

A Review on Brucellosis in Cameroon: Diagnostic Approaches, Epidemiology and Risk Factors for Infection

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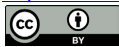
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Abstract

Brucellosis is a neglected tropical zoonotic disease that threatens the food production and public health sectors. It is of considerable animal welfare and economic importance and is underreported in most parts of the world, especially in developing countries like Cameroon. Brucellosis has been reported in cattle, other domestic animals and humans in Cameroon. The burden of the disease is unclear, and the awareness remains questionable. It became necessary for this review to be carried out to highlight the diagnostic approaches used to confirm brucellosis in animals and humans, disease epidemiology and risk factors for infection. So far, reports of brucellosis in previous studies have been based on serology only. Seroprevalence data of *Brucella* antibodies in animals indicate the risk of human brucellosis in Cameroon. However, few investigations have been undertaken on human brucellosis, considering the different epidemiological settings. There is no report or unsuccessful attempts to identify *Brucella* species circulating in Cameroon. It could largely be attributed to a lack of standard laboratories for testing and the lack of consumables. The way forward will require a surveillance system for brucellosis in the country, educating all sectors affected and drafting a diagnostic protocol for high-risk individuals.

Keywords

Brucella, Brucellosis, Epidemiology, Diagnosis, Risk Factors, Cameroon

1. Introduction

Brucellosis is an anthroponotic disease caused by a group of bacteria of the ge-

nus *Brucella* [1]. It is widespread, endemic and neglected in low-income countries and is recognised as the most common laboratory-acquired infection [2] [3]. It is classified as one of the eight neglected zoonotic diseases (NZD) worldwide [4] [5]. It is usually considered an occupational disease because it occurs mainly in abattoir workers, veterinarians, laboratory technicians, hunters, farmers, and livestock producers [6].

In cattle, the disease is spread through ingesting contaminated feed, water, or milk, suckling or licking an infected placenta, newborn, or the genitalia of an infected female soon after it has been aborted or after birth [7]. The disease in cattle is associated with abortion, death of young ones, stillbirth, retained placenta or birth of weak calves, delayed calving, male infertility, and a marked reduction in milk yield [8]. It causes considerable economic losses to cattle, sheep, goats, and to a lesser extent, pigs farmers due to loss of milk yield and low productivity of infected animals in low-resource settings where livestock is a source of food security and income [1] [9] [10]. In the absence of control programs against brucellosis in livestock, humans remain at risk of infection, given that disease in cattle is an indication of human cases.

Humans become infected from consuming unpasteurised milk and dairy products, by direct contact with aborted fetuses, afterbirth and parturition fluids, and slaughter practices [11]. The disease symptoms include undulant fever, weight loss, night sweats, joint pain, enlarged lymph nodes and hepatosplenomegaly [12]. Given that these clinical manifestations of brucellosis are diverse and nonspecific, it is frequently under/misdiagnosed in febrile patients seeking treatment at healthcare centres. Misdiagnosis leads to the over-diagnoses of more recognisable febrile conditions like malaria [13].

Consequently, there is increased expenditure on medication. It also leads to complications of the infection which spreads to and affects other organs, resulting in debilitating illness and loss to work hours due to ill health, hence the ensuing economic losses [1]. Therefore, it is a disease of both public health and financial concern [14]. The absence of pathognomic symptoms of the disease makes clinical diagnosis challenging, requiring sensitive, specific and easy-to-use laboratory assays to confirm the disease.

Brucellosis is reported in domestic animals and humans in Cameroon. The domestic animals implicated are cattle, sheep, dogs, pigs and goats [1]. The seroprevalence in these animals ranges from 1.1% to 9.12% [1]. Studies on cattle alone report seroprevalence ranging from 2.3% to 30.8% [11] [15]-[22]. Identifying the bacteria in animals indicates the source of infection in humans. A high herd seroprevalence is compatible with a high human disease burden [11] [23]. The prevalence of human brucellosis ranges from 0.28% to 31.4 across different study populations [17] [24] [25].

The magnitude of the disease burden in Cameroon is unclear. It is thought to be undiagnosed, and some infections may be missed. The knowledge of the disease, risk factors, and treatment is insufficient, and hospitals have no diagnostic

protocol for the condition. This review seeks to elucidate the current situation of brucellosis in Cameroon. Emphasis is laid on epidemiology, the diagnostic techniques used, possible applications for routine diagnosis, and an evaluation of the risk factors in the country.

Etiologic agents of brucellosis

The genus *Brucella* includes twelve species: *Brucella abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. microti*, *B. pinnipedialis*, *B. ceti*, *B. inopinata*, *B. papionis*, and *B. vulpis* [26]. Three of these species have been further classified into biovars, *B. abortus* with eight, three for *B. melitensis* and five for *B. suis* [8] [26]. A summary of the *Brucella* species, their biovars and their primary host are presented in **Table 1**.

The primary hosts for various species and biovars are bovine (*B. abortus*), caprine (*B. melitensis*), swine (*B. suis*), ovine (*B. ovis*), camels, elk, bison (*B. abortus*), as well as marine mammals such as seals, porpoises, dolphins and whales (*B. pinnipedialis* and *B. ceti*), and also amphibians (*B. inopinata*) [27]. The disease in cattle is usually caused by *B. abortus* and sometimes *B. melitensis*. In humans, the most common *Brucella* spp. associated with human-to-human transmission (HHT) is *B. melitensis* [28]. Other species commonly related to the human disease include *B. abortus*, *B. suis* and *B. canis* [14] [29]. There is no available literature either on the isolation of *Brucella* spp. or on the molecular characterisation of the species in circulation in Cameroon. Identifying the species and strains is essential to understanding the disease patterns.

Epidemiology

Brucellosis has been reported in 86 different countries worldwide with more

Table 1. *Brucella* spp and their hosts [8] [26] [27] [30].

Species	Biovars	Primary host
<i>B. abortus</i>	1 - 7, 9	Cattle, camels, yaks, buffalo and humans
<i>B. melitensis</i> *	1 - 3	Sheep, goats and humans
<i>B. Suis</i>	1 - 3	Pigs and humans
	4 and 5	Small rodents and reindeer
<i>B. ovis</i>		Sheep
<i>B. canis</i>		Dogs and humans
<i>B. Maris</i> <i>B. ceti/B. cetaceae</i>		Whales
	<i>B. pinnipediae</i>	Seals
<i>B. neotomae</i>		Desert rats
<i>B. papionis</i>		Monkeys
<i>B. microti</i>		Voles
<i>B. inopinata</i>		Not reported
<i>B. vulpis</i>		Red fox

*Most pathogenic in humans.

than 500,000 documented cases reported annually [8] [31]. The incidence of human brucellosis varies widely. Typically, <1 case per 100,000 population is reported in developed countries where the disease has been eradicated from animals and most incidents occur in travellers or immigrants. In contrast, some Middle Eastern countries have a high prevalence of >100 cases per 100,000 population [32]. Countries with a higher incidence of brucellosis are Kenya (203.07/100,000), Yemen (89.96/100,000), Syria (47.26/100,000), Greece (42.96/100,000) and Eritrea (21.82/100,000) [33]. The epidemiological characteristics of human brucellosis have undergone great changes in the past ten years, and the overall incidence has shown a downward trend. However, there is a lack of reporting data in endemic areas of human brucellosis in some countries, such as Ethiopia and other African regions, Cameroon inclusive [33]. Estimates of the case fatality rate for untreated brucellosis are usually 1% - 2% or less, although rates as high as 5% have been reported in smaller series [32].

Prevalence of brucellosis in domestic animals

Cattle, sheep, dogs, pigs and goats are the domestic animals reported as having brucellosis in Cameroon [1]. The seroprevalence of 9.12% in cattle, 8.04% in sheep, 6.06% in dogs, 1.87% in pigs and 1.1% in goats indicate that these domestic animals could also be the source of infection in humans. The low prevalence of *Brucella* antibodies in goats and pigs could be explained by their large-scale slaughtering for meat consumption, a phenomenon that reduces the number of life-infected animals. Another reason could be the involvement of these animals in the intensive production systems in which they are not often in contact with infected animals or contaminated products [1].

Prevalence of Cattle brucellosis

The seroprevalence of brucellosis in cattle ranges from 2.3% to 30.8%. These values vary across the study population and diagnostic assays used (Table 2). The cattle were located at slaughterhouses, in ranches and in dairy herds from different geographical locations, with diverse risk factors associated with each site. Interpretation of this seroprevalence data is challenging due to the variability of the study population and diagnostic methods used. Despite this number of seroprevalence studies on cattle, there is no available information on the bacteria's isolation or molecular characterisation. There is also a need for a harmonised approach to screening, detecting and controlling this infection.

Human brucellosis

The prevalence of human brucellosis in Cameroon ranges from 0.28% to 31.4% in populations of pregnant women with a history of abortion and high-risk occupational groups (HROG) that include cattle rearers, abattoir workers, and butchers [17] [24] [25]. This seroprevalence was recorded from populations in the East, West and Adamawa Regions of Cameroon, where livestock farming is a significant economic activity (Table 3). There is no available literature on the isolation of bacteria from these populations or the molecular characterisation of the infection. An in-depth understanding of the disease patterns in the country

Table 2. Prevalence of cattle brucellosis in various geographic locations in Cameroon.

Geographic location	Cattle population (sample size used)	Prevalence of brucellosis (%)	Reference (s)
Adamawa and North regions	Cattle (1031) Herd (82)	5.4 25.6	[15]
Adamawa	Cattle (1377)	16 - 20	[16]
Ngaoundere	Cattle for slaughter (590)	3.6 - 5.9	[17]
Ngaoundere	Dairy herds (142)	2.8	[11]
Western highlands	Holstein cattle (266)	8.4	[18]
Bamenda	Dairy herds (100)	14	[11]
Bamenda	Cattle for slaughter (198)	4.04	[19]
Northwest region	Cattle (689)	5.2	[7]
WHPS* and GHS**	Cattle (1562) Herds	4.61 16	[20] [21]
Dschang	Cattle for slaughter (840)	4.9 - 9.6	[22]

*Western Highland Plateau Savannah (WHPS), **The Guinea Highland Savannah (GHS).

Table 3. Prevalence of human brucellosis in various geographic locations in Cameroon

Geographic location	Human population (sample size used)	Prevalence of brucellosis (%)	Reference (s)
Bertoua	Slaughterhouse workers	31.4	[24]
Ngaoundere	Abattoir personnel (107)	5.6-12.15	[17]
	Pregnant women (705)	0.28	[17]
Noun division (West Region)	High-risk occupational groups (273)	10.26 - 12.45	[26]

requires the use of molecular diagnostic approaches. Besides, this number of studies is grossly insufficient to portray a comprehensive situation on human brucellosis in the country where the disease is said to be endemic. Hence, there is a need for a harmonised approach to identifying the illness in HROGs throughout the national territory.

