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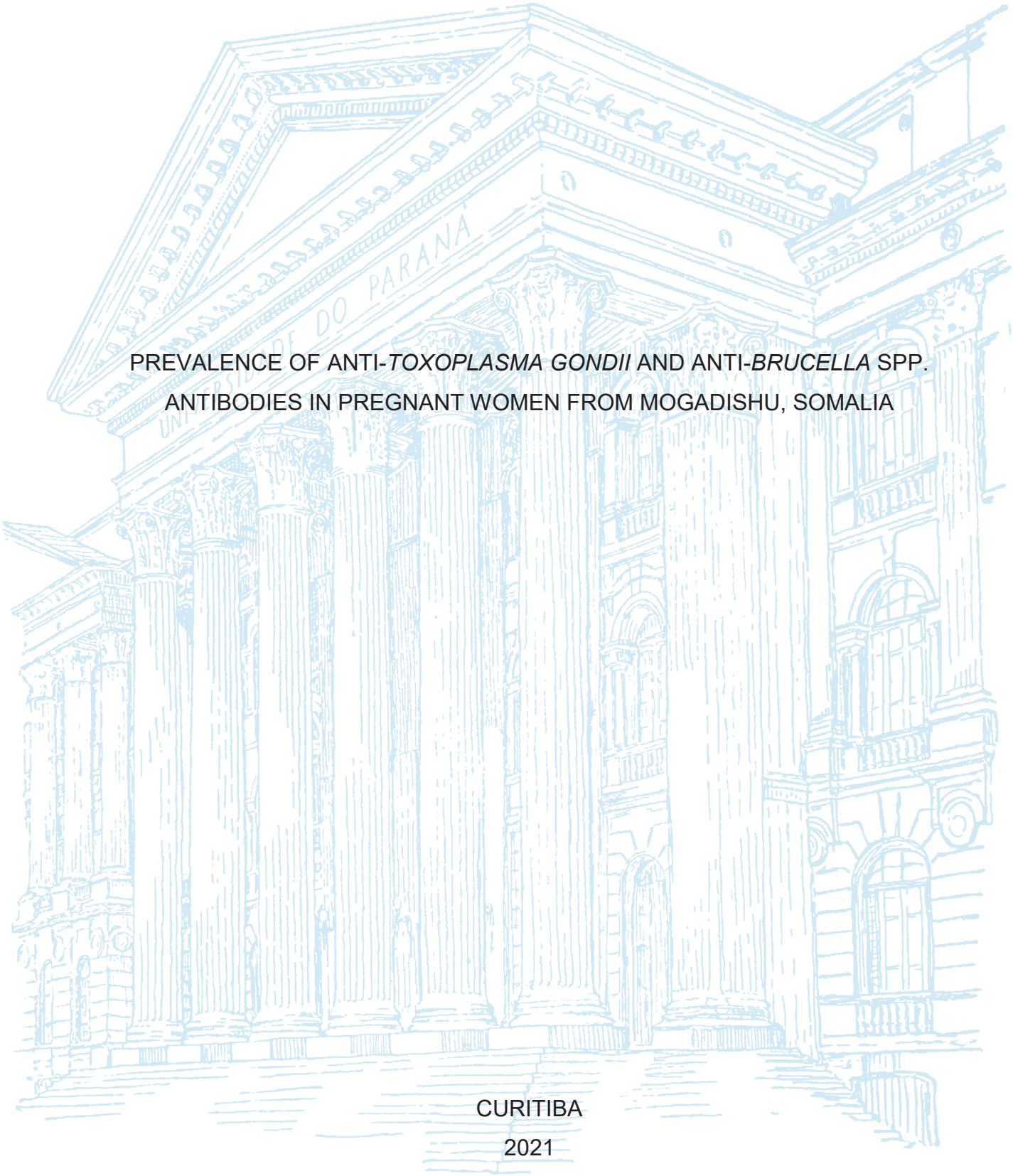
ABDULKARIM ABDULLE YUSUF



