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RESEARCH ARTICLE

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Diagnostic challenges of brucellosis in humans and livestock in Tanzania: A thematic review

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Abstract

Brucellosis is an endemic bacterial zoonosis in Tanzania, and is among the most prioritized zoonotic diseases in the country. Brucellosis affects public health and livestock production in developing countries. Most human and livestock cases are not detected by the existing surveillance systems resulting in significant underestimation of the disease burden, and poor management of human cases by using nonspecific antibiotics may potentially contribute to antimicrobial resistance. To quantify the factors related to underreporting and those associated with the challenges in the diagnosis of brucellosis in Tanzania, search terms including "Brucella" "diagnosis" and "challenges" were used to query in Google search engine and publisher databases such as MEDLINE, PUBMED, NCBI, Springer, Hindawi, and Elsevier. The search parameters were limited to publications between 1995 and 2020. The searches returned 319 publications and grey articles which were screened and 57 were eligible for inclusion in this study. Four main areas were identified that cause underreporting of brucellosis and hinder brucellosis diagnosis: (1) inadequate knowledge of brucellosis among stakeholders in the livestock value chain, (2) limited diagnostic capacity for brucellosis due to unawareness of diagnostic tests and lack of epidemiological background of brucellosis among human and livestock health service workers (3) challenges associated with diagnostic tests, which include unreliable availability of diagnostic tests and unskilled workers, and (4) the uneven distribution of brucellosis surveillance studies in the country. This study suggests that there is a need for (1) training on public health education and brucellosis awareness among stakeholders in the livestock value chain; (2) providing scheduled continuing professional education with regard to brucellosis and other zoonotic diseases to health and livestock workers; (3) future brucellosis surveillance studies must focus on unrepresented regions; and (4) lastly, we recommend that the rose Bengal plate test (RBPT) and competitive enzyme-linked immunosorbent assay (cELISA) should be considered in brucellosis diagnostic schemes as a complementary tool to hasten the implementation of an ongoing national strategy for the prevention and control of brucellosis in humans and livestock in Tanzania. We suggest that these recommendations be considered for inclusion in the national strategy for brucellosis control in Tanzania.

One Health Impact Statement

Brucellosis is a disease caused by bacteria of genus *Brucella* and is transmitted from animals to humans. Humans get infected through ingestion of infected animal products but also through contact with bacteria via broken skin or inhalation of aerosolized bacteria particles during culture in the laboratory. Humans working with animals or animal products and laboratory personnel are at higher risk of infection. Tanzania has recently established a One-Health desk in the Prime Minister's Office to address all matters related to One Health. However, the desk is in the infancy stage, more studies must be done to fill knowledge gaps so that working instruments became realistic. This review provides a synthesis of information that could be used by the One Health desk and other One Health stakeholders in the country on how to improve the existing brucellosis surveillance structures for improvement of One-Health service delivery in the country.

Keywords: brucellosis, zoonosis, diagnosis, serology, culture, knowledge, competence, relevance

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Introduction

Since its discovery by Dr. David Bruce and his team in the spleen of a British soldier in Malta in the nineteenth century (Bruce, 1887; Pappas and Papadimitriou, 2007), brucellosis has been recognized as endemic globally, affecting human and animal health (Corbel et al., 2006; Franc et al., 2018; Moreno, 2014). Because of its significant impact in low-and middle-income countries (LMICs), the World Health Organization (WHO) classified the disease as the leading neglected non-malarial febrile zoonotic illness (WHO, 2020). The control and elimination of *Brucella* require collaborative, cross-sectoral efforts of human and animal health systems and a multidisciplinary approach (Pappas et al., 2006; WHO, 2001). In high-income countries (HICs) *Brucella* has been eradicated in livestock through screening and vaccination campaigns but it continues to pose a significant economic threat in lower-and middle-income countries owing to inadequate resources to control the disease (Tadesse, 2016; Marcotty et al., 2009).

Currently, four *Brucella* species (*B. abortus, B. melitensis, B. canis, and B. suis*) are considered potentially infectious in humans and have important public health implications (Hadush and Pal, 2013; Xavier et al., 2009). In humans, *Brucella* spp. frequently isolated in cases of human brucellosis are *B. melitensis*, *B. abortus,* and *B. suis* (Pappas et al., 2006; Pappas, 2010), although less frequently *B. canis* has also been isolated (Marzetti et al., 2013). Among the frequent isolates, *B. melitensis* is the most pathogenic in humans followed by *B. abortus* (Corbel et al., 2006). The infective dose of *Brucella* spp. is estimated to be as low as 10–100 bacterial cells (Pappas and Papadimitriou, 2007; Glynn and Lynn, 2008).

Brucella can spread through direct and indirect contact in humans and ruminants. Transmission occurs through direct contact with bacteria from the placenta, fetus, vaginal discharge, or fetal fluid from infected animals entering through broken skin or mucus membranes (Tadesse, 2016; Godfroid et al., 2005; Ferrero et al., 2014; Poester et al., 2013). Indirect contact occurs through the ingestion of contaminated feeds/pastures, water, and milk (Yaeger and Holler, 2007). Some vectors have been shown to be responsible for transmission (Neglia et al., 2013; Dawson et al., 2008); however, they are not considered to play a significant role in the transmission and epidemiology of the disease (Moreno and Moriyón, 2006). In ruminants, *Brucella* can also be vertically transmitted to unborn offspring (Moreno, 2014; Rossetti et al., 2017).

Approximately 500,000 new human cases of brucellosis are reported globally each year (Pappas et al., 2006). However, the true incidence is estimated to be between 5,000,000 and 12,500,000 cases per annum (Hull and Schumaker, 2018). This discrepancy comes from the fact that there are a lot of cases missed by the existing surveillance systems, resulting in gross underestimation of the local, regional, and ultimately global disease burdens (Dean et al., 2012). In Chad, the incidence of 35 cases per 100,000 person-years is estimated from a seroprevalence of 3.8% (Rubach et al., 2013). Based on a retrospective cohort study in Turkey and joint report of WHO, the World Organization for Animal Health (WOAH) [formerly the International Office for Animal Health (OIE)], and Food and Agricultural Organization of the United Nation (FAO) on the brucellosis in humans and animals, the case fatality rate for brucellosis has been estimated to be less than 1% (Corbel et al., 2006; Buzgan et al., 2010). However, the socioeconomic impact of the disease on people is much higher due to healthcare costs, loss of productive years, physical pain, and emotional suffering, which together reduce the quality of life of the individual (Franc et al., 2018). Currently, there are no data showing the extent of quality-adjusted life years (QALY) caused by brucellosis, however, an estimate of the disability-adjusted life years (DALY) caused by brucellosis in Tanzania was 92,080–121,550 (Kunda et al., 2007). Generally, brucellosis contributes to food insecurity as a result of livestock production losses and the loss of international trade (Franc et al., 2018; Fensterbank, 1986).

In Tanzania, the first reported outbreak of brucellosis occurred in imported dairy cattle in Arusha in 1927 (Mahlau, 1967) and was first confirmed in 1928 (Kitalyi, 1984). However, later studies have shown that the disease affects all the production systems with individual animal seroprevalence ranging from 1% to 30% (Assenga et al., 2015). In humans, the disease has been reported in different regions and zones of Tanzania with seroprevalence estimates ranging from 0.7% to 20.5% (Assenga et al., 2015; Shirima, 2005; Swai and Schoonman, 2012). A study carried out in the Ngorongoro district showed that the risk of contracting infection among members of a household with a seropositive herd was 3.3 times higher than the households with seronegative herds (Shirima et al., 2010). This suggests that the disease is of great economic and public health importance in smallholder communities.

A proper diagnosis of brucellosis in both humans and animals is a key requirement for the control and elimination of the disease (Khan and Zahoor, 2018; Minda and Gezahegne, 2016). There are direct and indirect approaches to the diagnosis of brucellosis in both animals and humans. Direct methods include the isolation and identification of *Brucella* or its nucleic acid from the tissues or organs of an infected individual (Hull and Schumaker, 2018; URT, 2020). Indirect methods involve the detection of antibodies (immunoglobulins) produced by the host immune response against the bacterial immunodominant smooth lipopolysaccharide (S-LPS) during infection (Corbel et al., 2006).

Regardless of the wide spectrum of diagnostic tests available, underdiagnosis and underreporting of the disease in both humans and animals is a major problem not only in Tanzania but also in other resource-poor developing countries in sub-Saharan Africa (SSA). Therefore, the objective of the study was to review the published research articles and grey literatures that identified challenges associated with the diagnosis of brucellosis in humans and livestock in the Tanzania mainland.

