostly dependent on the age, sex and physiological condition of the animal. The *Brucella* entry sites are essentially the oral, nasopharyngeal, conjunctival and sexual mucosae. After penetration, a submucosal inflammatory reaction, characterised by infiltrates of mononuclear, polymorphonuclear and eosinophilic leucocytes, is produced. Invading bacteria are moved to regional lymph nodes through lymphatic drainage. Under experimental conditions, *Brucella* remains confined to the target lymph nodes close to its entry sites for two to three weeks. With the development of lymphadenitis, the bacteria reach the blood via the efferent lymph, and bacteraemia leads to a generalised infection of the reticulo-endothelial organs, lymph nodes distant from the entry sites, and genital and extragenital organs and accessory sexual glands. Brucellae can be isolated from the liver, kidney, spleen, testes, epididymis, vesicular glands, prostate, bulbo-urethral glands, uterus, mammary glands, bone marrow and most lymph nodes: submaxillary, parotid, retropharyngeal, prescapular, precrural, supramammary, iliac and genital lymph nodes. For this reason, excretion of the microorganism in milk, vaginal discharges in females and semen in males are the most important mechanisms for spreading the infection. Other organs, such as the brain, vertebral column and synovial structures, can also be found to be infected with *Brucella* in some animals (41, 81).

However, the prevalence of brucellosis in some wildlife species is very low and thus, besides the classical factors considered to be important in transmission (host susceptibility, shedding, survival in the environment, etc.), the behaviour of individuals and interactions between wildlife and livestock may actually be the most important drivers for transmission. For instance, the reclusive calving behaviour of elk, which keeps the elk cow and calf away from the herd for several days after calving, minimises the risks of *B. abortus* transmission to other animals and, in free-ranging settings, elk may be looked upon as a potentially dead-end host. On the other hand, in situations with important anthropic effects (i.e. intentional winter feeding of wild animals, or unintended co-mingling of wildlife on livestock feed grounds), the risk of infection increases dramatically, due to increasing density and enhanced risk of exposure to an infectious abortion near the feeding grounds, resulting in feed contamination. In the GYA, winter feeding changed the behaviour and density of free-ranging elk, probably converting what would otherwise be a dead-end host in nature to a maintenance host of *B. abortus* (93). As documented recently in the context of bovine tuberculosis due to *Mycobacterium bovis*, specific individuals with relatively high contact rates, in both cattle and badger populations, have the potential to act as hubs in the spread of disease through complex contact networks (9).

Clinical signs

Brucellosis in wildlife is characterised by reproductive disorders: abortion, retention of the placenta, metritis, subclinical mastitis, infertility, orchitis or epididymitis with frequent infertility or sterility, as well as articular and peri-articular hygromas, seen more frequently in chronic infections. Abortion is the main sign of clinical brucellosis, and often occurs during the second half of the gestation (41, 81). In 75% to 90% of cases, the animal will abort only once. Finally, a given number of animals born from *Brucella*-infected females may be latent carriers (approximately 5%) and can only be identified by immunological tests after their first or second pregnancy (47, 74).

Gross and microscopic pathology

In general, *Brucella* spp. infection in wildlife causes a similar pathology to the lesions in domestic animals (18). Brucellae have a predilection for the gravid uterus, udder, testicle and accessory male sex glands, lymph nodes, joint capsules and bursae. Infected animals develop placentitis, which results in abortion or premature or weak offspring and increased perinatal mortality. Placental retention also occurs in a significant proportion of animals. After abortion, the placenta may be oedematous and hyperaemic, and the fetus may have haemorrhagic fluid in the peritoneal space and subcutaneous tissues. Metritis sometimes occurs, and nodules and abscesses may be found in both the gravid and non-gravid uterus. Lesions in the uterus are frequent sequelae, being the main reason for infertility. Although the number of infected males with testicular alterations is not usually high, an important proportion of infected males excrete *Brucella* in semen. After necropsy, inflammatory lesions, abscesses or calcified foci may be seen in the testes and accessory sexual glands and organs, particularly the epididymis and seminal vesicles. Testicular atrophy and enlargement of the tail of the epididymis are characteristics of the chronic phase of disease in males. The macroscopic appearance of the testes is usually normal, but granulomas and calcification may be apparent on the cut surface. The affected epididymis appears firm, showing a white cut surface as a consequence of connective tissue proliferation. One or more abscesses filled with creamy or caseous substances may be observed in the thick connective tissue. Vesicular glands can show enlargement and altered cut surfaces with dilated ducts, either empty or filled with fluid.

