Brucellosis: A Neglected Zoonosis

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ABSTRACT

Brucellosis is a bacterial disease mainly of domestic animals. The infection is directly transmitted to humans by animals through breaks in the skin or by contact with infected materials like aborted foetuses and placenta. It can also be transmitted indirectly by ingestion of contaminated animal products as well as inhalation of the agent. It is an important zoonosis worldwide which accounts for about 500,000 reported human cases annually around the globe; particularly amongst agricultural and pastoral populations. It results in serious economic losses in animals due to abortion, reduced fertility, birth of weak offspring and reduced productivity. In humans, it leads to chronic debilitation resulting in low work output and subsequent negative economic impact. The paper reviews brucellosis in different species of animals. It highlights the aetiology, morphology, host

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range, pathophysiology, clinical signs, pathology and epidemiology of the disease in various species. Preventive and control measures against the disease, economic and public health implications have also been examined. It is concluded that, the eradication of brucellosis in animals may be achieved by long-term investment in surveillance programmes, including testing and culling of positive reactors. Vaccination of animal hosts may culminate in the eradication of the disease in human population.

Keywords: Brucellosis; zoonosis.

1. INTRODUCTION

Brucellosis is a highly contagious and one of the most important and prevalent zoonoses worldwide which is caused by bacteria of the genus *Brucella* [1]. The synonyms of the disease in humans are: Undulant fever, Constantinople fever as well as fevers of Malta, Naples, Cyprus, Crete, Crimea, Levant, Syria, Mediterranean, Gilbraltar, Gastric, milk, Remittent, Relapsing and Rock [2,3]. The disease in cattle is also known as infectious abortion contagious abortion, epizootic abortion and Bang's disease in tribute to the pioneer of the study of the disease in this species [2]. The burden of the disease is enormous but yet remains under-prioritised globally especially amongst the pastoralists and small-scale livestock farmers [4]. Humans can be infected by ingestion of food products such as milk and its products contaminated with the organism or by direct interaction with an infected animal or by aerosol inhalation [3]. It is mainly an occupational disease affecting veterinarians, abattoir workers, farmers and laboratory workers working with the organism. It is therefore recommended that, all laboratory work involving this organism should be carried out under biosafety level 3 [5].

2. AETIOLOGY

Organisms of the genus *Brucella* are part of the α-2 subgroup of the Proteobacteriaceae, they show a close genetic bond with some plant pathogens and symbionts of the genera *Agrobacterium* and *Rhizobium*, and also with animal pathogens (*Bartonella*) and opportunistic or soil bacteria (*Ochrobactrum*) [6]. *Brucella* organisms are Gram-negative, facultative intracellular bacteria [7,8]. They are strictly aerobic, non-encapsulated, and catalase and oxidase-positive [9]. They do not ferment carbohydrate and have variable urease activity. The *Brucella* organisms have a lipopolysaccharide coat that is much less pyrogenic than other Gram-negative organisms, which explains the reason for the rare presence of high fever in brucellosis [3,10]. There are six recognized species of the genus which include *B. abortus*, *B. melitensis*, *B. ovis*, *B. canis*, *B. neotome* and *B. suis* which are further divided into biotypes [11]. However, a marine species has been recently identified and was first classified as *B. maris* which was later divided into two species: *B. ceti* and *B. pinnipedialis*, denoting isolates from cetaceans and seals, respectively [12]. Furthermore, a new *Brucella* species which was first isolated in 2008 from systemically-infected common voles (*Microtus arvalis*) in South Moravia, Czech Republic, was named *B. microti* [13], a similar organism was isolated from the mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) in Austria. The latest species described is *B. inopinata*, which was isolated from infected human breast implant [14,15], though, the reservoir of this species of *Brucella* is not known. An unnamed strain (*Brucella spp*. NVSL 07-0026) has also been isolated lately from a baboon [16]). Consequently, this shows that there is more information to be explored with respect to the genus *Brucella* and its host range.
3. MORPHOLOGY

*Brucella* organisms are small, measuring about 0.5-0.7 by 0.6-1.5µm, non-motile, non-sporing, non-encapsulated, coccobacilli, which occur singly or occasionally in groups of short chains or in clusters [17]. They do not have flagella or pili and do not show bipolar staining. The cellular and colonial morphology of the species of *Brucella* are similar in most respects [18]. All *Brucella* species possess smooth lipopolysaccharide (SLPS) in their outer cell wall with the exception of *B. ovis* and *B. canis*, which have rough lipopolysaccharide (RLPS) and protein antigens [19]. Smooth lipopolysaccharide contains an immunodominant O polysaccharide (OPS) which has been chemically defined as a homopolymer of 4, 6-dideoxy-4-formamide-alpha-D mannose linked via glycosidic linkages.

4. HOST RANGE

Verger et al. [20] reported that, in spite of more than 94% similarity amongst members of the genus *Brucella*, the causative agents have inclinations for different hosts. It has also been advocated that *Brucella melitensis* can represent members of the genus with multiple biovars. The primary host plays an important role in the maintenance of the disease in nature, as most of them are reservoirs of infection for each particular species [21]. The alpine ibex in Italy and chamois in the French Alps have been found to be infected with *Brucella* organism. However, there is no evidence that these animals serve as reservoir hosts for livestock [7].

Sheep and goats are mainly infected by three biovars of *Brucella melitensis* [7]. Most breeds of goats are readily susceptible, but the susceptibility in breeds of sheep is variable. *Brucella melitensis*, the least species-specific species of *Brucella*, has been reported in cattle, camels and dogs, but rarely in horses and pigs. Infections in sheep and goats can spill over to wild ruminants. Sheep are relatively resistant to infections with *B. abortus* [22,23]. They can, however, assume a carrier state, capable of excreting the organism for up to 40 months post-infection [24]. The occurrence of natural infection with *B. abortus* is low in goats, thus making this animal species less important as a host [25].