Methods

SEARCH ENGINES AND DATABASES

A literature search was conducted to identify relevant journal articles published from 1995 to July 2020 in different databases using Google and Google Scholar search engines, different journals, and publisher databases visited were MEDLINE, PUBMED, NCBI, Springer, Hindawi, Elsevier, and others such as the university institutional repository, that is, Sokoine University of Agriculture website. Grey literatures from government departments and ministries were visited, such as the Ministry of Health and Social Welfare, Ministry of Livestock and Fisheries, Prime Minister's Office-Disaster Management Department, and relevant information were extracted.

SEARCHING STRATEGY

Different strings of terms were used in the search strategy, these included, ("brucellosis" OR "Brucella") AND ("diagnosis" OR "prevalence" OR "tests") AND ("challenges" OR "socioeconomic impact" OR "health seeking behavior") AND ("Tanzania" OR "sub-Saharan Africa" OR "developing countries" OR "developing world") AND ("human" OR "livestock"). For laboratory accreditation status in Tanzania, the independent search string terms used were ("accredited laboratories" OR "accredited veterinary laboratories") AND "Tanzania". Some of the articles were retrieved from the identified articles using a snowball-type approach.

SELECTION CRITERIA

After the identification of 319 articles, the next step was initial screening where 30 duplicate articles were removed from the lists to remain with 289 articles. During the second screening,

the language check was conducted on 289 articles by scanning the titles and abstracts, 2 articles were removed due to language as were written in Portuguese. The remaining 287 articles were screened for eligibility which included reading the titles and abstracts of each publication for scope, country, and relevance of information. During this eligibility stage, 230 articles that were not focused on Tanzania or were out of scope as they presented with socioeconomic data not related to brucellosis or those missing relevant information as they focused on diagnostic challenges of other diseases and laboratory accreditations were rejected to remain with only 57 articles. Therefore, fifty-seven (57) articles and grey literature passed the eligibility criteria, the full article was read, data extracted, and included in this review, as outlined in Figure 1.

DATA EXTRACTION

Information was extracted from relevant publications regarding the knowledge of brucellosis among stakeholders in the livestock value chain. In this context, stakeholders in the livestock value chain include livestock keepers, middlemen (businessman buying animals from livestock keepers), abattoir workers, butchers, and the general public.

The information collected from reviewed publications and summarized were as follows: first author, study region, target study group, method used to collect information, and key findings for each study.

Information was collected on the different diagnostic tests used in all reports and was summarized.

From studies reporting culturing, data were summarized with respect to the culturing method, species studied, study region, purpose of culturing, culturing results, and results comparable to those of serological tests.

Data extraction for polymerase chain reactions (PCR) methods included the laboratory facility where the technique was deployed, the purposes of the study, the method, molecular markers used in the identification of *Brucella* species in either humans or animals, and serological test results for comparison.

For serological studies of livestock, information regarding the study period, animal species studied, *Brucella* species tested, study purpose, serological tests used, test results, and relevance of the test used to the study purpose according to WOAH are summarized in the table format. The WOAH guidelines specify the tests to be used for different purposes, such as surveillance, confirmation of clinical cases, and testing of individuals or populations free from infection (International Organization for Animal Health (OIE), 2018).

The serological tests used in animals were classified according to the WOAH guidelines, as either screening (S) or confirmatory (C). They were further classified as recommended (+++) or suitable (++) or (+) which means that can be used in some situations considering the cost, reliability, and other factors that limit their application. The rose Bengal plate test (RBPT) as a screening test and competitive enzyme-linked immunosorbent assay (cELISA) as a confirmatory test were considered recommended (+++) and suitable (++) tests, respectively. The serum agglutination test (SAT) was ranked (+). Other tests such as the microscopic agglutination test (MAT) and lateral flow assay (LFA), were not included in the list and therefore were not classified (n/c) according to the WOAH.

For serological studies in humans, information was extracted regarding the study period, study purpose, case definition,

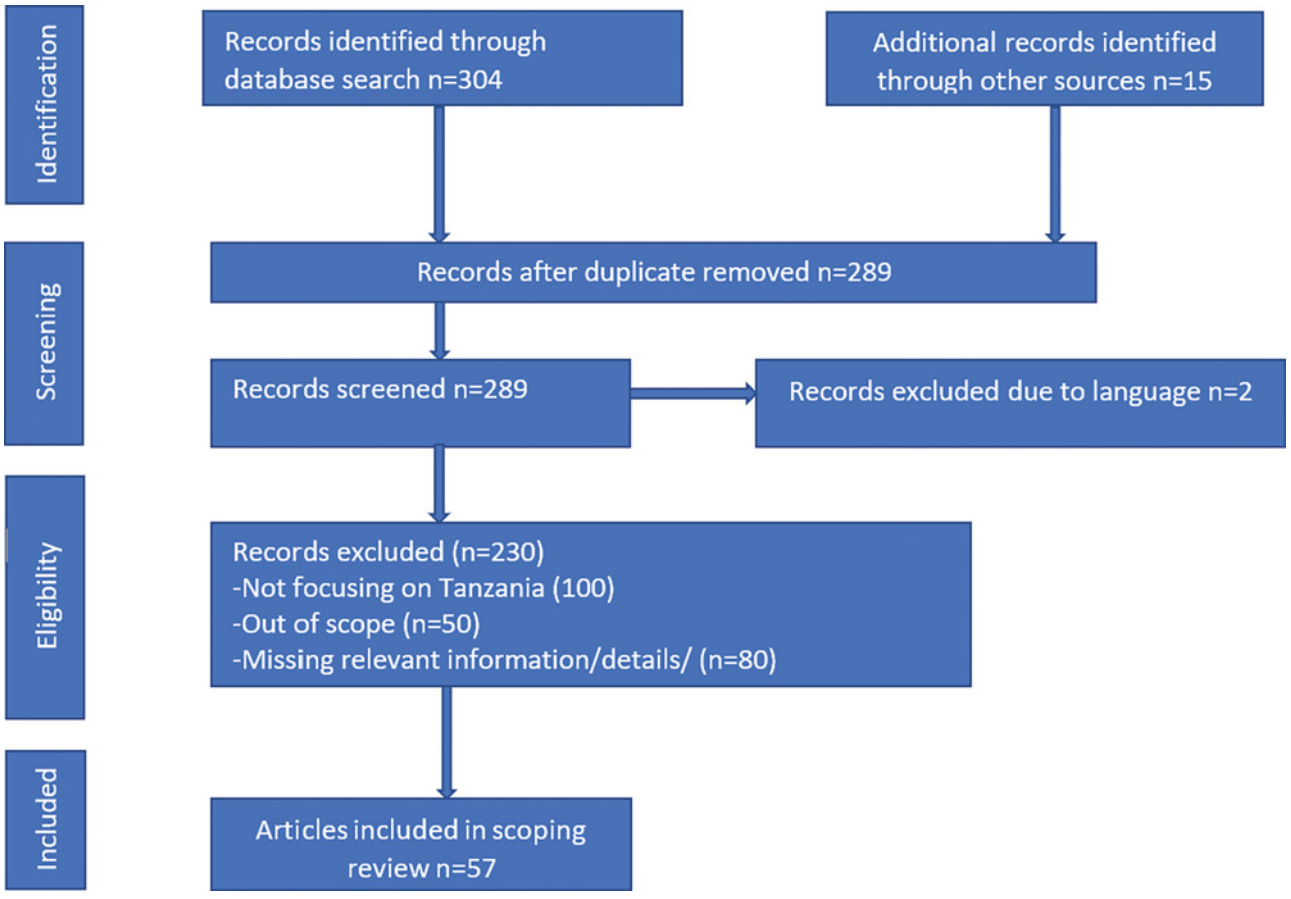

Figure 1. The schematic flow diagram of publications identified, screened, eligible and included in the review.

serological test used, results, and relevance of the test used to the study purpose according to WHO (2001). Serological tests were grouped into screening and confirmatory tests; RBPT is the recommended screening test, and ELISA, standard agglutination tests (SAT), and complement fixation test (CFT) are considered confirmatory tests.

The map for the regional distribution of brucellosis research studies was created with the assistance of: https://www.datanovia.com/en/blog/how-to-create-a-map-using-ggplot2/ and https://r-spatial.org/r/2018/10/25/ggplot2-sf-2.html.

