

Review Article

Camelid Brucellosis: A Review

Wernery U*

Central Veterinary Research Laboratory, Dubai, UAE

***Corresponding author:** U Wernery, Central Veterinary Research Laboratory, Dubai, UAE**Received:** December 16, 2015; **Accepted:** March 03, 2016; **Published:** March 05, 2016**Abstract**

Camel brucellosis has been diagnosed in all camel-rearing countries except Australia. In many countries the infection is on the rise in Old World camels (OWCs) due to the uncontrolled trade of live animals. Knowledge of camelid brucellosis has increased over the last decade through field investigations, experimental infection trials and comprehensive laboratory testing. Infection with *Brucella melitensis* is frequent in OWCs and rare with *B. abortus*. New World Camels (NWCs) rarely contract brucellosis. In East African countries the seroprevalence of brucellosis can reach 40% (herd level) and depends on the management system. The highest incidence is found when camels are kept together with infected small ruminants. Only a combination of serological methods can detect all serological reactors. However, many brucellosis antibody ELISAs for serum or milk are not suitable for diagnosis. Culturing the pathogen is still the preferred test method, although several assays based on polymerase chain reaction have been developed.

Keywords: Brucellosis; Camelid; Diagnosis; Epidemiology; Treatment

Introduction

Many countries, such as the United Kingdom, Australia and Japan, as well as parts of the United States of America (USA) and some countries in North Europe have succeeded in eradicating brucellosis through intensive health control measures, but elsewhere the disease remains widespread in domesticated and wild animal populations and presents a great economic problem for tropical animal husbandry [1]. Brucellosis is also one of the most important zoonoses in developing countries. Old World camels (OWCs) are frequently infected with brucellosis, particularly when they are in contact with infected ruminants [2-6]. The disease is rare in New World Camels (NWCs) but outbreaks with classical signs of brucellosis have been described [7].

Aetiology

Brucellosis is a contagious disease caused by bacteria of the genus *Brucella*. Taxonomically, the genus *Brucella* is divided into ten classified species and subdivided into biovars. The subdivision is based on biochemical reactions and agglutination with mono-specific sera. Recently, *Brucella* strains have been isolated from numerous marine mammal species; molecular typing methods have not been able to classify these isolates within the described species, and therefore they have received their own names: *Brucella cetacea* (dolphins) and *B. pinnipeda* (seals, fur seals, walruses). *Brucella* bacteria are Gram-negative coccobacillae that are non-motile and non-spore-forming. They grow aerobically and certain strains need a 5% to 10% carbon dioxide atmosphere. *Brucella* organisms grow slowly, but can be enhanced by using enriched media, such as Farrell's media supplemented with 5% horse serum and six added antibiotics.

The growth of *B. ovis* and *B. abortus*, biotype 2, always requires media enriched with serum or blood incubated in an atmosphere of 5% to 10% carbon dioxide.

Impact on Human Health

In humans, the disease, which is often referred to as 'undulant fever' or 'Malta fever' is a serious public health problem. Human brucellosis remains one of the most common zoonotic diseases worldwide, with more than 500,000 new cases annually (World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO), [8]. Infection prevalence in the animal reservoirs determines the incidence of human cases [9]. *Brucella* spp. are also potential agents of bioterrorism and are classified in group B (second-highest priority agent) of the Centres' for Disease Control and Prevention (CDC) in the USA. *Brucella melitensis* and *B. abortus* are the two species most commonly found in human cases, and *B. melitensis* is responsible for the most serious infections. Human brucellosis is mainly an occupational disease, and the main modes of transmission are contact through skin with animal tissues, blood, urine, vaginal discharge, aborted fetuses and, especially, placentas, and consumption of raw milk and other unheated dairy products. Airborne infections occur in animal pens, stables, laboratories [10] and abattoirs. Some cases have also occurred from accidental self-inoculation with live vaccines [11] World Organisation for Animal Health (OIE), [12]. Moreover, it was also shown by Bradenstein *et al.* [13], that Rev 1 vaccine strain can cause human infections. In their study humans became infected after consuming milk from vaccinated adult pregnant animals which excreted the vaccine strain in milk for a long period of time. The high and increasing herd and animal prevalence of camel brucellosis in many countries is of grave concern [14] therefore, veterinary authorities, consumers, camel owners and camel keepers, as well as responsible persons in the Ministry of Health and Agriculture of each country, should make every effort to address this issue.

During investigations conducted by Radwan *et al.* [15], it was found that brucellosis was diagnosed in 30% of the camel handlers

Table 1: Signs of brucellosis in humans.

Clinical signs	Patients affected in %
Fever	90–95
Malaise	80–95
Body ache	40–70
Sweats	40–90
Arthralgia	20–40
Splenomegaly	10–30
Hepatomegaly	10–70

and *milkers* and the same *B. melitensis* biovars were cultured from aborted sheep and goats sharing the same premises.

In humans, the incubation period lasts from five to 60 days, but can also be longer. Clinical signs are not specific and can be acute or chronic (Table 1) [16].

Brucella infections in pregnant women in early pregnancy may lead to high rates of fetal loss (up to 40%) and infection in men can lead to orchitis and epididymitis. *Brucella melitensis* DNA persists in human blood for many years after infection despite appropriate treatment and apparent recovery [17]. Humans are at risk through consumption of unheated milk or through handling *Brucella*-positive animals [8,15,18-19]. Shimol *et al.* [20] described a brucellosis outbreak that affected 15 people who consumed unpasteurized camel milk. Affected people suffered mainly from arthralgia and fever and 50% had positive blood culture for *B. melitensis*, whereas 60% had serum agglutination titres of 1:60 or higher.