Pathogenesis of brucellosis

Brucellosis is transmitted from animal to animal through the consumption of contaminated feed, milk and water, contact with aborted materials, and in utero [34] [35]. *Brucellae* spreads quickly between animals in close contact, especially when they are giving birth. Infection may cause occasional clinical cases if animals are not pregnant; however, reproductive losses can be high when *brucellae* are first introduced into a fully susceptible herd or kennel. Later, the losses usually decrease and may become sporadic or cyclical. Deaths are rare in domesticated animals [32].

Infection in humans could be acquired from cattle, dogs, pigs, sheep and goats [36]. Transmission is usually by contact with animal tissues, blood, urine, vaginal secretions, aborted fetuses, especially placentae [28] and consuming unpasteurised dairy products [37] [38]. It can also be acquired by inhalation, wherein laboratory personnel are at significant risk for infection [39]. Human-to-human transmission (HHT) is possible via a placental barrier, lactation, sexual and tissues such as blood (blood transfusion) and bone marrow [28].

In both animals and humans, *Brucellae* have strong tissue tropism for lymphoreticular and reproductive systems; specifically macrophages, dendritic cells (DCs) and placental trophoblasts, where they replicate within their vacuoles. The pathogen can also replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells [40]. Once in the host, they can invade epithelial cells of the host, allowing infection through mucosal surfaces; M cells in the intestine have been identified as a portal of entry for *Brucella* spp. [41]. When taken up by polymorphonuclear cells or macrophages [42], they survive and replicate, limiting exposure to innate and adaptive immune responses, sequestering the organism from the effects of antibiotics, and driving clinical disease manifestations and pathology [40]. They are then transported to local lymph nodes, where bacterial replication continues, before spreading to reticuloendothelial organs, including the liver, spleen, and bone marrow [31]. The bacteria secrete proteins that induce granuloma formation in these organs, and destructive changes in these and other tissues occur with advanced disease [39].

Its pathology has three phases: the incubation phase, the acute phase and the chronic phase [40]. In the incubation phase, clinical symptoms are not evident. In the acute phase, the pathogen invades and disseminates in host tissue. It is characterised by nonspecific influenza-like symptoms including pyrexia, diaphoresis, fatigue, anorexia, myalgia, and arthralgia. This stage is associated with adverse pregnancy outcomes and abortion in humans. Chronic infection results from the ability of the organism to persist in the host's cells. The lymphoreticular system distributes *Brucellae* to cause cardiovascular, hepatic, lymphoreticular, neurologic, and osteoarticular disease. It eventually results in severe organ damage and death of the host organism [40].

2. Diagnosis of Brucellosis

Clinical diagnosis

Cattle with brucellosis present with symptoms such as hygromas, abortions, infertility and arthritis, singly or in combination [43]. Other symptoms identified are decreased milk production and sterility in bulls [44]. Cattle rearers need to be educated on identifying these symptoms that point toward the disease and should be encouraged to seek veterinary intervention to prevent its spread to other cattle and the ensuing losses to the flock.

In humans, the presence of repeated fever episodes (undulant fever), a disabling flu-like syndrome, sweating, chills, myalgia, arthralgia, and fatigue are suspi-

cious of acute brucellosis infection [45]. At the same time, the chronic disease can be localised in any organ, such as the gastrointestinal tract, osteomyelitis, orchitis, respiratory tract symptoms, and, less commonly, cutaneous, neurologic, or cardiovascular manifestations [29]. Symptoms such as febrile illness, myalgia and arthralgia point toward other endemic diseases like malaria. Health personnel with poor brucellosis knowledge are prone to underdiagnose the condition. Human brucellosis lacks pathognomonic signs. Hence, laboratory tests are essential for diagnosis [46]. Health personnel require education on the clinical presentation of the disease amongst HROG. Education will facilitate the identification of symptoms, enhance the request for diagnostic tests and direct the treatment of cases. Associated morbidity of brucellosis will be reduced consequently. Detailed patient history is essential for clinical diagnosis, including travel locations, animal contact, and ingestion of unpasteurised milk and cheese products [47].

Laboratory diagnosis

Laboratory diagnosis entails various direct and indirect methods performed *in-vitro* (mainly on blood or milk) or *in vivo*; allergic tests [48] [49]. Direct diagnosis is mostly via culture and DNA-based methods, while indirect diagnosis uses the skin tests and serology assays. Serology includes: the Slow Agglutination Test or Slow Agglutination of Wright (SAT or SAW), Buffered *Brucella* antigen tests (BAT), Complement fixation test (CFT), Rose Bengal test or Rose Bengal Plate test (RBT/RBPT), Enzyme-Linked Immunosorbent Assay (ELISA), Fluorescence Polarization Assay (FPA), Lateral Flow Assays (LFAs), and Milk Ring Test-MRT [48]. Direct tests detect the presence of *Brucella* and are used in clinical situations where the affected animals or humans show clinical signs. In contrast, indirect tests are mainly used to detect subclinical conditions [50].

Diagnosis in Cameroon has been based on the use of serology with the RBT/RBPT, ELISA, CFT, LFAs, Slow Agglutination of Wright with EDTA (SAW-EDTA) and the Delayed Hypersensitivity Test to Brucellin (DHTB). **Figure 1** summarises the frequency of use of these assays in various publications on brucellosis in Cameroon.

3. Indirect Detection

Serologic assays

Serology tests for diagnosing brucellosis are broadly classified as those detecting antibodies to the S-LPS and those detecting antibodies to proteins. The former tests either utilise suspensions of *S. brucellae* as antigens or S-LPS extracts [51]. RBT, CFT, LFA and SAT utilise *S. brucellae* antigens to test for *Brucella* antibodies, while ELISA uses S-LPS extracts or its O-chain [51]. The sensitivity and specificity of these tests vary depending on their characteristics, the antigenic suspensions used, and the stage of infection [52].

Rose Bengal Plate Test (RBPT)

The RBPT is a highly sensitive assay, inexpensive, simple and rapid to perform,

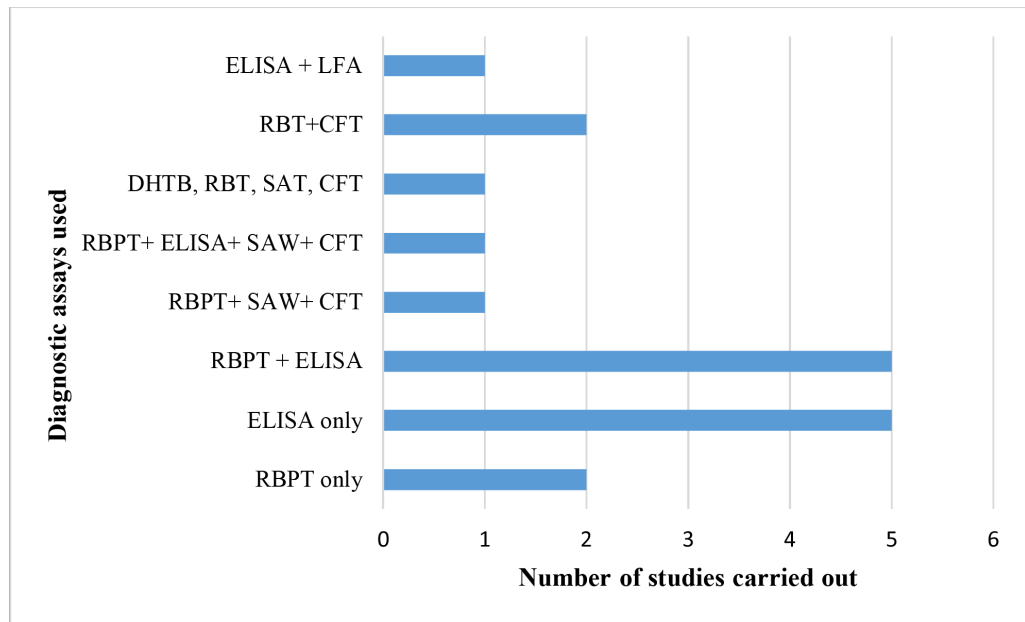


Figure 1. Diagnostic assays used for *Brucella* infections in Cameroon [Rose Bengal Test (RBT) or Rose Bengal Plate Test (RBPT), Enzyme-Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT), Lateral Flow Assays (LFAs), Slow Agglutination of Wright with EDTA (SAW-EDTA) and the Delayed Hypersensitivity Test to Brucellin (DHTB)].

which is used as a quick diagnostic test requiring essential laboratory equipment and expertise [52] [53]. However, it has a low specificity than the iELISA [54]. Adapting the serum dilutions in RBT increases its specificity, reducing the need for additional serological tests to confirm the diagnosis [46]. In cattle, false-negative results are usually obtained during the early stages of incubation or after an abortion. False-positive results are recorded amongst cattle vaccinated with the S19 vaccine. False-positive results in young stock could be recorded due to colostral antibodies [51] [55]. Even though it has been said to be used in diagnosing infection irrespective of the stage of the disease [53], it is not recommended for the diagnosis of chronic brucellosis since it mainly detects IgM [22]. As a result of its low specificity, confirmation of results RBT is required, using either CFT or ELISA [56].

Indirect Enzyme-Linked Immunosorbent Assay (iELISA)

This assay has high sensitivity and the possibility of measuring various antibody titres (IgG, IgM, and IgA), providing a better interpretation of the clinical situation [6]. The test is affordable, has shorter run times and requires less interpretation training than agglutination methodologies [47]. Compared to other assays, it is more sensitive than RBT but has a lower specificity, and it is more sensitive and specific than SAT [1] [8] [18] [55]. These make the iELISA suitable for screening purposes, diagnosing chronic cases of brucellosis and detecting incomplete antibodies [8] [55]. Hence, the ELISA procedure has been used individually or in combination with other assays, as illustrated in **Figure 1**.

Competitive Enzyme-Linked Immunosorbent Assay (cELISA)

Competitive ELISA replaced the complement fixation test (CFT) because of its

higher specificity and ease of automation [57]. It is species unspecific with lower sensitivity than iELISA [55] [58]. Its advantage over other tests includes its use in confirming brucellosis on various animal species. Also, poor quality samples such as hemolysed blood can be processed using this assay [57] [58].

Complement Fixation Test (CFT)

The complement fixation test (CFT) is a specific test that can detect IgM and IgG1 antibodies, incomplete antibodies, and the slightest changes in antibody titres [8] [44]. It is mainly used with RBT as a confirmatory test [55] [59] [60]. It has the following limitations: Antibodies of the IgG2 type impede complement fixation resulting in the exhibition of false-negative results [44]; it may not detect animals that have been recently infected naturally or experimentally [22]; it requires expertise for interpretation of results that may be affected by the sample quality and the standardisation of the antigen [61]. CFT is considered better for control and surveillance programs for brucellosis [44].