Result

THE REGIONAL DISTRIBUTION OF BRUCELLOSIS RESEARCH STUDIES ON THE TANZANIAN MAINLAND

The regional distribution of reports of brucellosis on the Tanzanian mainland based on the reviewed research articles is indicated in Figure 2 and Supplementary Material 1. Nineteen (19) out of twenty-five (25) regions (76%) on the Tanzanian mainland have reported animals exposed to *Brucella* spp. and one region (Njombe) reported one active case of abortion (Mathew et al., 2015). A total of 21 articles reported brucellosis in humans, with 15 articles reporting brucellosis in humans only and six articles reporting brucellosis in humans and animals. In addition, 25 articles reported brucellosis in animals alone, Supplementary Material 2. The number of publications from each region is shown in Figure 2, and the seroprevalence in humans and animals for each region are shown in Supplementary Material 1. The Arusha and Morogoro regions have seven publications each, which is the highest number of publications, followed by Mbeya, Tanga, and Manyara regions, which have four publications each. Other regions had between 1 and 3 publications. There are six regions of Tanzania where brucellosis has not been reported in humans or animals, Ruvuma and Mtwara in the southern zone, Tabora in the western zone, Shinyanga and Simiyu in the lake zone, and Singida in the central zone.

KNOWLEDGE OF BRUCELLOSIS AMONG STAKEHOLDERS IN THE LIVESTOCK VALUE CHAIN

Nine publications related to knowledge of brucellosis among stakeholders in the livestock value chain were reviewed. In this context, stakeholders in the livestock value chain include livestock keepers, middle person (business person buying animals from livestock keepers and selling meat to the butchers), abattoir workers, butchers, and general public. Nine out of twenty-one regions of Tanzania are represented in the studies assessing knowledge of brucellosis among stakeholders in the livestock value chain (Table 1). Different methods were used to gather information, including key informant interviews and face-to-face interviews using closed-ended questionnaires, and focus group discussions to gain insight into the knowledge gaps and zoonotic disease prioritization.

This review identified that stakeholders in the livestock value chain have varying levels of knowledge of brucellosis. For example, 74.1% of farmers' focus groups had no knowledge of the cause, clinical signs, and transmission of the disease in humans and animals. In Kigoma region, 90% of livestock keepers lack knowledge of brucellosis (Chitupila et al., 2015). Lack of knowledge of brucellosis cause patients with brucellosis to delay attending health facilities for more than 30 days (Kunda et al., 2007). Abattoir workers and butchers were also found to have limited knowledge of the disease (Swai and Schoonman, 2009) (Table 1).

THE RISK OF INFECTION AND SEROPREVALENCE OF BRUCELLOSIS AMONG STAKEHOLDERS IN LIVESTOCK VALUE CHAIN

This review has also found that stakeholders in the livestock value chain have varying risks of brucellosis and seroprevalence levels. Abattoir workers were found to have a 5–7 times higher risk of infection compared to other occupational groups in the livestock value chain (Swai and Schoonman, 2009). Butchers were also found to have higher seroprevalence (5.6%) than other occupational groups in the livestock value chain (Sagamiko et al., 2019) (Table 1).

LIMITED DIAGNOSTIC CAPACITY FOR BRUCELLOSIS

According to this review, two major limitations to brucellosis diagnostics in Tanzania were identified (Zhang et al., 2016; Bouley et al., 2012; Klemick et al., 2009; URT, 2017). First, there is a lack of technical knowledge and skills among laboratory personnel in Tanzania to perform the tests and a wider lack of resources available at the health or veterinary facilities to purchase and maintain testing kits and equipment.

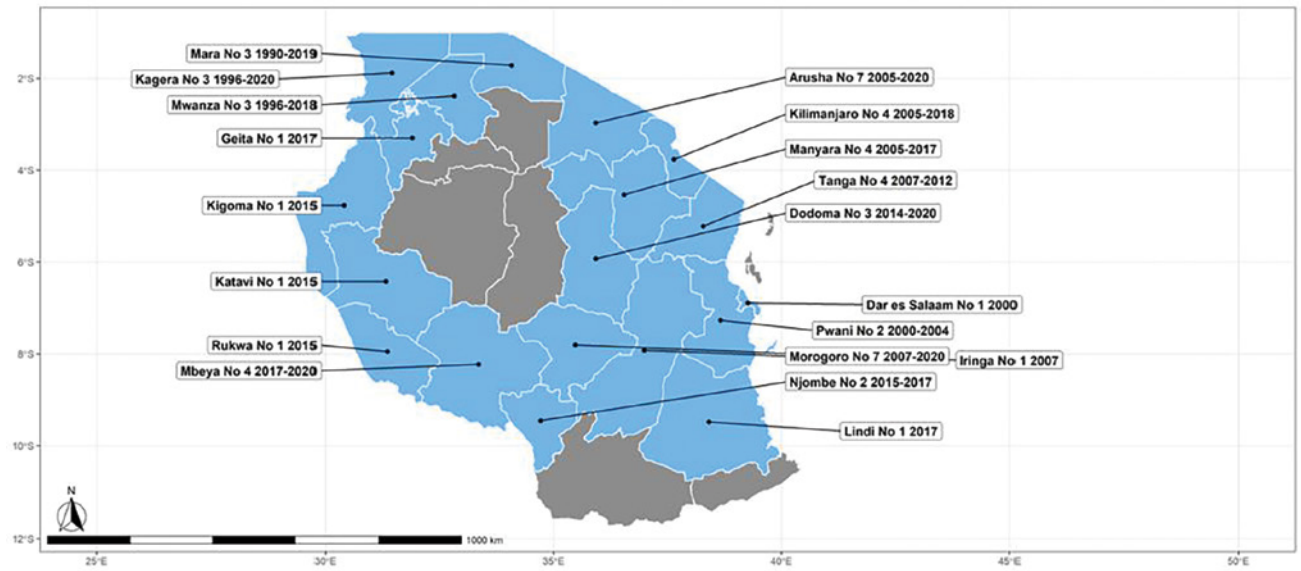

Figure 2. Map of Tanzania showing regions, blue regions reported to have *Brucella* infection in human and animals and gray regions had no report of *Brucella* infection in human and animals. The box in each region gives the region name, the number of articles reporting brucellosis and the year of publication.

Table 1. Summary of the studies reporting knowledge of brucellosis among key stakeholders in the livestock value chain in Tanzania.

Reference	YOS	Region	Target group	Method	Key findings
(Ntirandekura et al., 2018)	2017	Kagera	Farmers, admin leaders, religious leaders, and youth	FGDs KIIs	71.4% (5/7 groups) had low knowledge: causes, clinical signs, and transmission
(Chitupila et al., 2015)	2013–2014	Kigoma	Livestock keepers	Closed-ended questionnaire	90% of respondents were not aware of brucellosis.
(Kiputa et al., 2008)	2007	Kagera	Pastoral communities and livestock tradesmen	Closed-ended questionnaire	Low awareness regarding clinical signs (74%), transmission (42.3%), control (20.3), and zoonotic nature (21%).
(Swai et al., 2010)	2001–2002	Tanga and Arusha	Livestock keepers and livestock health officers	Closed and open-ended questionnaire	70% of livestock keepers had no knowledge of disease symptoms. Only 17% of respondents mentioned brucellosis as a zoonotic disease.
(Kunda et al., 2007)	2002–2003	Manyara and Arusha	Agropastoral communities	Questionnaire	Delays going to the hospital for more than 30 days due to lack of knowledge.
(Swai and Schoonman, 2009)	2004	Tanga	Abattoir workers, livestock keepers, animal health personnel, crop growers, and "others"	Closed-ended questionnaire	Abattoir workers (13.7% of occupational groups) not aware of brucellosis and are 5–7 times at risk of infection.
(Sagamiko et al., 2019)	2015–2016	Mbeya and Songwe	Livestock officers, herdsmen, butchers, milkers, and abattoir workers	Closed-ended Questionnaire	Butchers: lowest knowledge and highly prevalent (5.6%) when compared to other occupational groups
(Mngumi et al., 2016)	2008	Mwanza	Agropastoral communities	Closed-ended Questionnaire	15–24% of respondents lack knowledge of the disease transmission to humans through the placenta: by touching the placentas is 1–3 times higher risk compared to those who do not touch.
(Mengele et al., 2018)	2013	Dodoma	Agropastoral communities	Closed-ended Questionnaire	78% had low knowledge brucellosis and milk-borne zoonosis.

Key: YOS = year of study; FGDs = focus group discussions; and KIIs = key informant interviews.