Cattle are the preferential host for *B. abortus*. However, infections with this species of *Brucella* can occur in buffaloes, camels, deer, dogs, horses, goats, sheep, and man [2,26,27]. Turkeys, pigeons, pheasant, duck, goose, and chicken have also been reported to be susceptible [28,29].

Horses are susceptible to *B. abortus* and less frequently to *B. suis*. The organism has a preference for bursae, tendons, muscles and joints, where they cause arthritis, intermittent lameness and swelling of the carpal joint. However, they are normally found as a secondary invaders in cases of “fistulous withers” and “poll evil”, which is inflammation of the supraspinatous and supra-atlantal bursa, respectively [2,30].

Systemic infection leading to reproductive involvements in pigs is usually caused only by *Brucella suis* biovars and have been identified as an important re-emerging disease of domestic and wild pigs [31]. This pathogen may also affect other animal species such as cattle, horses, rabbits, hares, reindeers, dogs, and humans [3]. *Brucella suis* biovars 1 and 3, have been shown to also have pathogenic potential for humans [32]. In addition, biovars 4 and 5 are specifically associated with reindeer and rodents, respectively [33].
Dogs can be infected by four of the six species of *Brucella*, namely *B. canis, B. abortus, B. melitensis* and *B. suis*, excluding *B. ovis* and *B. neotomae* [34]. Canine brucellosis primarily caused by *B. canis* usually affect domestic dogs, wild carnivores, and seldom other domestic animals and man [35]. The disease could be presented as asymptomatic to mild, despite an on-going systemic infection [36].

The Office International des Epizooties [7] reported that camels are not known to be primary host for any species of *Brucella* organisms. Nevertheless, they are susceptible to both *B. abortus* and *B. melitensis*.

*Brucella neotomae* is known to infect only the desert wood rat under natural conditions and has not been reported to infect other species of animals [37].

The marine strain of *Brucella* has been isolated from various species of marine mammals, which include: seal (*Phoca vitulina*), dolphins (*Tursiops truncatus, Delphinus delphis, Lagenorhynchus acutus* and *Stenella coeruleoalba*), whale (*Balaenoptera acutorostrata, Balaenoptera physalus, Orcinus orca*) [38,39,40].

*Brucella melitensis, B. suis, B. abortus* are the major *Brucella* species responsible for human brucellosis with *B. melitensis* incriminated as the principal cause of the disease, a few organisms, ranging from 10 to 100 are sufficient to set off an incapacitating chronic infection [41]. Two recently-identified *Brucella* species isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, may cause human brucellosis [13].

5. INCUBATION PERIOD

There is a significant variation in the incubation period in infection with members of the genus *Brucella*. The period is mainly influenced by gestation, exposure dose, age, and vaccination status [42]. In cows, the incubation period is inversely proportional to the stage of gestation at the time of exposure and could range between 53-251 days [43]. The period of incubation of brucellosis in the bull has not been fully determined [44].

6. PATHOPYSIOLOGY

Members of the genus *Brucella* are intracellular pathogens that localize in lympho-reticular cells of the body and have a special affinity for sugar-rich organs such as the uterus during pregnancy, udder, testicles and the accessory sex glands of the female and male hosts, respectively [45]. After ingestion, the *Brucella* organism penetrates the epithelium of the gastro-intestinal tract, from where it is transported to the regional lymph nodes [46]. In a pregnant uterus, multiplication of the bacteria is supported by the presence of the sugar alcohol, erythritol, which is a foetal product concentrated in the chorion and cotyledons. Furthermore, the interaction of the bacteria with placental trophoblasts suggests that its ability to acquire iron is vital as the brucellae enter their acute replicative stage [47]. Mutants of *Brucellae*, unable to utilize erythritol, are severely attenuated in ruminant hosts. *In vitro* data suggest that *Brucella* metabolizing erythritol have a heightened requirement also for iron scavenged through siderophores such as 2, 3-dihydroxybenzoic acid or brucebactin. This may be linked with the requirement for effective iron acquisition for virulence in ruminant hosts. In a more advanced phase, *B. melitensis* can colonize the udder of lactating goats, resulting in acute mastitis with the production of clotted and watery milk and reduced milk yield [48]. The long-term survival and replication of *Brucella spp.* is enhanced by their ability
to modify the endosomal compartment of phagocytic cells; this they do by employing various mechanisms to make the host environment suitable. It was also reported by Xavier et al [49] that the Brucella organisms have the ability to persist and replicate within phagocytic cells of the reticulo-endothelial system as well as in non-phagocytic cells such as dendritic cells macrophages and trophoblasts. In these cells, they form an important source of the organisms for periodic re-infection. The major virulence factor for the organism is cell wall lipopolysaccharide, which is essential for the functional and structural integrity of the outer membrane of Gram-negative bacteria. Normal serum factors, including complement, are involved in opsonisation of the organisms to allow phagocytosis, polymorphonuclear leucocytes have limited ability to kill bacteria within phagocytes. Thus, Brucella organisms that escape are further ingested by macrophages, where they become localised and undergo multiplication within organs of the reticulo-endothelial system. There could be an involvement of any or multiple organs and systems of the body [50]. There is a surge in the level of humoral antibodies directed against lipopolysaccharide soon after an infection and other cell wall antigens are produced. However, the ability of the body to mount cell-mediated immunity is the most important mechanism of recovery from the illness. Survival of Brucella organisms in the host may be due to the production of adenine and guanine monophosphate. The production inhibits phagosomal fusion and oxidative burst activity by inhibiting the degranulation of peroxidase-containing granules and, consequently, the myeloperoxidase-peroxide-halide system of bacterial killing [51].

7. CLINICAL SIGNS OF BRUCELLOSIS

Brucellosis is a multi-systemic disease. The nature, infecting dose, animal host and the infecting Brucella species are determinants of the manifestation of clinical signs [2].