During a *B. melitensis* outbreak which occurred in a herd of alpacas in Peru, over 25% of the alpaca handlers were seropositive to brucellosis and some developed clinical signs [21].

Extreme care must be exercised when working with *Brucella* organisms in laboratories. It is estimated that up to 2% of all diagnosed brucellosis cases are laboratory-acquired infections, mainly through inhalation when handling diagnostic specimens [22].

Incidence of Camelid Brucellosis

Camelid brucellosis caused by *B. melitensis* and *B. abortus* has been reported in all camel-rearing countries except Australia and the incidence appears to be closely related to breeding and husbandry practices [23], which Omer *et al.* [24] were able to prove in Saudi Arabia. They compared the brucellosis seroprevalence of a female dromedary herd which was in close contact with small ruminants ($n = 165$) with a closed female dromedary herd ($n = 95$). The brucellosis prevalence in the open camel herd was 8.5%, whereas only one animal (1%) was diagnosed in the closed herd. The diagnostic tests used were the Rose Bengal test (RBT), serum agglutination test (SAT) and competitive enzyme-linked immunosorbent assay (cELISA). High animal and herd prevalences have been reported from many countries, which not only pose a severe risk to humans but also to other livestock. The infection rate in some regions of the former Union of Soviet Socialist Republics (USSR), where Bactrian camels were kept on large farms, was 15% [25], whereas in countries with more extensive forms of husbandry, such as Chad or Ethiopia, the brucellosis seroprevalence was 3.8% [26] and 5.5% [23], respectively.

Similar differences in seroprevalence have been reported from Saudi Arabia by Radwan *et al.* [27] and Ghoneim and Amjad [28]. They reported a higher incidence of camel brucellosis in intensively farmed camels than in free-grazing desert camels. In Sudan, prevalence varies according to the system of camel husbandry: agro pastoralists reported a higher prevalence of brucellosis (31.5%) than nomads (21.4%) [29-31]. A seroprevalence of dromedary brucellosis of 40% has been reported from Sudan [32], and the United Arab Emirates (UAE) has experienced a drastic increase of brucellosis in camel populations due to the uncontrolled import of dromedaries from East African countries. Also, introduction of camels into cattle, sheep and goat areas in the Darfur region of Sudan led to high incidence levels, as shown by Musa and Shigidi [33]. In another study in Sudan, conducted by the same authors, in 3,413 dromedaries that were intermingled with cattle and small ruminants, the herd infection rate was 45.5%, with prevalence rates of between 1.4% and 90%.

Moustafa *et al.* [34] reported on a serological survey in dromedaries and a brucellosis eradication campaign in the eastern regions of the UAE during a five-year period. The highest prevalence was in 1991, with a reactor rate of 5.8%, whereas the lowest was in 1996, with a rate of 0.01%. Since no camels had been culled due to brucellosis, it is believed that the reduction in camel brucellosis was caused by the reduction in brucellosis in sheep and goats.

Epidemiology

The disease has a worldwide distribution and affects cattle, pigs, sheep, goats, camelids, dogs and, occasionally, horses. *Brucella* infections have also been documented worldwide in a great variety of wildlife species and, more recently, in marine mammals. A spill over of infection from domestic animals to bison, elks or African buffalos may also be possible [35].

The infection occurs via the mucous membranes, including oral-nasopharyngeal, conjunctival and genital mucosa, and also through cutaneous abrasions. Animals become infected through feed, water, colostrum, contaminated milk and, especially, by licking or sniffing at placentas and aborted foetuses. The spread of brucellosis during sexual activity plays a subordinate role. The primary shedding routes of *Brucella* organisms remain uterine fluids (lochia) and placenta expelled from infected animals. In cattle it is known that abortion is associated with the shedding of 10^{12} to 10^{13} *Brucella* bacteria. Survival of the organisms in the environment is enhanced by cool temperatures and humidity; however, it was proven that two dromedaries in a *Brucella*-negative dromedary herd were infected with *B. melitensis* through contaminated dust particles from aborted camel foetuses 500 m apart, indicating that organisms can also survive in a hot desert environment. Many placental mammals, including herbivores, participate in placentophagy, with camelids as a noted exception, which may contribute to the spread of *Brucella* bacteria through wind. In bovines, shedding of up to 10^3 *B. abortus* bacteria/ml through milk following abortion may last for a period of up to three months, which is considered an important fact from an epidemiological point of view. The situation in camelids is unknown. Excretion of the pathogen through milk is intermittent [36]. However, in chronically infected (serologically positive) dromedaries from the UAE which gave birth to healthy off springs, no *Brucella* organisms were isolated from expelled placentas, and no shedding occurred

Table 2: *Brucella* species isolated from camelids in different countries.

