The University of Zambia

School of Veterinary Medicine

A Meta-Analysis on the Antibiotic Resistance Patterns of Brucella Strains in Humans.

A dissertation Submitted to the University of Zambia in Fulfillment of the Requirements of the Degree Master of Science Degree in Tropical Infectious Diseases and Zoonosis

By

Mwiza Munang'andu

Computer Number: 220005814

DECLARATION

I, Mwiza Munang'andu, do hereby declare that the contents of the dissertation being submitted herein are my original work, and has not been previously submitted to any University for the award of a degree or any other qualification.

Signature---------- Date----------

CERTIFICATE OF APPROVAL

This dissertation submitted by Mwiza Munang'andu is approved as fulfilling the requirements for the award of Master of Science in Tropical and Infectious Diseases and Zoonosis.

Professor John Bwalya Muma (PhD):	……	… ……..
Supervisor	Signature	Date
Examiner:	……	……..
	Signature	Date
Examiner:	……	……..
	Signature	Date
Examiner:	……	……..
	Signature	Date
Chairman (Board of Examiners):	……	… ……
	Signature	Date

ABSTRACT

Brucellosis is a zoonotic disease threatening livestock productivity and human health, especially in low-income regions. *Brucella*-induced infections present significant treatment challenges due to the limited intracellular penetration of antibiotics, leading to prolonged treatment durations and elevated risks of treatment failure and relapse. The emergence of antibiotic resistance further exacerbates these challenges, presenting an impending public health threat. This study aims to analyze the global prevalence of antibiotic resistant *Brucella* strains in humans in order to manage and control antimicrobial resistance. A meta-analysis was performed to assess the prevalence of antibiotic resistant *Brucella* strains. Peer-reviewed research publications were gathered from the 14th August, 2023 to 12th September, 2023, through a literature search utilizing the following keywords((*"Brucella*") AND "Antibiotic susceptibility," OR "Antibiotic resistance," OR "Antibiotic sensitivity," OR "Antibiotic activity" OR "Antimicrobial susceptibility" OR "Antimicrobial resistance " OR "Antimicrobial sensitivity " OR "Antimicrobial activity" "Antibacterial susceptibility" OR " Antibacterial resistance " OR "Antibacterial sensitivity" OR " Antibacterial activity" OR " Antimicrobial Susceptibility Testing" OR "Microbial Sensitivity" OR "Microbial resistance" OR "Antibiogram") in databases such as PubMed, Google Scholar, and Science-Direct. The study examined online research articles published from 2008 to 2022, specifically concentrating on English studies. A total of 19 eligible studies representing 10 countries and 1, 798 samples were included. *Brucella melitensis* was isolated in all the studies, with two other *Brucella* species found in two studies; *Brucella abortus* in Turkey and *Brucella suis* in Malaysia. The global pooled prevalence of antibiotic-resistant bacteria was 9% (95% CI: 6-13%). *Brucella* strains showed the highest antibiotic resistance to macrolides at a prevalence of 58% (95% CI: 0-100%) followed by ansamycins at 35% (95% CI: 17-56%), then beta-lactams at 7% (95% CI: 0-26%), sulfonamides at 4% (95% CI: 0-19%), aminoglycosides at 1% (0- 1%), and fluoroquinolones, tetracyclines, and cephalosporin at 0% (95% CI: 0-1%). The study observed significant bacterial resistance to macrolides and ansamycins specifically, azithromycin and rifampicin, suggesting the need for an alternate treatment considering that the combination of rifampicin with doxycycline is the recommended treatment of human brucellosis. The study also indicates moderate resistance to sulfonamides and beta-lactams whereas tetracyclines, cephalosporin, fluoroquinolones, and aminoglycosides remain extremely effective.

Keywords: Antibiotic Resistance, Brucellosis, *Brucella*, Prevalence, Antibiotic

DEDICATION

This project is dedicated to Mr. and Mrs. Munang'andu as well as to my loving and supportive husband, siblings and friends. Thank you for your loving support, and sacrifices for this study to reach this far. I shall forever remain indebted to you. May our good Lord Jehovah bless and reward you.

ACKNOWLEDGEMENTS

Firstly, I thank God for the knowledge, wisdom and the blessings for me to come this far with this research. Indeed, it is because of his favor, love and care that I have made it this far.

I would like to express my deep and sincere gratitude to my sponsors Africa Centre of Excellence of Infectious Diseases in Humans and Animals (ACEIHDA) for the financial support throughout the Master's program and funding this research.

Secondly, I would love to say thank you to my research supervisor Prof. John Bwalya Muma for his unconditional and unceasingly availability he showed towards my work, his empathy, friendship and sense of humor, it was such a great honor to work and study under his supervision. My special thanks to my co-supervisors Dr. Prudence Mpundu and Dr. Nozyechi Chidumayo for their contribution towards the success of this project. They offered their best guidance at each and every step of the project methodologies.

Lastly, I am extremely grateful to my parents for their love, care, prayers, sacrifices and financial support for me to accomplish this work.

TABLE OF CONTENTS

ABBREVIATIONS

B.- Brucella

CDC- Center of Diseases Control

WHO- World Health Organization

EU/EEA- European and Economic European Areas

List of Tables

List of Figures

List of Appendices

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Brucellosis is a zoonotic disease that poses a significant threat to both human health and livestock productivity worldwide (Lokamar *et al.* 2022). The estimated global loss according to (Singh *et al.*, 2015) is US $3.4 billion (with a range of US $2.8 to 4.2 billion) annually. Pérez-Sancho *et al.*, (2015) reported a prevalence of nearly 500,000 new cases occur annually. However, O'Callaghan, (2020) believes that the commonly reported figure of 500,000 new human cases each year maybe a vast underestimation. This is because many affected countries lack the infrastructure for accurate diagnosis, and brucellosis symptoms are non-specific (O'Callaghan 2020).

Brucellosis is a significant foodborne disease affecting people globally (Fritz, Nguyen, and Vugia 2021a). The World Health Organization (WHO) estimates that contaminated food is responsible for 0.83 million cases of brucellosis worldwide, leading to 333,000 chronic infections and 83,300 cases of orchitis (WHO, 2015). In Africa, the estimated disease burden varies widely from <0.9 to >8.43 per 100 000 populations (Frean *et al.* 2016). However, the disease burden varies greatly across different regions. In East Africa, the prevalence of human brucellosis ranges from 0% to 35.8% (Djangwani e*t al*. 2021), while in the sub-Saharan region, it ranges from 5% to 55% (Lokamar *et al*. 2022). Unfortunately, the lack of awareness, resources, and facilities for controlling the disease has led to over 250 outbreaks reported each year since 2003 (Ducrotoy *et al.*, 2017).

Despite implementing animal vaccination, Zambia is still facing challenges in controlling and preventing the spread of brucellosis (Mwinyi *et al.*, 2016). The prevalence rate of brucellosis is high in cattle herds, ranging from 21% to 58% (Mfune *et al.*, 2021), and in humans, ranges from 5.08% to 20.3% in the last decade (Muma *et al.*, 2013; Lysholm, Fischer, *et al.*, 2022). Financial constraints, lack of information on disease risks, and inadequate hygiene at slaughterhouses and smallholder markets contribute to brucellosis in Zambia (Lysholm, Fischer, *et al.*, 2022).

Currently, there is limited treatment for animal brucellosis, and infected animals must be culled (Khan and Zahoor 2018) . Although, preventive measures for humans exist, there is no human *Brucella* vaccine (Heidary *et al*. 2022a). Animal vaccination campaigns show potential, but

drawbacks like abortion induction and bacterial shedding, leading to human transmission impede efforts to eradicate brucellosis (O'Callaghan 2020).

Additionally, apart from the inadequate treatment availability of brucellosis, is the emergence of antimicrobial resistant *Brucella* strains leading to treatment failures (Elbehiry *et al.* 2022a). Antibiotic overuse contributes to the development of antibiotic resistance, with *Brucella* strains becoming resistant, and the incidence of antibiotic resistance continues to increase annually, especially in the Middle East and North Africa (Ma *et al*. 2023). A recent systematic review by (Wareth *et al.*, 2022a) revealed that both human and animal *Brucella* isolates (*B. melitensis* and *B. abortus)* have developed resistance to several antibiotics. The most prevalent antibiotics recorded were rifampicin, streptomycin, trimethoprim/sulfamethoxazole, azithromycin, and ceftriaxone.

Antibiotic resistance can be innate or acquired, resulting from microorganisms adapting to changing environmental conditions or a sudden selective pressure from antimicrobial treatment (Palma, Tilocca, and Roncada 2020). *Brucella* species have no evidence of plasmids or other means of horizontal gene transfer (Suárez-Esquivel et al. 2020), antimicrobial resistance occurs exclusively due to spontaneous mutations in the genome (Pereira *et al.* 2023). Nucleotide variations in the housekeeping gene rpoB are reliable markers for distinguishing lineages and biovars, and mutations in rpoB can confer resistance to rifampin resistance (Brangsch *et al.* 2023). In vitro, induction of fluoroquinolone resistance in *Brucella* strains has been observed through overexpression of efflux pumps and mutations in gyrA (D. J. Trott, Abraham, and Adler 2018).

There has been no research conducted to assess the worldwide occurrence of antimicrobial resistance in *Brucella* strains against medically significant drugs. The study aims to offer a thorough analysis of the resistance patterns of *Brucella* strains to antibiotics in humans.

1.2 Problem Statement

Brucellosis is a significant global threat to human health and livestock productivity (Franc et al. 2018). The disease is both an occupational and a food-borne disease (Fritz, Nguyen, and Vugia 2021b). According to WHO, there are approximately 0.83 million cases of brucellosis worldwide caused by consuming contaminated food. Additionally, individuals who work with

animals, in laboratories, or in abattoirs have a significantly higher risk of contracting *Brucella* species, which is 3.47 times greater than the general population (Pereira *et al.*, 2020). In Africa, brucellosis is prevalent in both animals and humans, with over 250 outbreaks reported annually since 2003 (Ducrotoy *et al.*, 2017).

Controlling and preventing the spread of brucellosis poses significant challenges for Zambia (Mfune *et al.* 2021). The prevalence rate in humans has increased tremendously from 5.03% in 2008 (Muma *et al.* 2008) to a current estimation of 20.3% (Mubanga *et al*. 2021).

Antibiotic-resistant *Brucella* strains are becoming prevalent, leading to treatment failures and disease relapse of (0% to 32%) in children aged between 7 months and 14 years (Bosilkovski *et al.*, 2015). Antibiotic resistance has been reported in various studies to commonly used antimicrobial drugs like trimethoprim/sulfamethoxazole and rifampicin (Ebani *et al*. 2023). In China, 100% of *Brucella* isolates were resistant to azithromycin, while 62.1%, 58.6%, and 62.1% of isolates in Egypt were resistant to ciprofloxacin, rifampicin, and imipenem, respectively (Ma *et al*. 2023). Drug resistance in brucellosis treatment is a significant issue in low-income developing countries where tuberculosis is endemic, raising concerns about long-term tuberculosis medication resistance (Gültekin *et al*. 2021a).

Amidst the growing concern of antibiotic resistant *Brucella* strains, the study's aim is to provide detailed prevalence data on antibiotic-resistant *Brucella* strains, and identify less effective antibiotics which is essential for developing new treatment strategies and guidelines. The study's results will aid in implementing public health interventions and policies to control brucellosis, particularly in resource-limited settings, prioritizing effective antibiotics and refraining from resistant antibiotics.

1.3 Significance of the Study

Brucellosis is a disease that is neglected on a global scale, contributing to increase in misdiagnosis and a limited understanding to its true disease impact (Moreno *et al.* 2022). With the rise of antibiotic resistance, it is crucial to determine the most effective antibiotics and the prevalence of antibiotic resistance in order to raise awareness and urgency in eradicating the disease. Infections caused by multidrug-resistant bacterial strains are among the main factors influencing morbidity and mortality (Prestinaci *et al.* 2015). This study aims to address this gap by investigating the prevalence of antibiotic resistant *Brucella* strains. By consolidating all relevant data on this matter, the study will enhance its statistical reliability and allow for broader application of the results. The discoveries from this research will aid in

the advancement of more effective treatment strategies for individuals who have been diagnosed with or are suspected of having brucellosis. This, in turn, will help mitigate the threat of outbreaks caused by multi-drug resistant strains or *Brucella* epidemics.

1.4 Research Questions

a. What is the prevalence of antibiotic resistant *Brucella* strains?

b. What are the most effective antibiotics against *Brucella* strains?

1.5 General Objective

- The objective of this meta-analysis is to evaluate of the resistance patterns of *Brucella* strains to antibiotics in humans.

1.6 Specific Objectives

a. To determine the prevalence of antibiotic resistant *Brucella* strains**.**

b. To evaluate the most effective antibiotics against *Brucella* strains

CHAPTER TWO

2. LITERATURE REVIEW

Brucellosis is a worldwide threat to both livestock productivity and human health (Franc et al. 2018), as it is a neglected zoonotic disease that can be transmitted naturally between animals and humans. Neglected tropical diseases are another type of disease that affects people mainly living in low-income regions of tropical and subtropical areas (Williams and Kovarik 2021). These diseases are termed "neglected" as they receive little attention despite their high prevalence and impact on poor and marginalized populations(WHO 2015).

The economic burden that brucellosis places specifically on low-income countries has attracted worldwide attention as one of the world's leading neglected zoonotic diseases of economic importance (Franc et al. 2018). According to (Singh et al., 2015), brucellosis results in a median loss of US $3.4 billion (with a range of US $2.8 to 4.2 billion), with 95.6% of the total losses attributed to the disease in cattle and buffalo. Per animal, the disease leads to a loss of US $6.8 for cattle, US $18.2 for buffalo, US $0.7 for sheep, US $0.5 for goats, and US $0.6 for pigs.

2.1 Etiology of Brucellosis

The genus *Brucella* resides within the family *Brucellaceae* with *Mycoplana* and *Ochrobactrum*, of the order *Rhizobiales* in the class *Alphaproteobacteria* of the phylum *Proteobacteria* (Ficht 2010). *Brucella* and *Ochrobactrum* are part of the *Rhizobiales* order within *Brucellaceae* family (Leclercq, Cloeckaert, and Zygmunt 2020). One of the most significant distinctive features of *Brucella* organisms is the smaller genome sizes (3.1–3.4 Mb) as compared with their closest *Ochrobactrum* relatives (4.7–8.3 Mb), a phenomenon linked to their different lifestyles. (Moreno et al. 2022).

Brucella are very small (0.5–0.7 μm × 0.6–1.5 μm), faintly stained gram-negative coccoid rods, with a microscopic appearance of ‘fine sand’ (Liu 2014). They are intracellular pathogens that survive and multiply in macrophages during infection, adapting to acidic conditions (Głowacka et al. 2018). *Brucella* species can be classified into two phenotypic types based on the structure of their lipopolysaccharide (LPS), which is the main component of the outer membrane of gram-negative bacteria. These types are referred to as "smooth" (S-type) and "rough" (R-type) (Kurmanov et al. 2022).

There are several types of *Brucella* bacteria that infect different hosts. *B. abortus* mainly infects cattle, *B. melitensis* preferentially infects sheep and goats, *B. suis* mostly infects pigs, *B. canis* affects dogs, *B. ovis* affects sheep, *B. neotomae* infects the desert wood rat, *B. microti* affects the common vole, *B. ceti* infects cetaceans, *B. pinnipedialis* infects seals, and *B. inopinata* was isolated from a human breast implant infection. In addition to these, *B. papionis* and *B. vulpis* were recently obtained from the baboon (*Papio* spp.) and red fox (*Vulpes vulpes*), respectively (De Massis et al. 2019). Additionally, there are several *Brucella* isolates that have been found in rodents, frogs, reptiles, fish, and bats, but have not yet been formally described in terms of their taxonomy (About et al. 2023).

2.1.1 Etiology of Brucellosis in Humans

In humans, the main cause of brucellosis is mostly attributed to four distinct species of *Brucella: B. melitensis, B. suis, B. abortus,* and *B. canis* (Pal et al. 2020). *B. melitensis* is the most potent species causing brucellosis in humans, followed by *B. suis,* while, *B. abortus* is the mildest form of the disease (Jin et al. 2023). Although human infection with *B. abortus* can be moderate, it is the most important zoonotic agent after *B. melitensis* and it can also lead to persistent and difficult-to-treat illness (Mirnejad et al. 2017a). A study conducted in Bangladesh identified *B. abortus* as the primary cause of brucellosis in occupationally exposed individuals. The study did not find any cases of *B. melitensis* and suggested that additional research should be conducted to investigate the presence of *Brucella melitensis* in Bangladesh (Rahman et al. 2017).

B. melitensis, B. suis, and *B. abortus* are divided into subtypes or biovars. Although *B. melitensis* is more infectious and causes disease in humans compared to *B. abortus*, there are no significant differences in disease presentation and severity (Osman et al. 2015). *B. abortus* has been classified into biovars 1, 2, 3, 4, 5, 6, 7, and 9, *B. melitensis* has biovars 1, 2, and 3, and *B. suis* biovars 1, 2, 3, 4, and 5 (Mathew et al. 2015). *B. melitensis,* the most pathogenic species among *Brucella* species, causes the most human infections, with all three biovars (bv1, bv2, bv3) being pathogenic to humans (Zange and Scholz 2023). *B. suis* biovars have varying zoonotic potential, with biovars 1, 3, and 4 being more pathogenic than *B. abortus* but less than *B. melitensis*. Other *B. suis* biovars have limited potential to infect humans (El-Sayed and Awad 2018).

2.1.2 Etiology of Brucellosis in Animals

The most important recognized *Brucella* species listed in descending order of pathogenicity are *B. melitensis, B. suis,* and *B. abortus* (Rowe 2014). The species *B. melitensis* biovars 1–3 have been observed in sheep and goats, *B. abortus* biovars 1–6 and 9 in cattle, and *B. suis* biovars 1–3 are known to infect pigs, while *B. suis* biovar 4 and 5 are more frequently associated with infection in reindeer and small rodents (Khan and Zahoor 2018). Nevertheless, *B. melitensis* biovar 1 is the predominant pathogen responsible for infection in sheep and goats (Akar and Erganis 2022), and *B. abortus* biovar 1 is the main culprit behind cattle brucellosis, accounting for 41 out of the reported 45 strains contrasting to the prevalence of biovar 3, as highlighted by (Bertu et al. 2015). Porcine brucellosis is caused by *B. suis* biovars 1, 2, or 3, with biovar 2 having a distinct host range, limited distribution, and pathology (Coelho, Díez, and Coelho 2015).

It is worth mentioning that *Brucella* species have a preference for specific animal species as hosts, however, they can infect other hosts, except for *B. ovis* (El-Sayed and Awad 2018). Cross infection can occur due to mixed husbandry systems, although at relatively low and controlled infection rates (Matle et al. 2021). A systematic review on brucellosis in Africa reported *B. abortus, B. melitensis, B. inopinata* and *B. suis* as the observed *Brucella* species in wildlife (Simpson et al. 2021a). Wildlife species such as bison and elk have been confirmed to be important reservoirs of *B. abortus* (Yang et al. 2019). *Brucella* infections that are recognized as sustainable in wildlife are *B. abortus* in buffalo and bison, *B. suis* biovar 2 in wild boar and European hare, *B. suis* biovar 4 in reindeer, *B. ceti* in cetaceans, *B. microti* in voles and red fox (González-Espinoza et al. 2021).