8. CATTLE

In cows, the disease usually becomes apparent, causing abortion occurring from the 5th to the 8th month of gestation. Though, abortion was observed in a three-month pregnant heifer 56 days after being fed with the cotyledons from an aborting cow as observed by Bang in 1906 [2]. According to Acha and Szfyres [52], abortions in pregnant females usually occur once, after which immunity is acquired. Afterwards, as an alternative to abortion, birth of premature foetuses, still-born or birth of weak calves may occur. Placental retention, which may follow abortion, could lead to metritis, which in turn may result in infertility. Some infected cows may not show symptoms of the disease and may give birth to normal calves [42]. Brucellosis in bulls mainly affects the reproductive tract, leading to orchitis, epididymitis, ampullitis and seminal vesiculitis. Orchitis is usually unilateral, but there is a possibility that both testicles may become affected.

8.1 Pigs

The most common manifestation of brucellosis in the sow is abortion, occurring at any time during gestation [53]. Vaginal discharge is often absent in chronically infected sows. The most economically important sequel of the disease is infertility rather than abortion. Megid [2] and Deyoe [54] reported that abortion rate is higher in sows and gilts exposed to B. suis via the genital tract at the time of breeding, and that abortions are influenced more by the time of exposure to the B. suis rather than by the stage of gestation. In addition, embryonic resorptions have been encountered as early as 17 days following natural mating by boars disseminating B. suis in their semen. Affected sows rarely abort more than once and those
infected before sexual maturity hardly ever abort [52]. Brucellosis could turn out to be more persistent causing lesions in the reproductive tract that often result in either temporary or permanent interference with sexual activities [53]. Furthermore, the organism can be discharged in the semen of infected boars without any apparent abnormality in the sex organs or interference with sexual activity. In both sexes, infection may result in swollen joints and tendon sheaths, which may subsequently manifest as lameness and, occasionally, posterior paralysis [55, 56]. There could also be abscesses of various sizes in various organs and tissues [57]. Boars that do not develop a genital infection seldom recover from the disease [54]. However, infections in accessory sex glands are very common in boars, but do not always affect fertility. Infection of the genital organs is usually short-lived in sows [52].

8.2 Sheep and Goats

There has not been evidence that involvement of different biovars of *Brucella melitensis* results in varying clinical signs in sheep and goats [58]. The principal clinical feature of *B. melitensis* infection in ewes and does are late term abortion, placental retention, and birth of weak offspring, who usually die during the peri-partum period [59]. Alton [60] observed that abortion in female goats occurred between 3 and 4 weeks after experimental infection with high doses of *B. melitensis*. However, abortion in ewes occurred 4 to 12 weeks after experimental infection with the same organism, which is an indication that ewes are relatively more resistant. Most acute natural infections with *Brucella melitensis* in does during pregnancy lead to colonisation of the udder, which lead to shedding of the organism in milk during subsequent lactations. Intermittent shedding of the organism in milk also occurs in animals with persistent udder infection, resulting in inflammation of the udder with subsequent reduction in milk production [61].

Acute phase of brucellosis in the ram results in epididymitis, which can be severe, with sperm stasis and secondary spermatocele formation, resulting in infertility. This phase is characterised by poor semen quality and distension of the scrotal sac by either haemorrhagic or fibrino-purulent exudates [48]. The principal clinical findings are palpable lesions in the epididymis and tunicae. However, lesions usually ensue after the acute syndrome has resolved and the latent period has elapsed. The chronic phase in infected bucks could, however, be characterised by hygromas and joint inflammation. It is worthy of note in infection with *Brucella ovis* that some infected rams, showing palpable lesions at one examination may be clinically normal a few weeks later, and that not all infected rams develop lesions in their external genital organs [62].

8.3 Horses

In horses, brucellosis can pass un-noticed or may be associated with clinical signs for as long as two years post-exposure [63]. Infection in this species most often involves *Brucella abortus*. The disease is mainly recognised as an inflammation of the supraspinous and supra-atlantal bursae. The syndromes are known as “fistulous withers” and “poll evil”, respectively. The bursal sac becomes obviously distended by a clear, viscous, straw-coloured exudate and develops a thickened wall. It may rupture, resulting in a secondary point of infection by secondary invaders. Excretion of the organism in vaginal discharges seems to be short-lived in mares. Other clinical signs reported in horses due to *B. abortus* infection are arthritis, intermittent lameness, lethargy and swelling of the carpal joint [64].
8.4 Dogs

Shin and Carmichael [36] reported that infection with B. canis results in variable clinical signs associated with the reproductive system. This infection can be asymptomatic to mild, despite an on-going systemic infection, with high morbidity and low mortality. The characteristic sign of brucellosis in the canine species as in most species of animals is late-term abortion, which can occur between 30-57 days of gestation. It is more common from 45 to 55 days of gestation in about 75% of the cases. Abortions are followed by mucoid, sero-sanguineous, brownish or grey vaginal discharge that persists for up to six weeks. The pattern of oestrus and breeding does not change in bitches with brucellosis [34]. The disease in the bitch can result in repeated abortions or birth of sick puppies that often die during the post-partum period. Resorption or early embryonic death within 2 to 3 weeks after breeding can also occur. Subsequently in the disease, birth of apparently normal off-springs can occur [34,36].

Clinical signs of Brucella abortus infection in dogs vary from mild fever to orchitis and testicular atrophy with shedding of the organisms in the urine [65]. Manthur et al [66] reported that dogs experimentally infected with B. suis were found to be afebrile and asymptomatic and do not present gross lesions. It was further reported that under natural conditions, weakness of the hind-limbs along with large and firm epididymitis could be associated with oligospermia and increased number of neutrophils in semen, similar to what is observed in infection with Brucella canis. Failure to achieve intromission due to pain, unwillingness to ejaculate or successful internal ties without pregnancy has been reported [34,67]. Brucella canis infection targets androgen-dependent organs, causing severe epididymitis, orchitis and prostatitis. Epididymitis usually begins at about five weeks after infection, and can be detected on physical examination by an acute onset of inflammation, pain and swelling. Scrotal dermatitis may ensue as a result of constant licking of the scrotum which may subsequently lead to oedema which might further be contaminated by non-haemolytic staphylococci [34].