Country	Author	Year	Species	Organs
Jordan	Al-Majali	2006	<i>B. melitensis</i> biovar 3	Aborted fetuses, vaginal swab
Russia	Solonitsyn	1949	<i>B. abortus</i>	n/a
	Pal'gov	1950	<i>B. abortus</i>	Bactrian, fetuses
Iran	Zowghi and Ebadi	1988	<i>B. melitensis</i> biovar 1	Lymph nodes
Kuwait	Zowghi and Ebadi	1988	<i>B. melitensis</i> biovar 3	Lymph nodes
Libya	Al-Khalaf and El-Khaladi	1989	<i>B. abortus</i> biovar 1	Foetal stomach
	Gameel <i>et al.</i>	1993	<i>B. melitensis</i> biovar 1	Milk
Saudia Arabia	Gameel <i>et al.</i>	1993	<i>B. melitensis</i> biovar 1	Milk, vaginal swab, aborted foetus
	Radwan <i>et al.</i>	1992	<i>B. melitensis</i> biovar 1 and 2	Milk
	Radwan <i>et al.</i>	1995	<i>B. melitensis</i> biovars 1, 2, 3	Milk
	Ramadan <i>et al.</i>	1998	<i>B. melitensis</i>	Carpal hygroma
	Al Dubaib	2007	<i>B. melitensis</i>	n/a
Sudan	Agab <i>et al.</i>	1996	<i>B. abortus</i> biovar 3	Teats, lymph nodes, vaginal swab, testis
	Musa <i>et al.</i>	2008	<i>B. abortus</i> biovar 6	Lymph nodes
			<i>B. melitensis</i> biovar 3	
	Omer <i>et al.</i>	2010a	<i>B. abortus</i> biovar 6	Lymph nodes, testis
Peru	Acosta <i>et al.</i> (alpacas)	1972	<i>B. melitensis</i>	Organs
UAE	Wernery <i>et al.</i> (camels from Sudan)	2007b	<i>B. melitensis</i> biovars 1 and 3	Milk, lymphnodes, placenta
	Moustafa <i>et al.</i>	1998	<i>B. melitensis</i>	Milk
Senegal	Verger <i>et al.</i>	1979	<i>Brucella abortus</i> biovars 1 and 3	n/a
Egypt	El-Seedy <i>et al.</i>	2000	<i>B. abortus</i> biovars 1 and 7	Organs
			<i>B. melitensis</i> biovar 3	

n/a: information not available.

UAE: United Arab Emirates.

through milk. Also, the blood of dromedary calves was negative in culture and Polymerase Chain Reaction (PCR). Interestingly, camel calves of serologically positive dams were all serologically negative, using RBT and cELISA techniques, at the age of six months. The calves therefore do not appear to be at risk for an acute brucellosis infection even after the disappearance of maternal antibodies. However, for confirmation of these findings, further investigations need to be performed [9]. Ostrovidov [37], and Solonitsyn and Pal'gov [38], proposed separating calves from their dams at the age of seven to eight months, when their maternal antibodies have disappeared. If this does not occur, they may contract infection from infected dams at the next parturition. The *Brucella*-negativity of female camel calves from chronically infected dams is controversially discussed among Dubai-based veterinarians and some researchers believe that confirmation of the *Brucella*-negativity can only be confirmed when camel calves remain serologically negative after parturition. In males, it is an even more complicated unsolved issue.

In general, abortions occur mainly during the first pregnancy and infected camelids are clinically well. The pathogen is found intracellular in mononuclear phagocytes, in which it also multiplies. In pregnant camels, the bacteria localize in the placenta and are most abundant in abortion material (up to 10^{13} bacteria) including the fetal stomach, vaginal discharge and colostrums [39]. *Brucella melitensis* and/or *B. abortus* organisms have been isolated from camel milk, aborted fetuses, the placenta, fetal stomach fluid, lymph nodes, vaginal swabs, testes and hygromas (Table 2).

It was also shown by Von Hieber [9] that, during a period of two years, 5% (n = 118) of the dams had fluctuating titres from positive to negative to positive and 20% of the serologically positive dams turned negative with RBT and cELISA (latent infection?). This indicates that the pathogens can conceal themselves, most probably in lymph nodes, and do not produce detectable antibodies in those intracellular hiding places. However, evidence of spontaneous recovery from brucellosis had also been described by Gatt Rutter and Mack [44] and Ostrovidov [37], with no further explanation. Further research by Wernery *et al.* [45], who investigated the question of where *Brucella* organisms were concealed in serologically positive lactating dromedaries which gave birth to healthy calves, revealed that they were in internal lymph nodes. They were mainly isolated in lung lymph nodes, indicating an inhalation infection route. These investigations in camelids clearly show that there are important epidemiological differences in dromedaries which abort (acute brucellosis) and chronically infected animals which do not abort. A chronic infection is certainly the most common occurrence, and in bovines it is known that 75% to 90% of cows abort once only [46].

Theoretically, the three *Brucella* species known to cause brucellosis in camels (*B. abortus*, *B. melitensis*, *B. ovis*) can cause infection anywhere [47]. However, it is surmised that *B. melitensis* is widespread in Africa and the Middle East and *B. abortus* is widespread in the former USSR. Solonitsyn [48] reported mixed infections with various *Brucella* species in Bactrian camels in Russia. (Table 2) demonstrates which *Brucella* species have been isolated from which

Table 3: Sensitivity and specificity of serological tests for brucellosis.

Serological test	Sensitivity in %	Specificity in %
SAT	81.5	98.9
CFT	90–91.8	99.7–99.9
RBT	87	97.8
cELISA	95.2	99.7
iELISA	97.2	97.1–99.8
FPA	96.6	99.1
MRT	88.5	77.4

cELISA: Competitive Enzyme-Linked Immunosorbent Assay; CFT: Complement Fixation Test; FPA: Fluorescence Polarisation Assay; iELISA: Indirect Enzyme-Linked Immunosorbent Assay; MRT: Milk Ring Test; RBT: Rose Bengal Test; SAT: Serum Agglutination Test.

organs in which country.