Abscesses or other purulent lesions can also be found in non-reproductive organs, particularly the lymph nodes, spleen, liver, kidneys, joint capsules, tendon sheaths, bones, mammary gland, urinary bladder and, occasionally, the

brain. Nodular splenitis, arthritis, bursitis and osteomyelitis of the vertebral bodies have been reported in some species. Swollen joints and tendon sheaths, accompanied by lameness and lack of coordination, can occur. Less common signs include posterior paralysis, spondylitis and abscess formation in various organs (100).

In fetuses infected with *Brucella* spp., a series of pathological changes occur, including pneumonia and sero-haemorrhagic lesions in body cavities and muscles. Other lesions, such as abnormal abomasal content, fibrinous pleuritis, vasculitis and meningitis, occur less frequently. Fetal bacteraemia occurs after replication of *Brucella* spp. in trophoblasts. Fetal viscera and placental cotyledons subsequently become heavily infected with *Brucella* spp. Histological lesions observed in aborted fetuses are not specific enough to be able to identify *Brucella* as the cause of abortion. In chronic cases, uni- and bilateral hygromas, mainly localised at the carpal joint, can be observed in a significant proportion of infected animals (100).

Diagnosis

Brucellosis signs are non-pathognomonic in livestock or wildlife, and definitive diagnosis depends on laboratory testing. Laboratory diagnosis includes indirect tests that can be applied to milk or blood, as well as direct tests (classical bacteriology and direct polymerase chain reaction or PCR-based methods). The choice of a particular testing strategy depends on the prevailing epidemiological situation of brucellosis in susceptible animals (livestock and wildlife) within a country or region. Isolation of *Brucella* spp. (or specific DNA detection by PCR) allows certainty of diagnosis. Biotyping provides important epidemiological information which allows tracing back to the source of infection in countries in which several biotypes are circulating. However, when one particular biovar is overwhelmingly the most frequently isolated strain in a country or region, classical typing techniques are of no help. In this context, new fingerprinting methods, such as Multiple Locus Variable [number of tandem repeat] Analysis (MLVA) and Multi Locus Sequence Analysis (MLSA), are gaining wider acceptance and are being routinely used for typing and fingerprinting of isolates for epidemiological purposes (54).

Direct diagnosis

Several techniques are available to identify *Brucella* spp. The staining of smears from pathological material is still often used, and can provide valuable information from abortive material (3). Bacterial isolation is nevertheless always preferable and often required for genotyping strains. New PCR techniques allowing the identification and typing of *Brucella* have been developed and are being implemented in specialised diagnostic laboratories (11, 54, 55), but the

performance of these molecular methods for direct diagnosis of brucellosis from field samples still needs to be improved.

Staining

Brucella is a coccobacillus, measuring 0.6 µm to 1.5 µm long and 0.5 µm to 0.7 µm wide. Morphologically, *Brucella* is generally present as individual bacteria, but can occasionally be observed in clumps of two or more. *Brucella* is a Gram-negative bacterium that can resist weak acidic treatment and therefore appears red after Stamp staining. However, this staining is not specific enough and other pathogens, such as *Chlamydomydia abortus* (formerly *Chlamydia psittaci*) and *Coxiella burnetii*, also stain in the same way and have a similar morphology (3).

Culture

The isolation of *Brucella* from a given animal is the only diagnosis of certainty. The choice of samples depends on the observed clinical signs. In the case of clinical brucellosis, adequate samples include: vaginal secretions, colostrum, milk, aborted fetuses (stomach, spleen and lung), fetal membranes, sperm and fluid collected from arthritis or hygroma. *Post mortem*, the preferred tissues are the genital organs, the spleen and the following lymph nodes: oropharyngeal, iliac, scapular, crural and supramammary/testicular lymph nodes. Bacteriological diagnosis of brucellosis is performed by culturing these animal samples (once properly homogenised) directly on both Farrell's (FM) and modified Thayer-Martin's (mTM) culture media. However, despite inhibiting most contaminating microorganisms, the FM also inhibits the growth of some *Brucella* strains. However, the mTM is adequate for all *Brucella* species but only partially inhibitory for contaminants. A new selective medium (CITA) is more sensitive than both mTM and FM for isolating all *Brucella* species from field samples (19). The simultaneous use of both FM and CITA media improves the diagnostic performance of the culture. Visible colonies may appear after three to four days, but cultures can only be considered negative after seven days of incubation. The presumptive identification of *Brucella* spp. is conducted by analysis of colonial morphology, Gram staining, and the catalase, oxidase and urease tests (3).