8.5 Camels

Camels are not known to be major host for any Brucella species [68]. They may however be infected with B. abortus and B. melitensis when they are pastured together with goats, cattle and sheep. In such instances, they shed the organisms in milk, thus posing a serious public health problem [69,70]. Camels are silent carriers of the disease as clinical signs of brucellosis in camels seem to be very rare.

8.9 Humans

Human brucellosis is a chronic and debilitating disease that may be presented with variable clinical signs [71]. Although the clinical presentations of the disease are variable, the most common clinical sign is undulant fever in which the temperature can vary from 37°C in the morning to 40°C in the afternoon. Other signs include night sweats with a peculiar odour, chills, arthralgia, myalgia and weakness [2]. Furthermore, malaise, insomnia, anorexia, headache, constipation, sexual impotence, nervousness, and depression are also common presentations. It has also been reported that brucellosis is capable of causing spontaneous abortion, congenital and neonatal infections in humans. This has been evidenced by a few cases in which Brucella species were isolated from fetal or placental tissues [72,73]. Depending on the internal organs affected, complications like spondylitis, arthritis, endocarditis, orchitis and prostatitis could be noticed [52]. Neurological complications like
meningitis, encephalitis, meningo-encephalitis, brain abscess, chorea, facial palsy, meningo-myeloencephalo-spondylosis, and ischaemic attacks have also been reported to occur during the onset of the disease, during the recuperation period or even well after recovery from an acute infection [74]. Though the illness can be debilitating and chronic, brucellosis is seldom fatal in humans, except for few untreated cases due to vegetative endocarditis [75]. A prolonged course of illness, often associated with suppurative destructive lesions, is associated with B. suis infections. Brucella abortus is associated with mild-to-moderate sporadic disease that rarely causes complications. Brucella canis infection has a disease course that is indistinguishable from B. abortus infection. Brucella canis infection has an insidious onset, causing frequent relapses, and does not commonly cause chronic disease in humans. Brucella pinnipediae and B. cetaceae have been recently reported to cause mainly neuro-brucellosis in humans [51].

9. PATHOLOGY DUE TO BRUCELLOSIS

9.1 General Pathology

Pathology due to brucellosis is usually generalized because various organs are affected, though they are mostly encountered in organs of the reproductive system [76]. The pathology may depend on the infecting Brucella species and the organs or tissues involved. Aborted foetuses may appear normal, autolysed or may have different degrees of subcutaneous oedema along with blood-stained fluid in their body cavities. Abortions in brucellosis are typically accompanied by placentitis with the cotyledons appearing red, yellow, normal or necrotic.

In cattle and small ruminants, the intercotyledonary region is characteristically leathery, appearing wet with focal thickening and exudates on the surface [6]. Histological findings in brucellosis infection are mainly lymphocytic infiltrations, granulomas with necrosis [77].

In adult animals, the reproductive tract, mammary gland, supramammary lymph nodes, other lymphoid tissues, bones, joints, and other organs may show granulomatous to purulent lesions. Mild to severe endometritis may be observed after an abortion [78]. In bulls, B. abortus results in barely visible swelling of the testicles. The pressure induced by the swelling, may result in necrotic foci that coalesce and lead to entire necrosis of the testicles with sequestration by inflammatory thickening of the tunica. It was reported by Nicoletti [79] that the accessory sex glands may be infected by B. abortus, and that the organism may also localize in the carpal and other bursae, where hygromas containing large number of bacteria may be found.

In sows, B. suis biovar 2 infections are associated with formation of nodule in internal organs, particularly seen more in the reproductive organs [53]. The lesions can also be seen in the spleen, liver, lungs, and most other organs, including the skin and subcutaneous tissues. Nodules often become purulent, but regardless of the presence of the nodules, the sow’s body condition may remain good. Catarrhal metritis is common and abscesses can frequently be found in the testes or seminal vesicles of the boar [80]. Histologic findings in brucellosis usually include mixed inflammatory infiltration with lymphocytes being prominent. Also, granulomas (in up to 55% of cases) and necrosis may be present.
9.2 Pathology Due to *Brucella melitensis* Infection

Aborted foetuses due to *B. melitensis* usually have a normal gross appearance. However, there can be bronchopneumonia, haemorrhagic fluid in the thoracic cavity, enlarged lymph nodes, liver and spleen [60]. Granulomatous inflammatory lesions are often found in lymphoid tissues and organs such as the reproductive organs, udder and supramammary lymph nodes. It may also be present in joints and synovial membranes. However, these lesions are not pathognomonic. There can also be an acute mastitis with palpable nodules and the production of clotted and watery milk in the udder of infected animals [61].

9.3 Pathology Due to *Brucella ovis* Infection

Lesions caused by *B. ovis* are generally restricted to the epididymis and accessory sex glands [81,82]. The epididymal enlargement may be unilateral or bilateral with the tail of the epididymis is being more affected. There is usually a fibrous atrophy of the testis with thickening of the tunica vaginalis, it may become fibrous with extensive adhesions. Semen of infected rams is usually of poor quality as characterized by decreased sperm concentration, and contains inflammatory cells [83]. Although placentitis is uncommon, it is occasionally seen in infected ewes [6].