Although camels appear to be very susceptible to *Brucella* infection, isolation of *Brucella* organisms from camel samples is rare. But attempts to isolate *Brucella* from milk have been successful. *Brucella abortus* biovars 1 and 3 were isolated from camels in Senegal [49]. Radwan *et al.* [3] were able to isolate *B. melitensis* biovars 1 and 2 26 times from a total of 100 milk samples from seropositive Saudi Arabian dromedaries. Gameel *et al.* [50] were also able to isolate *B. melitensis* biovar 1 five times from the milk of Libyan dromedaries and four times from aborted fetuses and vaginal swabs from a herd of 124 Libyan dromedaries. The authors did not mention from how many affected dromedaries the samples were taken. Zaki [51] inoculated guinea pigs with milk samples from seropositive dromedaries and cultured the milk samples *in vitro*. Both tests (SAT and culture) were negative. Al-Khalaf and El-Khaladi [52] examined cultures of 209 milk samples from Kuwaiti dromedaries. The samples were obtained from herds with an increased incidence of abortion. The results were culture-negative. However, the authors were successful in isolating *B. abortus* from the gastric fluids of five aborted fetuses. Pal'gov [53] was able to isolate *B. abortus* from Bactrian camels in Russia. In the herds examined, 2% of all animals aborted in the first half of the pregnancy. Fifteen percent of the herds were seropositive to brucellosis using the complement fixation test (CFT). Zowghi and Ebadi [54] cultured 3,500 lymph nodes from 300 slaughtered dromedaries from Iran for *Brucella* organisms. *Brucellosis melitensis* biovars 1 and 3 were isolated from these lymph nodes in 1% (3/300) of the camels. The authors are of the opinion that the *B. melitensis* infections in the dromedaries originated from neighbouring sheep and goat herds.

Radwan *et al.* [15] examined a large camel herd with 2,536 dromedaries in Saudi Arabia from which a 12% abortion rate had been reported. A *Brucella* seroprevalence of 8% was found with RBT and the standard buffered plate agglutination test (BPAT) of the United States Department of Agriculture (USDA). The authors also isolated *B. melitensis* biovars 1, 2 and 3 from aborted camel fetuses. *Brucella abortus* biovar 3 was recovered from an inguinal lymph node, three vaginal swabs and one supramammary lymph node obtained from free-ranging camels in eastern Sudan which had histories of abortion, presence of hygromas or testicular lesions [55]. It is worth mentioning that both isolates of *B. abortus* biovar 3 from Senegal and Sudan are the only oxidase-negative biovars reported in

the literature. Ramadan *et al.* [56] have recovered *B. melitensis* from a hygroma of an Indian camel. *Brucella melitensis* was isolated twice from two-quarters of milk samples from three seropositive camels in the UAE [36].

Brucellosis is not a major disease in NWCs, but severe outbreaks, such as the outbreak in Peru referred to earlier, have occurred from time to time. It was thought that sheep were the source of infection in this alpaca herd [21]. In an experimental infection trial in llamas in the USA, it was found that llamas are susceptible to *B. abortus* and that they develop positive serological titres. The authors used five conventional serological tests (CFT, standard tube test, standard plate agglutination test, RBT and BPAT) in addition to an ELISA developed at Iowa State University. The llamas also developed histological lesions similar to those found in cattle, sheep and goats [57].

Three llamas died at London zoo after they came into contact with camels which were newly imported from Moscow [58]. The authors claimed that the high serological titre (type of test not given) for *B. melitensis* was indicative of an acute infection.

Clinical Signs

Brucellosis is characterized by abortion and to a lesser extent by orchitis and infection of the accessory sex glands in males. According to various researchers, the clinical signs of brucellosis in breeding camelids are the same as those in bovines and small ruminants, although infection in breeding camelids causes fewer abortions than it does in bovines and small ruminants [8,15,21,55,59]. Infections may cause stillborn calves, retained placenta, foetal death, and mummification and reduced milk yield. Also, delayed service age and fertility have been reported [33]. A retained placenta is rare in Camelidae. This may be a result of the difference in the placental attachment [58]. Camelids possess a placenta diffusa like the horse and not a cotyledonary placenta.

Non-pregnant dromedaries (n = 6) artificially infected subcutaneously in the right lower back of the neck with two strains of *B. abortus* (four with S19, two with field bovine strain, $\times 10^6$ bacteria.) developed only mild clinical signs. Reduced appetite, slight lameness and bilateral lacrimation were observed. On necropsy the pathogen was re-isolated 45 to 65 days later from the cranial and genital lymph nodes. No clinical signs were observed in the four camels inoculated with S19, whereas slight non-specific signs were found in the dromedaries infected with the bovine *B. abortus* field strain. On necropsy no gross lesions were detected, but histological results revealed focal granulomas in the liver and a generalised lymphadenitis (supramammary lymph node). The pathogen was re-isolated from the lymph nodes of the genital tract and head [60].