Slow Agglutination Test (SAT)

This famous test used for routine diagnostic practice worldwide is usually complemented by the *Brucella* Coombs test [8] [52]. It was designed mainly to detect IgM and brucellosis on a herd basis [9] [22]. The downsides of this procedure are: It is slow and has low sensitivity and specificity [62]; it is not advisable for use in individual animals because of both false-negative or false-positive results which have been observed [9]; given that it mainly detects IgM, it is not suitable for the detection of chronic brucellosis [22].

Slow Agglutination of Wright with EDTA (SAW-EDTA)

The SAW-EDTA is a sensitive test for detection of IgM and a modification of SAT whose specificity is increased by pretreating the serum with ethylenediamine tetra-acetic acid (EDTA) [55]. It is a laborious and time-consuming method that has been replaced with the slide, plate and card agglutination tests [8].

Lateral Flow Assay (LFA)

It is a sensitive and specific test directly measuring Ab binding to the antigen [55]. Even though it is as sensitive as the RBT, it has a much higher specificity, providing a simple and inexpensive option for confirming RBT results [52]. Its ability to distinguish between IgM and IgG antibodies contributes to the clinical assessment of the stage of infection [52]. It is appropriate for rapid field or bedside testing in endemic areas where laboratories lack modern facilities. It is even more accurate and specific than the SAT in chronic and complex cases [8]. However, it is not suitable for extensive screening [63]. It can be used by small-holder herds to screen for and remove infected cattle or to reject milk from infected cattle [63].

4. Direct Detection

Delayed Hypersensitivity Test for Brucellin (DHTB)

Delayed hypersensitivity is known to appear before circulating antibodies and can be used in the early detection of the infection in cattle [64] [65]. It has a spe-

cificity of 96% - 99.3% in non-vaccinated cattle, while in vaccinated cattle, DHTB specificity decreased significantly to 78% [9] [64] [66], with a relative sensitivity of 33% [64]. Some naive cattle can serologically react to the injection of brucellin [9]. In the case of false-positive serology results, DHTB is more specific than RBT and CFT; hence it can be used at the herd level to confirm infection before slaughtering the cattle [66]. It is advisable to combine the results of DHTB and serological assays and consider an animal as positive if it is positive on one or both tests [65]. This way, the sensitivity will be high, although the specificity will be decreased [64].

Culture

Culture is the gold standard, providing the definitive diagnosis of brucellosis [14] [49]. It has good specificity, but in cases where the fever is intermittent, sensitivity is problematic [47]. The organism requires biosafety level 3 precautions and takes a minimum of 4 - 5 days to grow in culture [31] [67]. Patients with a long-standing disease may have negative culture results due to bacterial eradication without complete clinical recovery [49]. It is costly, and its application is not feasible in the routine diagnosis of the disease [50]. There is no available data on a microbiological study of *Brucella* species in Cameroon. Therefore, there is no knowledge of the species in circulation in this setting [61].

Polymerase Chain Reaction (PCR)

Nucleic acid amplification provides rapid detection and confirmation of *Brucella* with high specificity and low cost [68] [69] [70]. It requires minimum biological containment, providing results in a brief time [68]. It is more sensitive and safer than blood culture and more specific than serologic methods in diagnosing acute disease [50] [69] [70] [71]. It is applicable in assessing the treatment efficacy, species differentiation, and biotyping of isolates. It is convenient for diagnosing human brucellosis [6].

Challenges are faced in standardising extraction methods; infrastructure, equipment and expertise are still lacking [14]. The inhibition of DNA amplification in the presence of immunoglobulin G (IgG), proteins and polysaccharides in serum is another challenge [57]. The complexity of this method disfavours the routine use of PCR in laboratories [69]. The sensitivity of PCR-based methods can be reduced in chronic brucellosis, where it has a lower sensitivity than the ELISA method [72]. Furthermore, the sensitivity and accuracy of PCR-based methods are dependent on the DNA extraction method and the quality of extracted genomic DNA. This method is also subject to inhibition by substances such as phenol, EDTA, DNase and RNase [72]. The combined use of PCR and ELISA diagnostic tests can improve and overcome limitations in diagnosing brucellosis [72]. This PCR-ELISA molecular method is more sensitive than other methods, with a semi-quantitative ability [70]. There is no available data in Cameroon on using the PCR method to detect brucellosis infections and identify the species of the bacteria circulating within the country.

Sequencing

Sequencing gives an insight into the genetic basis for host preference, patho-

genesis, virulence, biotype differences and phylogenetic relationships [73]. It enables the identification of potential targets for the developing of vaccines and diagnostics to prevent and control brucellosis [74]. The equipment is expensive, not readily available and requires trained staff to carry out the procedures. Within Cameroon, a few research facilities with next-generation sequencing equipment are grossly insufficient to use in the country's research needs.

The laboratory diagnosis of *Brucella* requires a combination of several methods considering that there is no single test by which a bacterium can be identified unequivocally [50] [75]. Bacteriological and genomic limitations make serology the most practical and valuable tool for brucellosis diagnosis [14]. The strengths and limitations of assays applied in diagnosing brucellosis and possible applications are summarised in **Table 4**.

RBPT, ELISA, SAT, CFT, and LFA have been applied for routine diagnosis and screening of humans using blood samples. Although serology tests are easy to perform, their specificity is low in endemic areas. It is also low in persons who may be exposed to *Brucella* by their profession and in patients with relapse or a recent history of brucellosis [76]. Depending on the setting and purpose of the analysis, the assay should be chosen cautiously to achieve the target. It has been suggested that ELISA assays be used for screening while RBPT and CFT confirm the ELISA results [59] [60]. Drafting a diagnostic protocol for the disease by health officials will facilitate its applicability in various hospitals, particularly in areas inhabited by HROGs.

5. Risk Factors for Brucellosis

Risk factors in cattle

These risk factors are intrinsic (animal factors; age, sex and breed) and extrinsic (husbandry and environmental; ecological zone, herd size, herd management system, history of third-trimester abortion, interaction with wildlife, and interaction with sheep and goats during grazing) factors.

Extrinsic factors

Animal husbandry system

The pastoralist systems of nomadism and transhumance have been associated with higher seroprevalence in cattle [15] [77]. In contrast, an increased risk was associated with sedentary dairy herds, while transhumance was associated with a decreased risk [11] [78] [79]. Three farming systems have been associated with cattle production in Cameroon; the extensive or traditional system (which for centuries has been carried out by the Fulani pastoralists who live in areas of lower population density), the semi-intensive, and the intensive system [79]. Cows in the extensive system have been shown to have a higher infection rate than those in the semi-intensive system. This difference is explained by the fact that migratory herds contact other potentially infected herds during their movement into the different areas [7]. There is a need to carry out prevalence studies in larger herds and various farming systems. Within regions of high prevalence, the possible

Table 4. Strengths and limitations of various diagnostic approaches used for diagnosis of brucellosis.

Method	Strengths	Limitations	Recommendations
Indirect methods			
Serology			
Rose Bengal Plate Test (RBPT)	<ul style="list-style-type: none"> Highly sensitive, inexpensive, simple, and rapid to perform. Detects mainly IgM and requires essential laboratory equipment and expertise. 	<ul style="list-style-type: none"> Low specificity, false-negative and false-positive results and not recommended for diagnosing chronic brucellosis. 	<ul style="list-style-type: none"> Applicable as a rapid diagnostic test. Confirmation of results is required, using either CFT or ELISA.
Indirect Enzyme-linked immunosorbent assay (iELISA)	<ul style="list-style-type: none"> High sensitivity, is affordable, has shorter run times and requires less interpretation training. More sensitive than RBT. More sensitive and specific than SAT Measures various antibody titers (IgG, IgM, and IgA). 	<ul style="list-style-type: none"> Low specificity, lower than RBT. 	<ul style="list-style-type: none"> Appropriate in diagnosing various stages of infection, chronic brucellosis cases, and detecting incomplete antibodies. Ideal for screening purposes.
Competitive Enzyme-linked immunosorbent assay (cELISA)	<ul style="list-style-type: none"> High specificity. 	<ul style="list-style-type: none"> Lower sensitivity than iELISA and species unspecific. 	<ul style="list-style-type: none"> Applicable for various animal species. Can process poor quality samples such as hemolysed blood and use for the confirmation of brucellosis.
Complement fixation test (CFT)	<ul style="list-style-type: none"> Very specific, detecting IgM and IgG1 antibodies. Detects incomplete antibodies, as well as the slightest changes in antibody titres. 	<ul style="list-style-type: none"> False-negative results were recorded when IgG2 antibodies impede complement fixation. Not appropriate in detecting recent infections in cattle. Requires expertise for interpretation. The quality of its results is affected by the sample quality and the standardisation of the antigen. 	<ul style="list-style-type: none"> It is fitting for control and surveillance programs. It is used together with RBT as a confirmatory test.
Slow Agglutination Test (SAT)	<ul style="list-style-type: none"> Designed mainly to detect IgM. 	<ul style="list-style-type: none"> Low sensitivity and specificity, slow and not advisable for use in individual animals. Not applicable for the detection of chronic brucellosis 	<ul style="list-style-type: none"> Suitable in detecting brucellosis on a herd basis. Use in routine diagnosis, complemented with <i>Brucella</i> Coombs test.
Slow agglutination of Wright with EDTA (SAW-EDTA)	<ul style="list-style-type: none"> Sensitive, designed for the detection of IgM. 	<ul style="list-style-type: none"> Laborious and time-consuming. 	<ul style="list-style-type: none"> It has been replaced by slide, plate and card agglutination tests.
Lateral flow assay (LFA)	<ul style="list-style-type: none"> Sensitive and specific, as sensitive as the RBT but having a much higher specificity. Can distinguish between IgM and IgG antibodies. More accurate and specific than the SAT in chronic and complex cases. 	<ul style="list-style-type: none"> Not suitable for large-scale screening. 	<ul style="list-style-type: none"> Appropriate for rapid field or bedside testing in endemic areas and where laboratories lack modern facilities. Suitable in the confirmation of RBT results. It can be used by smallholder herds to screen for and remove infected cattle or to reject milk from infected cattle.