2.2 Epidemiology of Brucellosis

Brucellosis, also known as “undulant fever”, “Mediterranean fever”, “Malta fever”, “Gibraltar fever”, “thousand face disease”, “raging fever” or “melitococci disease” (Mirnejad et al. 2017b) is a reportable zoonotic disease and re-emerging in some countries. The WHO reported in (2020) that brucellosis is typically contracted by humans through direct contact with infected animals, ingestion of contaminated animal products, or inhalation of airborne agents. Of which, the majority of cases are caused by consuming unpasteurized milk or cheese from infected goats or sheep. Therefore, incidence of the disease in humans relates to the frequency of brucellosis in the local livestock population (Holt et al. 2021). In addition, *Brucella* species are highly infectious through the aerosol route, making them a potential agent of biological

weapons and bioterrorism (Lai et al. 2017). Unfortunately, in many parts of the world, the non-specific clinical signs of the disease often lead to misdiagnosis as malaria or typhoid (Zerfu et al. 2018), resulting in significant under-reporting.

The geographical distribution of animal brucellosis is constantly evolving, with new foci emerging in infected areas or re-emerging in previously free areas, potentially leading to new cases (De Massis et al. 2019). For insistence, Canada has effectively eliminated bovine brucellosis; however, *B. abortus* still persists in wild bison herds in certain regions (Corbel 2020). *Brucella* species are found in higher concentrations within the uterus of pregnant animals, and aborted fetuses, placental membranes and uterine discharges act as the main source of infection (Khurana et al. 2021). *Brucella* species can thrive in various environmental conditions, including climatic irregularities, temperature changes, and humidity, providing the necessary conditions for their long-term survival (Faramarzi et al. 2019). Animals can contract the infection through ingestion, contact with contaminated feed and water, inhalation, and natural or artificial insemination from one herd to another (Sharma et al. 2024).

2.2.1 Transmission of Brucellosis

In humans, brucellosis almost always originates from an animal reservoir (J Godfroid et al. 2013). Human-to-human transmission of brucellosis is rare (CDC 2019) but not impossible. A systematic study conducted by (Tuon et al., 2017) described human-to-human transmission occurring through the trans placental route, breastfeeding, sexual intercourse, and tissue such as blood and bone marrow. A few cases described in literature such as the transplantation of solid organs could be difficult to differentiate between interhuman and environmental transmissions because family members share the same endemic area (Tuon et al., 2017).

In animals, *Brucella* transmission occurs through ingestion of contaminated feeds and water, inhalation of aerosolized bacteria, mating, and direct contact with infected placenta and uterine discharges (Efrem et al. 2023). Animal infection occurs during communal herding and grazing, the addition of infected animals to a herd, transhumance movement and mixing at livestock markets (Oyetola et al. 2021).

2.2.2 Pathogenesis of Brucellosis

Brucella species cause brucellosis through their unique ability to penetrate and persist within host cells, and bypassing immune defenses, leading to chronic infections (Qureshi et al. 2023). The bacterium infects the host through four crucial steps: adherence, internalization, intracellular growth, and dissemination within the host (Dadar et al. 2021). *Brucella* enters

the host, moves across the mucosal epithelium layer, is endocytosed by macrophages and dendritic cells, survives and replicates inside professional phagocytic cells, modulates host immune response, and disseminates to preferred tissues (De Figueiredo et al. 2015). *Brucella* strains bind to epithelial cell surface receptors containing sialic acid and sulfated residues (Castañeda-Roldán et al. 2004). The binding process activates small guanosine triphosphate (GTPases), triggering a signaling cascade that rearranges the actin cytoskeleton, enhancing invasion and entry (Rossetti, Drake, and Adams 2012). They move across the epithelium by subverting the mucosal epithelial barrier function (Rossetti et al. 2013). Once translocated through the epithelium *Brucella*, are engulfed by mucosal phagocytic cells, with less than 10% survive. They delay immune system recognition by modifying or cloaking their pathogen-associated molecular patterns (De Figueiredo et al. 2015). Then *Brucella*, reside in a specific vacuole within mononuclear phagocytic cells, modify intracellular trafficking, and transform the vacuole into a replicative compartment (Köhler et al. 2002). Infected mononuclear phagocytic cells undergo extensive transcriptional changes during the adaptation stage, returning to normal after 12 hours (Billard, Dornand, and Gross 2007). However, *Brucella* uses strategies like inhibiting apoptosis, preventing dendritic cell maturation, reducing antigen presentation, and activating naive T cells to establish and maintain chronic infection (De Figueiredo et al. 2015).

Dendritic cells are antigen-presenting cells that can be a safe haven for *Brucella* growth, as they can inhibit antigen processing and presentation, bypassing the host immune response (Huy et al. 2022). *Brucella* can infect other cells such as neutrophils, lymphocytes, and erythrocytes, but they lack efficient intracellular replication due to their role in bacterial dispersion (González-Espinoza et al. 2021). Furthermore, *Brucella* can cause abortion by infecting animal placenta, particularly in trophoblasts where erythritol is produced, and its virulence factor is its ability to replicate (Huy et al. 2022).

2.2.3 Brucellosis in Humans

Brucellosis infections are caused by direct or indirect contact with infected animals or animal materials, and is linked to disease prevalence, socioeconomic status, eating habits, poor hygiene, and consumption of contaminated unpasteurized milk and meat (Adesokan, Alabi, and Ogundipe 2016).

It is a sub-acute or chronic disease with nonspecific clinical manifestations, presenting classically as an influenza-like syndrome (About et al. 2023). Of the twelve *Brucella* species, *Brucella melitensis* is the most prevalent cause of human brucellosis (Hashim et al. 2014). *Brucella* can cause common signs and symptoms such as fever, asthenia, myalgia, arthralgia, sweats, lymphadenopathy, hepatomegaly, and splenomegaly of which osteoarticular manifestations are the most common forms of localized disease (Alhabbab 2018). It can also cause neuro-brucellosis which has a prevalence that ranges from 5% to 7% and the reported frequency of liver involvement such as granulomas, inflammatory infiltrations, and parenchymal necroses ranges from 5% to 52% (Baldi and Giambartolomei 2013).

Antimicrobial resistance (AMR) is a global public health issue with a projected 10 million deaths per year by 2050 (Tang, Millar, and Moore 2023). It's primarily caused by overuse and misuse of antibiotics in clinical treatment, agriculture, animal healthcare, and the food system (Llor and Bjerrum 2014). Misuse and overuse of antibiotics lead to the selection of resistant strains, with an estimated 50% of prescriptions being deemed inappropriate (Samreen et al. 2021). Antibiotics in animal husbandry are a major contributor to antibiotic resistant as antibiotic resistant genes can cross species barriers and enter human food sources, introducing new resistant bacterial strains (Harris et al. 2023). Non-prescription antibiotic use is still common, accounting for 19% of total consumption and even up to 100% in some cases (Sohail et al. 2016). Hospitals are hotspots for horizontal gene transfer, facilitating the transfer of antibiotic resistance determinants and virulence factors between species, contributing to the acquisition, maintenance, and spread of antibiotic resistant genes within bacterial communities (Samreen et al. 2021).

2.2.4 Brucellosis in Animals

The negative impact of brucellosis is not limited to livestock, as it also affects wildlife. In fact, (Wiethoelter et al. 2015) identified brucellosis as one of the top 10 diseases at the wildlife-

livestock interface. *Brucella melitensis*, primarily common in goats and sheep, *B. abortus*, affects cattle, and *B. suis*, in swine, these are the most prevalent *Brucella* species that impact livestock (De Massis et al. 2019). These species can be transmitted between animals both vertically and horizontally, leading to abortion and infertility in their primary natural hosts (Plumb, Olsen, and Buttke 2013). Moreover, *Brucella* species can cross-infect not only their preferred hosts but also other domestic and wild animal species (Moreno 2021). As a result, these species can act as reservoirs for the disease for other animal species and humans (Díaz Aparicio 2013).

Notably, not all instances of *Brucella* infections in wildlife are caused by spillovers from livestock (González-Espinoza et al. 2021). For instance, (Jacques Godfroid 2018) reported that, *B. abortus* in buffalo and bison, as well as *B. melitensis* in Alpine ibex, are examples of infections that can occur in wildlife independent of livestock. In areas where livestock farming is scarce, such as the Arctic, *B. suis* biovar 4 infections have been observed in reindeer and caribou, while *B. ceti* and *B. pinnipedialis* infections have been primarily detected in different cetacean and seal species. There have been a few reports of human infections resulting from *B. suis* biovar 4 in Russia, Canada, and Alaska, and less than five cases of natural *B. pinnipedialis* infections in humans (Jacques Godfroid 2018). Anthropogenic ecological changes have led to a significant increase in the wildlife-livestock-human interface (Jones et al. 2013), resulting in emerging and re-emerging *Brucella* infections or diseases.

Antimicrobial resistance (AMR) is a growing issue affecting food animals due to antibiotic and antimicrobial use, inadequate local sanitation, pollution, and nonuse factors (Graham et al. 2019). Veterinary medicines are used as prophylactic agents, feed additives, and growth promoters, leading to the selection of mutations that confer antibiotic resistance (Samreen et al. 2021). A study on Vietnamese chicken farms found that 84% of antibiotics were used for prophylaxis, 12% for disease management, and 3.8% for combined use (Carrique-Mas et al. 2015). Poor regulation of antibiotic access, including access without prescription, poor awareness, inappropriate use, and inappropriate prescribing, in poultry contributes to the spread of antibiotic resistance (Mudenda et al. 2023). Biocides used in veterinary settings at sublethal concentrations can lead to the selection of mutations causing antibiotic resistance, similar to the selection of antibiotic resistance genes at low antibiotic concentrations (Singer et al. 2016).

2.3 Global Prevalence of Brucellosis

Human brucellosis, a foodborne disease caused by *Brucella* species, affects a significant number of people worldwide. According to (Pérez-Sancho et al. 2015), nearly 500,000 new cases are reported annually; however, underreporting and misdiagnosis result in a vast underestimate of its true prevalence. Every person, regardless of age or sex, is at risk of contamination through food. (Dadar et al. 2020) found a higher prevalence of Brucella species in raw milk (17%) than in cheese (7%), while (Dadar et al. 2022) reported a prevalence of 6.83% in male and 9.64% in female camels. In swine, the prevalence of Brucella species is 2.1%, with a higher infection rate in Europe (17.4%) (Gong et al. 2021). The WHO estimates that there are 0.83 million brucellosis cases worldwide resulting from the consumption of contaminated food, including 333,000 chronic infections and 83,300 cases of orchitis (WHO 2015).

In brucellosis patients, the prevalence of developing osteoarticular disease varies from 27% in low-risk regions to 36% in high-risk regions (Adetunji et al. 2019). The global prevalence of brucellosis is 14%, which is further compounded by the additional occupational risk faced by livestock-related personnel, with North America and Africa reporting the highest prevalence rates (Mia, Hasan, and Pory 2022). Despite this, several countries have taken measures to reduce the incidence and prevalence of brucellosis. For instance, within the EU/EEA, 26 Member States actively monitor brucellosis, while 20 countries have comprehensive surveillance systems that analyze both laboratory and epidemiological data (European Centre for Disease Prevention and Control 2022). As a result, the European Centre for Disease Prevention and Control (2022) reported a steady decrease in brucellosis cases from 2016 to 2020.

According to the (CDC 2012), Canada and Japan have successfully eradicated brucellosis by implementing expensive and lengthy programs that involved animal vaccination and culling of infected animals at later stages (O'Callaghan 2020). Countries such as Kenya reporting (203.07 cases per 100 000), Yemen (89.96), Syria (47.26), Greece (42.96) and Eritrea (21.82), have made improvements in their high incidence rates since 2006 (Wang and Jiang 2020). However, increased international trade, migration, tourism and lack of awareness threaten the prevention efforts to curb the prevalence of brucellosis and contribute to the emergence of new hotspots, especially in Africa and the Middle East (Wang and Jiang 2020).

2.4 Prevalence of Brucellosis in Africa

Brucellosis is a widespread zoonotic disease in Africa (Bamaiyi 2016), with a prevalence of 8% in livestock (Suresh et al. 2022). In Sub-Saharan Africa, the prevalence of animal brucellosis ranges from 10.2% to 25.7% (Mehari, Zerfu, and Desta 2021), while in the East Africa Center, cattle, goats, and sheep had a prevalence rate of 0.0% to 20.0%, 0.0% to 13.8%, and humans ranged mostly from 0.0% to 35.8% (Djangwani et al. 2021).

Africa lacks adequate information on the prevalence of brucellosis in both humans and animals, making it difficult to accurately assess the extent of the disease burden (Govindasamy 2020). The lack of facilities, budget, and low prevalence data has led to the absence of strategies to control brucellosis at regional levels in Africa (Racloz et al. 2013). However, the available data suggests that Africa has one of the highest rates of brucellosis in the world. For instance, Kenya's incidence rate is 203.07 cases per 100,000 individuals (Wang and Jiang, 2020), Ethiopia's pooled seroprevalence is 3.0% (Tesfaye et al. 2021), and Uganda has a national prevalence rate of 10% in cattle and 5.5% in cattle keepers (Baruho 2022). Additionally, a meta-analysis conducted by Pereira *et al.*, (2020) found that individuals who work with animals, in laboratories, or in abattoirs have a 3.47 times higher risk of contracting *Brucella* species compared to those who do not have any exposure to possible sources of infection. In Tanzania, 6.1% of febrile human illnesses requiring outpatient hospital care are caused by Brucella species (Bodenham et al. 2020).

While several countries in southern Africa have implemented measures such as surveillance, movement control, and stamping out or vaccinations for bovine brucellosis, only South Africa has made sustained efforts to control and eradicate the disease (Ducrotoy et al. 2017). Control and eradication programs for animal brucellosis are common in developed countries, but resources are often not allocated to such programs in developing countries (Bamaiyi 2016).

In addition to resources, lack of awareness is a major problem in Africa with regards to brucellosis. The pooled awareness level of the zoonotic nature of brucellosis in the African population is only 17.8%, with higher awareness among livestock owners (15.4%) compared to dairy farmers and abattoir workers (2.6%) (Zhang et al. 2010). A study conducted in Rwanda showed that 57.5% of respondents were unaware of brucellosis as a disease and 82.5% did not remove brucellosis seropositive animals from the herd (Ndazigaruye *et al.*, 2018). As a result of these challenges, brucellosis has not been eradicated, and the situation is worsening with over 250 outbreaks per year reported since 2003 (Ducrotoy et al. 2017).

2.5 Prevalence of Brucellosis in Zambia

There is a limited number of research in Zambia discussing the prevalence of brucellosis (Muma et al. 2007). Many of the research papers published from 2000 onwards focus on seroprevalence instead. Therefore, because of the lack of data on prevalence, this study will highlight on the seroprevalence of brucellosis in Zambia.

Around 80% of Zambia's national livestock belongs to the traditional sector, with *Brucella* seroprevalence in cattle herds ranging from 21% to 58% and small ruminants (sheep and goats) ranging from 0% to 1.65% (Muma et al. 2013). The prevalence of *Brucella* seroprevalence in Southern and Western Provinces of Zambia has remained stable over the past decade at 21.14% (Mfune et al. 2021). Meanwhile, in humans, the seroprevalence has increased from 5.08% to around 20.3% in the last decade (Mubanga et al. 2021).

Although Zambia has implemented animal vaccination against brucellosis, the program is not free and limited financial resources hinder control and prevention efforts, according to (Mwinyi et al. 2016). Moreover, there are no current national control programs or vaccination campaigns for goat diseases in Zambia, as reported by (Lysholm, Lindahl, et al. 2022). The continuous circulation of *Brucella* species in the country is attributed to suboptimal procedures and hygiene at slaughterhouses and smallholder markets, as well as the lack of information on disease risks (Lysholm, Fischer, et al. 2022).

Apart from the already high risk of brucellosis from livestock, Zambians are facing an additional threat from wildlife. The disease has taken hold in the Kafue lechwe antelope (*Kobus leche kafuensis*), with a prevalence of 43%, even in the absence of contact with livestock (Simpson et al. 2021b).

2.6 Treatment, Prevention, and Control of Brucellosis in Humans

According to (Dadar et al. 2021), controlling and preventing brucellosis in livestock could decrease the incidence of human brucellosis. Unfortunately, treatment of animal brucellosis is not feasible, and all infected animals must be culled (Wareth et al. 2022a). To prevent the spread of the disease, measures such as consuming pasteurized milk, handling meat, animal carcasses, and internal organs correctly, and disposing of afterbirths properly are recommended for agricultural and livestock personnel (WHO 2020).

2.6.1 Control and Treatment of Brucellosis in Humans

In settings with limited resources, following the traditional treatment for brucellosis (aminoglycosides plus doxycycline) can be difficult because of the potential for hepatitis and HIV transmission through syringes (Ducrotoy et al. 2017). Human brucellosis treatment usually requires a course of oral combination antimicrobial medication lasting at least 6 weeks, with doxycycline and rifampin being frequently prescribed (Villate et al. 2020). It is recommended to use combination therapies to lower relapse rates (Ranjbar et al. 2020). When comparing doxycycline and rifampin combinations with co-trimoxazole, relapse rates varied, with higher relapse rates of 1.96 observed with co-trimoxazole plus rifampin compared to co-trimoxazole with doxycycline (Khurana et al. 2021). Unfortunately, no human *Brucella* vaccine is currently available (Darbandi et al. 2022) and because of efficacy and safety concerns on the currently available *Brucella* vaccines (Dorneles, Sriranganathan, and Lage 2015).

2.6.2 Control and Treatment of Brucellosis in Animals

Antibiotic treatment of brucellosis in domestic animals is often unsuccessful owing to intracellular survival of *Brucella* and its adaptability in macrophages (Khurana et al. 2021). In livestock, treatment of brucellosis is not attempted because all positive cases are slaughtered (Wareth et al. 2021).Test-and-slaughter policies are the most effective (Bahmani and Bahmani 2022), but in endemic developing countries they can contribute to the spread of infection when identified seropositive animals are sold instead of slaughtered (Rubach, Halliday et al., 2013). Vaccination campaigns including *Brucella melitensis* Rev. 1 and *Brucella abortus* S19 have been successful in controlling brucellosis worldwide (Heidary et al. 2022b). However, none of the available vaccines are perfect and can cause abortion in animals, leading to the transmission of brucellosis to humans (O'Callaghan 2020).

2.7 Recurrence and Antibiotic Resistance of Brucellosis

The diagnosis of brucellosis is a complex task that necessitates a high level of suspicion, clinical and laboratory evidence (Shenoy, Jaiswal, and Vinod 2016). Due to variable clinical symptoms, microbiological laboratory is crucial for identifying and managing human cases using direct diagnosis by culture, indirect diagnosis by serological tests, and rapid diagnosis by molecular polymerase chain reaction -based methods (Di Bonaventura *et al.* 2021). The

isolation of *Brucella* species is considered as the gold standard technique for the diagnosis of brucellosis (Etemadi et al. 2019). Isolation from body fluids, exudates, blood, and tissues is 100% test-specific and absolute evidence for infection, but detection of cultures decreases with chronic infection (Legesse et al. 2023). The risk of laboratory infection varies from 40% to 100%, influenced by factors like laboratory accidents and aerosolization during routine identification activities (K. Singh 2009).