Pathology

Little is known about the pathological changes caused by *Brucella* organisms in camelids. These bacteria have a predilection for the pregnant uterus, udder, testicles, accessory male sex glands, lymph nodes, joint capsules and bursae. Lesions may be found in these tissues. Nada and Ahmed [61] described lesions in non-pregnant dromedaries. They found inflammation of the uterus lining with reddening, oedema and necrotic foci in the uterus epithelium, as well as fibrosis of the endometrium and atrophy of the uterine glands. The

authors also observed an increased number of ovario-bursal adhesions and hydrobursae. The adhesions occurred between the bursa ovarica and the ovary and in several cases also between the bursa ovarica and the salpinges, causing a severe induration of the latter. Hydrobursitis was often observed in brucellosis-positive dromedaries causing an enlargement of the bursa, which was then filled with a clear amber-coloured fluid. No lesions have been described so far in aborted camelids and in brucellosis-positive camelid males except orchitis and epididymitis. The testes and epididymis of 360 dromedaries were examined for gross and histopathological lesions. Around 12% of the tested organs originated from seropositive camel bulls. From the investigations it is not clear if the epididymitis, orchitis or testicular degeneration was caused by *Brucella* infection or was a normal pathological feature [62]. A pregnant llama was experimentally infected by inoculating viable *B. abortus* bacteria into the conjunctival sac. Forty-three days post inoculation; the llama aborted an eight-month-old fetus. *Brucella abortus* was isolated from the placenta and all fetal specimens, including the brain, small and large intestines, spleen, kidney, liver, stomach fluid, heart blood and lung. Bacteria were also isolated from numerous mammary gland lymph nodes in the dams. Histologically there was a moderate, multifocal, lymphocytic and histiocytic, subacute placentitis, with a marked loss of trophoblastic epithelial cells. The chorioallantoic stroma contained abundant necrotic and mineralised debris and the swollen capillaries were expanded by large numbers of *Brucella* organisms [57,63].

Abu Damir *et al.* [64] as well as Wernery *et al.* [36] described only a few lesions in non-pregnant *B. abortus*-infected dromedaries and in lactating dromedaries that were seropositive for *B. melitensis* (*B. melitensis* was also isolated from milk samples). Cranial and genital lymph nodes from which the pathogen was isolated showed marked sinusoidal oedema and follicular hyperplasia of cortical and paracortical areas, with active germinal centres and histiocytosis. There were no lesions in the reproductive tract.

In Saudi Arabia, pathological and histopathological studies of non-pregnant dromedaries naturally infected with *B. melitensis* biovar 3 [24] revealed the following alterations in the following organs:

- lymph node (especially supramammary): oedema, enlargement, lymphoid hyperplasia, granulomatous reaction in the cortical area of the lymphoid follicle
- spleen: enlargement with granular surface in some cases, depletion of some lymphoid follicles, proliferation of fibrous tissue, histiocytosis
- mammary gland: granulomatitis in some cases, proliferation of interlobular fibrous connective tissue
- uterus: moderate amount of mucous and ulceration of endometrial mucosa, endometrial stroma showed oedema and diffuse and heavy infiltration (mainly of macrophages and lymphocytes in the lamina propria), blood vessels were dilated and congested.

Diagnosis

The morphology of the *Brucella* bacterial colonies is associated with the presence of lipopolysaccharides (LPS) in the external

membrane of the bacterium.

Smooth (S-LPS) and rough (R-LPS) phenotypes are differentiated. The S-LPS phenotype is found in most *Brucella* species, only *B. canis* and *B. ovis* possess the R-LPS. Some proteins of *Brucella* are responsible for serological cross-reactions between *Brucella* spp. and other bacterial species [65]. Cross-reactivity exists to:

- *Yersinia enterocolitica* O: 9
- *Escherichia hermannii*
- *E. coli* O: 157
- *Francisella tularensis*
- *Stenotrophomonas maltophilia*
- *Vibrio cholera* O: 1
- *Salmonella* serotypes group N

Therefore, difficulties may arise in the diagnosis of brucellosis. Abortion and reduced fertility in the camel frequently have other causes, such as salmonellosis, trypanosomosis, or infections with *Campylobacter* or *Tritrichomonas fetus* [66-68], making laboratory testing essential. An incorrect diagnosis of brucellosis may occur when based on serology alone.

Culture

Brucellosis is usually diagnosed in the laboratory by culture of blood, milk or tissue or the detection of antibodies in sera. *Brucella* organisms can be recovered from the placenta, but, more conveniently, in pure culture from the stomach and lungs of aborted fetuses. It should be stressed that only fresh material is suitable for culture to avoid overgrowth by a number of opportunistic bacteria. Culture of *Brucella* spp. is still the gold standard but also time consuming, expensive, difficult and dangerous.

For isolation, the recommended medium is Farrell's medium, which contains six antibiotics. But other selective *Brucella* media are also in use for the growth of this pathogen from fresh camel milk and camel tissue samples [15]. During intensive investigations using selective media it was found that on a camel farm in Saudi Arabia 34% of all *Brucella* seropositive milking dromedaries were *Brucella* shedders. The high number suggests that it is preferable to use selective media.

Tissue specimens from *Brucella*-positive dromedaries were examined by Omer *et al.* [24] with the immunoperoxidase test, with very good results. *Brucella* organisms were detected in the cytoplasm of macrophages (visible as brown granules), in the lymphocytes of the lymph nodes and spleen, within the epithelial lining of the endometrium and endothelium of blood vessels, and within mononuclear cells around blood vessels.