Continued

Direct detection

Cellular test Delayed hypersensitivity test for brucellin (DHTB)	<ul style="list-style-type: none"> • High specificity in non-vaccinated cattle. It detects antigens before the circulating antibodies appear and are more specific than RBT and CFT. 	<ul style="list-style-type: none"> • Lower specificity in vaccinated cattle. Low sensitivity and may sensitise cattle that have not been exposed to the infection. 	<ul style="list-style-type: none"> • Results should be combined with serology for confirmation at herd levels before the slaughter of infected cattle.
Culture	<ul style="list-style-type: none"> • The gold standard provides the definitive diagnosis of the disease with good specificity. 	<ul style="list-style-type: none"> • It requires biosafety level 3 precautions, takes a minimum of 4 - 5 days to grow in culture and is costly. • Sensitivity is problematic in cases where the fever is intermittent. • In chronic disease, culture results may be negative due to bacterial eradication without complete clinical recovery. 	<ul style="list-style-type: none"> • Application is not feasible in the routine diagnosis of the disease. • Useful in research for identifying the species in circulation.
Polymerase chain reaction (PCR)	<ul style="list-style-type: none"> • Provides rapid detection and confirmation of <i>Brucella</i> with high specificity and low cost. • It is more sensitive and safer than blood culture and more specific than serologic methods in diagnosing acute disease. • The PCR-ELISA molecular method is more sensitive than other methods, with a semi-quantitative ability. 	<ul style="list-style-type: none"> • Sensitivity and accuracy are dependent on the DNA extraction method and the quality of extracted genomic DNA. • Challenges are faced in standardising extraction methods; infrastructure, equipment and expertise are still lacking. • It has a lower sensitivity than ELISA, and sensitivity can also be reduced in chronic brucellosis. • Its complexity contributes to the limited application in routine laboratory practice. 	<ul style="list-style-type: none"> • It is applicable in assessing the treatment efficacy, species differentiation and biotyping of isolates and is convenient for diagnosing human brucellosis. • The combined use of PCR and ELISA diagnostic tests can improve and overcome limitations in diagnosing brucellosis.
Sequencing	<ul style="list-style-type: none"> • It gives an insight into the genetic bases for host preference, pathogenesis, virulence, biotype differences and phylogenetic relationships. • Enables the identification of potential targets for the development of vaccines and diagnostics to prevent and control brucellosis. 	<ul style="list-style-type: none"> • Equipment is expensive, not readily available, and requires trained staff to carry out the procedures. 	<ul style="list-style-type: none"> • Applicable for research on genetic variability, geographical distribution and host preferences.

causes of the spread of infection should be determined, and appropriate measures should be put in place to curb the spread.

Mixing of herds and contact with other animals

The mixing of cattle with other livestock during grazing, watering, at livestock markets, and during veterinary interventions has also been associated with high

seroprevalence [11] [17] [80]. Contact with small ruminants, wildlife and seeing buffalos around grazing cattle are also associated with an increased risk of [37] [61] [81]. The close interaction of different animals at the interphase mentioned above facilitates the transmission of the disease [7]. Keeping cattle separate from other livestock is essential in minimising the risk of cross-transmission of infection from one species to another [82]. Meanwhile, pastoral households often keep a diverse composition of livestock species as part of a coping mechanism for uncertainties and risks [7]. However, this approach favours inter and intra-species transmission of *Brucella* [1] [83]. It is incumbent to educate the population on these risky practices and suggest vaccination and screening programs to curb the spread of the disease.

Geographical location and seasons

Seroprevalence varies across various geographic locations of the herds, with different studies providing variable prevalence data according to the site of collection [11] [18] [19] [21] [83]. A major setback in this setting is the heterogeneous nature of the studies. Therefore it is impossible to conclude prevalence according to geographic locations [7] [61]. Seasonal variation determines the pastoralist activities, thereby influencing the prevalence values. Bovine brucellosis infection rates are higher in the dry season than in the rainy season [7]. Sedentary cattle are taken out of their shelter for pasture and water during the dry season. The interaction between domestic animals, cattle and humans in this interphase favours the acquisition and spread of the disease from wildlife to cattle and then to humans [81]. Understanding the role of the geographical location and seasonal differences in the spread of the disease will require objective studies on these aspects.

Regular purchase of animals or sale and purchase of infected animals

The regular purchase of animals is a risk factor for brucellosis because most cows sold for slaughter by herders are not doing well [11] [61] [84]. “Not doing well” parameters coincide with poor reproductive performance [74]. Studies in the Western Highlands of Cameroon reveal that approximately 70% of the cattle sold for slaughter in the abattoirs were on the verge of death [7] [61]. These cattle are introduced for sale to mitigate the financial losses associated with animal death within the flock. The introduction of sick animals into the community of healthy animals before their sale favours interaction between these animals. Thus, facilitating the transmission of the infection and increasing the seroprevalence of brucellosis in healthy animals [7] [17].

On the other hand, the healthy cattle (potentially exposed to the *Brucella*) which were not sold are returned to their respective herds from the cattle markets, serving as a reservoir for infection of the disease within the herd [7]. To interrupt this transmission cycle, the screen and slaughter approach to eradicate the condition is a laudable approach. Educating the herders on the risks of transmitting the diseases infected animals to humans could change their attitudes.

Intrinsic risk factors

Age of cattle

Older cattle have been associated with higher seroprevalence than younger ones [7] [11] [37] [82]. This pattern is substantiated because the older the cattle, the greater their exposure to the bacteria from various sources [85]. In the same light, seropositivity is higher amongst sexually mature cattle than in the younger sexually immature cattle [85]. When testing cattle for the infection, older animals should be tested first, and separating the young flock from the older ones will decrease the exposure of young cattle.

Sex of cattle

Cows are at greater risk of *Brucella* infections than bulls because cows are kept longer in the herd for reproduction and, therefore, are more exposed to disease than bulls kept for relatively shorter durations [1]. The stress associated with pregnancy and calving tends to lower the immunity of female animals, also explaining the observed difference [85]. Vaccination programs should target female cattle kept longer in the herd and have a greater risk of being infected.

Breed

The cattle reared in Cameroon are the Zebu, White Fulani, Red Fulani, Djafoun, Goudali and other cross breeds. The breed contributes to the susceptibility to infection. The Red Fulani and Goudali have higher seroprevalence than other breeds; Red Fulani (11.48%), Goudali (9.61%) and White Fulani (7.21%) [1] [7]. At the same time, another study reported higher seroprevalence in White Fulani than in other breeds [20]. Another report showed minimal differences between the White Fulani (16.96%) and Red Fulani (16.67%) breeds [17]. Establishing clear patterns between breed and seroprevalence within the country will guide the choice of cattle for the local market and priorities for control programs.

Cattle with a history of abortion

Seropositivity is higher in herds with a history of third-semester abortion [20]. The presence of erythritol, the growth stimulant for *B. abortus*, increases the cows' susceptibility to *Brucella* infection, particularly during early pregnancy, and such conditions can result in late-term abortion [86]. After the first abortion, these animals can give birth without complications [21]. However, a certain proportion of infected cows may not abort [87]. Therefore, any history of abortions highlights the possibility of *Brucella* infection. Cattle that suffered an abortion should be isolated from the others and be tested for brucellosis to rule out the cause of the abortion.

Risk factors in humans

Occupational exposure

The high-risk occupational groups in the Noun Division had seroprevalence ranging from 10.24% to 12.45% and 5.6% amongst abattoir workers in the Ngaoundere abattoir [1] [17]. The high-risk populations for human brucellosis include livestock herders, butchers, slaughterhouse workers, veterinarians, meat and milk sellers [1]. Abattoir workers are at most the significant risk of infection

through open wounds on bare hands, splashing of infected fluids in the conjunctiva and inhalation of aerosols in the slaughtering area [88]. Laboratory personnel are also at high risk, considering that brucellosis is the most common laboratory-acquired infection in the world. Therefore, care must be exercised in handling these cultures [89]. High-risk individuals ought to be sensitised about the disease and encouraged to use personal protective clothing while at work to avoid contamination. Health personnel should also be sensitised about the condition and should be able to diagnose the high-risk individuals appropriately.

Longevity in the abattoir environment

In humans, seropositivity has been associated with longevity in the abattoir environment [17] [90] [91] [92]. Working at the abattoir exposes an individual to activities that increase the risk of picking up the bacteria, such as direct contact with infected tissues and inhalation of droplets [1]. There is a need for an annual screening of abattoir staff for early diagnosis and treatment of these occupational diseases.

Being a meat or offal processor

Dressing slaughtered animals is also a risk factor for acquiring the infection [17]. Individuals involved include meat carvers and cleaners exposed daily to blood, viscera and abortion products [24]. This exposure is aggravated by the failure to use personal protection equipment (boots, aprons, goggles, bibs, gloves and helmet) and disregard for proper hygiene measures [25] [93]. The management of slaughterhouses ought to impose on workers' personal protective equipment and wound dressing for those who have cuts on their skin to avoid contamination through the wounds.

Assisting in animal delivery and abortions

Humans handling aborted fetuses, and assisting in abortion and delivery without using personal protective clothing, have been associated with higher seroprevalence rates [1] [17] [85] [92] [93] [94]. *Brucella* has a tropism for tissues rich in erythritol, their preferred source of carbon/energy, promoting their massive growth [29]. High concentrations of erythritol are present in animals' breast, uterine, epididymal, and fetal tissues. In addition, the placenta produces progesterone, which enhances *in vitro Brucella* growth [95]. Secretions in these tissues contain high concentrations of bacteria, which are at their highest level in the vagina immediately after abortion or birth. Hence, products of abortion and birthing materials are the primary source of contagion [96]. Direct and regular contact with these tissues is a high risk for infection. Pregnant women who had contact with infected animals were seropositive for brucellosis [17] [97]. This scenario emphasises the importance of using personal protective clothing while assisting cattle and disposing of their aborted fetuses or placenta.

Consumption of raw milk

Consumption of raw milk by cattle rearers and individuals within their communities predisposes them to *Brucella* infection [7] [17] [22]. Breast tissues are rich in erythritol, the preferred energy source for *Brucella*. Infected cattle

will shed the bacteria in milk and the consumption of unpasteurised milk is a risk factor for infection in humans. Within some dairy farms in the country's western highlands, the milk produced is home consumed, sold in informal markets or collected by a processing plant. However, the health status of this milk is unknown [18]. There is also a need to assess the milk quality in terms of health hazards to ensure the safety of consumers before and after the production process [18]. Since the control measures for the disease have not been firmly established, avoiding the consumption of raw milk will control the spread of the infection.

Exposure to animals outside the abattoir

Abattoir workers exposed to other animals outside the abattoir have an increased risk of being seropositive [17] [98]. The presence of the disease in other domestic animals could increase their risk of infection, as demonstrated by Kamga *et al.* in 2020 [1]. Abattoir workers who own domestic animals should be more cautious even while at home to prevent infection.

Burying dead animals

The inappropriate disposal of infected animals or their products contaminates the environment, posing a health risk to the human and animal populations. In the North West region of Cameroon, only 10% of farmers buried dead animals, while 20% slaughtered the sick animals, and 70% sold the terminally ill animals [7]. Those who do not bury probably dispose of carcasses into the environment leading to soil contamination with the bacteria and serving as risk factors for infection [24] [99]. Thus, this emphasises the importance of environmentalists in controlling this disease. In line with the One Health approach, the Ministry of the Environment and Nature Protection should sensitise the communities on the dangers of poor disposal of animal carcasses and body parts. That way, contact with contaminated soil will not be a problem in this setting.