PREVALENCE OF ANTI-*TOXOPLASMA GONDII* AND ANTI-*BRUCELLA* SPP. ANTIBODIES IN PREGNANT WOMEN FROM MOGADISHU, SOMALIA

CURITIBA

2021

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PREVALENCE OF ANTI-*TOXOPLASMA GONDII* AND ANTI-*BRUCELLA* SPP. ANTIBODIES IN PREGNANT WOMEN FROM MOGADISHU, SOMALIA

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como um requerimento parcial para obtenção do título de Mestre em Ciências Veterinárias

Orientador: Prof. Dr. Rafael Felipe da Costa Vieira
Co-orientador: Prof. Dr. Abdalla Mohamed Ibrahim

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÂRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ABDULKARIM ABDULLE YUSUF** intitulada: **Prevalence of Anti-Toxoplasma gondii and anti-Brucella spp. antibodies in pregnant women from Mogadishu, Somalia,** sob orientação do Prof. Dr. RAFAEL FELIPE DA COSTA VIEIRA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Assinatura Eletrônica
13/05/2021 17:04:25.0
RAFAEL FELIPE DA COSTA VIEIRA
Presidente da Banca Examinadora (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
12/05/2021 22:30:42.0
IVAN ROQUE DE BARROS FILHO
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
12/05/2021 21:34:22.0
LUIZ DANIEL DE BARROS
Avaliador Externo (UNIVERSIDADE ESTADUAL DE LONDRINA)

DEDICATION

I would like to dedicate this work with great appreciation to the soul of my dad,

Abdulle Yusuf.

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“Experience tells you what to do; Confidence allows you to do it”

(Stan Smith)

RESUMO

A toxoplasmose e a brucelose são doenças zoonóticas de distribuição mundial. Ambos causam aborto e infertilidade em humanos e animais. Dados limitados estão disponíveis sobre esses patógenos em Somalis e seus animais. Assim, este estudo avaliou a prevalência de anticorpos anti-*Toxoplasma gondii* e anti-*Brucella* spp. em mulheres grávidas em Mogadíscio, Somália. Amostras de soro de 307 mulheres grávidas de Mogadíscio, Somália, foram testadas para anticorpos anti-*T. gondii* pelo Teste de Aglutinação de Látex (LAT) e anticorpos anti-*Brucella* spp. pelo Teste Rosa Bengala (RBPT) e um ELISA competitivo comercial (cELISA). Um total de 159/307 (51,79%; IC 95%: 46,2–57,35%) gestantes foram sororreativas para *T. gondii* em diferentes estágios da gravidez. Para *Brucella* spp., quando RBPT e cELISA foram combinados 4/307 (1,30%; IC 95%: 0,36 - 3,30%) mulheres grávidas eram sororreativas, sendo 2/307 (0,65%; IC 95%: 0,18 - 2,34%) por RBPT e 3/307 (0,98%; IC 95%: 0,33 - 2,83%) por cELISA. Duas mulheres foram sororreativas para ambos os agentes. O presente estudo é uma evidência da existência de alta positividade de anticorpos anti-*T. gondii* e baixa soropositividade de anti-*Brucella* spp. anticorpos entre mulheres grávidas de Mogadíscio, Somália. Como a conscientização sobre esses patógenos abortivos zoonóticos nos Somalis é muito baixa, as pessoas neste país correm um grande risco para a saúde pública. Além disso, mais esforços médicos e veterinários são necessários para maximizar a taxa de investigação de toxoplasmose e brucelose em humanos e animais no país, evitando assim o reconhecimento de qualquer associação entre doenças zoonóticas e pacientes imunocomprometidos, incluindo mulheres grávidas. Portanto, é necessário promover o conceito de Saúde Única entre os profissionais multissetoriais para um desenvolvimento integrado de saúde melhor e sustentável e a implementação de estratégias eficazes de controle contra essas doenças zoonóticas transmitidas por alimentos.

Palavras-chave: Patógenos de origem alimentar, toxoplasmose, brucelose, aborto, Saúde Unica, Somália.