Polymerase Chain Reaction

The isolation of *Brucella* organisms is still the preferred method of diagnosis. This method also allows typing of the isolated strains. However, new PCR techniques are now being implemented for both identification and phenotypic bio typing [35]. These PCRs can discriminate between *Brucella* species, and between wild and vaccine strains, but do not discriminate between *Brucella* biovars. So far, only

monoclonal antibodies against different epitopes of the *Brucella* LPS can be used for biovar differentiation.

PCR-based assays have been developed for brucellosis diagnosis and are based on the detection of specific sequences of the pathogen, such as genes of the locus 16S – 23S, the IS711 insertion sequence or the *bcs*p 31 gene encoding for a protein of 31kDa. Von Hieber [9], who used a PCR assay designed with hybridisation probes and primers targeting the insertion sequence of IS711 of the BMEI 1162 gene, has shown reliable results in the amplification of pure target DNA in bacterial dilutions, but the assay was less sensitive when tissue samples were tested. The reasons for this may be explained by the extraction method used, the intracellular presence of the pathogen and the distribution pattern of *Brucella* organisms [69].

Serology

The majority of studies on camelid brucellosis use serological methods for diagnosis, but none of the serological brucellosis tests are validated for use in camels yet, as acknowledged by the World Organisation of Animal Health (OIE). Similarly, none of the tests have been validated for the diagnosis of human brucellosis [70]. However, it was found that a combination of different serological tests can increase diagnostic efficacy in camels, although none of the serological tests can differentiate between a *B. abortus* or *B. melitensis* or *B. ovis* infection. Sunaga *et al.* [71] reported that five dromedaries imported into Japan were positive in the CFT and SAT. The animals were immediately slaughtered. No *Brucella* organisms were isolated; however, *Yersinia enterocolitica* serotype 0:9 was identified. It is known that false-positive (unspecific) reactions with various other bacterial species can occur [72-73].

Many authors regard the CFT as being the most sensitive and specific test for brucellosis because CFT antibodies remain in the serum for longer than SAT antibodies [25,44,74-75]. Shumilov [76] determined that the CFT was four times more sensitive than the SAT. He tested Bactrian's in Mongolia, where brucellosis is widespread among camels. He examined two herds with the following results:

- Herd 1: 3751 camels: CFT 4.3% and SAT 0.6%
- Herd 2: 54,673 camels: CFT 3.7% and SAT 1.0%.

In the SAT an end titre of 1:20 (40 IU) was regarded as suspicious by different researchers [53,77-80], Fayed *et al.* [80] Salem *et al.* [81], and El-Sawally *et al.* [82] believe that the SAT or tube agglutination test (TAT) detect a higher percentage of reactors to brucellosis than other assays due to their greater sensitivity to immunoglobulin M (IgM) than immunoglobulin G (IgG). In order to eliminate unspecific reactions in the SAT, Wernery and Wernery [83] utilised a 5% solution of phenol sodium chloride, which increases the specificity of the test and reduces the cross-reactivity. The specificity is also increased by adding mercaptoethanol, dithiotreitol or a chelating agent such as ethylenediaminetetraacetic acid (EDTA) to the antigen.

In addition to cross-reactivity with other bacteria that makes the serological diagnosis of brucellosis more difficult, Zhulobovski and Pal'gov [84] observed prozones in some sera of Bactrian camels in Russia, as did Nada [85] in dromedaries from Egypt. The absence of a visual positive reaction in low dilutions has also been observed in 1.5% of all positive dromedary sera in the UAE [86].

Nearly 30% of the 1,449 alpacas tested in Peru had a positive plate agglutination titre [21].

Other researchers have used ELISA for the detection of *Brucella* antibodies, not only in camel sera [2,87], but also in camel milk [88]. The camel milk ELISA seems to be an important alternative to the conventional serodiagnosis of camelid brucellosis. It must be noted that none of the commercially available brucellosis ELISAs (direct, indirect or competitive) for serum or milk has been evaluated for the diagnosis of camelid brucellosis. Our unpublished newest research clearly indicates that none of the tested ELISAs is suitable due to many false positive results and it is therefore highly recommended to establish a suitable camelid brucellosis antibody ELISA for milk and serum. False positive results may have their reasons in a poor cut-off level and /or the use of an anti-ruminant conjugate instead of a homologous system. It has been shown that dromedary IgG has 74.3% sequence identity to porcine and 73.1% to both equine and bovine, whereas anti-goat IgG has a much lower sequence identity of only 61.6% [89].

Several researchers have evaluated the different serological tests for the diagnosis of camel brucellosis [2,23,90-92]. It was concluded that the elimination of non-specific reactions to *Brucella* in camelid sera is essential for the correct diagnosis. It is also important to apply more than one test, one of which must be the TAT using 5% NaCl phenolised solution. Atwa [93] and Abou-Zaid [2] found a good agreement between five different serological tests (SAT using 5% NaCl-phenol lysed solution, SAT with 11.4% phenol-NaCl, BPAT, RBT, mercapto-ethanol test, and ELISA), ranging between 80.6% and 95.6%.