LIMITED KNOWLEDGE OF DISEASE AMONG HUMAN AND LIVESTOCK HEALTH CARE WORKERS

Only three publications addressed different aspects of brucellosis awareness among human and livestock health workers (Table 2). The methods used to collect information from the target groups were face-to-face interviews and observations using a questionnaire with a list of questions and a predetermined set of responses for each question (closed-ended questionnaire) and an observational checklist. Generally, these studies showed that both human and livestock health care workers had limited though varying levels of knowledge of the disease. In Kilimanjaro region, a study carried out in hospitals and livestock centers found that 73% of the interviewed health workers in hospitals had no knowledge of brucellosis and 69% did not know the brucellosis diagnostic tests for humans, and for the interviewed livestock workers, 98% did not know the brucellosis diagnostic tests for animals (Zhang et al., 2016). Twenty-five percent of health workers believed every fever was malaria and sixty-nine percent (69%) of the brucellosis patients were not provisionally diagnosed with brucellosis at the health facility (Bouley et al., 2012). The availability of skilled health workers in health facilities was another challenge when clinicians failed to perform 48% of the tasks found in official guidelines for the proper diagnosis and treatment of brucellosis (Klemick et al., 2009).

LACK OF AVAILABILITY OF DIAGNOSTIC TESTS AND LIMITED COMPETENCY OF HEALTH WORKERS

Data on the availability of diagnostic supplies and consumables and the lack of skilled staff to use them in Tanzania are summarized in Table 3. Different methods were used in these studies to elucidate the skills of service providers and the availability of diagnostic tests, including item response theory (checklist), interviews (questionnaire), and surveys in health facilities. In item response theory, the health worker was provided with a case, and a checklist was used to score their performance during brucellosis diagnosis. Interviews were also used to probe their awareness of diagnostic tests using a questionnaire with a set of questions and predetermined responses (closed-ended questionnaire) and the surveys were carried out in health facilities to reveal the challenges of providing brucellosis diagnostic services.

According to the national health policy document, there was unreliable availability of diagnostic supplies and consumables and a paucity of diagnostic instruments in the health facilities (URT, 2017). The unreliable availability of diagnostic tests was further confirmed by 81.4% of health workers who reported a lack of diagnostic tests for zoonosis in their health facilities (Zhang et al., 2016). In livestock, 100% of livestock workers reported a lack of diagnostic tests for zoonosis at their facilities (Zhang et al., 2016). Limited competency of health workers has also been found to significantly impair diagnostic capacity. Data showed that technicians demonstrated poor diagnostic skills, with only 48% correctly implementing procedures for diagnosis and only 10% correctly diagnosing the cases (Klemick et al., 2009). Furthermore, clinicians failed to diagnose 93.8% of patients who were later diagnosed with brucellosis (Bouley et al., 2012) (Table 3).

CHALLENGES ASSOCIATED WITH DIFFERENT TYPES OF DIAGNOSTIC TESTS

Different types of brucellosis diagnostic tests are available in the global market to provide wider room for selection and use during human and livestock health service provision which is important for definitive diagnosis and correct treatment. This section summarizes

Table 2. Summary of the studies reporting the human and livestock health services workers' knowledge of brucellosis in Tanzania.

Publication	YOS	Region	Target group	Method	Key findings
(Zhang et al., 2016)	2016	Kilimanjaro	Human and livestock health care workers	Closed-ended Questionnaire	Health: No knowledge of disease (73%), 25% Think any fever is malaria. Health: 69% unaware of diagnostic tests of brucellosis. Livestock: 98% unaware of diagnostic test for brucellosis.
(Bouley et al., 2012)	2012	Kilimanjaro	OP and Health workers	Closed-ended Questionnaire	Health workers: Failed to clinically diagnose brucellosis, 69% brucellosis patients were not provisionally diagnosed.
(Klemick et al., 2009)	2001–2002	Rural Arusha	Health workers	Checklist	Unskilled clinicians: did not perform 48% of tasks in the checklist for proper diagnosis and treatment of the disease.

Key: OP = outpatients; and YOS = year of study.

Table 3. Summary of the availability of diagnostic resources and skills shortage among human and livestock health service workers in Tanzania.

Publication	YOS	Region	Target group	Method	Key findings
(Klemick et al., 2009)	2002/2003	Arusha	Health workers	Checklist	Limited competency of clinicians and personnel: exhibit poor diagnostic skills: implemented correctly only 41–48% of items in checklist, 10% correctly diagnosed the case study.
(United Republic of Tanzania (URT), 2017)	2017	Tanzania	Health facilities	Survey	Unreliable availability of diagnostic supplies and consumables, weak public health laboratory services, Weak biosafety, and biosecurity containment, paucity of diagnostic instruments.
(Zhang et al., 2016)	2012/2014	Kiliman-jaro	Human and livestock health care workers	Closed and open-ended questionnaire	Knowledge of diagnostic test's names for brucellosis: 62% of respondents in health do not know, 90% in livestock do not know. Lack of diagnostic resources for testing zoonosis: 81.4% of respondents (38/43) in health reported absence at their work facility and 100% in livestock.
(Bouley et al., 2012)	2007/2008	Kiliman-jaro	OP and Health workers	Interview	Health workers: Failed to clinically diagnose brucellosis. 93.8% of patients later diagnosed with brucellosis were diagnosed with malaria only.

Key: YOS = year of study; OP = outpatient; and MoHSW = ministry of health and social welfare (national health policy).

the reviewed articles reporting the different types of tests used, how often the test was used and the challenges indicating why other WOAH/WHO recommended tests were not used.

BACTERIAL ISOLATION AND IDENTIFICATION

Four articles reported using culturing for isolation and identification of *Brucella* organisms Table 4.

Results showed that three out of four studies (3/4) reported culturing using BacT/ALERT technology in laboratory facilities in Tanzania. BacT/ALERT is a closed automated system for bacterial identification. The conventional bacterial culture which involves growing bacteria on a selective media on a petri dish was reported by one article and was done outside Tanzania (Mathew et al., 2015).

In an animal study that reported culturing from milk and aborted materials, *Brucella* was only detected in organs from the aborted fetus (Mathew et al., 2015). In this study, there were animals that were positive by serological tests, but *Brucella* was not detected by culture (Table 4).

In the three human studies, there were differences in the number of clinical cases detected by culture versus serological testing (Table 4). Only in one study, 3.5% of samples were culture positive (Bodenham et al., 2020) whereas the other two studies detected none (0%) (Bouley et al., 2012; Cash-Goldwasser et al., 2018). This is in contrast to the number of confirmed cases detected by serology (Table 4). However, none of the studies reported culturing as a method for the routine diagnosis of brucellosis and was only used for research purposes.

MOLECULAR TECHNIQUES (POLYMERASE CHAIN REACTION METHOD)

Several molecular techniques using PCR have been developed for the identification of *Brucella* spp and are being used for routine diagnosis in high-income countries (HICs). In contrast, low- and middle-income countries (LMICs) such as Tanzania, report using molecular techniques for research purposes rather than routine diagnosis.

Seven articles were reviewed, and the results are summarized in Table 5. This indicates that PCR has only been used for research

Table 4. Summary of the publications reporting results of culturing, clinical, and serological tests used in clinical cases in Tanzania.

Publication	Species	Region	YOS	Number of participants	Clinical diagnosis of Brucellosis	Culturing method and results	Serological results: Probable—single sample Confirmed—paired samples
(Mathew et al., 2015)	Animals	Mbeya	2012–2013	N = 20 culture N = 200 cattle N = 50goats N = 35 sheep N = 6 dog	1/1-abortion case	1/20 (5%)—Conventional	96/200 (48%) confirmed 1/50 (2%) confirmed 2/35 (5.7%) confirmed 0/6 (0%) probable
(Bodenham et al., 2020)	Human	Arusha	2016–2017	N = 228 (culture) N = 230 (serology)	16/230 (7%)	8/228 (3.5%)—Bact/ALERT	11/230 (4.8%) probable 1/230 (0.4%) confirmed
(Bouley et al., 2012)	Human	Moshi	2007–2008	N = 870 culture N = 830 serology	0/16	0/870 (0%)—Bact/ALERT	4/830 probable 16/455 confirmed
(Cash-Goldwasser et al., 2018)	Human	Moshi	2012–2014	N = 1382 culture N = 1293 serology	50/562	0/1382 (0%)—Bact/ALERT	39/562 confirmed 11/562 probable

N = number of study participants/samples; and YOS = year of study.

Table 5. Summary of the facility where the diagnostic technique was used, the purpose of the original study and the molecular markers used to identify *Brucella* spp. using PCR in Tanzania.