9.4 Pathology Due to *Brucella canis* Infection

There may be generalized lymphadenitis involving mostly the retropharyngeal and inguinal lymph node. The spleen is frequently enlarged, and may be firm and nodular. Hepatomegaly may also be seen. Scrotal oedema and scrotal dermatitis, epididymitis, orchitis, prostatitis, testicular atrophy and fibrosis may occur in some infected males, while metritis and vaginal discharge may be seen in infected females. Less commonly reported lesions include discospondylitis, meningitis, focal non-suppurative encephalitis, osteomyelitis, uveitis and abscesses in various internal organs [34]. There are no obvious lesions from aborted foetus in the canine species. However, the organism can be isolated from the lungs and liver [84]. Carmicheal and Joubert [85] reported that semen from infected dogs usually contains large numbers of abnormal sperm and inflammatory cells, especially during the first three months following infection and chronically infected males may have azospermia, or reduced numbers of immature sperm cells.

9.5 Pathology Due to *Brucella neotome* Infection

Lesions due to brucellosis in marine animals occur only in a few species, and they include: meningoencephalitis, subcutaneous abscesses, placentitis, abortion, epididymitis, chronic purulent or granulomatous orchitis, lymphadenitis, mastitis, spinal discospondylitis, peritonitis, and a mineralized lung granuloma [40]. Others are hepatic abscesses, hepatic and splenic necrosis. There is also the presence of macrophage and or histiocytic cell infiltrations in the liver, spleen and lymph nodes. The lesions in dolphins with meningoencephalitis are usually severe, chronic and widespread along with non-suppurative meningitis, which is most severe in the brainstem. The meningitis is usually accompanied by periventricular encephalitis [86,87].
10. EPIDEMIOLOGY OF BRUCELLOSIS

10.1 Distribution of Brucellosis

*Brucella* organisms have a worldwide distribution. However, it is more common in countries with poorly standardized or ineffective animal and public health programmes [88]. Rust [89] reported that, brucellosis is a zoonotic infectious illness to which humans became exposed as a result of domestication of animals and the establishment of animal husbandry as an important element following civilisation. The prevalence of zoonotic disease globally is usually and also a reflection of indigenous cultures in husbandry systems. The epidemiology of brucellosis is therefore dynamic as changes in the causative agent, host or environment may affect the complex interrelationship that exists between them thereby influencing the epidemiology of the disease. Thus, control measures of this disease have been difficult because of the involvement of livestock and humans in its spread [90]. The Mediterranean countries of Europe, Northern and Eastern Africa, Near East countries, India, Central Asia, Mexico, and Central and South America are particularly affected by this disease [91].

The herding of different species of livestock together significantly promotes interspecies transmission of brucellosis [92,93]. It was reported by Esuruoso [94] that generally, brucellosis is more prevalent in government-owned farms than in nomadic herds due to intensive system of management. Similarly, it was reported that the prevalence of the disease could result in variations, which may be attributed to some factors like sampling technique, diagnostic method used, vaccination status of the animals, climatic conditions, geography, species, sex and age [93,95].

Of the known *Brucella* species, *Brucella melitensis* is the least species-specific and is the major cause of human brucellosis [96]. It is one of the most important zoonoses worldwide, accounting for an annual incidence of more than half a million cases [97,98]. The risk of infection is proportional to the degree of contact with *Brucella*-infected animals, their excreta, or their edible by-products, particularly milk or cheese. Pasteurisation of milk and care in the slaughtering of animals for meat and assurance of adequate cooking of meat are of great importance in preventing human brucellosis. Eradication is usually defined as the absence of any reported cases for at least five years. Countries reported to have eradicated the disease include: Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom [99]. The fact that brucellosis is considered a re-emerging problem for countries like Israel, Kuwait, Saudi Arabia, Brazil and Colombia is of great concern [98].

10.2 Susceptibility of Animals to *Brucella* organisms

All domesticated animals are susceptible to *Brucella* organisms, except the cat [100]. Ko and Splitter [21] reported that, the primary host is an important agent in the maintenance of the disease as most of the other hosts serve as reservoirs of the infection for each particular species. There is the possibility for cross infection among animal species, especially when they are kept in close contact. Adult animals are considerably more susceptible to infection by *Brucella* spp. than younger ones [93]. Such adults may be infected and may not show any clinical sign, but generally show only a weak and transient serological response [33]. Young animals are relatively resistant, though it should be noted that, latent infections can occur and such animals may be hazardous at maturity. Susceptibility increases during pregnancy, and animals get more susceptible with the advancement of pregnancy due to the presence
of erythritol in the uterus. Also, bulls are relatively more resistant than sexually matured heifers and they are less resistant than sexually immature heifers [2]. Conversely, in the swine industry, boars are more likely to be sources for introducing Brucella organisms into a herd [52].

Corbe and Brinley-Morgan [101] reported that dairy breeds of sheep are more susceptible to brucellosis than those kept for meat production. Other than natural immunity, management is also a key factor that could possibly account for the lower incidence of brucellosis in males than in females. This is because, most dairy cows and many beef bulls are maintained separately from the general herd and their exposure to infection is therefore lessened [102].

10.3 Resistance of Brucella Organisms to Environmental Factors and Chemicals

The ability of members of the genus to persist outside the mammalian hosts is relatively high compared to most other non-sporing pathogenic bacteria, they can survive for a long time in both hot and cold environments, particularly with high moisture content. Brucella organisms can survive in tap water for several months at 4ºC to 8ºC, 2.5 years at 0ºC, and several years in frozen tissues or medium [103]. Brucellae can also survive for up to 60 days in damp soil, and for up to 144 days at 20ºC, 40% relative humidity and at pH greater than 4. They can survive freezing and thawing and also several weeks in non-fermented milk [104]. These account for the persistence of infection in tropical Africa where husbandry and management practices are poor and tend to encourage the survival of the organism.