Mohammed [94] evaluated the RBT, the TAT, and the CFT for the diagnosis of brucellosis in camels. He found that the RBT and the CFT demonstrated equal ability in detecting positive and negative sera as well as prozone reactions. However, for optimal sensitivity, the RBT has to be used with serum-antigen at a 3:1 dilution. When using the CFT, the 1:10 diluted sera have to be inactivated at 54°C for 30 min and the cold fixation technique has to be applied. Using the TAT, the classical neutral pH antigen has to be replaced by a buffered (pH 3.5) antigen to achieve optimal results. As mentioned earlier, none of these tests have been validated for use in camel brucellosis and the results are therefore difficult to compare.

Radwan *et al.* [15] examined a large camel farm comprising 2,536 dromedaries in Saudi Arabia for *Brucella* antibodies. The authors used a combination of two tests to identify seropositive dromedaries – the RBT and the standard USDA BPAT. With these two methods, the authors successfully eradicated the disease from the farm, where it had caused abortion in 12% of female camels. The authors adopted these tests due to their sensitivity, simplicity and applicability in the field.

The use of serological tests is the core of the control or eradication of brucellosis. Many such tests are available but, they must be used in accordance with strict standardisation rules and meet the requirements laid down by the OIE. For bovine brucellosis the OIE recommends the RBT, the BPAT the CFT, the ELISA and the Fluorescence Polarisation Assay (FPA). The activity of immunoglobulins during infection in the different serological tests allows the distinction between acute and chronic infection. Hence, the presence of both IgM and IgG indicates

an acute brucellosis, whereas chronic brucellosis is characterised by the presence of IgG alone. Details of the sensitivity and specificity of the various serological tests are summarised in (Table 3).

The tests mentioned in (Table 3) have all been used for the detection of camelid brucellosis. The CFT, which was often used as a confirmatory test, is now progressively being replaced by ELISAs and more recently also by FPA.

The FPA is based on a physical principle and when antibodies against *Brucella* are present in a serum, a fluorescent complex is formed and expressed in milli-polarisation units (mP); in a negative sample the antigen remains uncomplexed [35]. ELISAs have a high sensitivity but their specificity is quite low. Reactions towards different bacterial species, especially *Y. enterocolitica* 0: 9, are known to occur to all serological tests. Results by Alshaikh *et al.* [95] clearly showed the SAT's limited reliability for chronically infected dromedaries. This was also demonstrated by Omer *et al.* [32], who reported that the RBT was suitable for screening camel sera for brucellosis, but the cELISA detected 2.1% more positives. More recent investigations by Von Hieber [9] and Gwida *et al.* [89] on hundreds of brucellosis-positive dromedaries imported into the UAE from Sudan compared several diagnostic tests. There was good agreement between the results of the CFT, RBT and SAT, proven by calculating kappa values, but the sensitivity of all three tests was low compared to the results by FPA or serum real-time PCR. Serum real-time PCR was not validated, but had a high diagnostic sensitivity, as it was able to detect as little as 23 femtograms of *Brucella* DNA per reaction, with a probability of 95% [89]. Therefore, it is advisable to combine real-time PCR with a serological test such as RBT, which would increase the sensitivity to 100% [96].

Detection of brucellosis in camel sera by PCR has been described by Alshaikh *et al.* [95] in Saudi Arabia. This is a very reliable diagnostic tool, which can even differentiate between *B. melitensis* and *B. abortus* brucellosis.

The FPA and a cELISA were used to test a total of 336 sera obtained from llamas and alpacas in Chile which came from a brucellosis negative herd. The results were compared with conventional tests such as the RBT, SAT and CFT. Only two sera were found positive with the FPA and cELISA (92), and none with the conventional tests. However, both sera had low titres.

In contrast to cattle milk, camel milk cannot be used to detect lacteal brucellosis antibodies using the conventional milk ring test (MRT), because camel milk lacks the agglutinating substance required to cluster fat globules [88]. It is also known that camel milk fat globulins are tiny micelles which, therefore, do not cream up to produce a surface fat layer. Van Straten *et al.* [88] established an MRT that can also be used to detect antibodies in camel milk. The researchers named this test a modified MRT because *Brucella*-negative cow milk is added to the camel milk, producing a typical blue-coloured creamy ring when antibodies to *Brucella* bacteria are present. The test is not highly sensitivity, but it is cheap to use (Figure 1).

Skin Test

Brucellosis skin tests have been tried by some researchers, particularly on Bactrian camels in the former USSR, using different



Figure 1: Modified camel milk MRT.

allergens [97]. The skin test is highly specific but its sensitivity is low, making it a good herd test. The antigen does not sensitise the animal's immune system and therefore will not induce interference in the diagnosis of the disease.

Control and Treatment

Brucella has been eradicated in many regions of the world, but in others it is widespread and an economically important disease. Many cases of human brucellosis are found in regions where the disease has not been eliminated in livestock. Different strategic options can be adopted to first decrease the prevalence of brucellosis to an acceptable level (brucellosis control) and secondly to remove the foci of infection (brucellosis eradication). The choice of control strategy depends on a number of considerations, such as infection prevalence in different animal species, human clinical incidence and the capacity of Veterinary Services. However, a pre-requisite for any control programme is the implementation of an efficient animal disease surveillance network. Eradication in small ruminants has never been achieved [98] and may be also very difficult to achieve in OWCs due to the complexity and expense of treating animals across widespread areas. In cattle and small ruminants, when prevalence is low (between 3% and 5%), vaccination comes first followed by slaughter (WHO and FAO of the United Nations, [8]). Abbas and Agab [31] suggest whole-herd vaccination in low-prevalence countries, and test-and-slaughter followed by vaccination in high-prevalence countries. In camel-racing countries, the culling method cannot be applied because racing dromedaries are often extremely valuable animals and play a very important role in Bedouin culture. Therefore, it is preferable to castrate all *Brucella*-positive bulls, not to breed positive females, and to vaccinate. No compromise should be made when it comes to camel dairy farms. They must be free of brucellosis.