Serological diagnostics for brucellosis use an indirect approach, examining a patient's immune response for antibodies indicating prior exposure or contact with the *Brucella* pathogen, such as immumoglobin M and immunoglobin G, which provides evidence of previous or ongoing infection (Qureshi et al. 2023). Molecular techniques such as polymerase chain reaction is a widely used method to detect brucellosis in serum and blood specimens, with a sensitivity ranging from 50 to 100% (Legesse et al. 2023).

Antibiotic susceptibility is the minimum concentration needed to inhibit bacteria or fungi growth (Syal et al. 2017). *Brucella* species are susceptible to various antibiotics, including doxycycline, rifampicin, streptomycin, gentamicin, and trimethoprim-sulfamethoxazole (Głowacka *et al.*, 2018; Gültekin *et al.*, 2021). Treatment typically involves dual-antibiotic therapy, varying based on the severity of the infection (WHO, 2020).

The rise in antibiotic-resistant *Brucella* strains has resulted in treatment failures and the reappearance of the disease. A study in Malaysia collected *Brucella* isolates from 2010 to 2011 and found that all 41 isolates were susceptible to the tested antimicrobial drugs except for rifampicin (Hashim et al. 2014). An examination of 87 isolates of *B. melitensis* in Turkey revealed that all isolates were sensitive to doxycycline and streptomycin, with intermediate susceptibility to rifampicin (Gültekin et al. 2021b). A study conducted by (Arapović et al. 2022) in 2022 in Bosnia and Herzegovina found a significant resistance rate of *B. melitensis* to azithromycin. A systematic review conducted in Middle Eastern and North African countries on *Brucella* isolates (*B. melitensis*, *B. abortus*, and *B. ovis*) from animal and human samples revealed resistance to rifampicin, streptomycin, trimethoprim/sulfamethoxazole, azithromycin, and ceftriaxone (Wareth et al. 2022b). Research on antibiotic resistance of *Brucella* strains in Zambia is insufficient. Several studies have documented antibiotic resistance in different bacterial species, but there is currently no research available on antimicrobial resistance specifically in *Brucella* species. The existing studies on brucellosis document the seroprevalence.

Brucellosis is known for recurrence, therapeutic failure, and chronicity despite the recommended treatment options by the WHO(Nematollahi et al. 2017). The disease can recur, and in 5 to 15% of cases, recurrence occurs despite treatment (Keramat et al. 2021). The relapse rate of Brucellosis is substantial, affecting 5-30% of individuals (Abd elbaser and Mohammed 2018). One study in the United Arab Emirates found a relapse rate of 10% (Al Nokhatha et al. 2021), while another study showed relapse rates ranging from 0% to 32% in children aged 7 months to 14 years (Bosilkovski et al. 2015). The doxycycline-streptomycin combination has shown the most effective treatment response and the lowest relapse rate of approximately 5–7% (Majzoobi et al. 2018).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study Design

This meta-analysis evaluates the prevalence of antibiotic resistant *Brucella* strains, following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) framework. The protocol was registered with PROSPERO under the ID CRD42023448388 and a thorough literature search was conducted using databases like PubMed, Google Scholar, and Science-Direct.

3.2 Study Frame

This study involved the analysis of various research articles on the antibiotic resistance of *Brucella* species.

3.2.1 Inclusion Criteria

Research articles inclusive of laboratory based cross-sectional studies published online between 2008 and 2022, utilizing cultural and/or molecular techniques to determine the antibiotic susceptibility of *Brucella* strains in humans. The studies were in English, with completed prevalence data and the full text-version was available online.

3.2.2 Exclusion Criteria

Research articles that did not meet the above inclusion criteria were excluded.

3.2.3 Search Strategy

A systematic search was performed for studies published from the 1st January, 2008 to the 31st December, 2022 in Google Scholar, PubMed, and ScienceDirect databases using a set of keywords. The keywords used were: (("Brucella") AND "Antibiotic susceptibility," OR "Antibiotic resistance," OR "Antibiotic sensitivity," OR "Antibiotic activity" OR "Antimicrobial susceptibility" OR "Antimicrobial resistance " OR "Antimicrobial sensitivity " OR "Antimicrobial activity" "Antibacterial susceptibility" OR " Antibacterial resistance " OR "Antibacterial sensitivity" OR " Antibacterial activity" OR " Antimicrobial Susceptibility Testing" OR "Microbial Sensitivity" OR "Microbial resistance" OR "Antibiogram").

a) PubMed Search Strategy

The advanced search was used for each individual keyword combined with AND *Brucella*. The following were the selected settings:

1. Query- ALL FIELDS.
2. Year- Custom Range; 01 January 2008 to 31 December 2022.

   Date searched: 14th August, 2023.

b) GoogleScholar Search Strategy

The advanced search was used for each individual keyword AND *Brucella*. The following were the selected settings:

1. Filter- All in title or Title.
2. Year- Custom Range; 2008-2022

   Date searched: 23rd August, 2023.

c) ScienceDirect Search Strategy

The advanced search was used for each individual keyword combined with AND *Brucella*. The following were the selected filter settings:

1. Year- 2008-2020
2. Article Type- Research Articles
3. Subject Area- Immunology and Microbiology.

   Date searched: 12th September, 2023.

3.2.4 Selection Process

The retrieved articles were saved onto an Excel® spreadsheet, and duplicates were removed. Subsequently, the articles were then subjected to title-based screening to eliminate unrelated studies. Following this, abstract and full-text screening in accordance with the inclusion criteria was done, which led to the exclusion of research articles that did not meet the specified requirements. Figure 4.1 summarizes the selection of articles.

Eligible studies were subjected to a quality assessment that contained eleven modified questions (Munn et al. 2014) (Additional file 1) and only studies that scored at least 80% (9 out of 11) were included in the analysis (Twohig-Bennett and Jones 2018). The score system used was 1 for “yes” and 0 for “no” (Supplementary Table1).

3.2.5 Data Extraction

Specific information was extracted from the selected articles, which included first author, year of publication, country of study, origin of sample (human only), study design,

sampling technique, sample duration (fresh or archived samples), type of sample, *Brucella* species isolated, sample size, total number of positive *Brucella*, total number of *Brucella* isolates tested, class of antibiotic, specific antibiotic, type of antibiotic susceptibility test, and proportion or total number of *Brucella* isolates susceptible, intermediate or resistant.

3.2.6 Study Risk of Bias Assessment

The Doi plot was utilized to investigate publication bias. Publication bias describes the increase in the likelihood of publishing significant positive results, leading to skewed meta-analysis results, and potentially over or underestimating an effect (Woodyard et al. 2024). It can affect the validity and generalization of conclusions (Lin and Chu 2018).

The Doi plot was analyzed using the Luis-Kanamori (LFK) index, which is used to distinguish between simulated publication bias asymmetry and chance or no asymmetry (Furuya-Kanamori, Barendregt, and Doi 2018). An LFK index with scores of ±1, between ±1 and ±2, or ±2 indicates "no asymmetry," "minor asymmetry," and "major asymmetry," respectively (Chen et al. 2022).

The Cochrane's Q which is equivalent to the P-value of a Chi-Square statistic (Kulinskaya and Dollinger 2015) and I2 statistic were used to determine heterogeneity in the analysis. According to the Cochrane Handbook for Systematic Reviews of Interventions (Chandler, 2022), the interpretation of the I2 statistic is as follows;

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity
- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: considerable heterogeneity

3.2.7 Effect Measure

The effect measure used in this study was the prevalence rate of the total number of resistant *Brucella* isolates and the sample size.

3.3 Data Analysis

Articles that satisfied the requirements of the screening and quality assessment criteria were transferred to a separate Excel® sheet for subsequent data extraction. Utilizing MetaXL version 5.3, a quantitative meta-analysis was conducted using the quality effect model.

Relevant details such as the article reference (first author and publication year), sample size, proportion of resistant *Brucella* isolates, and quality assessment scores were compiled into a table within a separate Excel® workbook.

The quality effect model was used to calculate the global pooled prevalence of antibiotic-resistant *Brucella* strains, as well as the Cochran's Q and I2 statistics. The analysis yielded a Cochran's Q value of 74.29 and an I2 of 76% (Table 4.2). Given that Cochran's Q is analogous to the P-value of the Chi-square statistic (with a standard threshold of P=0.05), and an I2 between 75% and 100% (Chandler, 2022) indicates considerable heterogeneity, the results indicated significant heterogeneity beyond chance alone.

To explore this heterogeneity, a subgroup analysis (Table 4.2) was conducted by grouping research articles based on their respective locations (countries). Interestingly, the subgroup analysis revealed insignificant heterogeneity, suggesting that the observed variation in the overall analysis could be attributed to geographical differences.

3.4 Ethical Considerations

No human or animal subjects was required to conduct this study, as it was a meta-analysis. Therefore, ethical approval was not required.

CHAPTER FOUR

4. RESULTS

4.1 Study Characteristics

The meta-analysis included 19 studies (Figure 4.1), of which the countries; Bosnia and Herzegovina, China, Egypt, Iran, Kazakhstan, Malaysia, Mongolia, Peru, Qatar, and Turkey were represented. Turkey had six studies which was the highest, followed by Iran and China with three studies each, while the rest had only one article each.

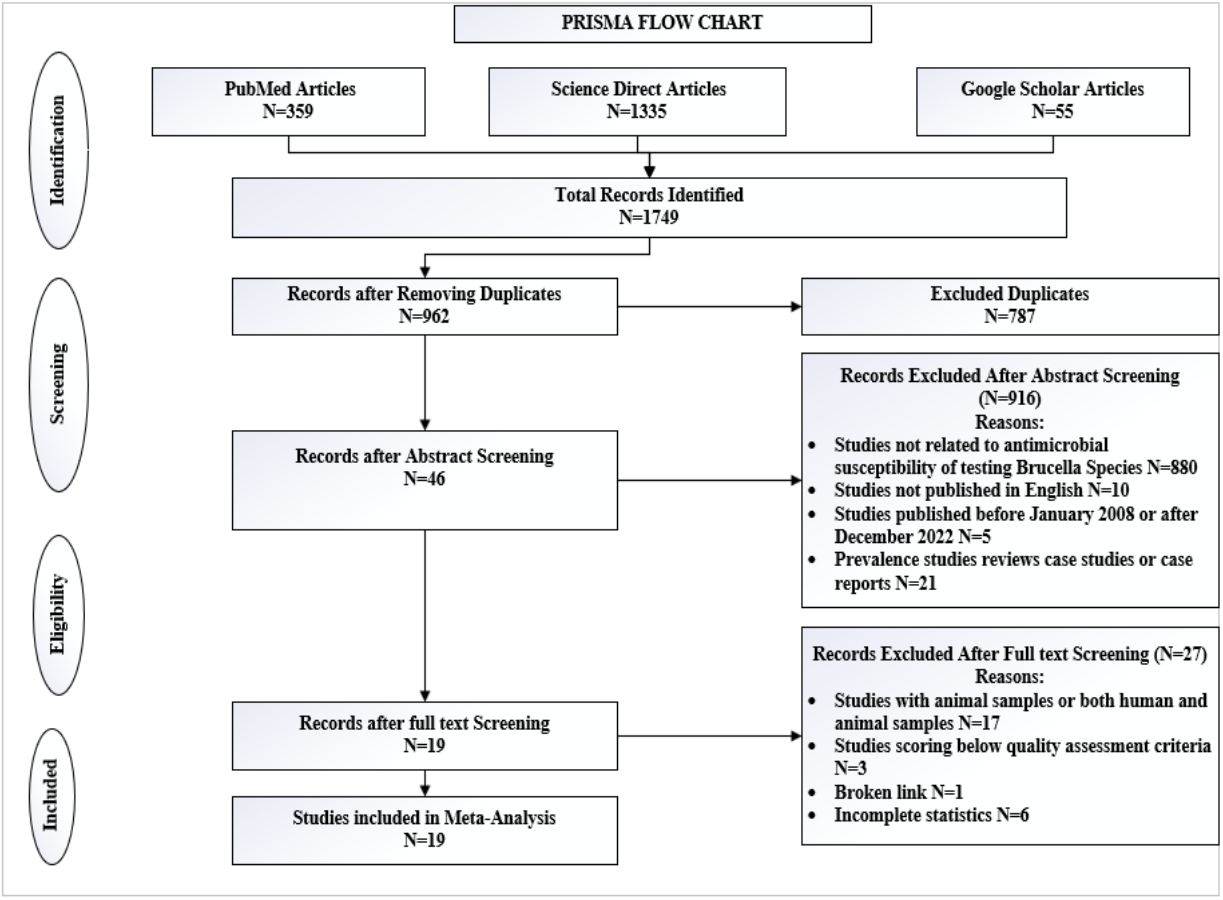


Figure 4.1 Preferred Items for Systematic Reviews and Meta-Analysis (PRISMA) Flow Chart

Across the 19 studies, a total of 24 antibiotics were documented, representing 10 antibiotic classes. Table 4.1 summarizes the distribution of antibiotics.

Table 4.1 Distribution of Antibiotics

Antibiotic Classes	**Number of studies (Class)**	**Specific Antibiotics**	**Number of studies (Antibiotics)**
Aminoglycosides	17	Streptomycin Gentamicin Amikacin	14 11 2
Ansamycin	19	Rifampicin	19
Beta-Lactams	3	Ampicillin Ceftazidime-avibactam Ampicillin-sulbactam Amoxicillin Clavulanic acid	2 1 1 1
Cephalosporin	5	Ceftriaxone	5
Chloramphenicol	1	Chloramphenicol	1
Fluoroquinolones	13	Ciprofloxacin Moxifloxacin Levofloxacin Norfloxacin Ofloxacin Sparfloxacin	13 4 3 1 2 1
Macrolides	6	Clarithromycin Azithromycin	6 1
Sulfonamides	14	Cotrimoxazole Trimethoprim-Sulfamethoxazole	3 12
Tetracyclines	16	Doxycycline Tetracycline Minocycline	16 8 1
Tigecycline	1	Tigecycline	1

In all the 19 studies, *B. melitensis* was identified as the most isolated species. In two studies, other *Brucella* species were found in addition to *B. melitensis*. One Turkish study isolated two *B. abortus* strains (Parlak et al., 2013), while another study in Malaysia identified a single strain of *B. suis* (Hashim et al. 2014).

Three distinct antibiotic susceptibility approaches were utilized. Four out of the 19 studies utilized the broth micro dilution method (Jiang et al., 2010a; Kaya, O. et al. 2012; Rohaidah Hashim et al., 2014; Arapović et al., 2022). Of the four, a study conducted in Bosnia and Herzegovina employed three different types of media for the broth micro dilution: *Brucella* broth, Cation-Adjusted Mueller–Hinton Broth with 4% lysed Horse Blood, and Cation-Adjusted Mueller–Hinton Broth with 5% defibrinated sheep blood (Arapović et al. 2022). While disk diffusion was equally utilized by only a single study (Alamian et al. 2019), the other 14 studies employed the E-test gradient approach.

4.2 Meta-Analysis Results

Table 4.2 Pooled Prevalence of Resistant *Brucella* Isolates

	Number of Studies	Total Sample Size	Pooled Prevalence Resistant Isolates	95% LCL	95% HCL	Cochran's Q	I2	
	19	1798	0.09	0.06	0.13	74.29	76.00	
Subgroup Analysis by Countries								
Countries	**Number of Studies**	**Sample Size**	**Prevalence of Resistant Isolates**	**95% LCL**	**95% HCL**	**Cochran's Q**	**I2**	**Brucella Species**	**Reference**
Bosnia and Herzegovina	1	108	0.21	0.14	0.30			*B. melitensis*	Arapović, J. *et al* . (2022)
China	3	75	0.20	0.11	0.31	2.37	16%	*B. melitensis*	*Xu, X. L. et al. (2013), Jiang, H. et al.(2010), Yuan H. T. et al.* (2020)
Iran	3	165	0.04	0.02	0.08	0.6	0%	*B. melitensis*	*Torkaman Asadi, F. et al. (2017), Alamian, S. et al.(2019),*
Kazakhstan	1	329	0.13	0.10	0.17	0.05	0%	*B. melitensis*	*Shevtsov, A. et al. (2017)*
Eygpt	1	355	0.09	0.06	0.13			*B. melitensis*	*Abdel-Maksoud, M. et al. (2012)*
Malaysia	1	41	0.12	0.04	0.24			*B. melitensis, B. suis*	*Hashim, R. et al. (2014)*
Mongolia	1	85	0.05	0.01	0.10			*B. melitensis*	*Liu, Z. et al. (2018).*
Peru	1	48	0.00	0.00	0.04			*B. melitensis*	*Maves, R. C. et al. (2011).*
Qatar	1	231	0.07	0.04	0.11			*B. melitensis*	*Deshmukh, A. et al. (2015).*
Turkey	6	361	0.06	0.06	0.13	18.86	73%	*B. melitensis, B. abortus*	*Sayan, M. et al. (2008), Sayan, M. et al. (2012), Kaya O. et al. (2012), Etiz P. et al. (2015), Gultekin, E. et al (2011), Parlak, M. et al. (2013)*

The analysis in Table 4.2 showed the global prevalence of antibiotic resistant *Brucella* strains to be 9% (95% CL: 6- 13%), with an estimated I^2 of 76%. A subgroup analysis based on

countries revealed insignificant heterogeneity, suggesting that the variation could be attributed to geographical differences (see Additional file 2 for forest plot). The observed prevalence of antibiotic resistance in individual countries was: Bosnia and Herzegovina 21% (95% CL: 14-30%), China 20% (95% CL: 11-31%), Egypt 9% (95% CL: 7-11%), Iran 4% (95%CL: 2-7%), Kazakhstan 13% (95%CL: 10-17%), Malaysia 12% (95%CL: 4-24%), Mongolia 5% (95% CL: 1-10%), Peru 0% (95%CL: 0-4%), Qatar 7% (95%CL: 4-11%), and Turkey 6% (95%CL: 6-12%).

4.2.1 Antibiotic Resistance Results

Table 4.3 Antibiotic Performance against *Brucella* Strains

Antibiotic Class	Number of Studies	Sample Size	Prevalence of Resistant Isolates	95% LCL	95% HCL	Cochran's Q	I^2
Aminoglycosides	17	1,683	0.01	0	0.01	18.07	11%
Ansamycins	19	1,798	0.35	0.17	0.56	711.32	97%
Beta-Lactam	3	129	0.07	0	0.26	19.6	90%
Cephalosporins	6	636	0	0	0.01	1.17	0%
Fluoroquinolones	13	1,232	0	0	0.01	3.85	0%
Macrolides	6	333	0.58	0	1	425.07	99%
Sulfonamides	14	1,233	0.04	0	0.19	622.24	98%
Tetracyclines	16	1,663	0	0	0	2.66	0%

Table 4.3 shows *Brucella* strains' antibiotic performance, with Macrolides having the highest resistance prevalence at 58% (95% CI: 0-100%) followed by ansamycins at 35% (95% CI: 17-56%), then beta-lactams at 7% (95% CI: 0-26%), sulfonamides at 4% (95% CI: 0-19%), aminoglycosides at 1% (0-1%), and fluoroquinolones, tetracycline, cephalosporin at 0% (95% CI: 01%) resistance.