Brucellae can survive for 30 days in urine, 75 days in aborted foetuses and more than 200 days in uterine exudates. They are however destroyed at 56ºC - 61ºC within 4-5 hours in beddings contaminated with infected faecal material [105]. Brucella species are susceptible to dyes like thionin, basic fuscin methylviolet, pyronin, and safranin-O at standard concentration of 20g/ml and the susceptibility varies between biovars [61]. They grow in a minimal medium containing sodium chloride, sodium thiosulphate, ammonium sulphate, glucose, nicotinic acid, thiamine, panthotenic acid and biotin [106]. Their growth can be enhanced by the addition of serum or blood.

Brucella species are readily killed by most commonly available disinfectants like hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene. However, presence of organic matter and low temperatures decrease the efficacy of these disinfectants. Sodium hypochlorite at 2.5%, 2-3% caustic soda, 20% freshly slaked lime suspension, and 2% formaldehyde solution are capable of destroying Brucella on contaminated surfaces following one hour of exposure [34].

10.4 Effects of Management Practices

The system of husbandry greatly influences the spread of infection due to Brucella species. Animals on institutional farms that abort due to brucellosis have high chances of infecting other animals within the flock than free range nomadic type of husbandry animals which have less chances of remaining or returning to the contaminated environment [107]. However, a more accepted view is that, the prevalence is higher in pastoral production systems, where there is free mixing of large number of animals of different species and lowest for the confined farms [108-111]. Lambing or kidding in dark or crowded enclosures
favours the spread of the organism, while open-air parturition in a dry sunny environment results in decreased transmission [78].

10.5 Transmission of Brucellosis

Most infections due to members of the genus Brucella result from ingestion of the organism from diseased animals, contaminated feedstuffs and drinking points. Aborted foetuses, placental membranes or fluids, and other vaginal discharges present after an infected animal has aborted or parturated are very rich sources of the organism. The habit of feeding aborted materials to dogs and also burning of such materials by pastoralists favour transmission of the disease [93]. Contamination of abraded skin and mucosal surfaces are possible ways of transmission. So also is through aerosol, thus making this organism a potential biological weapon [97].

Natural breeding can result in infection in swine and dogs and, to a lesser extent, sheep and goats [33]. Generally, brucellosis is carried from one herd to another by an infected animal. However, transplacental transmission can be possible occasionally [112]. The purchase of animals from unscreened sources, sharing of male breeding stock and extensive system of management, which permits indiscriminate matting are means by which susceptible animals can be exposed to infection. The use of pooled colostra for feeding newborn calves may also be responsible for the transmission of the infection [33].

It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active will shed Brucella organisms in the semen during the acute phase of the disease and can therefore help in the spread of the disease [103]. Shedding, however, may cease or become intermittent [113]. In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus [114].

In small ruminants, Brucella infection is usually transmitted by contact with the placenta, foetus, foetal fluids, aborted materials and vaginal discharges from infected animals. Small ruminants are capable of shedding the organism after either abortion or full-term parturition [6]. Goats usually shed B. melitensis in vaginal discharges for at least 2 to 3 months, while shedding usually ends within three weeks in sheep. Furthermore, B. melitensis can be found in milk and semen and infected ewes and does may shed the organism in milk and semen for prolonged periods, leading to infection in lambs and kids. Additionally, infection in sheep occurs mainly through the nasopharyngeal route and can also be transmitted from dam to kid/lamb in uterus or via the colostrum or milk.

Transmission of brucellosis in pigs occurs through venereal and oral routes, with B. suis being available in large numbers for long periods especially in afterbirth as well as in uterine discharges and milk in females or in semen from infected boars [60].

Brucella ovis transmission can occur by direct contact between rams kept in the same premises due to sniffing at one another, it can also be transmitted through ewes that have mated with an infected ram prior to a susceptible one during the same mating season. They thus serve as mechanical transmitters [115,116]. In ewes, B. ovis can uncommonly cause abortion associated with placentitis beginning at 30 days of gestation. Infected ewes may give birth to weak lambs and there may be high neonatal mortality rate [117]. Ewes do not play an important role in the transmission of Brucella ovis, since they do not remain permanent carriers of the disease.
Transmission of brucellosis in dogs usually occurs by breeding, sniffing or ingestion of contaminated placental tissues, aborted foetuses or vaginal secretions from infected bitches. *Brucella canis* may be shed for long periods in semen or vaginal secretion post-abortion [118].

Garner et al [119] reported that the means by which *Brucella spp* are transmitted among marine mammals is poorly understood. However, the almost restricted localisation of these bacteria within the intestinal lumen and/or uterus of the lungworm (*Parafi laroides, Phocoena*) in the pulmonary systems of an infected Pacific harbour seal and harbour porpoise suggests the strong possibility that these may play a role in transmission of the organism. On the other hand, Foster et al. [120] reported that transmission of brucellosis in marine animals may occur through mucosa and injured skin, direct contact, or by the oral route due to ingestion of other infected marine mammals. Vertical or horizontal transmission to the foetus is a possible means of infection since *Brucella* organisms have been isolated from foetal tissues and in milk of dolphins [87].

Brucellosis is primarily a disease of animals in which man is an accidental host [121]. Human exposure to this disease could be occupational with the organism gaining entry through the mucus membrane or abraded skin. Farmers, shepherds, hunters, butchers, laboratory workers, veterinarians, and slaughterhouse workers are at a greater risk of contracting the disease through this means [47,122]. The non-occupational sources of exposure include ingestion of infected meat and contaminated dairy products [47,123,124]. In addition to the food-borne and occupational infection, brucellosis is linked to travel and bioterrorism. *Brucella* is considered as a biological weapon in the category B pathogen [9, 125]. Recently, other routes of transmission have been identified to include infection through breast milk [74], sexual intercourse [126] and blood transfusion [127]. The role of flies and ticks in the transfer of *Brucella* organisms from infected to uninfected herd/flock has not been established [78].