Antibiotics

Brucella organisms are Gram-negative coccobacilli which are sensitive to many broad-spectrum antibiotics, but the use of antibiotics is forbidden in many countries because of the uncertainty related to the infective status of the treated animals and because of the spread of antibiotic resistance. Treatment is unlikely to be cost-efficient or therapeutically effective because of the intracellular sequestration of the organisms, mainly in the lymph nodes. However, cure rates between 65% and 100% have been reported in infected goats by daily intraperitoneal injection of 500 mg and 1,000 mg tetracyclines [3]. Radwan *et al.* [15] also treated 202 seropositive dromedaries with

Table 4: Serology results of 23 seropositive dromedaries 36 months after antibiotic treatment against brucellosis. (According to Wernery, unpublished data).

Tests	Positive	Negative
cELISA	20	3
CFT	16	7
RBT	14	9
SAT	7	16

cELISA: Competitive Enzyme-Linked Immunosorbent Assay; CFT: Complement Fixation Test; RBT: Rose Bengal Test; SAT: Serum Agglutination Test

a combination of oxytetracycline (25 mg/kg body weight) every two days for 30 days and streptomycin (25 mg/kg body weight) every two days for 16 days. In addition to this parenteral treatment, milking camels received 10 ml of oxytetracycline as intramammary infusions in each teat every two days for eight days. This regimen of treatment was effective in eliminating the shedding of *Brucella* organisms through milk. All treated dromedaries also became serologically negative within 16 months of treatment. But the single untreated control camel remained positive over the same period of time. Using antibiotics may be a way to save valuable animals (e.g. racing camels) from being culled, but it is doubtful if antibiotic treatment on a herd-level basis can be successful. It is not clear from this investigation whether or not the shedding would have stopped anyway, without any antibody treatment, because the study did not include any untreated controls. However, the author's unpublished treatment protocol clearly demonstrated that dromedary brucellosis is not treatable with antibiotics, although it is claimed otherwise. Twenty-three seropositive dromedaries were treated with antibiotics according to Radwan *et al.* [15] with the following results 36 months later (Table 4).

Vaccination

Because of the grave medical and economic consequences of brucellosis, serious efforts have been made to prevent the infection through the use of vaccines. In OWCs, both inactivated and attenuated *Brucella* vaccines have been used successfully. Dromedaries were vaccinated with *B. abortus* strain S19 [99] and with *B. melitensis* Rev 1 [15]. Young (three months) dromedaries received a full dose of the vaccine and adults (10 years) a reduced dosage. Both groups developed *Brucella* antibodies with titres of between 1:25 and 1:200 using the standard USDA BPAT, two to four weeks after vaccination. They receded after eight months in young stock and after three months in adult camels. Agab *et al.* [100] vaccinated five dromedaries with a reduced dose (5×10^8 cfu in 2 ml) of *B. abortus* strain S19. All five camels seroconvert after one week and their antibodies declined six to seven weeks later. The dromedaries tested negative 14 weeks later. So far, no challenge infections have been performed after vaccination. In cattle, the optimum age for vaccination is between four and eight months of age. Serum agglutination test returns negative by the time the bovines are of breeding age, except in 6% of cases [101]. It is obvious that post-vaccination titres increase with increasing age and therefore cattle vaccination is recommended only in young stock. Vaccination of bulls with S19 is of no value because it often resulted in the development of orchitis and the presence of strain S19 in semen [35]. Very little is known about the optimal vaccination age in camels and their serological response. Before vaccination is started in

dromedaries, thorough investigations are paramount in order to find out if animals are naturally infected by *B. abortus* or *B. melitensis* and this can only be determined by culture or PCR.

The attenuated vaccine *B. melitensis* Rev 1 is used worldwide and is effective in sheep and goats by the conjunctival route ($1 \times 10^9 - 2 \times 10^9$ cfu/animal). It gives full immunity. An eradication campaign in camelids may also be based on vaccination and 'test and slaughter' policy for dairy herds and 'test and no breeding' for racing herds. Vaccinations alone would not suffice for success. The main approach in a long term control strategy of brucellosis is to vaccinate only female replacement camels less than 1 year old (maturity in OWCs begins with 4 years). This strategy will after several years establish an immunized herd and will not induce abortions and excretion of the vaccinal strain through milk. It will also protect these herds from brucellosis threat by surrounding positive sheep and goat farms.

Conclusion

Brucellosis in OWCs is on the rise and needs the urgent intervention of all those concerned, including camel owners, to avoid further spread. Camel brucellosis has a severe impact on human health in camel-rearing countries. In brucellosis-endemic countries eradication can only be achieved by control, prevention and surveillance. In most countries where camels are reared they possess an important value for the owner, not only economically but also culturally. The value of dromedaries can be very high, especially in camel-racing countries. Most of the brucellosis-positive camels are clinically healthy animals and owners do not allow their *Brucella* serologically positive animals to be culled. Therefore, the author proposes that the best way to halt the spread of the disease is to castrate serologically positive bulls, never breed positive females and start vaccination in positive herds, especially when they are used for dairy.

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