Biotyping

Definitive identification and biotyping of *Brucella* colonies are performed by using various classical microbiological tests. The most important are: the dependence on serum and CO₂ for growth, the production of H₂S, growth in the presence of some dyes, agglutination with specific antisera and lysis by specific phages (3). To conduct these tests proficiently, submitting the isolated strains to an internationally recognised laboratory for definitive typing is recommended.

Molecular identification and typing methods

Molecular identification

Several PCR-based methods have been developed. The best are based on the detection of specific sequences of *Brucella* spp., such as the 16S-23S genes, the IS711 insertion sequence (which has, so far, only been detected in *Brucella* spp.) or the *bcbp31* gene encoding for a 31 kilodalton (kDa) protein (6, 70). These techniques have been used on human samples but have become more widely applied in veterinary medicine. However, their relatively low sensitivity, high cost and the lack of standardisation have limited their application to specific problems, such as wildlife studies. It is difficult to provide sensitivity and specificity estimates for these techniques because the test protocols described in the published literature differ greatly. As a general rule, these techniques show a significantly lower diagnostic sensitivity than culture but, depending on the samples and animal species considered, their specificity is close to 100% (10).

Molecular typing

The AMOS PCR has been used to type *Brucella* spp. (12). (AMOS is an acronym for the *Brucella* species: *B. abortus*, *melitensis*, *ovis* and *suis*.) This test allows discrimination among *Brucella* species and between vaccine and wild-type strains but does not allow differentiation between the biovars of a given *Brucella* species. However, not all the biovars of *B. abortus* (biovars 3, 5, 6, 7, 9) and *B. suis* (biovars 2, 3, 4, 5), some of which are epidemiologically important in Europe, can be identified in this assay. A newly developed multiplex PCR assay (named Bruce-ladder) can, for the first time, identify and differentiate between all the *Brucella* species and the vaccine strains in the same test (55).

New techniques appear promising, such as single nucleotide polymorphism signatures (SNPs), aimed at detecting DNA sequence variations that occur when a single nucleotide in the genome differs between members of a species; MLSA, aimed at directly measuring the DNA sequence variations in a set of housekeeping genes and characterising strains by their unique allelic profiles; and MLVA, which analyses the variability of loci presenting repeated sequences (54, 96).

Indirect diagnostic tests

A number of serological studies have been performed on brucellosis in wildlife, as well as in zoo collections, with the goal of assessing the prevalence of *Brucella* spp. within different wild species. Brucellosis serology in wildlife is usually performed using the same antigens as in domestic ruminants because the *Brucella* immunodominant antigens are associated with the surface 'smooth' lipopolysaccharide (sLPS), which is shared by all the naturally occurring biovars of *B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. microti*, *B. ceti*, *B. pinnipedialis* and *B. inopinata*. It is important to

note that the remaining species of the genus (*B. ovis* and *B. canis*) present on their surface a 'rough' LPS, which is antigenically different from the sLPS. This means that infections by these two rough *Brucella* species cannot be detected by the classical serological tests used for detecting antibodies against sLPS. Tests detecting rough LPS *Brucella* infections will not be discussed further because of their limited importance in wildlife.

It should be emphasised that it is impossible to determine which species of *Brucella* has induced antibodies against sLPS in the host and thus the gold standard in brucellosis diagnosis is the isolation of the bacteria. A sound testing strategy is first to estimate the apparent prevalence of brucellosis in wildlife by serology, and then attempt to isolate the bacteria from a selected subset of samples.

Wildlife brucellosis ecology can be divided into infection rate, attack rate (progress to become clinical) and mortality/morbidity rate (chronic carrier state) (25). With the shift to the detection of a pre- or subclinical disease (as in the context of brucellosis control and eradication programmes in livestock) and the identification of brucellosis in wildlife populations, the ecology of wildlife brucellosis has become more and more important when analysing serological results, and the emphasis should be put on the predictive value of test results, i.e. the clinical, ecological and epidemiological usefulness of the results (52).