4.2.2 Publication Bias Results

The study used a Doi plot to assess publication bias, revealing a Luis-Kanamori (LFK) index of 0.03 (Additional File 3) , indicating no publication bias, according to (Chen et al. 2022). In the absence of asymmetry, it would be expected that a perpendicular line to the X-axis from the tip of the Doi plot would divide the plot into two regions with similar areas (Furuya-Kanamori and Doi 2021). Indicating that the studies are equally distributed and not skewed to one specific area of interest.

CHAPTER FIVE

5. DISCUSSION

This study was done to determine the prevalence of antibiotic resistant *Brucella* strains and to evaluate the most effective antibiotics against *Brucella*. The study included 20 research articles representing the countries; Bosnia and Herzegovina, China, Egypt, Iran, Qatar, Peru, Mongolia, Malaysia, Turkey and Kazakhstan.

5.1 Antibiotic resistance

The study analyzed the resistance to macrolides (Additional file 4), specifically azithromycin and clarithromycin, in six studies. Azithromycin was found in all six studies, while clarithromycin was only recorded in one. The resistance rates ranged from 46% (Bayram et al. 2011), to 100% (Yuan et al. 2020; Jiang et al. 2010a) with only two studies reporting antibiotic susceptibility to azithromycin. Azithromycin's pharmacokinetic profile shows high concentrations in cells and tissues, but its activity against *Brucella* species is 6- to 8-fold lower at pH 5.0 compared to pH 7.0 (Solera et al. 2001). Considering that *Brucella* species grow and replicate in the phagolysosomes of macrophages, where the pH is 5.0 (Solera et al. 2001; Celli et al. 2003).

Brucella are still vulnerable to doxycycline and rifampicin, which are the most frequently used therapies for brucellosis in humans (D. Trott et al. 2018). Most primary studies on brucellosis susceptibility to antibiotics, show resistance to rifampicin (Additional file 5). In a recent study conducted in China, *B. melitensis* exhibited notable levels of antibiotic resistance, with 24.6% resistant to rifampin, 86.9% to azithromycin, 65.6% to cefepime, 27.9% to cefoperazone/sulbactam, 3.3% to cefotaxime, and 1.6% to meperidine/sulfamethoxazole (Ma et al. 2023). A comprehensive analysis comparing treatment failure rates between doxycycline plus rifampicin and doxycycline plus streptomycin concluded that the doxycycline plus rifampicin regimen was less effective, with a risk ratio (RR) of 1.91 and a recurrence rate of 2.39 (95% CI 1.17 to 4.86) (Yousefi-Nooraie et al. 2012). A study in Kazakhstan suggested that the resistance in rifampicin *Brucella* develops in conjunction to a rise in multidrug-resistant tuberculosis (Shevtsov et al. 2017)

This study comprised of 15 studies that conducted antibiotic susceptibility testing on *Brucella* strains with sulfonamides (Additional file 6). Out of them, two studies documented the presence of resistance. A study conducted in China discovered that all tested strains were

resistant to trimethoprim-sulfamethoxazole (Xu et al. 2013). Similarly, a separate study conducted in Bosnia and Herzegovina reported that 84.3% of *Brucella* isolates exhibited resistance to trimethoprim-sulfamethoxazole when tested using micro dilution: *Brucella* Broth (Arapović et al. 2022). Although several studies have found minimal or no resistance to sulfonamides, a few investigations have also reported resistance to sulfonamides (Xu et al. 2013; Arapović et al. 2022). A recent study conducted in Saudi Arabia revealed a resistance rate of 36.36% to trimethoprim-sulfamethoxazole (Elbehiry et al. 2022b). Furthermore, (Arapović et al. 2022) concluded that test isolates for susceptibility to trimethoprim-sulfamethoxazole and other sulfonamide drugs should be done using medium with low or no thymine and thymidine. Thymine and thymidine weaken the drugs' efficacy, making susceptible bacteria appear resistant. Therefore, more investigation into the antibiotic resistance of *Brucella* strains to sulfonamides is necessary.

Beta-lactams were examined in four investigations, with two demonstrating resistance and two showing complete antibiotic susceptibilities (Additional file 7). Although cephalosporin and beta-lactam showed little or no resistance, they are less efficient in vivo, resulting in brucellosis relapses not because of antimicrobial resistance of *Brucella* strains but due the bacteria's capacity to live in host cells away from antibiotics (Torkaman Asadi et al. 2017).

Tetracycline, fluoroquinolones, and cephalosporin exhibited 0% resistance (Additional file 8, 9 and 10), with the exception of aminoglycosides (Additional file 11). Two studies documented resistance to streptomycin and gentamycin, one from Egypt (Abdel-Maksoud et al. 2012) and the other Kazakhstan (Shevtsov et al. 2017) respectively. Gentamicin has broad antibacterial activity but low intracellular levels, making it less effective for brucellosis treatment. However, it is more active than streptomycin and has shown greater efficacy in combined treatment regimens (M. Alavi and Nokhodchi 2023). On the other hand, ceftriaxone, an antibiotic active against *B. melitensis*, has no specific clinical recommendations due to current treatment based on small case series and anecdotal reports, but positive outcomes have been reported with mild adverse reactions (Fatani et al. 2019). Quinolones alone in brucellosis treatment are considered cautious due to bacterial resistance risk, and further trial work is needed to determine their effect (S. M. Alavi and Alavi 2013).

5.2 Prevalence of Antibiotic Resistance per Country

The study revealed a 21% antibiotic resistance rate to brucellosis in China, which could be attributed to an increase in incidence from 63.7% in 2016 to 65.9% in 2019 (Tao et al. 2021). The incidence of brucellosis has not yet been effectively suppressed and the resistance

of *Brucella* to common antibiotics is increasing every year (Ma et al. 2023). Xi'an, the largest city in Northwest China, has seen a 20-fold increase in brucellosis incidence over the past decade, despite being a traditional non-epidemic region (Zhao et al. 2022) The spread of brucellosis in China is linked to animal farming practices and antibiotic resistance (Lai et al. 2017).

In this study, Turkey has one of the lowest resistance rates of 6% to brucellosis, despite having the most representation. Brucellosis is an endemic in Turkey due to under-diagnosis and under-reporting of the disease (Yumuk and O'Callaghan 2012). However, Turkey has experienced a decrease in brucellosis prevalence due to animal disease control programs, vaccination campaigns and a new national brucellosis control project that was initiated in Turkey in 2009, following European Community Council directives (Gül et al. 2014). Turkey's rich economy, advanced healthcare system, and top universities provide ample opportunities for comprehensive research on human brucellosis physiology and immune pathology (Yumuk and O'Callaghan 2012). Additionally, Iran also had a low antibiotic resistance rate of 4%. This low resistance mirrors findings from a systematic study on drug-resistant *Brucella* in Iran conducted by Khademi et al. in 2018 (Khademi et al. 2018).

The prevalence of antibiotic resistance in countries like Bosnia and Herzegovina, Kazakhstan, Malaysia, Egypt, Qatar, Mongolia, and Peru may be inaccurately estimated based on a single primary study recorded for each country. This could lead to significant overestimation or underestimation, particularly for Bosnia and Herzegovina at 21% and Peru at 0%.

5.3 Limitations and Challenges

Underreporting of brucellosis is a significant issue in accurately assessing the true global prevalence of antibiotic resistance of the disease. The underreporting of brucellosis can be attributed to several key factors. The livestock value chain encounters difficulties in comprehending brucellosis due to insufficient knowledge, limited diagnostic capabilities, challenges with diagnostic tests, and uneven distribution of surveillance studies (Mengele et al. 2023). The primary reason for the underreporting of brucellosis is the presence of nonspecific signs and symptoms, which often result in misdiagnosis (Facciolà et al. 2018).

Antibiotic categories like tigecycline and chloramphenicol were only featured in one study each. Therefore, a meta-analysis was not possible for these antibiotic classes due to the

limited number of study records available to calculate the total antibiotic resistance prevalence.

Antimicrobial susceptibility testing for *Brucella* species is hardly performed because of the high infectiousness and hazardous nature of the bacteria (Arapović et al., 2022). As a result of this factor, there is a scarcity of research that specifically examines the antibiotic resistance of *Brucella,* particularly in underdeveloped nations where there is lack of adequate laboratory facilities that can safely handle hazardous bacteria. In addition, brucellosis is a neglected disease, and the research on its antibiotic resistance patterns is not given priority in most countries. The scarcity of research conducted on the antimicrobial resistance of *Brucella* pathogens, was the primary restriction of the study. Another limitation encountered in this study was lack of completed statistics in the some of the available studies on the antibiotic resistance of *Brucella* pathogens.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the study highlights the increasing prevalence of antibiotic resistant *Brucella* strains. The results show significant resistance to macrolides, ansamycins, and sulfonamides, while tetracycline and aminoglycosides remain the most effective antibiotics. *Brucella* strains showed little or no resistance to fluoroquinolones and cephalosporins but very little research has been done clinically to explore their efficacy in the treatment of brucellosis.

6.2 Recommendations

In view of the finding in this study, it is recommended that an alternative to rifampicin be considered as a primary antibiotic for treating brucellosis, given the potential risk of multi-drug resistance that could extend to other diseases, such as tuberculosis, which also rely on this drug. Tetracyclines and aminoglycosides are suggested as viable alternatives, due to their continued effectiveness against *Brucella* strains.

Further research is needed to investigate the antibiotic resistance of *Brucella* on a worldwide scale and within individual countries. Most importantly, implementing more advanced surveillance systems, enhancing diagnostic skills, increased international collaboration and implementing comprehensive public health control programs will help curb the rising prevalence of antibiotic resistant *Brucella* strains.

7. REFERENCES

Abd elbaser, Elsayed, and Heba Mohammed. 2018. “Brucellosis Relapse: A Retrospective Study of Risk Factors among Saudi Patients.” *Afro-Egyptian Journal of Infectious and Endemic Diseases* 8 (3). https://doi.org/10.21608/aeji.2018.13526.

Abdel-Maksoud, Mohamed, Brent House, Momtaz Wasfy, Bassem Abdel-Rahman, Guillermo Pimentel, Gehan Roushdy, and Erica Dueger. 2012. “In Vitro Antibiotic Susceptibility Testing of Brucella Isolates from Egypt between 1999 and 2007 and Evidence of Probable Rifampin Resistance.” *Annals of Clinical Microbiology and Antimicrobials* 11 (August). https://doi.org/10.1186/1476-0711-11-24.

About, Frédégonde, Theo Pastre, Mathilde Boutrou, Alex Yahiaoui Martinez, Alessia Melzani, Sandrine Peugny, Céline Michaud, et al. 2023. “Novel Species of Brucella Causing Human Brucellosis, French Guiana.” *Emerging Infectious Diseases* 29 (2): 333–40. https://doi.org/10.3201/eid2902.220725.

Adesokan, H. K., P. I. Alabi, and M. A. Ogundipe. 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 (3).

Adetunji, Shakirat A., Gilbert Ramirez, Margaret J. Foster, and Angela M. Arenas-Gamboa. 2019. “A Systematic Review and Meta-Analysis of the Prevalence of Osteoarticular Brucellosis.” *PLOS Neglected Tropical Diseases* 13 (1): e0007112. https://doi.org/10.1371/JOURNAL.PNTD.0007112.

Akar, Kadir, and Osman Erganis. 2022. “Evaluation of the Genetic Profiles of Brucella Melitensis Strain from Turkey Using Multilocus Variable Number Tandem Repeat Analysis (MLVA) and Multilocus Sequence Typing (MLST) Techniques.” *Veterinary Microbiology* 269. https://doi.org/10.1016/j.vetmic.2022.109423.

Alamian, Saeed, Maryam Dadar, Afshar Etemadi, Davoud Afshar, and Mohammad Mehdi Alamian. 2019. “Antimicrobial Susceptibility of Brucella Spp. Isolated from Iranian Patients during 2016 to 2018.” *Iranian Journal of Microbiology* 11 (5): 363–67. https://doi.org/10.18502/ijm.v11i5.1953.

Alavi, Mehran, and Ali Nokhodchi. 2023. “Micro- and Nanoformulations of Antibiotics

against Brucella." *Drug Discovery Today*. https://doi.org/10.1016/j.drudis.2023.103809.

Alavi, Seyed Mohammad, and Leila Alavi. 2013. "Treatment of Brucellosis: A Systematic Review of Studies in Recent Twenty Years." *Caspian Journal of Internal Medicine*.

Alhabbab, Rowa Yousef. 2018. "Stained Brucella Suspensions," 41–48. https://doi.org/10.1007/978-3-319-77694-1_6.

Arapović, Jurica, Gordan Kompes, Kanita Dedić, Sanela Teskeredžić, Maja Ostojić, Maja Travar, Nijaz Tihić, et al. 2022. "Antimicrobial Resistance Profiles of Human Brucella Melitensis Isolates in Three Different Microdilution Broths: The First Multicentre Study in Bosnia and Herzegovina." *Journal of Global Antimicrobial Resistance* 29 (June): 99–104. https://doi.org/10.1016/j.jgar.2022.02.005.

Bahmani, Nasrin, and Afshin Bahmani. 2022. "A Review of Brucellosis in the Middle East and Control of Animal Brucellosis in an Iranian Experience." *Reviews and Research in Medical Microbiology* 33 (1): E63–69. https://doi.org/10.1097/MRM.0000000000000266.

Baldi, P. C., and G. H. Giambartolomei. 2013. "Pathogenesis and Pathobiology of Zoonotic Brucellosis in Humans." *OIE Revue Scientifique et Technique* 32 (1). https://doi.org/10.20506/rst.32.1.2192.

Bamaiyi, PH. 2016. "Prevalence and Risk Factors of Brucellosis in Man and Domestic Animals: A Review." http://ir.kiu.ac.ug/handle/20.500.12306/1533.

Baruho, J. 2022. "Knowledge Attitude and Practices towards Prevention of Brucellosis among Cattle Keepers Aged (15-45) Years in Lyantonde General Hospital Lyantonde District. A." *Sjhresearchafrica.Org*. https://sjhresearchafrica.org/index.php/public-html/article/view/157.

Bayram, Yasemin, Hanifi Korkoca, Cenk Aypak, Mehmet Parlak, Aytekin Cikman, Selcuk Kilic, and Mustafa Berktas. 2011. "Antimicrobial Susceptibilities of Brucella Isolates from Various Clinical Specimens." *International Journal of Medical Sciences* 8 (3): 198–202. https://doi.org/10.7150/ijms.8.198.

Bertu, Wilson J., Marie J. Ducrotoy, Pilar M. Muñoz, Virginie Mick, Amaia Zúñiga-Ripa, Ward Bryssinckx, Jacob K.P. Kwaga, et al. 2015. "Phenotypic and Genotypic Characterization of Brucella Strains Isolated from Autochthonous Livestock Reveals the Dominance of B. Abortus Biovar 3a in Nigeria." *Veterinary Microbiology* 180 (1–2).

https://doi.org/10.1016/j.vetmic.2015.08.014.

Billard, Elisabeth, Jacques Dornand, and Antoine Gross. 2007. “Brucella Suis Prevents Human Dendritic Cell Maturation and Antigen Presentation through Regulation of Tumor Necrosis Factor Alpha Secretion.” *Infection and Immunity* 75 (10). https://doi.org/10.1128/IAI.00637-07.

Bodenham, Rebecca F., Abdul Hamid S. Lukambagire, Roland T. Ashford, Joram J. Buza, Shama Cash-Goldwasser, John A. Crump, Rudovick R. Kazwala, et al. 2020. “Prevalence and Speciation of Brucellosis in Febrile Patients from a Pastoralist Community of Tanzania.” *Scientific Reports* 10 (1). https://doi.org/10.1038/S41598-020-62849-4.

Bonaventura, Giovanni Di, Silvia Angeletti, Andrea Ianni, Tommasangelo Petitti, and Giovanni Gherardi. 2021. “Microbiological Laboratory Diagnosis of Human Brucellosis: An Overview.” *Pathogens*. https://doi.org/10.3390/pathogens10121623.

Bosilkovski, Mile, Ljiljana Krteva, Sonja Caparoska, Nikola Labacevski, and Mile Petrovski. 2015. “Childhood Brucellosis: Review of 317 Cases.” *Asian Pacific Journal of Tropical Medicine* 8 (12): 1027–32. https://doi.org/10.1016/j.apjtm.2015.11.009.

Brangsch, Hanka, Vassilios Sandalakis, Maria Babetsa, Evridiki Boukouvala, Artemisia Ntoula, Eirini Makridaki, Athanasia Christidou, et al. 2023. “Genotype Diversity of Brucellosis Agents Isolated from Humans and Animals in Greece Based on Whole-Genome Sequencing.” *BMC Infectious Diseases* 23 (1): 529.

Carrique-Mas, Juan J., Nguyen V. Trung, Ngo T. Hoa, Ho Huynh Mai, Tuyen H. Thanh, James I. Campbell, Jaap A. Wagenaar, Anita Hardon, Thai Quoc Hieu, and Constance Schultsz. 2015. “Antimicrobial Usage in Chicken Production in the Mekong Delta of Vietnam.” *Zoonoses and Public Health* 62 (s1). https://doi.org/10.1111/zph.12165.

Castañeda-Roldán, Elsa I., Fabiola Avelino-Flores, Monique Dall’Agnol, Enrique Freer, Lilia Cedillo, Jacques Dornand, and Jorge A. Girón. 2004. “Adherence of Brucella to Human Epithelial Cells and Macrophages Is Mediated by Sialic Acid Residues.” *Cellular Microbiology* 6 (5). https://doi.org/10.1111/j.1462-5822.2004.00372.x.

CDC. 2012. “Areas at Risk | Risk of Exposure| Brucellosis | CDC.” Cdc. 2012. https://www.cdc.gov/brucellosis/exposure/areas.html.

———. 2019. “Transmission | Brucellosis | CDC.” Centers of Disease Control and

Prevention. 2019. https://www.cdc.gov/brucellosis/transmission/index.html.

Celli, Jean, Chantal De Chastellier, Don Marc Franchini, Javier Pizarro-Cerda, Edgardo Moreno, and Jean Pierre Gorvel. 2003. "Brucella Evades Macrophage Killing via VirB-Dependent Sustained Interactions with the Endoplasmic Reticulum." *Journal of Experimental Medicine* 198 (4). https://doi.org/10.1084/jem.20030088.

Chandler J, J Cumpston M Li T Page M J Welch V A (editors) Higgins J P T Thomas. 2022. *Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (Updated February 2022). The Cochrane Collaboration*.

Chen, Xi, Jiyao Chen, Meimei Zhang, Rebecca Kechen Dong, Jizhen Li, Zhe Dong, Yingying Ye, et al. 2022. "Meta-Regression on the Heterogenous Factors Contributing to the Prevalence of Mental Health Symptoms During the COVID-19 Crisis Among Healthcare Workers." *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2022.833865.

Coelho, Ana Cláudia, Juan García Díez, and Adosinda Maria Coelho. 2015. "Risk Factors for Brucella Spp. in Domestic and Wild Animals." In *Updates on Brucellosis*. https://doi.org/10.5772/61325.

Corbel, Michael J. 2020. "Brucellosis: Epidemiology and Prevalence Worldwide." In *Brucellosis: Clinical and Laboratory Aspects*. https://doi.org/10.1201/9781003068518-5.

Dadar, Maryam, Yadolah Fakhri, Youcef Shahali, and Amin Mousavi Khaneghah. 2020. "Contamination of Milk and Dairy Products by Brucella Species: A Global Systematic Review and Meta-Analysis." *Food Research International* 128 (February): 108775. https://doi.org/10.1016/J.FOODRES.2019.108775.