No knowledge of brucellosis

Inadequate knowledge is associated with a high prevalence of the disease in humans [1] [7] [93] [100] [101]. A high proportion of farmers (89.5%) in the North West region of Cameroon are recorded to have not known brucellosis [7]. Lack of knowledge is also associated with education, where the risk of being in contact with *Brucella* significantly increases in participants with no formal education [1]. Awareness of the disease enhances PPE use at work, proper handling and cleanliness [88]. It does not rule out that some individuals, although aware of the disease, choose not to respect the safety measures at work [100]. It could be because they know the disease presentation in humans but have no knowledge of the method of transmission and prevention of the disease [102]. Education on the mode of transmission, clinical presentation and preventive practices through sensitisation programs amongst high-risk occupational groups as a Public Health measure will contribute to the control of infections. Good knowledge of the disease and its symptoms by workers in the animal and human sectors enhances the diagnosis and appropriate treatment of the disease [103].

6. Control Measures

There are no records of the implementation of brucellosis control programs in Cameroon [1] [11]. Control measures for cattle brucellosis include: the regular screening and systematic slaughter of infected animals, vaccination of cattle against *Brucella*, and artificial insemination since *Brucella* is transmitted between animals through sexual intercourse [18] [98]. Eradication of brucellosis by test-and-slaughter is impracticable in developing countries. Due to limited resources to compensate farmers whose animals are slaughtered during such screening programs [102]. In the absence of these control measures, long-term chronic infections could be expected, thus providing a steady supply of infectious organisms [63]. The institution of a tracking system to trace cattle origin is a laudable approach for effectively eradicating the disease. Cattle rearers also need education on identifying the signs of infection in cattle (hygromas and abortions) and seeking veterinary assistance when these symptoms appear. They must know the risk factors for disease, the symptoms and the complications in humans who get infected.

Encouraging boiling milk before consumption, and avoiding the consumption of raw or undercooked animal products, including bone marrow, will contribute to the control of the infection [6] [18] [72]. The practice of good hygiene is essential in avoiding skin contamination. Using protective clothing/equipment by HROGs is critical in preventing inhalation and accidental ingestion of organisms. The accidental ingestion of the bacteria could occur while assisting at the birth, carrying out a necropsy, or butchering an animal. Special precautions should be taken when handling an aborted foetus or its membranes and fluids [6]. Surveillance information is the cornerstone of epidemiologic decision-making, and is needed to direct policy makers, public health authorities, and veterinary services to appropriate actions [61]. Veterinarians, public health authorities and other community leaders need to collaborate to control the disease in animals and manage human exposure [99].

7. Prevention

Brucella vaccines successfully protected cattle against infection and abortion worldwide. Strain 19 (S19) and RB51 are the approved *B. abortus* vaccine strains most commonly used to vaccinate cattle [103]. In Cameroon, however, there are no records of the vaccination of the cattle [18] [21] [22]. A vaccine trial was performed in the 1990s in the North of Cameroon using the *B. abortus* B19 vaccine. This vaccine was made from the *B. abortus* strain isolated in the National Veterinary Laboratory in Bokle, Garoua. It was administered in one dose subcutaneously at high doses of 5×10^{10} to 8×10^{10} colony forming units (CFU). The vaccine's efficacy depended on environmental factors and the breed of the animals [64]. Since then, no further study has been reported or evaluated for other available cattle vaccines.

An ideal vaccine against *Brucella* in cattle should: 1) prevent *Brucella* infec-

tion in both genders, 2) not provoke disease in immunised animals, 3) prevent abortion, 4) confer long-term protection with only one dose, 5) not interfere with LPS-based serological tests, 6) be biologically stable and not present the risk of virulence reversion, 7) not be pathogenic to humans, and 8) not contaminate the derivatives of the vaccinated animals [6]. There is presently no safe and protective human vaccine against brucellosis. Therefore, animal vaccination could be a critical factor for controlling and eradicating animal and human brucellosis [6].

The way forward

- Prevalence studies should be carried out in dairy herds, abattoirs receiving cattle from endemic areas and the abattoir personnel, who are at greater risk of occupational exposure to *Brucella*. It is mainly the case for the Littoral region, which has one of the three modern abattoirs in the country and one of the highest numbers of cattle slaughtered per day in the country.
- Studies and surveys should be done regularly to understand the actual disease burden and reduce the economic losses in the cattle sector.
- Knowledge, attitude and practices studies should be carried out to understand the knowledge gap in the population, followed by massive sensitisation of workers in the livestock sector and health personnel on the prevalence of this disease. That will enhance the implementation of adequate preventive measures, proper diagnosis and treatment of the infections and other control measures.
- There have been no microbiological or molecular characterisations of *Brucella* species in Cameroon in 37 years. Thus, there is no knowledge of the species in circulation. In cognisance of the time required and the risks involved in isolating the bacteria, molecular methods should be used to identify the species of the bacteria in circulation in Cameroon. Sequencing will further compare the strains in circulation to those of neighbouring and other sub-Saharan countries.
- Brucellosis control programs should be instituted, such as mass vaccination of cattle in the affected areas and separating pregnant cows and cattle that have undergone abortion from the rest of the flock to decrease disease transmission.
- Rapid diagnostic kits should be made available for clinical diagnosis of the human population to limit under-diagnosis and decrease the human suffering from the diseases.

8. Conclusion

The number of studies on brucellosis in Cameroon is insufficient. They do not portray a comprehensive human and cattle brucellosis situation where the disease is endemic. Accurate microbiological, molecular and epidemiological evidence of brucellosis within the country is lacking. Hence, the need for a nationwide survey amongst HROGs using the standardised methodology to understand

the disease burden in the country.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Kanga, R.N., Silatsa, B.A., Farikou, O., Kuate, J.-R. and Simo, G. (2020) Detection of *Brucella* antibodies in Domestic Animals of Southern Cameroon: Implications for the Control of Brucellosis. *Veterinary Medicine and Science*, **6**, 410-420. <https://doi.org/10.1002/vms3.264>
- [2] Hull, N.C. and Schumaker, B.A. (2018) Comparisons of Brucellosis between Human and Veterinary Medicine. *Infection Ecology and Epidemiology*, **8**, Article ID: 1500846. <https://doi.org/10.1080/20008686.2018.1500846>
- [3] Bakri, F.G., Alqadiri, H.M. and Adwan, M.H. (2018) The Highest Cited Papers in Brucellosis: Identification Using Two Databases and Review of the Papers' Major Findings. *Biomed Research International*, **2018**, Article ID: 9291326. <https://doi.org/10.1155/2018/9291326>
- [4] Fèvre, E.M., De Glanville, W.A., Thomas, L.F., Cook, E.A.J., Kariuki, S. and Wamae, C.N. (2017) An Integrated Study of Human and Animal Infectious Disease in the Lake Victoria Crescent Small-Holder Crop-Livestock Production System, Kenya. *BMC Infectious Diseases*, **17**, Article No. 457. <https://doi.org/10.1186/s12879-017-2559-6>
- [5] Elelu, N., Aiyedun, J.O., Mohammed, I.G., Oludairo, O.O., Odetokun, I.A., Mohammed, K.M., Bale, J.O. and Nuru, S. (2019) Neglected Zoonotic Diseases in Nigeria: Role of the Public Health Veterinarian. *Pan African Medical Journal*, **32**, Article No. 36. <https://doi.org/10.11604/pamj.2019.32.36.15659>
<https://www.panafrican-Med-Journal.com/content/article/32/36/full>
- [6] Golshani, M. and Buozari, S. (2017) A Review of Brucellosis in Iran: Epidemiology, Risk Factors, Diagnosis, Control, and Prevention. *Iranian Biomedical Journal*, **21**, 349-359.
- [7] Bayemi, P.H., Mah, G.D., Ndamukong, K., Nsongka, V.M., Leinyuy, I., Unger, H., Ndoumbe, N.M., Webb, E.C., Achukwi, M.D., Hakoue, F. and Luogbou, N.D. (2015) Bovine Brucellosis in Cattle Production Systems in the Western Highlands of Cameroon. *International Journal of Animal Biology*, **1**, 38-44.
- [8] Khan, M.Z. and Zahoor, M. (2018) An Overview of Brucellosis in Cattle and Humans, and Its Serological and Molecular Diagnosis in Control Strategies. *Tropical Medicine and Infectious Disease*, **3**, Article No. 65. <https://doi.org/10.3390/tropicalmed3020065>
- [9] Bercovich, Z. (2000) The Use of Skin Delayed-Type Hypersensitivity as an Adjunct Test to Diagnose Brucellosis in Cattle: A Review. *Veterinary Quarterly*, **22**, 123-130. <https://doi.org/10.1080/01652176.2000.9695040>
- [10] Carugati, M., Biggs, H.M., Mazebe, M.J., Stoddarde, R.A., Cash-Goldwasser, S., Hertz, J.T., Halliday, J.E.B., Saganda, W., Lwezaulah, B.F., Kazwalai, R.R., Cleaveland, S., Maro, V.P., Rubach, M.P. and Crumpa, J.A. (2018) Incidence of Human Brucellosis in the Kilimanjaro Region of Tanzania in the Periods 2007-2008 and 2012-2014. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **112**, 136-143. <https://doi.org/10.1093/trstmh/try033>