### 10.6 Immune Response to *Brucella* Infection

The *Brucella* organisms have developed adaptive measures for survival and multiplication within their hosts. They include the ability to control phagocyte apoptosis, while another is to affect the expression of cytokines, which is necessary for a normal protective function of the immune response [128]. Immune response to *B. abortus* will be used as an example because it has been most studied in detail [129]. *Brucella* infection in cattle consists of early IgM isotype which usually appears 5-15 days post-exposure, but may sometimes be delayed. Appearance of this antibody depends on the route of exposure, infecting dose of the bacteria and the immune status of the host animal [130]. Production of IgM isotype is followed shortly by production of IgG1 isotype and subsequently IgG2 and IgA in little quantities [131,132]. Indicator of exposure, therefore, depends on identification of IgM antibody isotype. However, a number of other microorganisms contain antigens with similar epitopes to those of *Brucella*, and the main antibody response to these cross reacting antigens is IgM. Therefore, measurement of IgM antibody isotype sometimes give false positive serological reactions leading to low assay specificity [129]. Production of IgG2 and IgA isotypes occurs later in infection and so measurement of these antibodies would generally lower assay sensitivity. Therefore, the most reliable antibody measurement for serological tests for brucellosis is IgG1 [133]. In addition to cross reactions, residual antibodies in vaccinated animals sometimes cause diagnostic problems due to false positive reactors. *Brucella abortus* S19 is a widely used vaccine which is antigenically indistinguishable from pathogenic strains of *B. abortus* [130]. However, administration of the
vaccine to young animals, usually between 3 and 8 months of age (calfhood vaccination), generally allows the antibody response to wane sufficiently to eliminate some diagnostic problems at sexual maturity [134,135].

11. TREATMENT OF BRUCELLOSIS

The fact that Brucella species are intracellular Gram negative pathogens that infect host macrophages makes their contact with antimicrobial agents difficult. Therefore, for any antibiotic to be effective in the treatment of brucellosis, it must have the ability to adequately penetrate macrophages of the host animal and act in the acidic intracellular environment [123,136]. There is a general need for a drug combination since all monotherapies are characterized by unacceptably high relapse rates. Concurrent treatment with chlorotetracycline and streptomycin has effected some level of cure in sheep, though it is usually not economically feasible except in valuable rams [123,137]. Moreover, fertility may remain low, even if the organism is eliminated. Treatment of the disease in cattle is generally unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland and the reproductive organs. Besides, failure in treatment of brucellosis in animals has also been attributed to reasons like the use of incorrect dose of antibiotics, inadequate duration of treatment, high cost of medication, and failure to cure udder infection which, could lead to a relapse [138]. In humans, there has been a long term quest for a reliable regimen for antibiotic therapy for brucellosis; however, the issue of relapse still remains a major setback. This makes the optimum therapy inconclusive till date [139]. Treatment of brucellosis is aimed at shortening the course of the clinical symptoms, prevent relapse and complications like arthritis, sacroilitis, spondylitis, encephalitis, endocarditis, epididymoorchitis, and abortion [123]. To combat the high rates of relapse in monotherapies, a combination of two antibiotics has been resorted to. Some combination of antibiotics used are; doxycycline plus rifampicin, doxycycline plus streptomycin, quinolones plus rifampicin or doxycycline plus gentamycin [140]. Surgical interventions have also been employed in complicated cases like vegetative endocarditis, drainage of pyogenic joint effusions or paraspinal abscesses [141].

12. PREVENTION AND CONTROL OF BRUCELLOSIS

The fact that brucellosis is a disease of major economic and zoonotic importance, a strategy for its control in animals, especially livestock, is vital especially in endemic areas. The primary focus of the control strategy should be the reduction of the infection in animal population to such a level that the impact of the disease on human health and animal production is minimized. The B. abortus S19 and B. melitensis Rev. 1 vaccines are the cornerstones of control programme in cattle and small ruminants, respectively [142]. Prevention and control of brucellosis in animals essentially include test and culling of positive reactors, quarantine, zoning and tracing.

Serological testing of livestock should be routinely carried out using approved serological tests. All animals reacting positively to the tests should be destroyed or consigned for immediate slaughter in an approved abattoir following strict hygienic procedures. The supra mammary, medial, internal iliac, retropharyngeal, parotid and prescapular lymph nodes and spleen from such animals should be submitted for Brucella culture [78]. However, there is a limitation to eradication by test and slaughter as some cases of latently-infected young animals remain serologically negative to standard tests until late into their first pregnancy. Time of quarantine should be long enough to ensure that all pregnant animals complete their
gestation without any evidence of infection. In rams, the prevalence of *B. ovis* can be decreased by routine examination and culling of rams with palpable abnormalities before breeding [33]. However, it is worthy of note that palpable lesions are not always found in all infected rams and, therefore, laboratory testing of rams should also be considered.

12.1 Quarantine and Movement Controls

In case of any suspicion of brucellosis, quarantine must be immediately imposed on the affected herd or flock to ensure the containment of the infection, after which such herds/flocks will require repeated sero-monitoring to confirm their status. Movement of latently-infected animals presents the greatest risk, and the potential for movement of infected material by dogs or birds should not be ignored. In a brucellosis endemic country, clear demarcation of diseased and disease-free zones should be established. Thereafter, tight control on the movement of animals between these zones should be enforced. When an infection is suspected or confirmed, trace-back and trace-forward of animal movements is essential to identify the index case and other potentially infected or exposed herds to forestall future spread of the disease [143]. Surveillance should be carried out in neighbouring herd/flock to identify any infected herd/flock, not already identified by tracing. Surveillance has also been reported to be an effective tool in monitoring the progress and effectiveness of the control programme, after the cessation of vaccination [142].

Infected animals at term or having a vaginal discharge pose a disease risk to personnel and in contact animals. They should, therefore, preferably be culled immediately. It has been reported that the level of infection is at its peak 4 days to calving or abortion, and 14 days afterwards in cattle [144]. However, culling and depopulation of infected animals have serious economic impacts such as the availability of compensation from government, which is an important incentive to ensure that owners promptly report any evidence of infection.