Dadar, Maryam, Sharaf S. Omar, Youcef Shahali, Yadolah Fakhri, Jacques Godfroid, and Amin Mousavi Khaneghah. 2022. "The Prevalence of Camel Brucellosis and Associated Risk Factors: A Global Meta-Epidemiological Study." *Quality Assurance and Safety of Crops & Foods* 14 (3): 55–93. https://doi.org/10.15586/QAS.V14I3.1088.

Dadar, Maryam, Ruchi Tiwari, Khan Sharun, and Kuldeep Dhama. 2021. "Importance of Brucellosis Control Programs of Livestock on the Improvement of One Health." *Https://Doi.Org/10.1080/01652176.2021.1894501* 41 (1): 137–51. https://doi.org/10.1080/01652176.2021.1894501.

Darbandi, Atieh, Maryam Koupaei, Tahereh Navidifar, Soheila Shahroodian, Mohsen Heidary, and Malihe Talebi. 2022. “Brucellosis Control Methods with an Emphasis on Vaccination: A Systematic Review.” *Expert Review of Anti-Infective Therapy* 20 (7): 1025–35. https://doi.org/10.1080/14787210.2022.2066521.

Díaz Aparicio, E. 2013. “Epidemiology of Brucellosis in Domestic Animals Caused by Brucella Melitensis, Brucella Suis and Brucella Abortus.” *Revue Scientifique et Technique - Office International Des Épizooties* 32 (1).

Djangwani, Juvenal, George Ooko Abong’, Lucy Gicuku Njue, and Dasel W.M. Kaindi. 2021. “Brucellosis: Prevalence with Reference to East African Community Countries – A Rapid Review.” *Veterinary Medicine and Science* 7 (3): 851–67. https://doi.org/10.1002/VMS3.425.

Dorneles, Elaine M.S., Nammalwar Sriranganathan, and Andrey P. Lage. 2015. “Recent Advances in Brucella Abortus Vaccines.” *Veterinary Research* 46 (1). https://doi.org/10.1186/s13567-015-0199-7.

Ducrotoy, M., W. J. Bertu, G. Matope, S. Cadmus, R. Conde-Álvarez, A. M. Gusi, S. Welburn, R. Ocholi, J. M. Blasco, and I. Moriyón. 2017. “Brucellosis in Sub-Saharan Africa: Current Challenges for Management, Diagnosis and Control.” *Acta Tropica* 165 (January): 179–93. https://doi.org/10.1016/J.ACTATROPICA.2015.10.023.

Ebani, Virginia, Maryam Dadar, Saeed Alamian, Hanka Brangsch, Mohamed Elbadawy, Ahmed R Elkharsawi, Heinrich Neubauer, and Gamal Wareth. 2023. “Determination of Virulence-Associated Genes and Antimicrobial Resistance Profiles in Brucella Isolates Recovered from Humans and Animals in Iran Using NGS.” *Mdpi.Com*. https://doi.org/10.3390/pathogens12010082.

Efrem, Ghebremeskel Habteyohannes, Bereket Mihreteab, Michael K. Ghebremariam, Tekeste Okbamichael, Yosief Ghebresilasie, Siobhan M. Mor, and Gezahegne Mamo. 2023. “Prevalence of Brucellosis and Associated Risk Factors in Dairy Cattle in Maekel and Debub Regions, Eritrea.” *Frontiers in Veterinary Science* 10. https://doi.org/10.3389/fvets.2023.1177572.

El-Sayed, Amr, and Walid Awad. 2018. “Brucellosis: Evolution and Expected Comeback.” *International Journal of Veterinary Science and Medicine*. https://doi.org/10.1016/j.ijvsm.2018.01.008.

Elbehiry, Ayman, Musaad Aldubaib, Osamah Al Rugaie, Eman Marzouk, Marwan Abaalkhail, Ihab Moussa, Mohamed H. El-Husseiny, Adil Abalkhail, and Mohammed Rawway. 2022a. “Proteomics-Based Screening and Antibiotic Resistance Assessment of Clinical and Sub-Clinical Brucella Species: An Evolution of Brucellosis Infection Control.” *PLoS ONE* 17 (1 January). https://doi.org/10.1371/journal.pone.0262551.

———. 2022b. “Proteomics-Based Screening and Antibiotic Resistance Assessment of Clinical and Sub-Clinical Brucella Species: An Evolution of Brucellosis Infection Control.” *PLoS ONE* 17 (1 January). https://doi.org/10.1371/JOURNAL.PONE.0262551.

Etemadi, Afshar, Rezvan Moniri, Heinrich Neubauer, Yasaman Dasteh Goli, and Saeed Alamian. 2019. “Laboratory Diagnostic Procedures for Human Brucellosis: An Overview of Existing Approaches.” *Jundishapur Journal of Microbiology*. https://doi.org/10.5812/jjm.91200.

European Centre for Disease Prevention and Control. 2022. “Annual Epidemiological Report for 2020 Surveillance Report.” European Centre for Disease Prevetion and Control. 2022. https://www.ecdc.europa.eu/en/publications-data/brucellosis-annual-epidemiological-report-2020.

Facciolà, Alessio, Maria A.R. Palamara, Giuseppa D’Andrea, Fernanda Marano, Domenico Magliarditi, Giovanni Puglisi, Isa Picerno, Angela Di Pietro, and Giuseppa Visalli. 2018. “Brucellosis Is a Public Health Problem in Southern Italy: Burden and Epidemiological Trend of Human and Animal Disease.” *Journal of Infection and Public Health* 11 (6). https://doi.org/10.1016/j.jiph.2018.07.007.

Faramarzi, Hossein, Maryam Nasiri, Mahmood Khosravi, Abouzar Keshavarzi, and Ahmad Reza Rezaei Ardakani. 2019. “Potential Effects of Climatic Parameters on Human Brucellosis in Fars Province, Iran, during 2009-2015.” *Iranian Journal of Medical Sciences* 44 (6). https://doi.org/10.30476/ijms.2019.44968.

Fatani, Daniah F., Walaa A. Alsanoosi, Mazen A. Badawi, and Abrar K. Thabit. 2019. “Ceftriaxone Use in Brucellosis: A Case Series.” *IDCases* 18. https://doi.org/10.1016/j.idcr.2019.e00633.

Ficht, Thomas. 2010. “Brucella Taxonomy and Evolution.” *Future Microbiology*. https://doi.org/10.2217/fmb.10.52.

Figueiredo, Paul De, Thomas A. Ficht, Allison Rice-Ficht, Carlos A. Rossetti, and L. Garry Adams. 2015. “Pathogenesis and Immunobiology of Brucellosis: Review of Brucella-Host Interactions.” *American Journal of Pathology*. https://doi.org/10.1016/j.ajpath.2015.03.003.

Franc, K. A., R. C. Krecek, B. N. Häsler, and A. M. Arenas-Gamboa. 2018. “Brucellosis Remains a Neglected Disease in the Developing World: A Call for Interdisciplinary Action.” *BMC Public Health* 18 (1). https://doi.org/10.1186/S12889-017-5016-Y.

Frean, John, Alicia Cloete, Jenny Rossouw, and Lucille Blumberg. 2016. “Brucellosus in South Africa - A Notifiable Medical Condition.” *DAFF* 16 (3).

Fritz, Curtis L., Alyssa Nguyen, and Duc J. Vugia. 2021a. “Epidemiology of Brucellosis in California, 1993-2017: A Continuing Foodborne Disease Risk for Older Latinos.” *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 73 (11): 2023–30. https://doi.org/10.1093/CID/CIAB551.

———. 2021b. “Epidemiology of Brucellosis in California, 1993-2017: A Continuing Foodborne Disease Risk for Older Latinos.” *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 73 (11): 2023–30. https://doi.org/10.1093/CID/CIAB551.

Furuya-Kanamori, Luis, Jan J. Barendregt, and Suhail A.R. Doi. 2018. “A New Improved Graphical and Quantitative Method for Detecting Bias in Meta-Analysis.” *International Journal of Evidence-Based Healthcare* 16 (4). https://doi.org/10.1097/XEB.0000000000000141.

Furuya-Kanamori, Luis, and Suhail AR Doi. 2021. “LFK: Stata Module to Compute LFK Index and Doi Plot for Detection of Publication Bias in Meta-Analysis.” *Statistical Software Components*.

Głowacka, Patrycja, Dorota Zakowska, Katarzyna Naylor, Marcin Niemcewicz, and Agata Bielawska-Drózd. 2018. “Brucella – Virulence Factors, Pathogenesis and Treatment.” *Polish Journal of Microbiology* 67 (2): 151–61. https://doi.org/10.21307/pjm-2018-029.

Godfroid, J, B Garin-Bastuji, … C Saegerman - Revue Scientifique et, and undefined 2013. 2013. “Brucellosis in Terrestrial Wildlife.” *Orbi.Uliege.Be* 32 (1): 27–42. https://orbi.uliege.be/handle/2268/168850.

Godfroid, Jacques. 2018. “Brucella Spp. at the Wildlife-Livestock Interface: An Evolutionary

Trajectory through a Livestock-to-Wildlife 'Host Jump'?" *Veterinary Sciences* 5 (3). https://doi.org/10.3390/vetsci5030081.

Gong, Qing Long, Yu Han Sun, Yang Yang, Bo Zhao, Qi Wang, Jian Ming Li, Gui Yang Ge, et al. 2021. "Global Comprehensive Literature Review and Meta-Analysis of Brucella Spp. in Swine Based on Publications From 2000 to 2020." *Frontiers in Veterinary Science* 8 (May): 405. https://doi.org/10.3389/FVETS.2021.630960/BIBTEX.

González-Espinoza, Gabriela, Vilma Arce-Gorvel, Sylvie Mémet, and Jean Pierre Gorvel. 2021. "Brucella: Reservoirs and Niches in Animals and Humans." *Pathogens*. https://doi.org/10.3390/pathogens10020186.

Govindasamy, K. 2020. "Human Brucellosis in South Africa: A Review for Medical Practitioners." *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde* 110 (7): 646–51. https://doi.org/10.7196/SAMJ.2020.V110I7.14538.

Graham, David W., Gilles Bergeron, Megan W. Bourassa, James Dickson, Filomena Gomes, Adina Howe, Laura H. Kahn, et al. 2019. "Complexities in Understanding Antimicrobial Resistance across Domesticated Animal, Human, and Environmental Systems." *Annals of the New York Academy of Sciences*. https://doi.org/10.1111/nyas.14036.

Gül, Serdar, Özgün Kiris Satilmis, Baris Ozturk, Mehmet Ilker Gökçe, and Ferit Kuscu. 2014. "Seroprevalence of Brucellosis among Children in the Middle Anatolia Region of Turkey." *Journal of Health, Population and Nutrition* 32 (4).

Gültekin, Esra, Muhammet Hamidullah Uyanık, Ayşe Albayrak, and Selçuk Kılıç. 2021a. "Investigation of Antibiotic Susceptibilities of Brucella Strains Isolated from Various Clinical Samples in Eastern Turkey." *European Journal of Medical Research* 26 (1). https://doi.org/10.1186/s40001-021-00527-5.

———. 2021b. "Investigation of Antibiotic Susceptibilities of Brucella Strains Isolated from Various Clinical Samples in Eastern Turkey." *European Journal of Medical Research* 26 (1): 1–6. https://doi.org/10.1186/s40001-021-00527-5.

Harris, Mohammed, Tracy Fasolino, Diana Ivankovic, Nicole J. Davis, and Noel Brownlee. 2023. "Genetic Factors That Contribute to Antibiotic Resistance through Intrinsic and Acquired Bacterial Genes in Urinary Tract Infections." *Microorganisms*. https://doi.org/10.3390/microorganisms11061407.

Hashim, Rohaidah, Norazah Ahmad, Jama ' Ayah, Mohamed Zahidi, B Y Tay, Azura Mohd

Noor, Sakina Zainal, et al. 2014. “Identification and In Vitro Antimicrobial Susceptibility of Brucella Species Isolated from Human Brucellosis.” https://doi.org/10.1155/2014/596245.

Heidary, Mohsen, Shirin Dashtbin, Roya Ghanavati, Marzie Mahdizade Ari, Narjess Bostanghadiri, Atieh Darbandi, Tahereh Navidifar, and Malihe Talebi. 2022a. “Evaluation of Brucellosis Vaccines: A Comprehensive Review.” *Frontiers in Veterinary Science* 9 (July): 925773. https://doi.org/10.3389/FVETS.2022.925773.

———. 2022b. “Evaluation of Brucellosis Vaccines: A Comprehensive Review.” *Frontiers in Veterinary Science* 9 (July): 902. https://doi.org/10.3389/FVETS.2022.925773/BIBTEX.

Holt, Hannah R., Jasbir Singh Bedi, Paviter Kaur, Punam Mangtani, Narinder Singh Sharma, Jatinder Paul Singh Gill, Yogeshwar Singh, et al. 2021. “Epidemiology of Brucellosis in Cattle and Dairy Farmers of Rural Ludhiana, Punjab.” *PLOS Neglected Tropical Diseases* 15 (3): e0009102. https://doi.org/10.1371/JOURNAL.PNTD.0009102.

Huy, Tran X.N., Trang T. Nguyen, Heejin Kim, Alisha W.B. Reyes, and Suk Kim. 2022. “Brucella Phagocytosis Mediated by Pathogen-Host Interactions and Their Intracellular Survival.” *Microorganisms*. https://doi.org/10.3390/microorganisms10102003.

Jiang, Hai, Ling ling Mao, Hong yan Zhao, Lan yu Li, Dong ri Piao, Wen qing Yao, and Bu yun Cui. 2010a. “MLVA Typing and Antibiotic Susceptibility of Brucella Human Isolates from Liaoning, China.” *Transactions of the Royal Society of Tropical Medicine and Hygiene* 104 (12): 796–800. https://doi.org/10.1016/j.trstmh.2010.08.002.

———. 2010b. “MLVA Typing and Antibiotic Susceptibility of Brucella Human Isolates from Liaoning, China.” *Transactions of the Royal Society of Tropical Medicine and Hygiene* 104 (12): 796–800. https://doi.org/10.1016/j.trstmh.2010.08.002.

Jin, Min, Zixu Fan, Ruifang Gao, Xingnan Li, Zhixiang Gao, and Zhanli Wang. 2023. “Research Progress on Complications of Brucellosis.” *Frontiers in Cellular and Infection Microbiology* 13 (March): 372. https://doi.org/10.3389/FCIMB.2023.1136674.

Jones, Bryony A., Delia Grace, Richard Kock, Silvia Alonso, Jonathan Rushton, Mohammed Y. Said, Declan McKeever, et al. 2013. “Zoonosis Emergence Linked to Agricultural Intensification and Environmental Change.” *Proceedings of the National Academy of Sciences of the United States of America* 110 (21): 8399–8404.

https://doi.org/10.1073/PNAS.1208059110/SUPPL_FILE/ST03.DOCX.

Kaya, Onur, Füsun Zeynep Akçam, and Güler Yayli. 2012. “Investigation of the in Vitro Activities of Various Antibiotics against Brucella Melitensis Strains.” *Turkish Journal of Medical Sciences* 42 (1). https://doi.org/10.3906/sag-1009-1129.

Keramat, Fariba, Mojgan Mamani, Maryam Adabi, Salman Khazaei, Zahra Shivapoor, and Manoochehr Karami. 2021. “Establishment of Brucellosis Relapse and Complications Registry: A Study Protocol.” *Journal of Preventive Medicine and Hygiene* 62 (2). https://doi.org/10.15167/2421-4248/jpmh2021.62.2.1422.

Khademi, Farzad, Arshid Yousefi-Avarvand, Amirhossein Sahebkar, Behnam Mohammadi-Ghalehbin, Mohsen Arzanlou, and Hadi Peeridogaheh. 2018. “Drug Resistance of Clinical and Environmental Isolates of Brucella Species in Iran: A Meta-Analysis.” *Reviews and Research in Medical Microbiology* 29 (4): 166–72. https://doi.org/10.1097/MRM.0000000000000148.

Khan, Muhammad Zahoor, and Muhammad Zahoor. 2018. “An Overview of Brucellosis in Cattle and Humans, and Its Serological and Molecular Diagnosis in Control Strategies.” *Tropical Medicine and Infectious Disease* 3 (2). https://doi.org/10.3390/TROPICALMED3020065.

Khurana, Sandip Kumar, Anju Sehrawat, Ruchi Tiwari, Minakshi Prasad, Baldev Gulati, Muhammad Zubair Shabbir, Rajesh Chhabra, et al. 2021. “Bovine Brucellosis–a Comprehensive Review.” *Veterinary Quarterly*. Taylor and Francis Ltd. https://doi.org/10.1080/01652176.2020.1868616.

Köhler, Stephan, Vincent Foulongne, Safia Ouahrani-Bettache, Gisèle Bourg, Jacques Teyssier, Michel Ramuz, and Jean Pierre Liautard. 2002. “The Analysis of the Intramacrophagic Virulome of Brucella Suis Deciphers the Environment Encountered by the Pathogen inside the Macrophage Host Cell.” *Proceedings of the National Academy of Sciences of the United States of America* 99 (24). https://doi.org/10.1073/pnas.232454299.

Kulinskaya, Elena, and Michael B. Dollinger. 2015. “An Accurate Test for Homogeneity of Odds Ratios Based on Cochran’s Q-Statistic.” *BMC Medical Research Methodology* 15 (1): 1–19. https://doi.org/10.1186/S12874-015-0034-X/FIGURES/16.

Kurmanov, Berzhan, Diansy Zincke, Wanwen Su, Ted L. Hadfield, Alim Aikimbayev, Talgat

Karibayev, Maxat Berdikulov, Mukhit Orynbayev, Mikeljon P. Nikolich, and Jason K. Blackburn. 2022. “Assays for Identification and Differentiation of Brucella Species: A Review.” *Microorganisms*. https://doi.org/10.3390/microorganisms10081584.

Lai, Shengjie, Hang Zhou, Weiyi Xiong, Marius Gilbert, Zhuojie Huang, Jianxing Yu, Wenwu Yin, et al. 2017. “Changing Epidemiology of Human Brucellosis, China, 1955-2014.” *Emerging Infectious Diseases* 23 (2). https://doi.org/10.3201/eid2302.151710.

Leclercq, Sébastien O., Axel Cloeckaert, and Michel S. Zygmunt. 2020. “Taxonomic Organization of the Family Brucellaceae Based on a Phylogenomic Approach.” *Frontiers in Microbiology* 10. https://doi.org/10.3389/fmicb.2019.03083.

Legesse, Abinet, Aregitu Mekuriaw, Esayas Gelaye, Takele Abayneh, Belayneh Getachew, Wubet Weldemedhin, Takele Tesgera, Getaw Deresse, and Kenaw Birhanu. 2023. “Comparative Evaluation of RBPT, I-ELISA, and CFT for the Diagnosis of Brucellosis and PCR Detection of Brucella Species from Ethiopian Sheep, Goats, and Cattle Sera.” *BMC Microbiology* 23 (1). https://doi.org/10.1186/s12866-023-02962-2.

Lin, Lifeng, and Haitao Chu. 2018. “Quantifying Publication Bias in Meta-Analysis.” *Biometrics* 74 (3). https://doi.org/10.1111/biom.12817.