- [11] Musallam, I. Ndour, A.P., Yempabou, D., Ngong, C.C., Dzousse, M.F., Mouiche-Mouliom. M-M., Feussom, J.M.K., Ntirandekura, J.B., Ntakirutimana, D., Fane, A., Dembele, E., Doumbia, A., Ayih-Akakpo, A.A.P.-H.S., Pato, P., Pali, M., Tapsoba, A.S.R., Compaore, G.M., Gagaro, H., Garba, A.I., Prakashbabu, B.C., Craighead, L., Takahashi, E., McGiven, J., Nguipdop-Djomo, P., Mangtani, P., Alambédji-Bada, R., Akakpo, A.J. and Guitian, J. (2019) Brucellosis in Dairy Herds: A Public Health Concern in the Milk Supply Chains of West and Central Africa. *Acta Tropica*, **197**, Article ID: 105042. <https://doi.org/10.1016/j.actatropica.2019.105042>
- [12] Luo, J., Yang, H., Hu, F., Zhang, S., Wang, T., Zhao, Q., Wang, R. and Zhen, Q. (2019) Associated Factors in Distinguishing Patients with Brucellosis from Suspected Cases. *BMC Infectious Diseases*, **19**, Article No. 1038. <https://doi.org/10.1186/s12879-019-4662-3>
- [13] Asante, J., Noreddin, A. and El Zowalaty, M.E. (2019) Pathogens Systematic Review of Important Bacterial Zoonoses in Africa in the Last Decade in Light of the “One Health” Concept’. *Pathogens*, **8**, Article No. 50. <https://doi.org/10.3390/pathogens8020050>
- [14] Muloki, H.N. Erume, J., Owiny, D.O. Kungu, J.M., Nakavuma, J., Ogeng, D. and Nasinyama, G.W. (2018) Prevalence and Risk Factors for Brucellosis in Prolonged Fever Patients in Post-Conflict Northern Uganda. *African Health Sciences*, **18**, 22-28. <https://doi.org/10.4314/ahs.v18i1.4>
- [15] Awah-Ndukum, J., Mouiche, M.M.M., Bayang, H.N., Ngwa, V.N., Assana, E., Feussom, K.J.M., Manchang, T.K. and Zoli, P.A. (2018) Seroprevalence and Associated Risk Factors of Brucellosis among Indigenous Cattle in the Adamawa and North Regions of Cameroon. *Veterinary Medicine International*, **2018**, Article ID: 3468596. <https://doi.org/10.1155/2018/3468596>
- [16] Scolamacchia, F., Handel, I.G., Fèvre, E.M., Morgan, K.L., Tanya, V.N. and de C. Bronsvooort, B.M. (2010) Serological Patterns of Brucellosis, Leptospirosis and Q Fever in Bos Indicus Cattle in Cameroon. *PLOS ONE*, **5**, e8623. <https://doi.org/10.1371/journal.pone.0008623>
- [17] Awah-Ndukum, J., Mouiche, Mohamed Moctar Mouliom, Kouonmo-Ngnoyem, L., Bayang, H.N., Manchang, T.K., Poueme, R.S.N., Kouamo, J., Ngu-Ngwa, V., Assana, E., Feussom, K.J.M. and Zoli, A.P. (2018) Seroprevalence and Risk Factors of Brucellosis among Slaughtered Indigenous Cattle, Abattoir Personnel and Pregnant Women in Ngaoundéré, Cameroon. *BMC Infectious Diseases*, **18**, Article No. 611. <https://doi.org/10.1186/s12879-018-3522-x>
- [18] Bayemi, P.H. Webb, E.C., Nsongka, M.V., Unger, H. and Njakoi, H. (2009) Prevalence of *Brucella abortus* Antibodies in Serum of Holstein Cattle in Cameroon. *Tropical Animal Health and Production*, **41**, 141-144. <https://doi.org/10.1007/s11250-008-9184-8>
- [19] Kong, A.T., Nsongka, M.V., Itoe, S.M., Hako, A.T. and Leinyuy, I. (2016) Seroprevalence of *Brucella abortus* in the Bamenda Municipal Abattoir of the Western Highlands, of Cameroon. *Greener Journal of Agricultural Sciences*, **6**, 245-251. <https://doi.org/10.15580/GJAS.2016.8.082216135>
- [20] Ojong, B. Ojong, Macleod, E., Ndip, M.L., Zulianid, A. and Piasentier, E. (2016) Brucellosis in Cameroon: Seroprevalence and Risk Factors in Beef-Type Cattle. *International Journal of Infectious Diseases*, **53**, 64. <https://doi.org/10.1016/j.ijid.2016.11.161>
- [21] Ojong, B.W. (2015) Situation of Brucellosis in Beef-Type Cattle Raised under Different Husbandry Systems in Cameroon. Establishment of a Multi-Sectorial Strategy for the Control of Brucellosis in the Main Peri-Urban Dairy Production Zones of

West and Central Africa Project Outline, Dakar.

- [22] Shey-Njila, Daouda, Nya, E., Zoli, P.A., Walravens, K., Godfroid, J. and Geerts, S. (2005) Serological Survey of Bovine Brucellosis in Cameroon. *Revue D'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, **58**, 139-143.
<https://doi.org/10.19182/remvt.9925>
- [23] Marvi, A., Asadi-Aliabadi, M., Darabi, M., Abedi, G., Siamian, H. and Rostami-Maskopae, F. (2018) Trend Analysis and Affecting Components of Human Brucellosis Incidence During 2006 to 2016. *Medical Archives*, **72**, 17-21.
<https://doi.org/10.5455/medarh.2018.72.17-21>
- [24] Ndoe, M.G., Damdam, F.B.H., Marat, M.K.I., Djoulde, I., Octavie, F.M.D. and Ntungwen, C.F. (2016) Assessment of Safety and Hygiene Measures about the Slaughtering System of Slaughterhouses with Regard to the Human Brucellosis. *Journal of Analytical & Pharmaceutical Research*, **3**, Article No. 62.
<https://doi.org/10.15406/japlr.2016.03.00062>
- [25] Kanga, R.M.N. Assongo, B.S., Magang, E.M.K., Fouapon, A.A., Salihou, M.M., Kuate, J.-R. and Simo, G. (2021) Seroprevalence of *Brucella* Antibodies and Risk Factors Associated with Human Brucellosis in High-Risk Occupational Groups of the Noun Division in the West Region of Cameroon. *Journal of Biosciences and Medicines*, **9**, 105-123. <https://doi.org/10.4236/jbm.2021.93011>
- [26] Paul, S., Peddayelachagiri, B.V., Gogoi, M., Nagaraj, S., Ramlal, S., Konduru, B. and Batra, H.V. (2020) Genome-Wide Unique Insertion Sequences among Five *Brucella* species and Demonstration of Differential Identification of *Brucella* by Multiplex PCR Assay. *Scientific Reports*, **10**, Article No. 6368.
<https://doi.org/10.1038/s41598-020-62472-3>
- [27] Celli, J. (2019) The Intracellular Life Cycle of *Brucella* spp. *Microbiology Spectrum*, **7**, 101-111. <https://doi.org/10.1128/microbiolspec.BAI-0006-2019>
- [28] Tuon, F.F. Cerchiari, N., Cequinel, J.C., Droppa, E.E.H., Moreira, S.D.R., Costa, T.P., Navarro, A.P.B., Handar, A.M. and Souza, M.N. (2017) Guidelines for the Management of Human Brucellosis in the State of Paraná, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, **50**, 458-464.
<https://doi.org/10.1590/0037-8682-0319-2016>
- [29] Murray, P.R., Rosenthal, K.S. and Pfaller, M.A. (2016) Medical Microbiology. Elsevier Inc., Amsterdam. <http://evolve.elsevier.com/Murray/microbiology/>
- [30] Cooke, F.J. and Slack, M.P.E. (2017) Gram-Negative Coccobacilli. In: Cohen, J., Powderly, W.G. and Opal, S.M., Eds., *Infectious Diseases*, Vol.2, 4th Edition, Elsevier Ltd., Amsterdam, 1611-1627.e1.
<https://doi.org/10.1016/B978-0-7020-6285-8.00183-0>
- [31] Harrison, E.R. and Posada, R. (2018) Brucellosis. *Pediatrics in Review*, **39**, 222-224.
<https://doi.org/10.1542/pir.2017-0126>
- [32] Rovid, S.A. (2018) Brucellosis.
<http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php>.
- [33] Wang, X. and Jiang, H. (2020) Epidemiological Characteristics of Human Brucellosis in Hubei, 2010-2016. *Chinese Journal of Epidemiology*, **41**, 1717-1722.
- [34] Ogugua, A.J., Akinseye, V.O., Cadmus, E.O., Emmanuel, A. Jolaoluwa Awosanya, Alabi, P.I., Idowu, O.S., Akinade, S.A., Dale, E.J., Perrett, L., Taylor, A., Ignocio, M., Simeon, I.B. and Cadmus, S.I.B. (2018) Prevalence and Risk Factors Associated with Bovine Brucellosis in Herds under Extensive Production System in Southwestern Nigeria. *Tropical Animal Health and Production*, **50**, 1573-1582.
<https://doi.org/10.1007/s11250-018-1597-4>

- [35] Corbel, M.J. (2006) *Brucellosis in Humans and Animals*. WHO Press, World Health Organization, Geneva.
- [36] World Health Organization (2011) *Brucellosis*.
<https://www.who.int/news-room/fact-sheets/detail/brucellosis>
- [37] Mazeri, S., Scolamacchia, F., Handel, I.G., Morgan, K.L., Tanya, V.N. and deC. Bronsvooort, B.M. (2013) Risk Factor Analysis for Antibodies to *Brucella*, *Leptospira* and, *C. burnetii* among Cattle in the Adamawa Region of Cameroon: A Cross-Sectional Study. *Tropical Animal Health and Production*, **45**, 617-623.
<https://doi.org/10.1007/s11250-012-0268-0>
- [38] Rahmanian, V., Rahmania, K., Mansoorian, E., Jahromi, A.S. and Madani, A. (2018) Epidemiological Characteristics and Temporal Trend of Human and Bovine Brucellosis Cases, Southern Iran, 2009-2016. *Pakistan Journal of Medical and Health Sciences*, **12**, 488-495.
- [39] Murray, P.R., Rosenthal, K.S. and Pfaller, M.A. (2016) *Medical Microbiology*. Elsevier Inc., Amsterdam.
- [40] De Figueiredo, P., Ficht, T.A., Rice-Ficht, A., Rossetti, C.A., Adams, L.G. and Adams, G. (2015) Pathogenesis and Immunobiology of Brucellosis Review of *Brucellae*-Host Interactions. *The American Journal of Pathology*, **185**, 1505-1517.
<https://doi.org/10.1016/j.ajpath.2015.03.003>
- [41] Pizarro-Cerdá, J., Moreno, E. and Gorvel, Jean, P. (2000) Invasion and Intracellular Trafficking of *Brucella abortus* in Nonphagocytic Cells. *Microbes and Infection*, **2**, 829-835. [https://doi.org/10.1016/S1286-4579\(00\)90368-X](https://doi.org/10.1016/S1286-4579(00)90368-X)
- [42] Neta, A.V.C., Mol, J.P.S., Xavier, M.N., Paixão, T.A., Lage, A.P. and Santos, R.L. (2010) Pathogenesis of Bovine Brucellosis. *Veterinary Journal*, **184**, 146-155.
<https://doi.org/10.1016/j.tvjl.2009.04.010>
- [43] Domenech, J., Lucet, P., Vallat, B., Stewart, C., Bonnet, J.B. and Bertaudière, L. (1980) La brucellose bovine en Afrique centrale: II. Etude clinique et épidémiologique: Particularités régionales et problèmes de l'élevage semi-intensif. *Revue D'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, **33**, 277-284.
- [44] Khurana, S.K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M.Z., Chhabra, R., Karthik, K., Patel, S.K., Pathak, M., Yattoo, M.I., Gupta, V.K., Dhama, K., Sah, R. and Chaicumpa, W. (2021) Bovine Brucellosis—A Comprehensive Review. *Veterinary Quarterly*, **41**, 61-88.
<https://doi.org/10.1080/01652176.2020.1868616>
- [45] Nejadid, R.B., Krecek, R.C., Khalaf, O.H., Hailat, N. and Arenas-Gamboa, A.M. (2020) Brucellosis in the Middle East: Current Situation and a Pathway Forward. *PLOS Neglected Tropical Diseases*, **14**, e0008071.
<https://doi.org/10.1371/journal.pntd.0008071>
- [46] Diaz, R., Casanova, A., Ariza, J. and Moriyón, I. (2011) The Rose Bengal Test in Human Brucellosis: A Neglected Test for the Diagnosis of a Neglected Disease. *PLOS Neglected Tropical Diseases*, **5**, e950.
<https://doi.org/10.1371/journal.pntd.0000950>
- [47] Welch, R.J. and Litwin, C.M. (2010) A Comparison of *Brucella* IgG and IgM ELISA Assays with Agglutination Methodology. *Journal of Clinical Laboratory Analysis*, **24**, 160-162. <https://doi.org/10.1002/jcla.20382>
- [48] Godfroid, J., Nielsen, K. and Saegerman, C. (2010) Diagnosis of Brucellosis in Livestock and Wildlife. *Croatian Medical Journal*, **51**, 295-305.
<https://doi.org/10.3325/cmj.2010.51.296>
- [49] Alsubaie, S.A., Turkistani, S.A., Zeaiter, A.A. and Thabit, A.K. (2021) Lack of Cor-