Most serological brucellosis tests used in wildlife brucellosis studies have been directly transposed, without any previous validation, from their use in domestic livestock populations (in which, quite often, unfortunately, these tests have not been validated either). Moreover, some of these assays rely on species-specific reagents that are not commercially available.

To determine the apparent prevalence of brucellosis in wildlife, simple classical tests, such as the Rose Bengal test, may be recommended. However, this classical test requires high-quality serum and, unfortunately, gathering high-quality serum samples from wildlife is frequently impossible. Therefore, recent brucellosis studies in wildlife have been based on enzyme-linked immunosorbent assays (ELISAs) (99) and fluorescence polarization assays (34). The degree of haemolysis and the 'quality' of serum samples do not significantly affect the diagnostic performance of these tests. The lack of availability of polyclonal or monoclonal antibodies that detect immunoglobulins in different wildlife species can be overcome in indirect ELISA, by using either Protein A or Protein G as conjugates (46, 61, 64).

Bacteria such as *Yersinia enterocolitica* O:9 induce serological cross-reactions in the brucellosis serological tests that are almost indistinguishable from true brucellosis serological reactions (39, 62, 82, 95). Other cross-reactive bacteria exist in wildlife, for example, *Francisella tularensis*, an important pathogen in hares (50). Thus, false-positive serological

reactions should always be taken into account for a sound interpretation of serological results in the absence of bacterial isolation. The parallel use of sLPS-based tests and tests detecting specific antibodies directed against *Brucella* cytoplasmic proteins, as documented in moose (21) and elk (53), could be of some help to differentiate cross-reactions from antibody responses due to true *Brucella* spp. infection.

Another approach could be based on assessing cell-mediated immunity, through *in vivo* (skin) tests or *in vitro* tests (proliferation assays or cytokine detection assays) against specific *Brucella* cytoplasmic proteins. Some of these approaches have been applied in domestic animals, particularly cattle (76, 82), and their efficacy could be estimated in wildlife. It is also important to acknowledge that, although brucellosis tests are usually adequate to detect an infected group of animals, they can have important limitations in detecting infected individuals.

Management and control

In Europe, wildlife brucellosis does not receive the attention it is currently receiving in the USA, where there is a wildlife reservoir of *B. abortus* in bison and elk in the GYA.

Notwithstanding the fact that the use of vaccine in wildlife should always be carefully considered, there is no brucellosis vaccine currently available that gives satisfactory results in terms of safety and efficacy in wildlife (17). However, in bison, calfhood RB51 vaccination conferred some reduced protection, as compared to the protection conferred in cattle (67). Vaccination is unlikely to eradicate *B. abortus* from Yellowstone bison, but could be an effective tool for reducing the level of infection (92). Therefore, a combination of novel brucellosis management strategies and public education to balance conservation, economic and public health issues will be required (75). However, the isolation of the S19 cattle vaccine from feral pigs (90) highlights the fact that, before any prospective use of a brucellosis vaccine in wildlife, the release of such a vaccine must be fully investigated, particularly in non-target species (17). Although some tools are currently available for use in the wildlife reservoirs, the development of new vaccines, diagnostics and management procedures will most likely be needed to gain effective control of brucellosis (66).

Nowadays, the control of brucellosis in wildlife should be based almost exclusively on good management practices, and it is thus vital to assess them. As dramatically shown in the GYA, elk (considered to be dead-end hosts when ranging freely) are becoming maintenance hosts for *B. abortus* when winter feeding is practised (93). As a general rule, management practices enhancing the wildlife/livestock interface should not be implemented.

In Europe, brucellosis in terrestrial wildlife is nowadays mostly restricted to *B. suis* biovar 2 infections in wild boar and hares. Given the ecology and geographical distribution of wild boar in Europe, *B. suis* biovar 2 infection in wild boar is of major concern for outdoor pigs in the EU. Moreover, *B. suis* biovar 2 can also spill over into cattle, as recently reported in Belgium (98).

The importance of *B. microti*, for which soil may be the reservoir, still needs to be assessed and its pathogenicity for wild rodent species and foxes remains to be properly determined.