Liu, Dongyou. 2014. “Brucella.” *Molecular Medical Microbiology*, January, 1781–88. https://doi.org/10.1016/B978-0-12-397169-2.00101-3.

Llor, Carl, and Lars Bjerrum. 2014. “Antimicrobial Resistance: Risk Associated with Antibiotic Overuse and Initiatives to Reduce the Problem.” *Therapeutic Advances in Drug Safety* 5 (6): 229. https://doi.org/10.1177/2042098614554919.

Lokamar, Peter N., Moses A. Kutwah, Elly O. Munde, Dickens Oloo, Harrysone Atieli, Sussy Gumo, James M. Akoko, and Collins Ouma. 2022. “Prevalence of Brucellosis in Livestock Keepers and Domestic Ruminants in Baringo County, Kenya.” *PLOS Global Public Health* 2 (8). https://doi.org/10.1371/journal.pgph.0000682.

Lysholm, Sara, Klara Fischer, Johanna F. Lindahl, Musso Munyeme, and Jonas Johansson Wensman. 2022. “Seropositivity Rates of Zoonotic Pathogens in Small Ruminants and Associated Public Health Risks at Informal Urban Markets in Zambia.” *Acta Tropica* 225 (January): 106217. https://doi.org/10.1016/J.ACTATROPICA.2021.106217.

Lysholm, Sara, Johanna F. Lindahl, George Dautu, Elin Johansson, Pernilla Karlsson Bergkvist, Musso Munyeme, and Jonas Johansson Wensman. 2022. “Seroepidemiology

of Selected Transboundary Animal Diseases in Goats in Zambia." *Preventive Veterinary Medicine* 206 (September): 105708. https://doi.org/10.1016/J.PREVETMED.2022.105708.

Ma, Han Rui, Hui Jiao Xu, Xin Wang, Zhao Yang Bu, Teng Yao, Zun Rong Zheng, Yang Sun, Xue Ji, and Jun Liu. 2023. "Molecular Characterization and Antimicrobial Susceptibility of Human Brucella in Northeast China." *Frontiers in Microbiology* 14. https://doi.org/10.3389/fmicb.2023.1137932.

Majzoobi, Mohammad Mahdi, Seyyed Hamid Hashemi, Mojgan Mamani, Fariba Keramat, Jalal Poorolajal, and Hamid Reza Ghasemi Basir. 2018. "Effect of Hydroxychloroquine on Treatment and Recurrence of Acute Brucellosis: A Single-Blind, Randomized Clinical Trial." *International Journal of Antimicrobial Agents* 51 (3): 365–69. https://doi.org/10.1016/J.IJANTIMICAG.2017.08.009.

Massis, Fabrizio De, Katiuscia Zilli, Guido Di Donato, Roberta Nuvoloni, Sandro Pelini, Lorena Sacchini, Nicola D'Alterio, and Elisabetta Di Giannatale. 2019. "Distribution of Brucella Field Strains Isolated from Livestock, Wildlife Populations, and Humans in Italy from 2007 to 2015." *PLOS ONE* 14 (3): e0213689. https://doi.org/10.1371/JOURNAL.PONE.0213689.

Mathew, C., M. Stokstad, T. B. Johansen, S. Klevar, R. H. Mdegela, G. Mwamengele, P. Michel, L. Escobar, D. Fretin, and J. Godfroid. 2015. "First Isolation, Identification, Phenotypic and Genotypic Characterization of Brucella Abortus Biovar 3 from Dairy Cattle in Tanzania." *BMC Veterinary Research* 11 (1). https://doi.org/10.1186/s12917-015-0476-8.

Matle, Itumeleng, Betty Ledwaba, Karabelo Madiba, Lavhelesani Makhado, Kudakwashe Jambwa, and Nombasa Ntushelo. 2021. "Characterisation of Brucella Species and Biovars in South Africa between 2008 and 2018 Using Laboratory Diagnostic Data." *Veterinary Medicine and Science* 7 (4). https://doi.org/10.1002/vms3.483.

Mehari, Sintayehu, Biruk Zerfu, and Kassu Desta. 2021. "Prevalence and Risk Factors of Human Brucellosis and Malaria among Patients with Fever in Malaria-Endemic Areas, Attending Health Institutes in Awra and Gulina District, Afar Region, Ethiopia." *BMC Infectious Diseases* 21 (1): 1–8. https://doi.org/10.1186/S12879-021-06654-Y/TABLES/4.

Mengele, Isaac J., Gabriel M. Shirima, Barend M. Bronsvoort, Luis E. Hernandez-Castro,

and Elizabeth A.J. Cook. 2023. “Diagnostic Challenges of Brucellosis in Humans and Livestock in Tanzania: A Thematic Review.” *CABI One Health*. https://doi.org/10.1079/cabionehealth.2023.0001.

Mfune, Ruth Lindizyani, Melai Mubanga, Isaac Silwamba, Frederick Sagamiko, Steward Mudenda, Victor Daka, Jacques Godfroid, Bernard M. Hangombe, and John B. Muma. 2021. “Seroprevalence of Bovine Brucellosis in Selected Districts of Zambia.” *International Journal of Environmental Research and Public Health 2021, Vol. 18, Page 1436* 18 (4): 1436. https://doi.org/10.3390/IJERPH18041436.

Mia, M. Mukthar, Mahamudul Hasan, and Faija Sadia Pory. 2022. “Occupational Exposure to Livestock and Risk of Tuberculosis and Brucellosis: A Systematic Review and Meta-Analysis.” *One Health* 15 (December): 100432. https://doi.org/10.1016/J.ONEHLT.2022.100432.

Mirnejad, Reza, Faramarz Masjedian Jazi, Shayan Mostafaei, and Mansour Sedighi. 2017a. “Molecular Investigation of Virulence Factors of Brucella Melitensis and Brucella Abortus Strains Isolated from Clinical and Non-Clinical Samples.” *Microbial Pathogenesis* 109. https://doi.org/10.1016/j.micpath.2017.05.019.

———. 2017b. “Epidemiology of Brucellosis in Iran: A Comprehensive Systematic Review and Meta-Analysis Study.” *Microbial Pathogenesis* 109 (August): 239–47. https://doi.org/10.1016/J.MICPATH.2017.06.005.

Moreno, Edgardo. 2021. “The One Hundred Year Journey of the Genus Brucella (Meyer and Shaw 1920).” *FEMS Microbiology Reviews* 45 (1). https://doi.org/10.1093/FEMSRE/FUAA045.

Moreno, Edgardo, José María Blasco, Jean Jacques Letesson, Jean Pierre Gorvel, and Ignacio Moriyón. 2022. “Pathogenicity and Its Implications in Taxonomy: The Brucella and Ochrobactrum Case.” *Pathogens*. https://doi.org/10.3390/pathogens11030377.

Moreno, Edgardo, José María Blasco, and Ignacio Moriyón. 2022. “Facing the Human and Animal Brucellosis Conundrums: The Forgotten Lessons.” *Microorganisms* 10 (5): 942. https://doi.org/10.3390/MICROORGANISMS10050942/S1.

Mubanga, Melai, Ruth L. Mfune, John Kothowa, Ahmed S. Mohamud, Chitalu Chanda, John Mcgiven, Flavien N. Bumbangi, et al. 2021. “Brucella Seroprevalence and Associated Risk Factors in Occupationally Exposed Humans in Selected Districts of Southern

Province, Zambia." *Frontiers in Public Health* 9 (November): 1601. https://doi.org/10.3389/FPUBH.2021.745244/BIBTEX.

Mudenda, Steward, Flavien Nsoni Bumbangi, Kaunda Yamba, Musso Munyeme, Sydney Malama, Moses Mukosha, Mwendalubi Albert Hadunka, et al. 2023. "Drivers of Antimicrobial Resistance in Layer Poultry Farming: Evidence from High Prevalence of Multidrug-Resistant Escherichia Coli and Enterococci in Zambia." *Veterinary World* 16 (9). https://doi.org/10.14202/vetworld.2023.1803-1814.

Muma, J. B., K. L. Samui, J. Oloya, M. Munyeme, and E. Skjerve. 2007. "Risk Factors for Brucellosis in Indigenous Cattle Reared in Livestock-Wildlife Interface Areas of Zambia." *Preventive Veterinary Medicine* 80 (4). https://doi.org/10.1016/j.prevetmed.2007.03.003.

Muma, J. B., M. Syakalima, M. Munyeme, V. C. Zulu, M. Simuunza, and M. Kurata. 2013. "Bovine Tuberculosis and Brucellosis in Traditionally Managed Livestock in Selected Districts of Southern Province of Zambia." *Veterinary Medicine International* 2013. https://doi.org/10.1155/2013/730367.

Muma, J.B, I.K Samui, M Munyeme, A Lund, K Ielsen, H Chimana, C Chisenga, and Skjerve.E. 2008. "Brucellosis in Rural Communities in Zambia and Factors Associated with Increased Anti _ Brucellosis Spp . Antibody Presence Brucellosis in Rural Communities in Zambia and Factors Associated." UNZA Journal of Science and Technology. 2008. https://www.researchgate.net/publication/303818198_Brucellosis_in_Rural_Communities_in_Zambia_and_Factors_Associated_with_Increased_anti_Brucellosis_spp_antibody_Presence.

Munn, Zachary, Sandeep Moola, Dagmara Riitano, and Karolina Lisy. 2014. "The Development of a Critical Appraisal Tool for Use in Systematic Reviews Addressing Questions of Prevalence." *International Journal of Health Policy and Management* 3 (3): 123–28. https://doi.org/10.15171/IJHPM.2014.71.

Mwinyi, Mwinyi Omary, Kim Abel Kayunze, Doreen C Sitali, Martin C Simuunza, and John Bwalya Muma. 2016. "Socio-Economic Impact of Brucellosis on Livestock Farmers in Southern and Western Provinces, Zambia." *International Journal of Technical Research and Applications* 4 (6): 204–9. http://www.suaire.sua.ac.tz/handle/123456789/4750.

Nematollahi, Shahrzad, Erfan Ayubi, Manoochehr Karami, Salman Khazaei, Masoud

Shojaeian, Reza Zamani, Kamyar Mansori, and Behzad Gholamaliee. 2017. "Epidemiological Characteristics of Human Brucellosis in Hamadan Province during 2009–2015: Results from the National Notifiable Diseases Surveillance System." *International Journal of Infectious Diseases* 61 (August): 56–61. https://doi.org/10.1016/J.IJID.2017.06.002.

Nokhatha, Shamma Al, Fatima AlKindi, Shaima Al Yassi, and Rayhan Hashmey. 2021. "Musculoskeletal Brucellosis in Adults in the United Arab Emirates: A Retrospective Study." *European Medical Journal*. https://doi.org/10.33590/emj/20-00271.

O'Callaghan, David. 2020. "Human Brucellosis: Recent Advances and Future Challenges." *Infectious Diseases of Poverty* 9 (1). https://doi.org/10.1186/S40249-020-00715-1.

Osman, Amira E.F., Abdullahi N. Hassan, Ali E. Ali, Theresia H. Abdoel, and Henk L. Smits. 2015. "Brucella Melitensis Biovar 1 and Brucella Abortus S19 Vaccine Strain Infections in Milkers Working at Cattle Farms in the Khartoum Area, Sudan." *PLoS ONE* 10 (5). https://doi.org/10.1371/journal.pone.0123374.

Oyetola, Wilfried Délé, Kanny Diallo, Katharina Kreppel, Philippe Soumahoro Kone, Esther Schelling, Bassirou Bonfoh, and Rianatou Bada Alambedji. 2021. "Factors Influencing the Transborder Transmission of Brucellosis in Cattle Between Côte d'Ivoire and Mali: Evidence From Literature and Current Key Stakeholders." *Frontiers in Veterinary Science* 8. https://doi.org/10.3389/fvets.2021.630580.

Pal, Mahendra, Gemechu Berhanu, Chaltu Desalegn, and Venkataramana Kandi. 2020. "Human and Animal Brucellosis: A Comprehensive Review of Biology, Pathogenesis, Epidemiology, Risk Factors, Clinical Signs, Laboratory Diagnosis, Public Health Significance, Economic Importance, Prevention and Control." *American Journal of Infectious Diseases and Microbiology* 8 (4).

Palma, Ernesto, Bruno Tilocca, and Paola Roncada. 2020. "Antimicrobial Resistance in Veterinary Medicine: An Overview." *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms21061914.

Parlak, Mehmet, Hüseyin Güdücüoğlu, Yasemin Bayram, Aytekin Çikman, Cenk Aypak, Selçuk Kiliç, and Mustafa Berktaş. 2013. "Identification and Determination of Antibiotic Susceptibilities of Brucella Strains Isolated from Patients in Van, Turkey by Conventional and Molecular Methods." *International Journal of Medical Sciences* 10 (10): 1406–11. https://doi.org/10.7150/ijms.6565.

Pereira, Carine Rodrigues, Rodrigo Bentes Kato, Fabrício Almeida Araújo, Alessandra Lima da Silva, Roselane Gonçalves dos Santos, Thiago de Jesus Sousa, Raquel Costa Neia, et al. 2023. "Genomic Investigation of Antimicrobial Resistance in Brucella Abortus Strains Isolated from Cattle in Brazil." *Gene Reports* 31. https://doi.org/10.1016/j.genrep.2023.101777.

Pérez-Sancho, Marta, Teresa García-Seco, Lucas Domínguez, and Julio Álvarez. 2015. "Control of Animal Brucellosis — The Most Effective Tool to Prevent Human Brucellosis." *Updates on Brucellosis*. https://doi.org/10.5772/61222.

Plumb, G E, S C Olsen, and D Buttke. 2013. "Brucellosis: 'One Health' Challenges and Opportunities." *Rev. Sci. Tech. Off. Int. Epiz* 32 (1): 271–78.

Prestinaci, Francesca, Patrizio Pezzotti, and Annalisa Pantosti. 2015. "Antimicrobial Resistance: A Global Multifaceted Phenomenon." *Pathogens and Global Health*. https://doi.org/10.1179/2047773215Y.0000000030.

Qureshi, Kamal A., Adil Parvez, Nada A. Fahmy, Bassant H. Abdel Hady, Shweta Kumar, Anusmita Ganguly, Akhtar Atiya, et al. 2023. "Brucellosis: Epidemiology, Pathogenesis, Diagnosis and Treatment–a Comprehensive Review." *Annals of Medicine*. https://doi.org/10.1080/07853890.2023.2295398.

Racloz, V, E Schelling, N Chitnis, F Roth, and J Zinsstag. 2013. "Persistence of Brucellosis in Pastoral Systems." *Rev. Sci. Tech. Off. Int. Epiz* 32 (1): 61–70. www.cbd.int.

Rahman, A. K.M.A., C. Saegerman, D. Berkvens, F. Melzer, H. Neubauer, D. Fretin, E. Abatih, N. Dhand, and M. P. Ward. 2017. "Brucella Abortus Is Prevalent in Both Humans and Animals in Bangladesh." *Zoonoses and Public Health* 64 (5). https://doi.org/10.1111/zph.12344.

Ranjbar, Mitra, Marzieh Nojomi, Maria T. Mascellino, Mitra Ranjbar, Marzieh Nojomi, and Maria T. Mascellino. 2020. "New Insight into Brucella Infection and Foodborne Diseases." *New Insight into Brucella Infection and Foodborne Diseases*, January. https://doi.org/10.5772/INTECHOPEN.77695.

Rossetti, Carlos A., Kenneth L. Drake, and L. Garry Adams. 2012. "Transcriptome Analysis of HeLa Cells Response to Brucella Melitensis Infection: A Molecular Approach to Understand the Role of the Mucosal Epithelium in the Onset of the Brucella Pathogenesis." *Microbes and Infection* 14 (9).

https://doi.org/10.1016/j.micinf.2012.03.003.

Rossetti, Carlos A., Kenneth L. Drake, Prasad Siddavatam, Sara D. Lawhon, Jairo E.S. Nunes, Tamara Gull, Sangeeta Khare, Robin E. Everts, Harris A. Lewin, and Leslie Garry Adams. 2013. "Systems Biology Analysis of Brucella Infected Peyer's Patch Reveals Rapid Invasion with Modest Transient Perturbations of the Host Transcriptome." *PLoS ONE* 8 (12). https://doi.org/10.1371/journal.pone.0081719.

Rowe, M. T. 2014. "Brucella: Problems with Dairy Products." In *Encyclopedia of Food Microbiology: Second Edition*. https://doi.org/10.1016/B978-0-12-384730-0.00050-1.

Rubach, MP, JEB Halliday, and … S Cleaveland. 2013. "Brucellosis in Low-Income and Middle-Income Countries." *Ncbi.Nlm.Nih.Gov*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3888775/.

Samreen, Iqbal Ahmad, Hesham A. Malak, and Hussein H. Abulreesh. 2021. "Environmental Antimicrobial Resistance and Its Drivers: A Potential Threat to Public Health." *Journal of Global Antimicrobial Resistance*. https://doi.org/10.1016/j.jgar.2021.08.001.

Sharma, V, P Kaur, R S Aulakh, R Sharma, R Verma, and B B Singh. 2024. "Is Brucella Excreted in Cattle Faeces? – Evidence from Punjab, India." *Comparative Immunology, Microbiology and Infectious Diseases* 104: 102099. https://doi.org/https://doi.org/10.1016/j.cimid.2023.102099.

Shenoy, Bhaskar, Anupam Jaiswal, and Anuradha Vinod. 2016. "Lab Diagnosis of Brucellosis." *Pediatric Infectious Disease* 8 (1): 40–44. https://doi.org/10.1016/J.PID.2016.03.006.

Shevtsov, Alexandr, Marat Syzdykov, Andrey Kuznetsov, Alexandr Shustov, Elena Shevtsova, Kalysh Berdimuratova, Kasim Mukanov, and Yerlan Ramankulov. 2017. "Antimicrobial Susceptibility of Brucella Melitensis in Kazakhstan." *Antimicrobial Resistance and Infection Control* 6 (1). https://doi.org/10.1186/s13756-017-0293-x.

Simpson, Gregory, Peter N. Thompson, Claude Saegerman, Tanguy Marcotty, Jean Jacques Letesson, Xavier de Bolle, and Jacques Godfroid. 2021a. "Brucellosis in Wildlife in Africa: A Systematic Review and Meta-Analysis." *Scientific Reports 2021 11:1* 11 (1): 1–16. https://doi.org/10.1038/s41598-021-85441-w.

———. 2021b. "Brucellosis in Wildlife in Africa: A Systematic Review and Meta-Analysis." *Scientific Reports 2021 11:1* 11 (1): 1–16. https://doi.org/10.1038/s41598-021-85441-w.

Singer, Andrew C., Helen Shaw, Vicki Rhodes, and Alwyn Hart. 2016. “Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators.” *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2016.01728.

Singh, B. B., N. K. Dhand, and J. P.S. Gill. 2015. “Economic Losses Occurring Due to Brucellosis in Indian Livestock Populations.” *Preventive Veterinary Medicine* 119 (3–4): 211–15. https://doi.org/10.1016/J.PREVETMED.2015.03.013.

Singh, Kamaljit. 2009. “Laboratory-Acquired Infections.” *Clinical Infectious Diseases* 49 (1): 142–47. https://doi.org/10.1086/599104/2/49-1-142-TBL003.GIF.