Publication	YOS	Species	Study Region	Laboratory	Purpose	Method	Marker	Serological tests results	Key findings by PCR
(Ntirandekura et al., 2020)	2017–2018	Human, Cattle Goats	Kagera	SUA-Morogoro	Research	PCR	Vdcc	77/125 SAT positive	*Brucella* spp. identified in 47/125 (77 were serological positive)
(Mathew et al., 2015)	2012–2013	Cattle	Njombe	Outside Tanzania	Research	PCR	IS711	96/200 iELISA	*B. abortus* identified in 1/1 aborted fetus and materials
(Assenga et al., 2015)	2012–2013	Human, Cattle Goats, Buffalo Lion, Zebra	Katavi and Rukwa	SUA-Morogoro	Research	PCR	IS711	79/340 cELISA	*B. abortus* identified in 8/231 milk samples
(Kassuku, 2017)	2016–2017	Goats	Morogoro	TVLA-Dar Es Salaam	Research	PCR	not mentioned	1/478 iELISA	*B. abortus* identified in 18/ 27 random serum samples.
(Kayombo et al., 2017)	2013–2014	Cattle	Manyara	SUA-Morogoro	Research	PCR	IS711	8/192 cELISA	*B. abortus* identified in 3/8 milk samples of cELISA+
(Mhozya, 2017)	2017	Cattle	Geita	SUA-Morogoro	Research	PCR	bcsp31	3/219 cELISA	*B. abortus* identified in 1/3 milk samples from cELISA positive cows.
(Sambu et al., 2019)	2018	Bufallos, Lions Wilde-beests Impala Zebra	Serengeti Ecosystem	SUA-Morogoro	Research	PCR	IS711	No results	*B. abortus.* *B. melitensis* identified 12/189 AMOS, 22/189 by qPCR

AMOS = AMOS LADDER = multiplex conventional PCR; and YOS = year of study.

purposes by research institutions such as the Sokoine University of Agriculture (SUA) and Tanzania Veterinary Laboratory Agency (TVLA). The most commonly used molecular marker was the insertion sequence IS711 in *Brucella* genome, which was used in four out of seven studies. In addition, PCR has only been performed in universities and research institutes, and no study has reported its use to diagnose brucellosis by health or veterinary facilities in the country as a routine diagnostic test. Mathew et al. used PCR to confirm active cattle abortion (Mathew et al., 2015); the rest of the studies used PCR for research purposes in apparently healthy animals (Assenga et al., 2015; Ntirandekura et al., 2020; Kassuku, 2017; Kayombo et al., 2017; Mhozya, 2017; Sambu et al., 2019).

Furthermore, Table 5 shows that 4/7 publications reported the use of PCR used samples that previously tested positive by serological tests and the results show that there were discrepancies in the number of positives between the PCR and serological test results. In addition, results show that two different PCR-based methods (AMOS LADDER and qPCR) yielded different results for the same samples. Among the *Brucella* species circulating in livestock, 6/7 studies identified *B. abortus*, only 1/7 study identified *B. melitensis* from wild animals, and surprisingly one study identified *B. abortus* from goat sera.

SEROLOGICAL TESTS

Forty-six articles were reviewed regarding the use of serological tests in Tanzania mainland, and the data were extracted and are summarized in Figure 3 and Supplementary Material 2. Ten different serological tests have been used to screen for and confirm brucellosis in Tanzania in both humans and animals. The results showed that RBPT was the most frequently used brucellosis diagnostic test in Tanzania (26/46 publications). The results also showed that cELISA is the most frequently used confirmatory test for brucellosis in Tanzania (19/46 publications). These two tests have been extensively used in Tanzania because of their available expertise, reliability, and user-friendliness. Other tests reported from Tanzania were rivanol, precipitation, fluorescent polarization assay, milk ring, and agglutination/precipitation tests but they do not appear to be widely used.

THE RELEVANT USES OF SEROLOGICAL TESTS IN LIVESTOCK STUDIES

This review identified 28 publications (Table 6) reporting seroprevalence studies (passive surveillance) aimed at establishing the status (prevalence) of brucellosis in different livestock populations. Only one study was conducted for outbreak investigation following an active abortion when a researcher was in the field for research samples collection (Mathew et al., 2015). Among the livestock species tested for brucellosis, cattle featured more frequently than other domestic species, and only one out of 28 studies reported brucellosis in goats (Table 6).

The results of this review show that, in livestock, the most common screening test used was the rose Bengal plate test (RBPT) and the cELISA as a confirmatory test, which are considered recommended (+++) and suitable (++) tests respectively by the WOAH (Table 6). Other tests such as the serum agglutination test (SAT), indirect enzyme-linked immunosorbent assay (iELISA), microscopic agglutination test (MAT), lateral flow assay (LFA), milk ring test (MRT), and buffered acidified plate test (BAPA) have been used to report brucellosis but relatively less frequently compared to RBPT and cELISA (Figure 3).

For the studies that used more than one test, one as screening and the other as confirmatory, the results show that there was a clear difference in the number of seropositive animals between the two test results (Table 6). However, 14/28 studies performed serological tests in series, where confirmatory tests were only performed on samples that tested positive for the screening test and not those that tested negative. The WOAH recommends that two parallel serological tests, screening, and confirmatory tests,

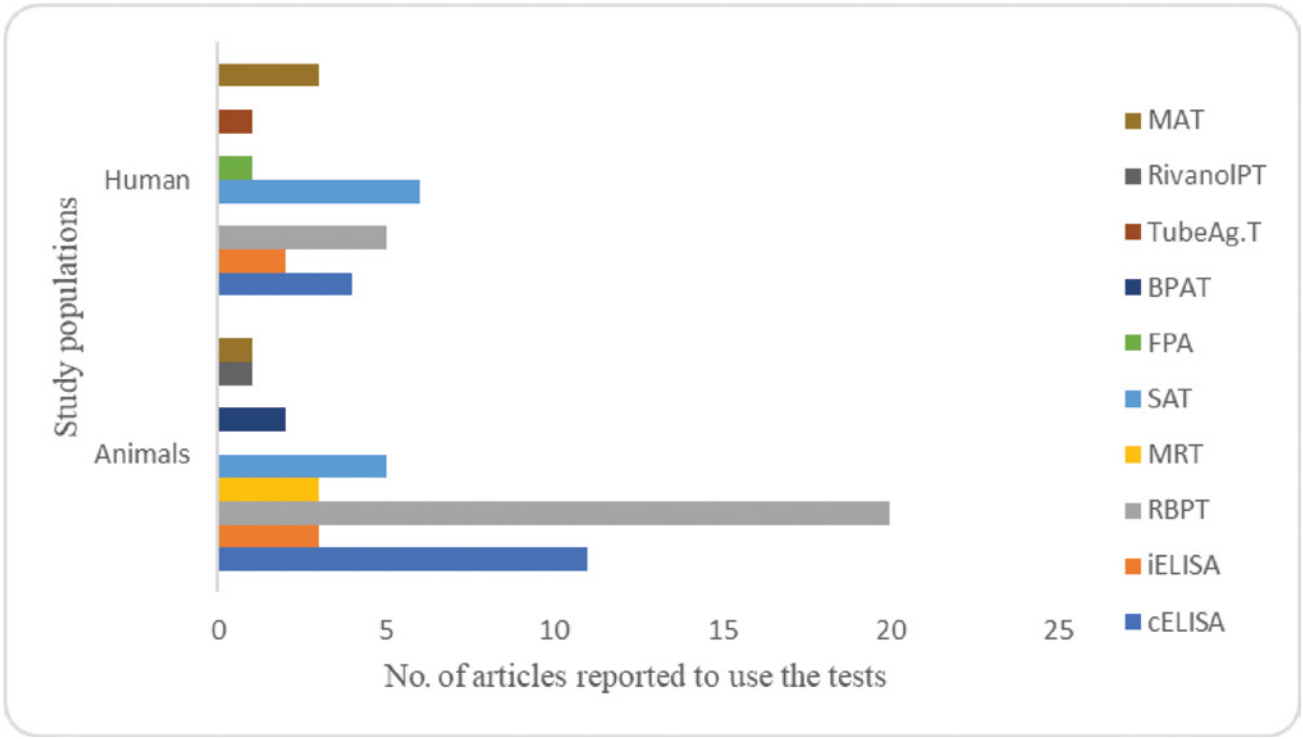

Figure 3. The frequency of use of different types of serological tests used to report brucellosis status in Tanzania both in human and animals. Key: LFA = lateral flow assay; MAT = microscopic agglutination test; RivanolPT = rivanol precipitation test; TubeAg.T = tube agglutination test; BPAT = buffered phosphate agglutination test; SAT = serum agglutination test; MRT = milk ring test; RBPT = rose Bengal plate test; iELISA = indirect enzyme linked immunosorbent assay; and cELISA = competitive enzyme linked immunosorbent assay.

Table 6. Summary of publications and their purposes, the test used, and the relevance of the test used according to the International Organization for Animal Health (OIE).