- relation of *Brucella* antibody Titers with Clinical Outcomes and Culture Positivity of Brucellosis. *Tropical Diseases, Travel Medicine and Vaccines*, **7**, Article No. 5. <https://doi.org/10.1186/s40794-021-00130-w>
- [50] Saavedra, M.J., Fernandes, C. and Queiroga, C. (2019) Laboratory Diagnosis of Brucellosis: Brucellosis in Goats and Sheep. Nova Science Publishers, Inc., Hauppauge, 151-180.
- [51] Muñoz, P., Marín, M.C.M., Monrea, D., González, D., Garin-Bastuji, B., Díaz, R., Mainar-Jaime, R.C., Moriyón, I. and Blasco, J.M. (2005) Efficacy of Several Serological Tests and Antigens for Diagnosis of Bovine Brucellosis in the Presence of False-Positive Serological Results Due to *Yersinia Enterocolitica* O:9. *Clinical And Diagnostic Laboratory Immunology*, **12**, 141-151. <https://doi.org/10.1128/CDLI.12.1.141-151.2005>
- [52] de Glanville, W.A. Conde-Álvarez, R., Moriyón, I., Njeru, J., Díaz, R., Cook, E.A.J., Morin, M., De, C. Bronsvoort, B.M., Thomas, L.F., Kariuki, S. and Fèvre, E.M. (2017) Poor Performance of the Rapid Test for Human Brucellosis in Health Facilities in Kenya. *PLOS Neglected Tropical Diseases*, **11**, e0005508. <https://doi.org/10.1371/journal.pntd.0005508>
- [53] Ruiz-Mesa, J.D., Reguera, J.M., Martín, L. and Lopez-Palmero, S. (2005) Rose Bengal Test: Diagnostic Yield and Use for the Rapid Diagnosis of Human Brucellosis in Emergency Departments in Endemic Areas. *Clinical Microbiology and Infection*, **11**, 221-225. <https://doi.org/10.1111/j.1469-0691.2004.01063.x>
- [54] Mathew, C., Stokstad, M., Johansen, T.B., Klevar, S., Mdegela, R.H., Mwamengele, G., Michel, P., Escobar, L., Fretin, D. and Godfroid, J. (2015) First Isolation, Identification, Phenotypic and Genotypic Characterisation of *Brucella abortus* Biovar 3 from Dairy Cattle in Tanzania. *BMC Veterinary Research*, **11**, Article No. 156. <https://doi.org/10.1186/s12917-015-0476-8>
- [55] Nymo, I.H., Godfroid, J., Åsbakk, K., Larsen, A.K., Das Neves, C.G., Rødven, R. and Tryland, M. (2013) A Protein A/G Indirect Enzyme-Linked Immunosorbent Assay for the Detection of Anti-*Brucella* Antibodies in Arctic Wildlife. *Journal of Veterinary Diagnostic Investigation*, **25**, 369-375. <https://doi.org/10.1177/1040638713485073>
<http://vdi.sagepub.com/content/early/2013/03/28/1040638713485073>
- [56] Swai, E.S. and Schoonman, L. (2010) The Use of Rose Bengal Plate Test to Assess Cattle Exposure to *Brucella* Infection in Traditional and Smallholder Dairy Production Systems of Tanga Region of Tanzania. *Veterinary Medicine International*, **2010**, Article ID: 837950. <https://doi.org/10.4061/2010/837950>
- [57] Perrett, L.L., McGiven, J.A., Brew, S.D. and Stack, J.A. (2010) Evaluation of Competitive ELISA for Detection of Antibodies to *Brucella* infection in Domestic Animals. *Croatian Medical Journal*, **51**, 314-319. <https://doi.org/10.3325/cmj.2010.51.314>
- [58] Poester, F.P., Ramos, E. and Thiesen, S. (2010) Diagnosis of Brucellosis. *The Open Veterinary Science Journal*, **4**, 46-60. <https://doi.org/10.2174/1874318801004010046>
- [59] Magwedere, K., Bishi, A., Tjipura-Zaire, G., Eberle, G., Hemberger, Y., Hoffman, L.C. and Dziva, F. (2011) *Brucellae* through the Food Chain: The Role of Sheep, Goats and Springbok (*Antidorcus marsupialis*) as Sources of Human Infections in Namibia. *Journal of the South African Veterinary Association*, **82**, 205-212. <https://doi.org/10.4102/jsava.v82i4.75>
- [60] Zerfu, B., Medhin, G., Mamo, G., Getahun, G., Tschopp, R. and Legesse, M. (2018) Community-Based Prevalence of Typhoid Fever, Typhus, Brucellosis and Malaria among Symptomatic Individuals in Afar Region, Ethiopia. *PLOS Neglected Tropical*

- Diseases*, **12**, e0006749. <https://doi.org/10.1371/journal.pntd.0006749>
- [61] Laine, C.G., Wade, A., Scott, H.M., Krecek, R.C. and Arenas-Gamboa, A.M. (2020) Scoping Review of Brucellosis in Cameroon: Where Do We Stand, and Where Are We Going? *PLOS ONE*, **15**, e0239854. <https://doi.org/10.1371/journal.pone.0239854>
- [62] Mutua, P.K. (2017) Pastoralists' Perceptions and Risk of Human Brucellosis in Kajiado County, Kenya. MSc. Thesis, University of Nairobi, Nairobi.
- [63] Bronsvort, B.M., Koterwas, B., Land, F., Handel, I.G., Tucker, J., Morgan, K.L., Tanya, V.N., Abdoel, T.H. and Smits, H.L. (2009) Comparison of a Flow Assay for Brucellosis Antibodies with the Reference CELISA Test in West African *Bos indicus*. *PLOS ONE*, **4**, e5221. <https://doi.org/10.1371/journal.pone.0005221>
- [64] Martrenchar, A., Njanpopb, B.M., Yayab, A., Njoyac, A. and Tulasne, J.J. (1993) Problems Associated with Tuberculosis and Brucellosis Skin-Test Methods in Northern Cameroon. *Preventive Veterinary Medicine*, **15**, 221-229. [https://doi.org/10.1016/0167-5877\(93\)90115-A](https://doi.org/10.1016/0167-5877(93)90115-A)
- [65] Bercovich, Z. and Muskens, J.A.M. (1999) The Efficacy of the Skin Delayed-Type Hypersensitivity Using a Brucellin Prepared from a Mucoid Strain of *Brucella abortus* to Detect Brucellosis. *Veterinary Journal*, **157**, 61-67. <https://doi.org/10.1053/tvj.1998.0252>
- [66] Pouillot, R., Garin-Bastuji, B., Gerbier, G., Coche, Y., Cau, C., Dufour, B. and Moutou, F. (1997) The Brucellin Skin Test as a Tool to Discriminate False Positive Serological Reactions in Bovine Brucellosis. *Veterinary Research*, **28**, 365-374.
- [67] Pal, D., Boby, N., Kumar, S., Kaur, G., Ali, S.A., Reboud, J., Shrivastava, S., Gupta, P.K., Cooper, J.M. and Chaudhuri, P. (2017) Visual Detection of *Brucella* in Bovine Biological Samples Using DNA-Activated Gold Nanoparticles. *PLOS ONE*, **12**, e0180919. <https://doi.org/10.1371/journal.pone.0180919>
- [68] Yu, L.W. and Nielsen, K. (2010) Review of Detection of *Brucella* spp. by Polymerase Chain Reaction Here. *Croatian Medical Journal*, **51**, 306-313. <https://doi.org/10.3325/cmj.2010.51.306>
- [69] Zamanian, M., Hashemi Tabar, G.R., Rad, M. and Haghparast, A. (2015) Evaluation of Different Primers for Detection of *Brucella* in Human and Animal Serum Samples by Using PCR Method. *Archives of Iranian Medicine*, **18**, 44-50.
- [70] Hasani, S.M., Mirnejad, R., Amani, J. and Vafadar, M.J. (2016) Comparing Rapid and Specific Detection of *Brucella* in Clinical Samples by PCR-ELISA and Multiplex-PCR Method. *Iranian Journal of Pathology*, **11**, 144-150.
- [71] Alikhani, M.Y., Hashemi, S.H., Naseri, Z., Farajnia, S. and Peeri-Dogaheh, H. (2013) Diagnosis of Human Brucellosis by Blood Culture (BACTEC) and PCR Method via Whole Blood and Serum. *Jundishapur Journal of Microbiology*, **6**, 248-251. <https://doi.org/10.5812/jjm.5073>
- [72] Mohseni, K., Mirnejad, R., Piranfar, V. and Mirkalantari, S. (2017) A Comparative Evaluation of ELISA, PCR, and Serum Agglutination Tests for Diagnosis of *Brucella* Using Human Serum. *Iranian Journal of Pathology*, **12**, 371-376. <https://doi.org/10.30699/ijp.2017.28293>
- [73] Halling, S.M., Peterson-Burch, B.D., Bricker, B.J., Zuerner, R.L., Qing, Z., Li, L.-L., Kapur, V., Alt, D.P. and Olsen, S.C. (2005) Completion of the Genome Sequence of *Brucella abortus* and Comparison to the Highly Similar Genomes of *Brucella melitensis* and *Brucella suis*. *Journal of Bacteriology*, **187**, 2715-2726. <https://doi.org/10.1128/JB.187.8.2715-2726.2005>