Sohail, Muhammad, Abid Rashid, Bilal Aslam, Muhammad Waseem, Muhammad Shahid, Muhammad Akram, Mohsin Khurshid, and Muhammad Hidayat Rasool. 2016. “Antimicrobial Susceptibility of Acinetobacter Clinical Isolates and Emerging Antibiogram Trends for Nosocomial Infection Management.” *Revista Da Sociedade Brasileira de Medicina Tropical* 49 (3). https://doi.org/10.1590/0037-8682-0111-2016.

Solera, Javier, José L Beato, Elisa Martínez-Alfaro, Juan C Segura, and Elena de Tomas. 2001. “Azithromycin and Gentamicin Therapy for the Treatment of Humans with Brucellosis.” *Clinical Infectious Diseases* 32 (3): 506–9. https://doi.org/10.1086/318503.

Suárez-Esquivel, Marcela, Esteban Chaves-Olarte, Edgardo Moreno, and Caterina Guzmán-Verri. 2020. “Molecular Sciences Brucella Genomics: Macro and Micro Evolution.” *International Journal of Molecular Sciences* 21 (October): 7749. https://doi.org/10.3390/ijms21207749.

Suresh, Kuralayanapalya P., Sharanagouda S. Patil, Akshata Nayak, Himani Dhanze, Shinduja Rajamani, Chandan Shivamallu, Charley A. Cull, and Raghavendra G. Amachawadi. 2022. “Prevalence of Brucellosis in Livestock of African and Asian Continents: A Systematic Review and Meta-Analysis.” *Frontiers in Veterinary Science* 9. https://doi.org/10.3389/fvets.2022.923657.

Syal, Karan, Manni Mo, Hui Yu, Rafael Iriya, Wenwen Jing, Sui Guodong, Shaopeng Wang, Thomas E. Grys, Shelley E. Haydel, and Nongjian Tao. 2017. “Current and Emerging Techniques for Antibiotic Susceptibility Tests.” *Theranostics* 7 (7): 1795. https://doi.org/10.7150/THNO.19217.

Tang, Ka Wah Kelly, Beverley C. Millar, and John E. Moore. 2023. “Antimicrobial Resistance (AMR).” *British Journal of Biomedical Science*.

https://doi.org/10.3389/bjbs.2023.11387.

Tao, Zhongfa, Qiulan Chen, Yishan Chen, Yu Li, Di Mu, Huimin Yang, and Wenwu Yin. 2021. “Epidemiological Characteristics of Human Brucellosis — China, 2016−2019.” *China CDC Weekly* 3 (6). https://doi.org/10.46234/ccdcw2021.030.

Tesfaye, Abebe, Haileyesus Dejene, Bemrew Admassu, Takele Adugna Kassegn, Destaw Asfaw, Gashaw Getaneh Dagnaw, and Abebe Belete Bitew. 2021. “Seroprevalence of Bovine Brucellosis in Ethiopia: Systematic Review and Meta-Analysis.” *Veterinary Medicine: Research and Reports* Volume 12 (January): 1–6. https://doi.org/10.2147/VMRR.S289721.

Torkaman Asadi, Fatemeh, Seyyed Hamid Hashemi, Mohammad Yousef Alikhani, Abbas Moghimbeigi, and Zahra Naseri. 2017. “Clinical and Diagnostic Aspects of Brucellosis and Antimicrobial Susceptibility of Brucella Isolates in Hamedan, Iran.” *Japanese Journal of Infectious Diseases* 70 (3): 235–38. https://doi.org/10.7883/yoken.JJID.2016.133.

Trott, Darren J., Sam Abraham, and Ben Adler. 2018. “Antimicrobial Resistance in Leptospira , Brucella , and Other Rarely Investigated Veterinary and Zoonotic Pathogens.” *Microbiology Spectrum* 6 (4). https://doi.org/10.1128/microbiolspec.arba-0029-2017.

Trott, DJ, S Abraham, B Adler - Microbiology Spectrum, and undefined 2018. 2018. “Antimicrobial Resistance in Leptospira, Brucella, and Other Rarely Investigated Veterinary and Zoonotic Pathogens.” *Am Soc Microbiol* 6 (4). https://doi.org/10.1128/microbiolspec.ARBA-0029-2017.

Tuon, Felipe F., Regina B. Gondolfo, and Natacha Cerchiari. 2017. “Human-to-Human Transmission of Brucella – a Systematic Review.” *Tropical Medicine & International Health* 22 (5): 539–46. https://doi.org/10.1111/TMI.12856.

Twohig-Bennett, Caoimhe, and Andy Jones. 2018. “The Health Benefits of the Great Outdoors: A Systematic Review and Meta-Analysis of Greenspace Exposure and Health Outcomes.” *Environmental Research* 166. https://doi.org/10.1016/j.envres.2018.06.030.

Villate, Sara Consuelo Arias, Julio Cesar García Casallas, Sara Consuelo Arias Villate, and Julio Cesar García Casallas. 2020. “Update of Antibiotic Therapy of Brucellosis.” *New Insight into Brucella Infection and Foodborne Diseases*, January.

https://doi.org/10.5772/INTECHOPEN.86325.

Wang, X. H., and H. Jiang. 2020. “Global Prevalence of Human Brucellosis.” *Zhonghua Liu Xing Bing Xue Za Zhi = Zhonghua Liuxingbingxue Zazhi* 41 (10): 1717–22. https://doi.org/10.3760/CMA.J.CN112338-20191022-00751.

Wareth, Gamal, Maryam Dadar, Haytham Ali, Mahmoud E.R. Hamdy, Ahmad M. Al-Talhy, Ahmed R. Elkharsawi, Ashraf A.Abd El Tawab, and Heinrich Neubauer. 2022a. “The Perspective of Antibiotic Therapeutic Challenges of Brucellosis in the Middle East and North African Countries: Current Situation and Therapeutic Management.” *Transboundary and Emerging Diseases* 69 (5): e1253–68. https://doi.org/10.1111/tbed.14502.

———. 2022b. “The Perspective of Antibiotic Therapeutic Challenges of Brucellosis in the Middle East and North African Countries: Current Situation and Therapeutic Management.” *Transboundary and Emerging Diseases* 69 (5): e1253–68. https://doi.org/10.1111/TBED.14502.

Wareth, Gamal, Mohamed El-Diasty, Nour H. Abdel-Hamid, Katharina Holzer, Mahmoud E.R. Hamdy, Shawky Moustafa, Momtaz A. Shahein, et al. 2021. “Molecular Characterization and Antimicrobial Susceptibility Testing of Clinical and Non-Clinical Brucella Melitensis and Brucella Abortus Isolates from Egypt.” *One Health* 13 (December): 100255. https://doi.org/10.1016/J.ONEHLT.2021.100255.

WHO. 2015. “WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015.” World Health Organization. 2015. https://apps.who.int/iris/handle/10665/199350.

———. 2020. “Brucellosis.” *World Health Organisation.Factsheet.* https://www.who.int/news-room/questions-and-answers/item/brucellosis.

Wiethoelter, Anke K., Daniel Beltrán-Alcrudo, Richard Kock, and Siobhan M. Mor. 2015. “Global Trends in Infectious Diseases at the Wildlife-Livestock Interface.” *Proceedings of the National Academy of Sciences of the United States of America* 112 (31): 9662–67. https://doi.org/10.1073/PNAS.1422741112/SUPPL_FILE/PNAS.1422741112.SD01.XLSX.

Williams, Victoria L., and Carrie L. Kovarik. 2021. “Global Dermatology and Telemedicine.” *Dermatologic Clinics* 39 (1). https://doi.org/10.1016/s0733-

8635(20)30080-2.

Woodyard, Kiersten C., Elise Hogan, Douglas R. Dembinski, Jules Madzia, Lane Guyton, Christopher F. Janowak, Brian S. Pan, and Ryan M. Gobble. 2024. “A Review of Meta-Analyses in Plastic Surgery: Need for Adequate Assessment of Publication Bias.” *Journal of Surgical Research*. https://doi.org/10.1016/j.jss.2023.06.052.

Xu, Xiu Li, Xiao Chen, Pei Hong Yang, Jia Yun Liu, and Xiao Ke Hao. 2013. “In Vitro Drug Resistance of Clinical Isolated Brucella against Antimicrobial Agents.” *Asian Pacific Journal of Tropical Medicine* 6 (11): 921–24. https://doi.org/10.1016/S1995-7645(13)60165-0.

Yang, Anni, Juan Pablo Gomez, Catherine G. Haase, Kelly M. Proffitt, and Jason K. Blackburn. 2019. “Effects of Brucellosis Serologic Status on Physiology and Behavior of Rocky Mountain ELK (Cervus Canadensis Nelsoni) in Southwestern Montana, USA.” *Journal of Wildlife Diseases* 55 (2). https://doi.org/10.7589/2018-01-011.

Yousefi-Nooraie, Reza, Sameh Mortaz-Hejri, Mehdi Mehrani, and Parham Sadeghipour. 2012. “Antibiotics for Treating Human Brucellosis.” *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.cd007179.pub2.

Yuan, Hai Tao, Cheng Ling Wang, Li Na Liu, Dan Wang, Dan Li, Zhen Jun Li, and Zhi Guo Liu. 2020. “Epidemiologically Characteristics of Human Brucellosis and Antimicrobial Susceptibility Pattern of Brucella Melitensis in Hinggan League of the Inner Mongolia Autonomous Region, China.” *Infectious Diseases of Poverty* 9 (1). https://doi.org/10.1186/s40249-020-00697-0.

Yumuk, Zeki, and David O’Callaghan. 2012. “Brucellosis in Turkey - an Overview.” *International Journal of Infectious Diseases*. https://doi.org/10.1016/j.ijid.2011.12.011.

Zange, Sabine, and Holger C Scholz. 2023. “Brucellosis BT - Zoonoses: Infections Affecting Humans and Animals.” In , edited by Andreas Sing, 411–50. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-27164-9_63.

Zerfu, Biruk, Girmay Medhin, Gezahegne Mamo, Gezahegn Getahun, Rea Tschopp, and Mengistu Legesse. 2018. “Community-Based Prevalence of Typhoid Fever, Typhus, Brucellosis and Malaria among Symptomatic Individuals in Afar Region, Ethiopia.” *PLOS Neglected Tropical Diseases* 12 (10): e0006749. https://doi.org/10.1371/JOURNAL.PNTD.0006749.

Zhang, Zhan, Zhi Qiang Liu, Peng Yuan Zheng, Fu Ai Tang, and Ping Chang Yang. 2010. "Influence of Efflux Pump Inhibitors on the Multidrug Resistance of Helicobacter Pylori." *World Journal of Gastroenterology* 16 (10): 1279–84. https://doi.org/10.3748/wjg.v16.i10.1279.

Zhao, Chenxi, Kun Liu, Chenghao Jiang, Xiao Wei, Shuxuan Song, Xubin Wu, Xiaohui Wen, et al. 2022. "Epidemic Characteristics and Transmission Risk Prediction of Brucellosis in Xi'an City, Northwest China." *Frontiers in Public Health* 10. https://doi.org/10.3389/fpubh.2022.926812.

8. APPENDICES

8.1 Institutional Approval

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE OFFICE OF THE ASSISTANT DEAN (POSTGRADUATE)

Telephone: 293727 P.O. Box 32379
Telegrams: UNZA LUSAKA Lusaka, Zambia
Telex: UNZALU ZA 44370
Fax: 293727/253952 Your Ref:
School Fax: 293727
Vet. Clinic Telephone: 291515 Our Ref:

==========

30th August 2022

Mwiza Munangándu
Department of Disease Control
School of Veterinary Medicine
University of Zambia
P. O. Box 32379
LUSAKA

Dear Mwiza

SUBJECT: **APPROVAL OF RESEARCH PROPOSAL**

At the meeting of the School Board of Graduate Studies held on 29th August, 2022, your research proposal entitled: ***"A Global Overview of Anti-Biotic Susceptibility of Brucella Strains"*** was tabled and discussed. I am therefore, pleased to inform you that the research proposal was subsequently approved by the Board. On behalf of the Board, I wish you success as you apply for ethical approval and carry on with your research activities.

Yours sincerely

Dr. Chisoni Mumba
ASSISTANT DEAN (PG), SCHOOL OF VETERINARY MEDICINE

Cc *Director, DRGS*
Dean, School of Veterinary Medicine
Head, Disease Control
File

Additional File 1: Quality Assessment Criteria

1. Experimental studies with techniques used to determine antibiotic susceptibility should be either cultural or molecular techniques.

2. The specific *Brucella* strain isolated and tested should be clearly stated

3. The samples collected should be human samples and clearly stated.

4. The location of sample collection and study should be stated.

5. The specific antibiotics and their respective concentrations used in the study should be clearly stated.

6. Number or proportion of isolates susceptible or resistant to a specific antibiotic should be clearly stated.

7. The sample size should be clearly stated.

8. The sample type collected should be stated.

9. How the samples were collected, prepared or stored, and duration of incubation and interpretation of results during analysis should be stated.

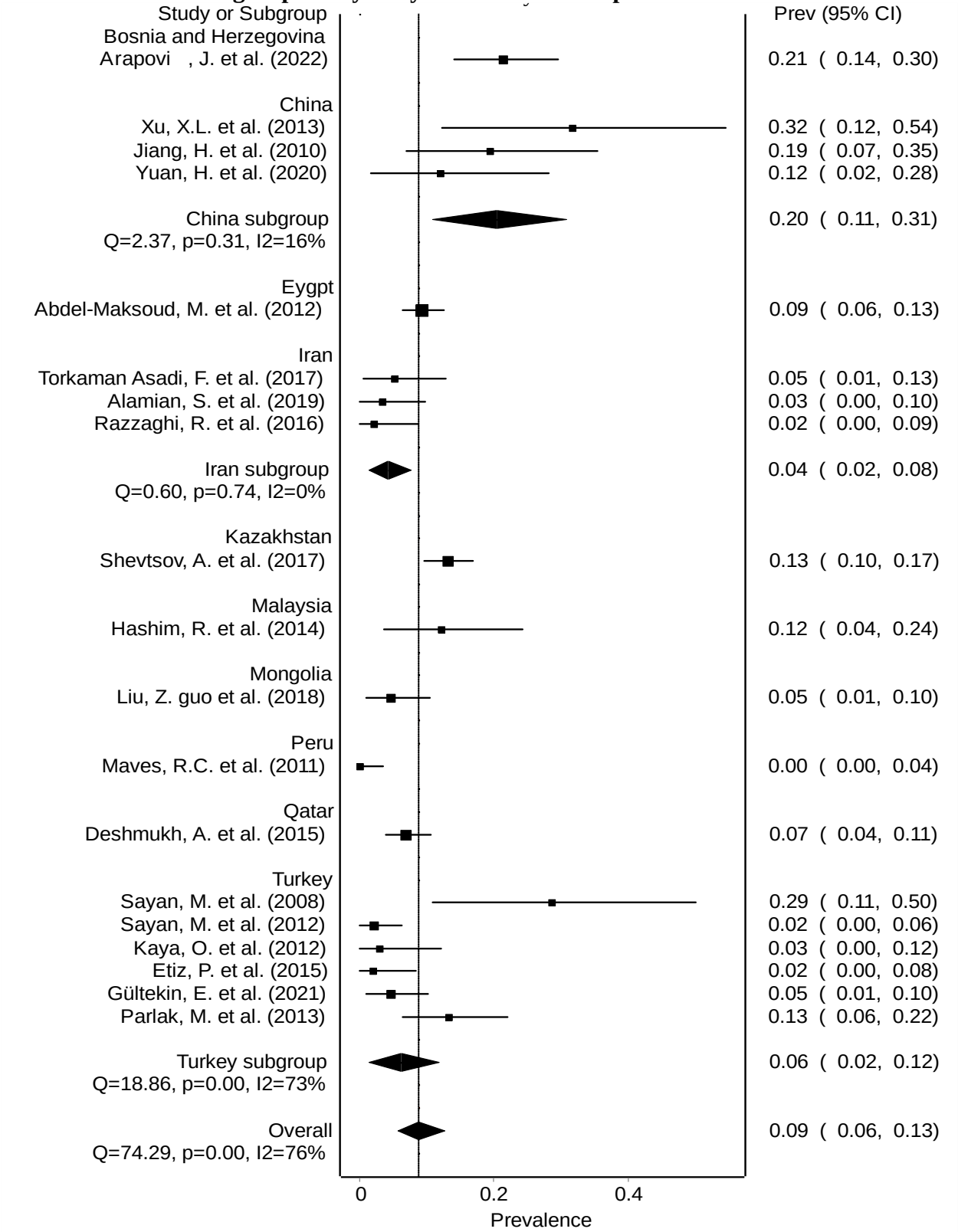
10. The sample collection method should be stated.

11. Does the study have complete prevalence data?

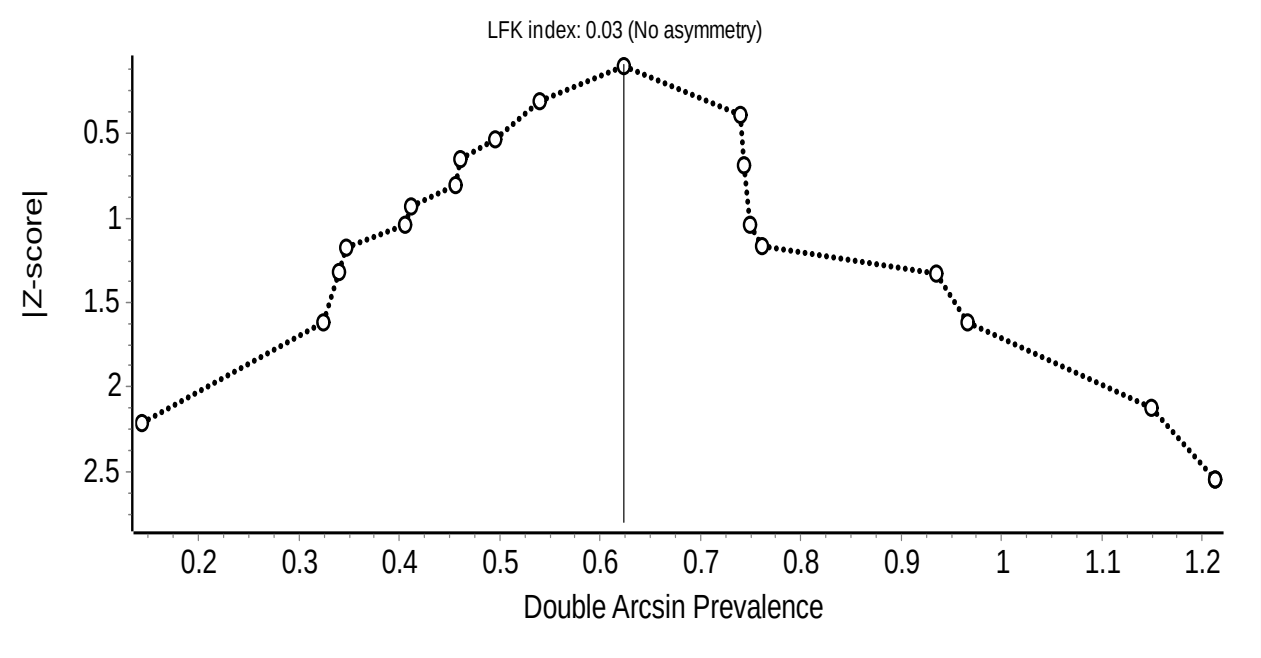
Supplementary Table 1: Quality Assessment Criteria Results

References	Q. 1	Q. 2	Q. 3	Q. 4	Q. 5	Q. 6	Q. 7	Q. 8	Q. 9	Q. 10	Q. 11	Score
Torkaman Asadi, F. *et al.* (2017)	1	1	1	1	1	1	1	1	1	0	1	10
Shevtsov, A. *et al*. (2017)	1	1	1	1	1	1	1	1	1	0	1	10
Xu, X.L. *et al*. (2013)	1	1	1	1	1	1	1	1	1	0	1	10
Arapović, J. *et al*. (2022)	1	1	1	1	1	1	1	1	1	0	1	10
Jiang, H. *et al*. (2010)	1	1	1	1	1	1	1	1	1	0	1	10
Alamian, S. *et al*. (2019)	1	1	1	1	1	1	1	1	1	0	1	10
Yuan, H. *et al.* (2020)	1	1	1	1	1	1	1	1	1	1	1	11
Gültekin, E. *et al.* (2021)	1	1	1	1	1	1	1	1	1	0	1	10
Parlak, M. *et al.* (2013)	1	1	1	1	1	1	1	1	1	0	1	10
Abdel-Maksoud, M. *et al*. (2012)	1	1	1	1	1	1	1	1	1	0	1	10
Liu, Z. guo *et al.* (2018)	1	1	1	1	1	1	1	1	1	0	1	10
Razzaghi, R. *et al.* (2016)	1	1	1	1	1	1	1	1	1	0	1	10
Sayan, M. *et al*. (2008)	1	1	1	1	1	1	1	1	1	0	1	10
Maves, R.C. *et al*. (2011)	1	1	1	1	1	1	1	1	1	0	1	10
Deshmukh, A. *et al.* (2015)	1	1	1	1	1	1	1	1	1	0	1	10
Kaya, P. *et al*. (2012)	1	1	1	1	1	1	1	1	1	0	1	10
Hashim, R. *et al*. (2014)	1	1	1	1	1	1	1	1	1	0	1	10
Etiz, P. *et al*. (2015)	1	1	1	1	1	1	1	1	1	0	1	10
Sayan, M. *et al.* (2012)	1	1	1	1	1	1	1	1	1	0	1	10
*Q. stands for Quality Assessment. Check the quality assessment checklist above.												