ABSTRACT

Toxoplasmosis and brucellosis are zoonotic diseases of worldwide distribution. They both cause abortion and infertility in human and animals. Limited data are available about these pathogens in Somali people and their animals. Hence, this study has evaluated the prevalence of anti-*Toxoplasma gondii* and anti-*Brucella* spp. antibodies in pregnant women in Mogadishu, Somalia. Serum samples from 307 pregnant women from Mogadishu, Somalia were tested for anti-*T. gondii* antibodies by Latex Agglutination Test (LAT) and anti-*Brucella* spp. antibodies by Rose Bengal Plate Test (RBPT) and a commercial competitive-ELISA (cELISA). A total of 159/307 (51.79%; 95% CI: 46.2–57.35%) pregnant women were seroreactive for *T. gondii* by LAT at different stages of pregnancy. For *Brucella* spp., when RBPT and cELISA were combined 4/307 (1.30%; 95% CI: 0.36 – 3.30%) pregnant women were seroreactive to anti-*Brucella* spp. antibodies, being 2/307 (0.65%; 95% CI: 0.18 – 2.34%) by RBPT and 3/307 (0.98%; 95% CI: 0.33 – 2.83%) by cELISA. Two women were seroreactive for both agents. The present study is an evidence of the existence of high seropositivity of anti-*T. gondii* antibodies and low seropositivity of anti-*Brucella* spp. antibodies among pregnant women from Mogadishu, Somalia. Since the awareness on these zoonotic abortifacient pathogens in Somalis is very low, the people in this country are at great public health risk. Additionally, more medical and veterinary efforts needed to maximize toxoplasmosis and brucellosis investigation rate in humans and animals in the country, thus preventing recognition of any association between zoonotic diseases and immunocompromised patients including pregnant women. Therefore, there is a need to promote the One Health concept among multi-sectoral professionals for better and sustainable integrated health development and implementing effective control strategies against these food-borne zoonotic diseases.

Keywords: Foodborne pathogens, Toxoplasmosis, Brucellosis, Abortion, One Health, Somalia.

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LIST OF ABBREVIATIONS

ARTC	- Abrar Research and Training Centre
AU	- Abrar University
CI	- Confidence interval
CNPq	- Conselho Nacional de Desenvolvimento Científico e Tecnológico
LAT	- Lat Agglutination Test
RBPT	- Rose Bengal Plate Test
cELISA	- Competitive Enzyme-linked immunosorbent assay
GOHi	- Global One Health initiative
OR	- Odds ratio
PCR	- Polymerase chain reaction
SAT	- Serum Agglutination Test
SPSS	- Statistical package for social sciences
UFPR	- Universidade Federal do Paraná

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1 GENERAL INTRODUCTION

1.1 BACKGROUND

Toxoplasmosis, caused by *Toxoplasma gondii,* and brucellosis, caused by *Brucella* spp., are important zoonotic diseases with worldwide occurrence (Corbel *et al.* 2006; Dubey and Jones, 2008; Tenter 2009). These zoonotic pathogens can be transmitted from animals to humans, including pregnant women, and lead to adverse health consequences (Kledmanee *et al.,* 2019).

Brucellosis in humans is commonly caused by *B. melitensis* or *B. abortus* and is characterized by inflammation of the genitals and foetal membranes, abortions, sterility, and lesions in the lymphatic system and joints (Awah-Ndukum *et al.,* 2018; Kledmanee *et al.,* 2019). Endocarditis and neurological outcomes, including motor deficits, cranial nerve deficits, sciatica, confusion and/or psychological disturbances, meningitis, and seizures, are severe clinical presentations of the disease (Dean *et al.,* 2012). Spontaneous miscarriage and intrauterine foetal death during the first trimesters have also been reported in pregnant women (Ali *et al.,* 2016; Awah-Ndukum *et al.,* 2018). The most common causes of human infection were linked to consumption of unpasteurized dairy products and labour conditions (veterinarians, slaughterhouse workers, and animal breeders) (Mufinda *et al.* 2017; Kledmanee *et al.,* 2019).