Publication	YOS	Species	Purpose	RBPT	cELISA	Other tests	Test used and Relevance of test (OIE)
(Chitupila et al., 2015)	2013/2014	Cattle	Seroprevalence	25/410	23/410		RBPT +++ cELISA ++
(Kiputa et al., 2008)	2007	Cattle	Seroprevalence	29/162		17/162	RBPT +++ SAT ++
(Swai et al., 2010)	2001/2002	Cattle	Seroprevalence	35/654			RBPT +++
(Karimuribo et al., 2007)	1999	Cattle	Seroprevalence	93/1350		84/1350	RBPT +++ SAT ++
(Shirima, 2005)	2000/2001	Ruminants	Seroprevalence	37/1596	37/1596		RBPT+++ cELISA ++
(Shirima et al., 2014)	2010	Cattle	Seroprevalence	163/487	141/487		RBPT +++ cELISA++
(Shirima et al., 2010)	2002/2003	Ruminants	Seroprevalence		155/2723		cELISA ++
(Shirima et al., 2018)	2012	Cattle	Seroprevalence Milk prevalence		83/390	138/390	cELISA ++ MRT+++
(Mengele et al., 2018)	2013	Cattle	Seroprevalence	57/545	5/545		RBPT +++ cELISA++
(Luwumba et al., 2019)	2018	Ruminants	Seroprevalence	14/190		9/190	RBPT+++ iELISA+++
(Mathew et al., 2015)	2012/2013	Cattle	Seroprevalence	43/200		96/200 43/200	RBPT+++ MRT+++ iELISA+++
(Mathew et al., 2019)	2012/2013	Animals	Seroprevalence	0/277		0/277	RBPT+++ BPAT+++
(Assenga et al., 2015)	2012/2013	Animals	Seroprevalence	83/1103	75/1103		RBPT+++ cELISA++
(Kassuku, 2017)	2016/2017	Goats	Seroprevalence	1/475	1/475		RBPT+++ iELISA+++
(Asakura et al., 2018)	2015	Cattle	Seroprevalence	1/667	5/667		RBPT+++ cELISA++
(Weinhäupl et al., 2000)	1995/1997	Cattle	Seroprevalence			48/342	SAT++
(Jiwa et al., 1996)	1974/1978	Cattle	Seroprevalence			227/3626	SAT++
(Kayombo et al., 2017)	2013/2014	Cattle	Seroprevalence	9/192	8/192	10/192	RBPT+++ BAPA+++ cELISA++
(Sijapenda et al., 2017)	2017	Cattle	Seroprevalence	18/200	16/200		RBPT+++ cELISA++
(Mathew et al., 2017)	2012/2013	Cattle	Seroprevalence			36/658	iELISA+++
(Mhozya, 2017)	2017	Cattle	Seroprevalence	3/219	3/219		RBPT+++ cELISA++
(Mdegela et al., 2004)	2001	Cattle	Seroprevalence		4/312		cELISA++
(Mtui-Malamsha, 2001)	1999/2000	Cattle	Seroprevalence	19/457		17/457	RBPT+++ SAT++
(Mellau et al., 2009)	2009	Ruminants	Seroprevalence	20/200		12/200	RBPT+++ MAT+++

Continued

Table 6. Continued.

Publication	YOS	Species	Purpose	RBPT	cELISA	Other tests	Test used and Relevance of test (OIE)
(Lyimo, 2013)	2012	Cattle	Seroprevalence		83/450	132/450	MRT^{+++} cELISA++
(Mfune, 2015)	2014/2015	Cattle	Seroprevalence	0/400	21/400	0/400	RBPT+++ LFA$^{n/c}$ cELISA++
(Sagamiko et al., 2018)	2015/2016	Cattle	Seroprevalence	—	113/1211		RBPT+++ cELISA++
(Swai et al., 2005)	2003	Cattle	Seroprevalence			51/417	SAT++

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; − = not appropriate for this purpose; n/c = no OIE comment; and YOS = year of study.

must be performed in all samples to increase sensitivity and increase the efficiency of eradication policies in infected herds or flocks.

THE RELEVANT USES OF SEROLOGICAL TESTS IN HUMAN CLINICAL STUDIES

Ten (10) studies have been carried out in Tanzania to establish the status of human brucellosis among patients who attended health facilities and among risk groups of people in the communities (Table 7). Different serological tests have been used to screen and confirm brucellosis in clinical cases. Prototypes of classical brucellosis case definitions were used to identify clinical cases, and the most common clinical sign was fever. Lack of consistency in defining a brucellosis case was a common and prominent feature among the reviewed studies.

There was a high rate of using screening tests alone to report the disease in clinical studies, which is contrary to WHO guidelines. Six out of ten studies used only screening tests to diagnose and report the disease, which means that the results should be interpreted with consideration of the intrinsic flaws of the screening tests, which include high sensitivity and low specificity (Padilla Poester et al., 2010). Four out of ten studies used two or more tests, one as a screening test and the other as a confirmatory test, which is in line with the WHO guidelines.

Generally, the results show that the tests used for the clinical diagnosis of brucellosis in humans were in accordance with WHO recommendations.

THE RELEVANT USES OF SEROLOGICAL TESTS IN HUMAN SURVEILLANCE (SEROPREVALENCE) STUDIES

Table 8 summarizes the seroprevalence of antibodies against *Brucella* spp. in humans. Different serological tests have been used to study the seroprevalence of brucellosis in different social and occupational groups. The selection criteria for the study population depended on the target groups, therefore, different selection criteria were used.

This review found that the tests which have been recommended for testing suspected clinical cases in humans have been used for surveillance (seroprevalence) studies, in addition, there are tests that have not been approved by WHO but have been used to study human brucellosis, such tests include Rivanol plate test (Riv.PT), fluorescence polarization assay (FPA), and buffered acidified plate assay (BAPA) (Table 8).

The FPA and BAPA have been recommended for use in animals by the WOAH to test individual or population freedom from brucellosis and to study herd/flock seroprevalence of brucellosis (surveillance). Although neither has been approved nor on the list of any international organization body, Riv.PT has also been used to test for brucellosis in humans and animals elsewhere (Kaltungo et al., 2014).

Only 6/11 research studies used screening and confirmatory tests, which was in line with WHO guidelines, and 5/11 research studies used only a screening test to report brucellosis (Table 8).

THE USE OF SEROLOGICAL TESTS TO DISTINGUISH *BRUCELLA* SPECIES

All data in Tables 6–8 showed different types of serological tests used to study brucellosis in humans and livestock. In livestock studies (Table 6), none of the 28 studies used a serological test (screening or confirmatory test) that was able to distinguish *Brucella* at species level. In humans, only one study (Orsel et al., 2015) out of 10 studies for clinical brucellosis (Table 7) used a serological test that claimed to differentiate *Brucella* to species level, this was contrary to human studies on exposure to brucellosis (Table 8) where two (Mirambo et al., 2018; Mngumi et al., 2016) out of 11 studies used a serological test (rapid agglutination test) which claimed to differentiate *Brucella* to *B. abortus* (Eurocell A) and *B. melitensis* (Eurocell M), the product of Euromedi equip LTD, UK. These rapid agglutination tests which claim to differentiate *B. melitensis* and *B. abortus* are called FBAT (febrile *Brucella* agglutination test) are variants of the rapid slide *Brucella* agglutination test (SAT).

Discussion

This review demonstrates that *Brucella* is widespread in cattle and humans in Tanzania. However, there are regions and zones of Tanzania where there are no published reports on brucellosis, such as the lake zone regions of Shinyanga and Simiyu, the western zone region, Tabora, the central zone region, Singida, and the southern zone regions of Ruvuma and Mtwara. The lake and western zone regions have the largest number of cattle in Tanzania (NBS, 2021), supporting pastoralist and agropastoral communities, and efforts must be made to fill the *Brucella* status gaps in these regions. The lack of published brucellosis reports in some regions potentially leads to brucellosis underestimation, which affects strategic efforts to control the disease.

In Tanzania, brucellosis among pastoral communities is commonly known in Swahili as “ugonjwa wa kutupa mimba” meaning the “disease of abortion” (Ntirandekura et al., 2018). Despite this, the review highlighted that there is generally low and varying knowledge of the disease with respect to the causative agent, modes of transmission, and control methods among stakeholders in the livestock value chain (Chitupila et al., 2015; Zhang et al., 2016; Cash-Goldwasser et al., 2018; Kiputa et al., 2008; Swai et al., 2010).

Table 7. Summary of the publications where human testing for clinical brucellosis was conducted in accordance with WHO guidelines showing study purpose, case definition, test used, prevalence established, and relevance of test.