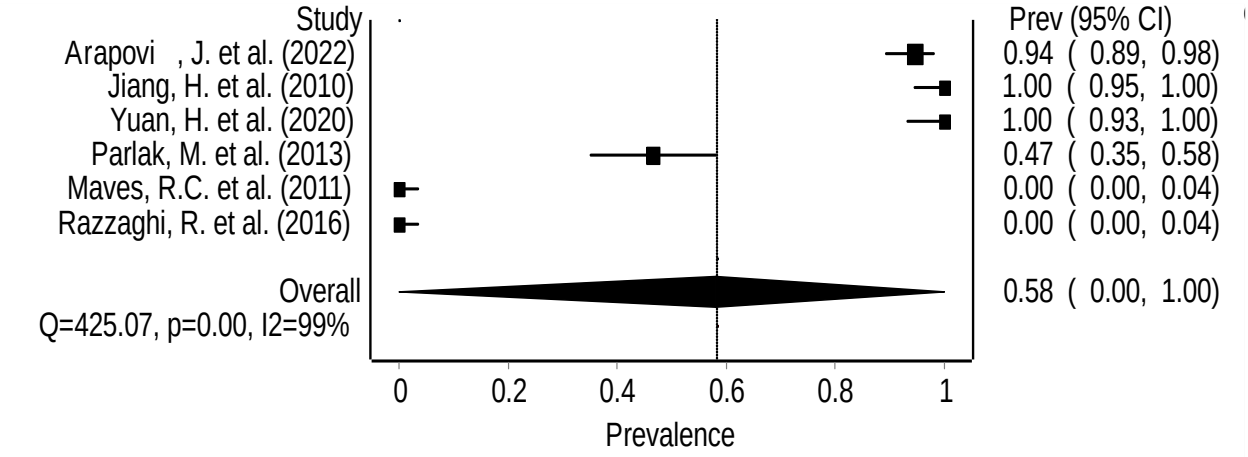
Additional File 2: Subgroup Analysis by Countries-Forest plot



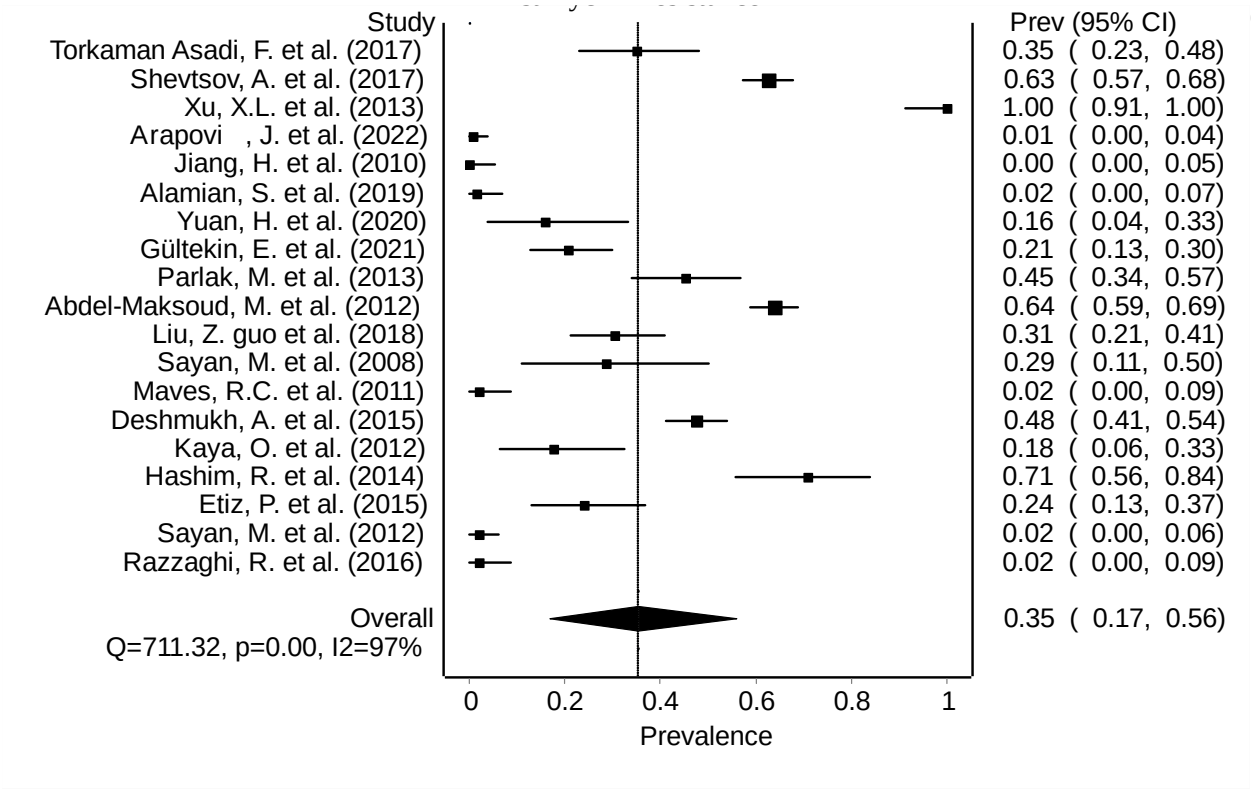
Additional File 3: Doi Plot



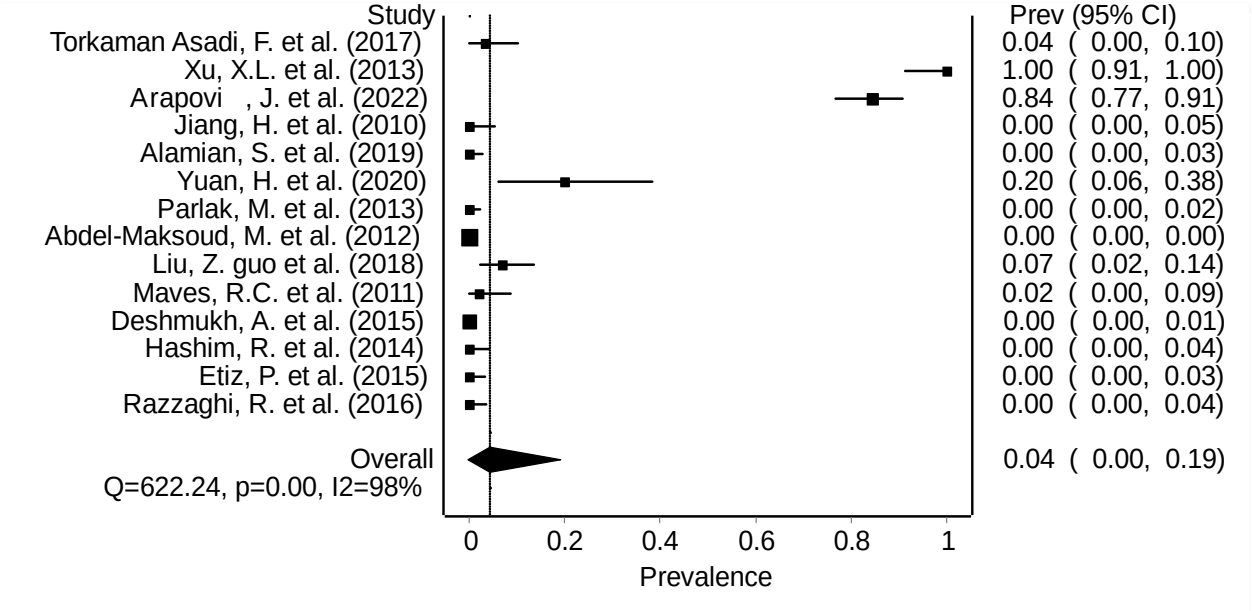
Additional File 4: *Brucella* Resistance to Macrolides-Forest Plot



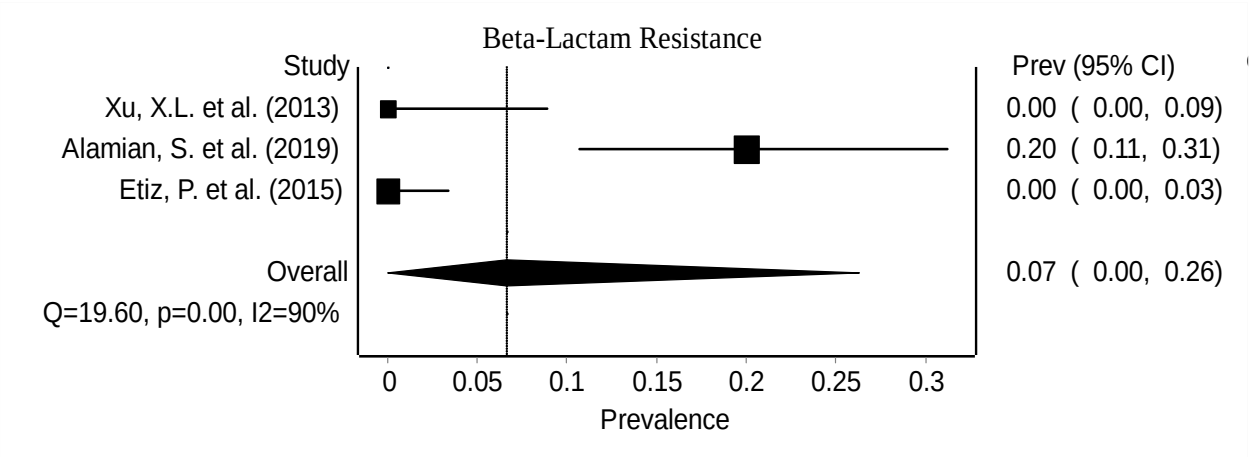
Additional File 5: *Brucella* Resistance to Ansamycin Forest Plot



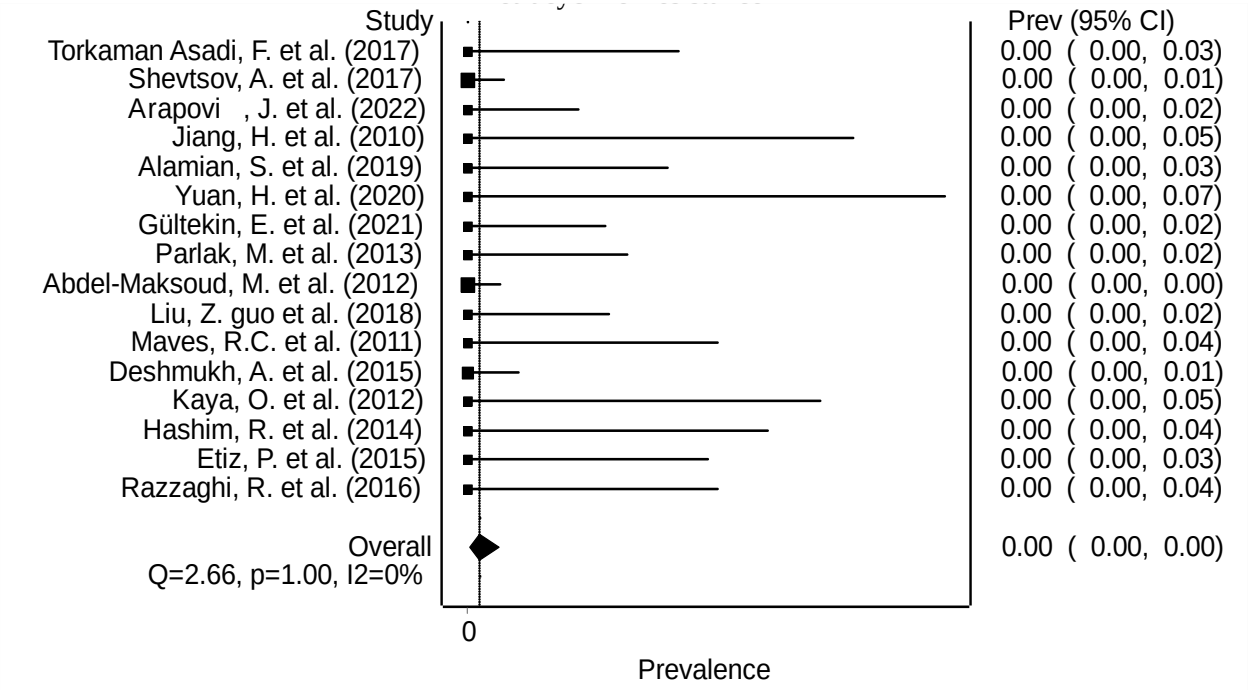
Additional File 6: *Brucella* Resistance to Sulfonamides-Forest Plot



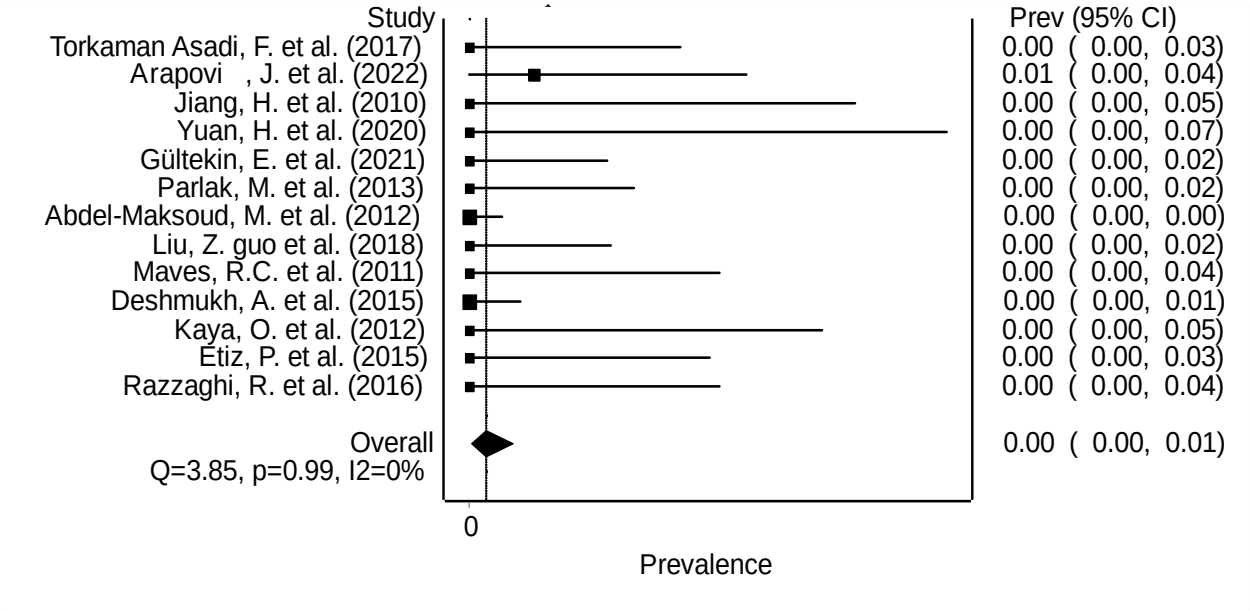
Additional File 7: *Brucella* Resistance to Beta-Lactam-Forest Plot



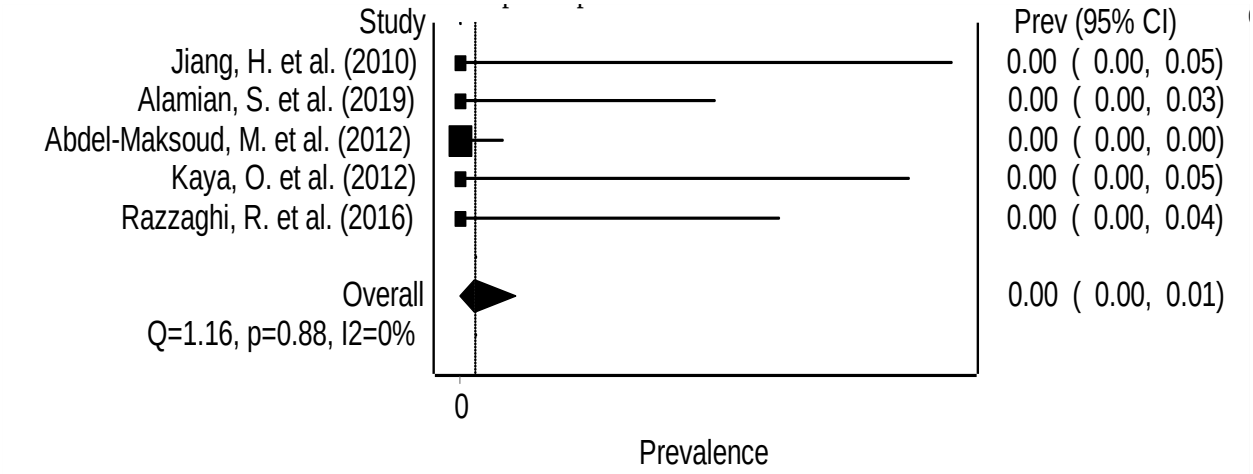
Additional File 8: *Brucella* Resistance to Tetracycline- Forest Plot



Additional File 9: *Brucella* Resistance to Fluoroquinolones- Forest Plot



Additional File 10: *Brucella* Resistance to Cephalosporin- Forest Plot



Additional File 11: *Brucella* Resistance to Aminoglycosides-Forest Plot