Zoonotic concern

Brucellosis is an ancient disease with a low fatality rate (less than 2% of untreated cases). Yet human brucellosis remains the most common zoonotic disease worldwide, with more than 500,000 new cases every year. It is associated with substantial residual disability and is an important cause of travel-associated morbidity (72). However, direct transmission of *Brucella* spp. from wildlife to humans seems to be restricted to the butchering of meat and the dressing of carcasses, for feral pig meat (20, 84) and African buffalo bushmeat (2). In the Arctic, there is a close tie between native communities and animals, especially reindeer/caribou, both for nutrition and as an important part of the communities' culture. In this respect, the consumption of raw bone marrow and internal organs from freshly killed caribou is an important risk factor for *B. suis* biovar 4 infection. In contrast to *B. suis* biovars 1, 3 and 4, *B. suis* biovar 2 has rarely been isolated from humans, and its zoonotic role is questioned (42).

Significance and implications for livestock and wildlife health

In 1991, Professor Paul Nicoletti, from the College of Veterinary Medicine at the University of Florida, said, during an interview: 'I encourage my veterinary students to pick a "survivor", a disease that will provide a lifetime of challenging and rewarding work. Brucellosis is notorious for being a survivor.' From the first description of *B. melitensis* as an agent of Malta fever by David Bruce in 1887 to the discovery of a new worldwide ecological niche of *Brucella* spp. in marine mammals, brucellosis has always been of concern (42).

In Europe, given the progress of national bovine brucellosis eradication programmes, it is unlikely that *B. abortus* will establish itself in free-ranging wild ungulates. The same can probably be said for *B. melitensis*, although foci of infection

in small ruminants remain important in some Southern European states and in the Balkans. In the future, probably only anecdotic spillover from small ruminants to wild ungulates will be reported in the EU.

Although brucellosis has been eradicated from the domestic pig population in Europe for decades, *B. suis* biovar 2 infection in wild boar (which is a sustainable infection in almost all European wild boar populations) is of major concern for pigs reared outdoors, should brucellosis control programmes in domestic pigs be implemented in the EU Member States.

With particular reference to wildlife brucellosis, it is amazing to see that the epidemiology and ecology of the infection are still so poorly understood. For decades, scientists speculated on whether brucellosis induced abortion in bison (71). Little is known about the pathology and biological significance of *B. suis* biovar 2 in wild boar (44). The geographical distribution of brucellosis in the

European brown hare is not known but data suggest that its prevalence is less important than it is in wild boar, and that foci of infections show a patchy pattern, most probably due to the ecology of hares (49). Nevertheless, translocation of infected hares, in the absence of brucellosis regulations, may contribute to the disease's dissemination in Europe. At present, there are many taxonomical issues related to *Brucella* isolates which may lead to the description of new species and/or biovars in the near future (96). Lastly, the recent discovery of *Brucella*-like organisms from non-human primates (85) and African bullfrogs (*Pyxicephalus edulis*) originating from Tanzania (22) poses new challenges for our understanding of the genus *Brucella* and brucellosis at the wildlife/livestock/human interface.

Brucellose dans la faune sauvage terrestre

J. Godfroid, B. Garin-Bastuji, C. Saegerman & J.M. Blasco

Résumé

Le lien épidémiologique entre brucellose dans la faune sauvage et brucellose des animaux domestiques et de l'homme est largement établi. Lors d'études sur la brucellose dans la faune sauvage, trois questions doivent être posées : (i) s'agit-il d'une infection résiduelle trouvant son origine chez les animaux domestiques ou bien s'agit-il d'une infection durable dans une ou plusieurs espèces sauvages ? (ii) la brucellose dans la faune sauvage constitue-t-elle un réservoir pour les animaux domestiques ? (iii) y a-t-il un risque zoonotique ? Nonobstant leur préférence d'hôtes, *Brucella abortus* et *B. suis* ont été isolées de nombreuses espèces sauvages, alors que *B. melitensis* est plus rarement rapportée dans la faune sauvage. La pathogénicité de *Brucella* spp. chez les animaux sauvages n'est pas bien connue. La prévalence de la brucellose chez certaines espèces sauvages est très faible et partant, le comportement individuel des animaux sauvages ainsi que les interactions entre faune sauvage et animaux domestiques sont les facteurs de risque les plus importants pour la transmission de l'agent pathogène. Les signes cliniques de la maladie n'étant pas pathognomoniques, le diagnostic définitif se fonde sur des tests de laboratoire incluant des tests indirects