Decontamination of equipment and environment can be achieved by exposure to sunlight, high temperatures, wiping down contaminated surfaces with a freshly-made (less than 7 days old) 1:10 aqueous dilution of household bleach and a range of chemicals [145]. Furthermore, other measures to reduce the likelihood of environmental survival of infective bacteria include draining wet areas and ploughing of the soil to improve the rate of desiccation of the organism.

12.2 Vaccination

Vaccination is an extremely important and effective facet of most control strategies of brucellosis, but has the disadvantage that its use may confuse diagnosis by stimulating the production of antibodies, notably IgG1. Nevertheless, it has been reported that live vaccines have until now proved superior to inactivated products for the prevention of animal brucellosis [134]. *Brucella abortus* S19 and *B. melitensis* Rev. 1 vaccines have proven effective against *B. abortus* in cattle and against *B. melitensis* and *B. ovis* in sheep and goats, respectively [134,146]. Both vaccines are capable of causing abortion in a proportion of pregnant animals, and of being pathogenic for humans in case of accidental injection. Furthermore, *Brucella melitensis* Rev.1 has also been evaluated for the vaccination of cattle in countries, where *B. melitensis* infection in sheep and goats is widespread. Experimental studies have shown that *B. melitensis* Rev.1 vaccine provided immunity to *B. melitensis* equal to or superior than the immunity induced by *B. abortus* S19 with a lower vaccine dose in cattle. However, despite the promising results, the use of *B. melitensis* Rev.1 in this
species of animals has been very limited [134]. Xin [147] reported that since 1971, a live-attenuated smooth strain of *B. suis* biovar 1 strain 2 has been used as an oral vaccine to control brucellosis in cattle, sheep, goats and pigs in China. The vaccine is safe when administered orally, and does not induce persistent antibody titres.

Although the S2 strain of *B. abortus* also gave a satisfactory protection rate in cattle [148], its efficacy against experimental *B. melitensis* infection in pregnant ewes or against *B. ovis* infection in rams was inferior to that of *B. melitensis* Rev.1 vaccine [19,20]. *Brucella abortus* strain 45/20 vaccine is normally administered as two doses, given 6 to 12 weeks apart, followed by an annual booster [149]. There have been reports that, the new attenuated vaccine, *Brucella* strain RB51, licensed by the United States Department of Agriculture, Animal and Plant Health Inspection Service for use in cattle in the USA does not stimulate production of antibodies detectable in standard diagnostic tests. However, it stimulates the production of other antibodies that can be detected with a special assay that indicates that the animal has been previously vaccinated. Vaccination against brucellosis in other species of animals like sheep, dogs horses and pigs have not been well established, except for pigs where vaccination against *B. suis* is said to be carried out in China [33].

Presently, there is no approved vaccine for brucellosis in humans [150,151]. It has however been reported that accidental exposure to RB51, S19, and Rev-1 strains of *Brucella* meant for immunization in domestic animals can result in development of clinical brucellosis in humans [152]. It is therefore noteworthy that personnel carrying out vaccination in animals should be aware of the risk therein.

12.3 Wild Animal Control

Feral animals, including buffalo and deer, may become infected with brucellosis, if they graze in the same area as domestic animals. They should, therefore, be controlled by mustering or field destruction [33].

12.4 Control of Brucellosis in Other Species

Control of brucellosis in other species of animals is similar to that for livestock. Housing of dogs in individual cages can help in reducing the spread of the organism. Repeated testing and the removal of seropositive or culture-positive animals, combined with quarantine and testing of newly-added dogs, have been used to eradicate brucellosis from some kennels [33]. Specific control methods have not been established for brucellosis in marine mammals, but the general principles of infection control in such animals include isolation, disinfection, and practice of good hygiene. Nevertheless, prevention of brucellosis in humans still depends on the eradication or control of the disease in animal hosts. The exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods like meat should be adequately considered [153].

12.5 Public Awareness

Farmers should be enlightened through the media or public campaigns on the importance of regular sero-surveillance in susceptible animals. Also, reports of abortions, birth of weak or dead foetuses, or infertility to appropriate authorities will be of assistance. Surveillance programmes that relieve the farmer costs of investigation are a useful strategy. Details of
any imposed movement controls need to be readily available and clearly explained to farmers. Highlighting the zoonotic implications of brucellosis, people at risk must be advised on appropriate occupational health and safety requirements, health authorities should be well informed on the potential for human infection [154].

13. ECONOMIC IMPACT OF BRUCELLOSIS

In developed counties, the economic implication associated with brucellosis are mainly as a result of slaughter of herds/flocks infected with *Brucella*, while in developing countries, it is due to actual abortion of foetus and resulting in decreased milk production, birth of weak offspring that most often do not survive the neonatal days, placental retention and subsequent infertility. Unlike the disease in animals, it is very difficult to quantify financial losses in human brucellosis, but it is definitely substantial. The economic losses encompass cost of treatment of affected individual and long term debilitation that renders the individual unproductive. Others are restrictions in international livestock trade and by diminished animal working power [95].

14. CONCLUDING REMARKS

Brucellosis is an important zoonosis affecting a wide range of animals. It is a neglected disease, with no official policy on its eradication in most developing countries. The eradication of brucellosis may be achieved through effective collaboration between animal health and public health sectors in the spirit of “One World, one Health”. This may require a comprehensive approach and long-term investment. Eradication programmes include: vaccination, serologic testing with isolation and culling of infected animals (test and slaughter), public awareness, health education activities, and providing sufficient financial resources for successful and continuous implementation especially in terms of compensation for culled animals. Test and slaughter approach alone may not be very effective in most developing countries, where most animals are managed under semi-intensive or extensive transhuman or nomadic systems. This approach should, therefore, be adequately complemented with vaccination programmes to effectively curtail the spread of the infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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