Reference	YOS	Purpose	Case definition	Test used	Prevalence %	Relevance of test (WHO)
(Kunda et al., 2007)	2002–2003	Clinical	Fever, headache, joint pain, malaise, back-ache, loss of appetite	RBPT cELISA	No results 6.2	+++ (S) +++(C)
(Bouley et al., 2012)	2007–2008	Clinical	Adult: oral temp ≥38°C. Infants: recta temp ≥38°C	MAT	3.5	+++, (S)
(Nonga and Mwakapeje, 2016)	2013–2016	Clinical	Fever, sweating, headache, backpain, fatigue, arthralgia, abortion	PAT	5.8	+++, (S)
(Bodenham et al., 2020)	2016–2017	Clinical	≥2 years old with fever (≥38°C) at present or past 72 hours.	SAT	6.1	+++, (S)
(Cash-Goldwasser et al., 2018)	2012–2014	Clinical	Axillary temperature of >37.5°C, or a tympanic, oral, or rectal temperature of 38.0°C at presentation.	MAT	8.9	+++, (S)
(Orsel et al., 2015)	2011	Clinical	Febrile symptoms	SAT/FBAT IgG ELISA IgM ELISA	5.7 34 2.5	n/c +++, (C) +++, (C)
(Carugati et al., 2018)	2007/2008 2012/2014	Clinical	Adult/pediatric: oral/axillary/rectal temperature ≥ 38.0°C or history of fever in the past 48–72 hrs.	MAT	2.2 (2007/08) 2.9 (2012/14)	+++ (S)
(Wankyo, 2013)	2018	Clinical	Presence or absence of fever	RBPT	23.9	+++, (S)
(Kunda et al., 2010)	2002/2003	Clinical	Febrile patients	RBPT cELISA	No results 7.7	+++, (S) +++, (C)
(Chipwaza et al., 2015)	2012	Clinical	Febrile children 2–13 years	IgG ELISA IgM ELISA	15.4 7	+++, (C) +++, (C)

RBPT = rose Bengal plate test; MAT = microscopic agglutination test; PAT = plate agglutinating test; SAT = serum agglutination test; cELISA = competitive enzyme-linked immunosorbent assay; IgG = immunoglobulin g ELISA; IgM = immunoglobulin m ELISA; +++ = recommended by WHO; ++ = no clear recommendation by WHO; n/c = no WHO comment; YOS = year of study; (S) = Screening test; (C) = confirmatory test; and FBAT = febrile *Brucella* agglutination test.

Inadequate knowledge, combined with poor practices may increase the risk of brucellosis among livestock-keeping communities (Cash-Goldwasser et al., 2018; Ntirandekura et al., 2018; Kiputa et al., 2008) with some livestock value chain workers such as butchers and abattoir workers, reported to have the highest seroprevalence of the disease (Swai and Schoonman, 2009; Sagamiko et al., 2019). Lack of knowledge in a combination with other factors such as distance to health or veterinary facilities, and treatment costs, may result in failure or delay in attending health facilities for proper diagnosis and treatment (Kunda et al., 2007; Klemick et al., 2009; URT, 2006). In this regard, the true incidence of brucellosis in humans and livestock is likely underreported.

There are limited brucellosis diagnostic capacities in laboratory facilities for both humans and livestock in Tanzania (Zhang et al., 2016; Bouley et al., 2012; Klemick et al., 2009). The studies summarized in this review identified inadequate knowledge about the disease and diagnostic test options among humans and livestock health workers. Addressing the issue of limited knowledge is the key to improving service provision, establishing a more accurate picture of the scale of the problem and successful management of brucellosis in Tanzania.

The lack of availability of diagnostic tests and trained staff at health or veterinary facilities is another limitation. Four articles in this review reported that limited trained clinicians, unreliable availability of diagnostic supplies and consumables, weak biosafety and biosecurity containment, and paucity of diagnostic instruments have been challenges for brucellosis diagnosis and management (Zhang et al., 2016; Bouley et al., 2012; Klemick et al., 2009; URT, 2017). However, some public health and veterinary facilities at regional and zonal levels have at least a rapid diagnostic test for brucellosis (Zhang et al., 2016; Pers-Communication, 2019). In Tanzania, human serological testing has been reported to be performed only in districts or designated district hospitals after treatment failure for other common febrile ailments such as malaria, typhoid, and even tuberculosis (Kunda et al., 2007). This challenge is locally available health and veterinary diagnostic facilities significantly affect the detection of cases and lead to a gross underestimation of the scale of the problem.

Different types of diagnostic tests for brucellosis have been used in Tanzania, including bacterial isolation and identification, molecular techniques, and serological tests. The isolation and identification of bacteria are considered the gold standard method. However, the

Table 8. Summary of the publications where human testing for brucellosis exposure was conducted according to WHO guidelines showing study purpose, case definitions, test used, prevalence established, and relevance of test.

Publication	YOS	Study purpose	Case definition/ Selection criteria	Test Used	Prevalence %	Relevance WHO.
(Swai and Schoonman, 2009)	2004	Seroprevalence	Broad occupational groups	RBPT	5.52	+++, (S)
(Shirima et al., 2010)	2002–2003	Seroprevalence	Livestock keeping households	cELISA	8.3	+++, (C)
(Shirima and Kunda, 2016)	2005–2006	Seroprevalence	Livestock keeping households	RBPT cELISA	0 0	+++, (S) +++, (C)
(Mngumi et al., 2016)	2008	Seroprevalence	Livestock keeping household	SAT/FBAT	14.1	n/c
(Sagamiko et al., 2019)	2015–2016	Seroprevalence	Individuals in cattle value chain	RBPT cELISA	1.41 1.41	+++, (S) +++, (C)
(Luwumba et al., 2019)	2018	Seroprevalence	Abattoir workers	RBPT iELISA	1.6 1.6	+++, (S) +++, (C)
(Makala et al., 2020)	2018	Seroprevalence	Pregnant women receiving antenatal care	RBPT IgG ELISA IgM ELISA	10.9 8.6 2.6	+++, (S) +++, (C) +++, (C)
(Ntirandekura et al., 2020)	2017–2018	Seroprevalence	Pregnant women from pastoral community.	RBPT FPA	21 21	+++, (S) n/c
(Mirambo et al., 2018)	2017	Seroprevalence	Abattoir workers and meat vendors.	SAT/FBAT	48.4	n/c
(Assenga et al., 2015)	2012–2013	Seroprevalence	Humans living in wildlife and livestock interface.	RBPT BAPA Riv.PT	1.5 0.6 0.6	+++, (S) n/c n/c
(Shirima, 2005)	2000–2001	Seroprevalence	Pastoral households	RBPT cELISA	No results 8.26	+++, (S) +++, (C)

RBPT = rose Bengal plate test; MAT = microscopic agglutination test; PAT = plate agglutinating test; SAT = serum agglutination test; cELISA = competitive enzyme-linked immunosorbent assay; IgG = immunoglobulin g ELISA; IgM = immunoglobulin m ELISA; +++ = recommended by WHO; ++ = no clear recommendation by WHO but clear by OIE; n/c = no WHO comment; YOS = year of study; BAPA = buffered acidified plate antigen test; Riv.PT = rivanol precipitation test; FPA = fluorescence polarization assay; (S) = screening test; (C) = confirmatory test; and FBAT = febrile *Brucella* agglutination test.

results of this review show that its use was limited to research and the results differ from those of serological results from the same individuals suggesting its poor sensitivity. This difference was because culturing may miss brucellosis patients whose bacteremia phase has passed. Clear guidelines and case definitions for brucellosis were required when the two tests, culture, and serology, were used in parallel and contradicting. Five studies reported the isolation and identification of brucellosis in Tanzania for research purposes (Mathew et al., 2015; Bouley et al., 2012; Bodenham et al., 2020; Cash-Goldwasser et al., 2018; Carugati et al., 2018). No study reported bacterial isolation and identification as a routine clinical diagnostic approach as the process is long, complex, and requires a high containment level. Tanzania has low biosafety and biosecurity capacity, as required by the Cartagena Protocol in biosafety environments (URT, 2017, 2009). Low capacity was due to a lack of skilled personnel, financial resources, infrastructure, and awareness (URT, 2009). Isolation and identification of pathogens are critical for case management, disease control, and understanding the epidemiology of the disease. Tanzania needs to improve its laboratory network to be able to carry out bacterial isolation and identification, not only for diagnosis but also for a broad understanding of the causative agent. Some universities and research institutions do have level 3 biosafety and biosecurity containment laboratories to conduct isolation and identification of *Brucella* but are not available for routine clinical service (URT, 2009). In Tanzania, similar to many other LMICs, efforts have been made to build this capacity in state institutions.