effectués sur le sang ou le lait ainsi que des tests directs (bactériologie classique ou des méthodes basées sur la réaction de polymérisation en chaîne [PCR]). Cependant, la sérologie ne permet pas de déterminer quelle espèce de *Brucella* a induit la présence d'anticorps chez l'hôte. Seul l'isolement de *Brucella* spp. (ou la détection d'une séquence d'ADN spécifique par PCR) permet un diagnostic définitif en utilisant des techniques classiques et moléculaires d'identification et de typage des souches. Il n'y a, actuellement, aucun vaccin anti-brucellique montrant un degré satisfaisant d'innocuité et d'efficacité pour la faune sauvage. C'est pourquoi le contrôle de la brucellose dans la faune sauvage doit reposer sur des techniques de gestion adaptées. À l'heure actuelle, la transmission de *Brucella* spp. de la faune sauvage à l'homme semble liée à la préparation et à la consommation de carcasses de sangliers ou de porcs sauvages infectés dans le monde développé et de viande de brousse (buffle africain) dans les pays en développement. Dans l'Arctique, la consommation traditionnelle de moelle osseuse et d'organes internes crus de caribous ou de rennes fraîchement abattus constitue un facteur de risque important.

Mots-clés

Bactériologie – *Brucella* spp. – Brucellose – Épidémiologie – Faune sauvage – Interface animaux sauvages/animaux domestiques – Sérologie.



Brucelosis en la fauna silvestre terrestre

J. Godfroid, B. Garin-Bastuji, C. Saegerman & J.M. Blasco

Resumen

La existencia de relaciones epidemiológicas entre las enfermedades de los animales domésticos y las que afectan a las especies silvestres es un hecho bien establecido. En el caso concreto de la brucelosis, tres cuestiones importantes son objeto de debate: (i) ¿la presencia de brucelosis en los animales silvestres se produce como consecuencia de la transmisión desde el reservorio doméstico, o bien existen reservorios silvestres en los que la enfermedad se mantiene de manera sostenida? (ii) ¿la fauna silvestre representa realmente un reservorio de *Brucella* para el ganado doméstico? y (iii) ¿la eventual presencia de brucelosis en la fauna silvestre representa un riesgo para la salud humana? Pese a poseer distintos hospedadores preferentes, tanto *B. abortus* como *B. suis* se han aislado en varias especies animales silvestres, lo que resulta mucho menos frecuente con *B. melitensis*. La patogénesis de *Brucella* spp. en las especies silvestres no se ha definido todavía con suficiente precisión y su prevalencia es generalmente baja o muy baja, por lo que el comportamiento individual y las interacciones entre fauna silvestre y ganado doméstico son los factores de mayor relevancia epidemiológica en la potencial transmisión de la enfermedad. Puesto que los signos clínicos de la infección no son patognomónicos, su diagnóstico definitivo dependerá del resultado de las pruebas laboratoriales directas o indirectas. Puesto que las pruebas indirectas, basadas normalmente en el diagnóstico serológico en muestras de sangre o leche, no pueden identificar la especie de *Brucella* involucrada, es preciso recurrir, siempre que sea posible, al diagnóstico bacteriológico utilizando técnicas clásicas o aquellas basadas en la biología molecular (reacción en cadena de la polimerasa o PCR). Sin embargo, las pruebas serológicas no son capaces de determinar contra qué

especie de *Brucella* están dirigidos los anticuerpos detectados en el hospedador. Solo el aislamiento de la bacteria (o la detección de su ADN específico por PCR) constituye un diagnóstico definitivo, recurriendo a técnicas clásicas o moleculares para identificar y caracterizar las cepas específicas.

Puesto que ninguna de las vacunas existentes para el ganado doméstico ha demostrado ser eficaz para prevenir la brucelosis en los animales silvestres, el control de la enfermedad en estas especies debe basarse en adecuadas prácticas de manejo, evitando en la medida de lo posible la interacción entre las especies domésticas y las silvestres. La transmisión de la enfermedad a la especie humana desde los animales silvestres es un evento raro, y que queda restringido normalmente al procesado y consumo de órganos de cerdo o jabalí infectados en el mundo desarrollado, o de órganos y leche del búfalo africano en los países en desarrollo. Por otra parte, el consumo tradicional de médula ósea u órganos internos crudos de reno y caribú puede representar un riesgo potencial de transmisión al hombre en el Ártico.

Palabras clave

Bacteriología – *Brucella* spp. – Brucelosis – Epidemiología – Fauna silvestre – Interfaz ganado doméstico/animales silvestres – Serología.



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