- [74] Islam, M.S., El Zowalaty, M.E., Van Vliet, A.H.M., Thakur, S., Khatun, M.M., Saha, S., Rahman, M.T., Noreddin, A. and Islam, M.A. (2019) First Genome Sequence of *Brucella abortus* Biovar 3 Strain BAU21/S4023, Isolated from a Dairy Cow in Bangladesh. *Microbiology Resource Announcements*, **8**, e00446-19. <https://doi.org/10.1128/MRA.00779-19>
- [75] Etemadi, A., Moniri, R., Neubauer, H., Dasteh Goli, Y. and Alamian, S. (2019) Laboratory Diagnostic Procedures for Human Brucellosis: An Overview of Existing Approaches. *Jundishapur Journal of Microbiology*, **12**, e91200. <https://doi.org/10.5812/jjm.91200>
- [76] Garshasbi, M., Ramazani, A., Sorouri, R., Javani, S. and Moradi, S. (2014) Molecular Detection of *Brucella* Species in Patients Suspicious of Brucellosis from Zanjan, Iran. *Brazilian Journal of Microbiology*, **45**, 533-538. <https://doi.org/10.1590/S1517-83822014005000048>
- [77] Akakpo, A.J. and Bornarel, P. (1987) Epidémiologie des brucelloses animales en Afrique tropicale: Enquêtes clinique, sérologique et bactériologique. *Revue Scientifique et Technique de l'OIE*, **6**, 981-1027. <https://doi.org/10.20506/rst.6.4.313>
- [78] Craighead, L., Meyer, A., Chengat, B., Musallam, I., Akakpo, J., Kone, P., Guitian, J. and Häsler, B. (2018) Brucellosis in West and Central Africa: A Review of the Current Situation in a Changing Landscape of Dairy Cattle Systems. *Acta Tropica*, **179**, 96-108. <https://doi.org/10.1016/j.actatropica.2017.12.026>
- [79] Bayemi, P.H., Bryant, M.J., Perera, B.M.A.O., Mbanya, J.N., Cavestany, D. and Webb, E.C. (2005) Milk Production in Cameroon: A Review. *Livestock Research for Rural Development*, **17**, 1-64.
- [80] Njeru, J., Melzer, F., Wareth, G., El-Adawy, H., Henning, K., Pletz, M.W., Heller, R., Kariuki, S., Fèvre, E. and Neubauer, H. (2016) Human Brucellosis in Febrile Patients Seeking Treatment at Remote Hospitals, Northeastern Kenya, 2014-2015. *Emerging Infectious Diseases*, **22**, 2160-2164. <https://doi.org/10.3201/eid2212.160285>
- [81] Kansime, C., Mugisha, A., Makumbi, F., Mugisha, S., Rwego, I.B., Sempa, J., Suzanne N Kiwanuka, N., Asimwe, B.B. and Rutebemberwa, E. (2014) Knowledge and Perceptions of Brucellosis in the Pastoral Communities Adjacent to Lake Mburo National Park, Uganda. *BMC Public Health*, **14**, Article No. 242. <https://doi.org/10.1186/1471-2458-14-242>
- [82] Bamaiyi, P.H. (2016) Prevalence and Risk Factors of Brucellosis in Man and Domestic Animals: A Review. *International Journal of One Health*, **2**, 29-34. <https://doi.org/10.14202/IJOH.2016.29-34>
- [83] Domenech, J., Philippe, L., Vallat, B., Stewart, C., Bonnet, J.B. and Alain, H. (1982) La brucellose bovine en Afrique centrale. III. Résultats statistiques des enquêtes menées au Tchad et au Cameroun. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, **35**, 15-22.
- [84] Profitós, H.M.J., Moritz, M. and Garabed, R.B. (2013) What to Do with Chronically Sick Animals? Pastoralists' Management Strategies in the Far North Region of Cameroon. *Pastoralism*, **3**, Article No. 8. <https://doi.org/10.1186/2041-7136-3-8>
- [85] Ayoola, M.C., Akinseye, V.O., Cadmus, E., Awosanya, E., Popoola, O.A., Akinyemi, O.O., Perrett, L., Taylor, A., Stack, J., Moriyon, I. and Cadmus, S.I. (2017) Prevalence of Bovine Brucellosis in Slaughtered Cattle and Barriers to Better Protection of Abattoir Workers in Ibadan, South-Western Nigeria. *Pan African Medical Journal*, **28**, Article No. 68. <https://doi.org/10.11604/pamj.2017.28.68.10925>
<https://www.panafrican-Med-Journal.com/content/article/28/68/full>

- [86] Gomo, C. De Garine-Wichatitsky, M., Caron, A. and Pfukenyi, D.M. (2012) Survey of Brucellosis at the Wildlife-Livestock Interface on the Zimbabwean Side of the Great Limpopo Transfrontier Conservation Area. *Tropical Animal Health and Production*, **44**, 77-85. <https://doi.org/10.1007/s11250-011-9890-5>
- [87] Yohannes, M., Degefu, H., Tolosa, T., Belihu, K, Cutler, R. and Cutler, S. (2013) Brucellosis in Ethiopia. *African Journal of Microbiology Research*, **7**, 1150-1157. <https://doi.org/10.5897/AJMR12.738>
- [88] Luwumba, D., Kusiluka, L. and Shirima, G. (2019) Occupational Hazards Associated with Human Brucellosis in Abattoir Settings: A Case Study of Dodoma Abattoir in Tanzania. *Journal of Veterinary Medicine and Animal Health*, **11**, 73-80. <https://doi.org/10.5897/JVMAH2019.0752>
- [89] Hull, N., Miller, J., Berry, D., Laegreid, W., Smith, A., Klinghagen, C. and Schumaker, B. (2018) Optimisation of *Brucella abortus* Protocols for Downstream Molecular Applications. *Journal of Clinical Microbiology*, **56**, e01894-17. <https://doi.org/10.1128/JCM.01894-17>
- [90] Aworh, M.K., Okolocha, E., Kwaga, J., Fasina, F., Lazarus, D., Suleman, I., Poggensee, G., Nguku, P. and Nsubuga, P. (2013) Human Brucellosis: Seroprevalence and Associated Exposure Factors among Abattoir Workers in Abuja, Nigeria—2011. *Pan African Medical Journal*, **16**, Article No. 103. <https://doi.org/10.11604/pamj.2013.16.103.2143>
- [91] Mirambo, M.M., Mgode, G.F., Malima, Z.O., John, M., Mngumi, E.B., Mhamphi, G.G. and Mshana, S.E. (2018) Seropositivity of *Brucella* spp. and *Leptospira* spp. Antibodies among Abattoir Workers and Meat Vendors in the City of Mwanza, Tanzania: A Call for One Health Approach Control Strategies. *PLOS Neglected Tropical Diseases*, **12**, e0006600. <https://doi.org/10.1371/journal.pntd.0006600>
- [92] Nasinyama, G., Ssekawojwa, E., Opuda, J., Grimaud, P., Etter, E. and Bellinguez, A. (2014) *Brucella* Sero-Prevalence and Modifiable Risk Factors among Predisposed Cattle Keepers and Consumers of Un-Pasteurised Milk in Mbarara and Kampala Districts, Uganda. *African Health Sciences*, **14**, 790-796. <https://doi.org/10.4314/ahs.v14i4.3>
- [93] Adesokan, H.K., Alabi, P.I. and Ogundipe, M.A. (2016) Prevalence and Predictors of Risk Factors for Brucellosis Transmission by Meat Handlers and Traditional Healers' Risk Practices in Ibadan, Nigeria. *Journal of Preventive Medicine and Hygiene*, **57**, E164-E171.
- [94] Ekere, S.O., Njoga, E.O., Onunkwo, J.I. and Njoga, U.J. (2018) Serosurveillance of *Brucella* antibody in Food Animals and Role of Slaughterhouse Workers in Spread of *Brucella* infection in Southeast Nigeria. *Veterinary World*, **11**, 1171-1178. <https://doi.org/10.14202/vetworld.2018.1171-1178>
- [95] González-Espinoza, G., Arce-Gorvel, V., Mémet, S. and Gorvel, J.P. (2021) *Brucella*: Reservoirs and Niches in Animals and Humans. *Pathogens*, **10**, Article No. 186. <https://doi.org/10.3390/pathogens10020186>
- [96] Ducrotoy, M.J., Ammary, K., Ait Lbacha, H., Zouagui, Z., Mick, V., Prevost, L., Bryssinckx, W., Welburn, S.C. and Benkirane, A. (2015) Narrative Overview of Animal and Human Brucellosis in Morocco: Intensification of Livestock Production as a Driver for Emergence? *Infectious Diseases of Poverty*, **4**, Article No. 57. <https://doi.org/10.1186/s40249-015-0086-5>
- [97] Rujeni, N. and Mbanzamihiho, L. (2014) Prevalence of Brucellosis among Women Presenting with Abortion/Stillbirth in Huye, Rwanda. *Journal of Tropical Medicine*, **2014**, Article ID: 740479. <https://doi.org/10.1155/2014/740479>

- [98] Workalemahu, B., Sewunet, T. and Astatkie, A. (2017) Seroepidemiology of Human Brucellosis among Blood Donors in Southern Ethiopia: Calling Attention to a Neglected Zoonotic Disease. *American Journal of Tropical Medicine and Hygiene*, **96**, 88-92. <https://doi.org/10.4269/ajtmh.15-0702>
- [99] Holt, H.R., Eltholth, M.M., Hegazy, Y.M., El-Tras, W.F., Tayel, A.A. and Guitian, J. (2011) *Brucella* spp. Infection in Large Ruminants in an Endemic Area of Egypt: Cross-Sectional Study Investigating Seroprevalence, Risk Factors and Livestock Owner's Knowledge, Attitudes and Practices (KAPs). *BMC Public Health*, **11**, Article No. 341. <https://doi.org/10.1186/1471-2458-11-341>
- [100] Mufinda, F., Boinas, F. and Nunes, C. (2015) A Survey on Knowledge and Practices of Brucellosis among Occupationally Exposed Livestock Workers in Namibe Province, Angola. *African Journal of Science and Research*, **4**, 29-38.
- [101] Tebug, S.F., Kanga-Waladjo, A.R., Ngoni Ema, P.J., Muyenzeza, C., Kane, O., Seck, A., Ly, M.T. and Lo, M. (2015) Cattle Farmer Awareness and Behavior Regarding Prevention of Zoonotic Disease Transmission in Senegal. *Journal of Agromedicine*, **20**, 217-224. <https://doi.org/10.1080/1059924X.2015.1010068>
- [102] Hassan-Kadle, A.A. (2015) A Review on Ruminant and Human Brucellosis in Somalia. *Open Journal of Veterinary Medicine*, **5**, 133-137. <https://doi.org/10.4236/ojvm.2015.56018>
- [103] Dorneles, E.M.S., Sriranganathan, N. and Lage, A.P. (2015) Recent Advances In *Brucella abortus* Vaccines. *Veterinary Research*, **46**, Article No. 76. <https://doi.org/10.1186/s13567-015-0199-7>