Polymerase chain reaction (PCR) was used by seven studies to report brucellosis in this review. The molecular marker commonly used for the identification of *Brucella* at the genus and species levels was insertion sequence 711 (IS711). This marker has been frequently used globally, and hence can reliably be used to identify *Brucella* spp. in Tanzania. However, all studies reporting brucellosis in the country by PCR were carried out for research purposes at universities and research institutes rather than for routine diagnostic service provision (Mathew et al., 2015; Ntirandekura et al., 2020; Kassuku, 2017; Kayombo et al., 2017; Mhozya, 2017; Sambu et al., 2019). This test is relatively expensive and requires some investment costs and skilled personnel to carry it out, therefore, at the moment it is used routinely in human and livestock health facilities (URT, 2009, 2017). More PCR-based studies are needed to identify and characterize *Brucella* spp. circulating in the country for proper strategic control of the disease.

Serological tests have been predominantly used to study brucellosis in both humans and animals. This is due to the fact that they are relatively affordable, faster, and safe when compared to other tests such as culturing and PCR. The use of screening tests alone and screening and confirmatory tests in series to report brucellosis has been widely practiced conventionally to accommodate financial

constraints associated with the diagnosis of brucellosis, even though it is contrary to WHO and WOAH guidelines. Ten different types of serological tests have been used (Figure 3) in Tanzania, most of which are in the approved list of WHO and WOAH. However, for human health, the WHO has a narrow list of approved serological tests for use in humans compared with WOAH (WHO, 2001), the list excludes some commonly used *Brucella* serological tests such as FBAT kits, BAPA, FPA, and Riv. PT. Furthermore, WOAH has recommended different serological tests for different purposes such as to study individual or population freedom from brucellosis, for confirmation of suspected or clinical cases, for surveillance (prevalence) studies, and including testing immune status following vaccination, which is not the case in the WHO list (International Organization for Animal Health (OIE), 2018). This study also found that brucellosis studies in human health were predominated by the use of non-specific *Brucella* agglutination tests only to report the disease. Six (6) studies out of 10 reported clinical brucelloses (Table 7) and two (2) studies out of 11 reported *Brucella* exposure status (Table 8) used non-specific *Brucella* agglutination tests alone. These tests are considered as screening tests that need further confirmatory test.

Two human studies of exposure status (Mirambo et al., 2018; Mngumi et al., 2016) and one human study in clinical diagnosis (Orsel et al., 2015) used agglutination tests that claim to differentiate *B. abortus* and *B. melitensis* (FBAT kits). Recent studies show that FBAT tests were commonly used in health facilities in East African countries including Tanzania but they had poor specificity (De Glanville et al., 2017; Lukambagire et al., 2022) there was also variation in testing practices among health facilities (Lukambagire et al., 2022). The FBAT kits have been shown to have poor performance in detecting brucellosis in humans (De Glanville et al., 2017). This finding from other studies suggests that the FBAT kits may not give valid and reliable results for the treatment and control of disease in humans and should not be used in isolation as a confirmatory test for brucellosis.

Most of these serological tests, particularly agglutination and precipitation tests, are based on smooth *Brucella* species with O-side chains that cannot capture rough *Brucella* species circulating in domestic animals (*B. canis* and *B. ovis*) (Nielsen, 2002). This indicates that there is no information regarding *B. canis* and *B. ovis* in dogs and sheep in Tanzania or the infection of people with *B. canis*. Generally, serological tests were not able to differentiate *Brucella* to species level. Therefore, this study recommends that there is a need for the WHO and WOAH to support brucellosis testing capacity in Tanzania clearly iterate the limitations of certain tests, categorize them to serve different purposes, and recommend the use of tests which are able to detect classical *Brucella* species circulating in livestock populations as previously proposed (Kalule et al., 2020) and to differentiate the source of infection in people.

Most research on brucellosis in livestock has focused on cattle (Table 6). Research should also focus on other livestock species such as small ruminants, pigs, and dogs, in order to obtain an accurate measure of the magnitude and diversity of diseases in the country. Neglecting brucellosis in other livestock will affect efforts toward controlling brucellosis because the real status of the disease in livestock is underreported.

Conclusions and recommendations

In Tanzania, like many other resource-poor developing countries, there are challenges associated with the diagnosis that cause under-reporting of the disease situation. This review reveals key areas related to brucellosis diagnostic challenges in humans and livestock. These include inadequate knowledge of brucellosis among stakeholders in the livestock value chain, limited diagnostic capacity for brucellosis, challenges associated with diagnostic tests, and uneven distribution of brucellosis surveillance studies in the country.

Understanding the true status of brucellosis in human and livestock populations is critical for the improvement of human and livestock health and is necessary for its control and elimination. To capture the true status of the disease in both humans and animals, stakeholders in the livestock value chain and the public must be motivated to attend health and veterinary facilities for proper diagnosis and treatment.

Inadequate knowledge of brucellosis among stakeholders in the livestock value chain including health and livestock workers can only be remedied through awareness and training. Organizing strategic public health education is critical for these groups to prevent disease transmission to humans and animals. Continuing professional education for health workers in humans and livestock is vital for improved service delivery and disease control.

Challenges associated with diagnostic test availability in Tanzania need to be addressed by national authorities. Bacterial isolation, identification, and molecular techniques require a higher initial investment to be used as a confirmatory diagnostic test in the country. Efforts must be made to improve the testing capacities at regional and district health facilities and zonal levels in livestock facilities.

There have been ad hoc use of serological tests in the country; however, RBPT and cELISA have been more frequently used in Tanzania than other tests. The Tanzanian authorities may consider recommending RBPT and cELISA as the preferred diagnostic tests to report brucellosis in the country in order to have comparable results and a standardized format of reporting brucellosis results at local and international levels and for strategic planning in controlling the disease. This approach may support the ongoing rolling-out of the national strategy for the prevention and control of brucellosis in the country.

There was an uneven distribution of brucellosis studies in regions of Tanzania. This review found that some regions had a higher number of studies while other regions had none, this causes misrepresentation of the disease status in the country. Future brucellosis studies on both humans and livestock must be directed to these unrepresented regions in the southern, central, western, and lake zones of Tanzania. Furthermore, there were more studies of brucellosis in livestock than in humans. More studies of brucellosis are required in humans and livestock to elucidate the magnitude of the disease in respective populations, with a balanced One-Health approach required for controlling the disease.

Although this review attempts to report on the diagnostic challenges of brucellosis in humans and animals the study has some limitations. The review focused on published (written) information which may not accurately reflect the practical situation, particularly with regard to clinical diagnosis. Studies using diagnostic tests for research purposes are over-represented in the review because researchers are more likely to publish or report their findings than clinicians. Understanding the challenges to clinical diagnosis may require a primary qualitative approach to appreciate the nuances regarding diagnostic test selection. There are also likely to be publication biases with regard to the region where research is conducted and the selection of diagnostic tests due to affordability, accessibility, and individual preferences. This review did not summarize the gaps related to validity, protocols, inappropriate use or treatments of tests, and their results which other studies might have reported. Finally, the review highlights the need for increased education and awareness of livestock owners, and human and animal health clinicians. However, the target audience is likely to be other researchers. Further efforts need to be made to educate the responsible authorities and one-health stakeholders to establish policies and practices to reveal the true status of the disease and this will help in the fight against brucellosis both in humans and animals.

Towards this end, the identified issues should be incorporated into the national strategy for the prevention and control of brucellosis

in humans and animals, which was inaugurated in 2018 and will be ended in 2022 (URT, 2018). This strategy recognizes the importance of diagnostic schemes for brucellosis in humans and livestock (URT, 2018). In addition, the "National One Health Strategic Plan" recognizes the need for multidisciplinary approaches for the control of zoonotic diseases; however, the control of these diseases has been challenging due to a number of factors, including a lack of adequate policies, technologies, and resources (URT, 2015).

COMPETING INTEREST DECLARATION

None of the authors have competing financial or non-financial interests in the writing of this manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This review provides evidence for the synthesis of existing research information and ethical approval is not required.

AUTHORSHIP

IJ. Mengele, GM. Shirima and EAJ. Cook made substantial contribution to the conception and design of this work. IJ. Mengele wrote the manuscript. IJ. Mengele, GM. Shirima, EA.J. Cook, LE. Hernandez-Castro, and BM. Bronsvoort made the substantial acquisition, organization, and interpretation of the data. IJ. Mengele and LE. Hernandez-Castro contributed substantially to the design and creation of the map. All authors have read and approved the submitted version of the manuscript and have agreed to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, were appropriately investigated and resolved, and the resolution documented in the literature.

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AVAILABILITY OF DATA AND SUPPLEMENTARY MATERIALS

The data supporting the findings of this study are contained in the manuscript and supplementary materials.

CONSENT FOR PUBLICATION

Not applicable.

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