Toxoplasma gondii is an important food and waterborne opportunistic pathogen that causes severe disease in immunocompromised individuals, including pregnant women, which may result in abortion, foetal anomaly, stillbirth, foetal growth restriction, and preterm birth (Robert-Gangneux and Dardé, 2012; Deng *et al.,* 2018; Kledmanee *et al.,* 2019). The acute phase of the disease during pregnancy can also cause congenital toxoplasmosis (Yamada *et al.,* 2011). Several risk factors have been associated with human toxoplasmosis, particularly cat ownership and history of raw meat consumption (Kledmanee *et al.,* 2019). Somali people do not usually keep pets, but cats are sometimes let into the houses, and some households perform their house activities on the ground with the possibility of a high risk of contamination (Hinda *et al.,* 1988). Hygienic conditions, socio-economic structure, food, and environment can collectively have a notable influence on the diffusion of *T. gondii* (Zardi *et al.*, 1980). Moreover, Dehkordi *et al.* (2013) reported the presence of *T. gondii* (51/889; 5.73%) in raw milk

samples from dairy herds in Iran. These risk factors of zoonotic agents were recently recorded in Somalia (Kadle *et al.,* 2017).

Somalia is a tropical developing country; climatic and living conditions favour many pathogens, including zoonotic pathogens. Despite the public and economic importance of toxoplasmosis and brucellosis, few data are available in Somali people (Hussein *et al.* 1978; Zardi *et al.* 1980; Hinda *et al.* 1988) and their animals (Andreani *et al.* 1982; Amina 1987; Ghanem *et al.,* 2009; Kadle *et al.* 2017). In addition, there are little or no concerted medical and veterinary efforts to maximize toxoplasmosis and brucellosis detection rates. Therefore, the present study aimed to assess the seroprevalence *T. gondii* and *Brucella* spp. in pregnant women in Mogadishu, Somalia.

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2 LITERATURE REVIEW

2.1 BRUCELLOSIS

2.1.1 History

Brucellosis is an ancient and one of the world's most widespread zoonotic diseases affecting both public health and animal production (Ariza *et al.*, 2007). The disease is caused by gram-negative, facultative intracellular bacterias of the genus *Brucella*. Historically, the disease has been recognized in the Mediterranean region since the 1850s (Radostits *et al.*, 2006). In 1887, David Bruce and several others working on Mediterranean fever isolated *Micrococcus melitensis* from diseased soldiers in the Mediterranean island of Malta. The Maltese medical doctor, Themistocles Zammit, had revealed in 1905 that the organism was transmitted through contaminated goat milk, and the banning of goat's milk would be effective (Radostits *et al.*, 2006; Wyatt, 2013).

In 1897, Danish scientist, Bernhard Bang, identified a different causative organism isolated from cattle, called *Bacillus abortus,* which was later named *Brucella abortus* (Radostits *et al.*, 2006). Finally, in 1920 Meyer and Shaw proposed to group these organisms under the genus "*Brucella*" in honour of Bruce to settle the nomenclature issues (Meyer and Shaw, 1920).

2.1.2 Taxonomy

Taxonomically, ten species are recognized within the genus *Brucella*, six of which are the classical species including *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis*, and B*. neotomae*. Recently, *Brucella* strains have been isolated from numerous mammalian species such as *B. ceti*, *B. pinnipedialis*, *B. microti,* and *B. inopinata* (Foster *et al.*, 2007; Godfroid *et al.*, 2010). This classification is based on biochemical reactions and agglutination with mono-specific sera (Godfroid *et al.*, 2010; Wernery, 2016). *Brucella ovis* and *B. canis* contain rough lipopolysaccharide (R-LPS) in their outer cell wall, whereas all the other species contain smooth lipopolysaccharide (S-LPS) (Nielsen and Yu, 2010). Under favourable conditions, Brucella survives for up to 6 days in urine and 4 months in milk, water, and damp soil (Bekele, 2004). However, they are generally susceptible to heat, direct sunlight, acidic conditions, and common disinfectant (Walker, 1999).

2.1.3 Transmission

Zoonotic transmission occurs most frequently by direct contact with *Brucella* infected animals and consumption of unpasteurized milk and milk products (WHO, 1986). Other routes are tissues or fluids associated with abortion during the occupation of farmers, veterinarians, medical doctors or laboratory workers, meat processing workers, and abattoir workers (Corbel, 2006). Accidental self-inoculation with live vaccines is also reported (WHO, 1986). Consequently, pastoralists in endemic areas are at high risk of infection by *Brucella* species during animal handling and given the traditional preference of raw camel milk consumption (Bekele, 2004). In animals, brucellosis is acquired via ingestion of contaminated food, water, or licking an infected placenta, calf, foetus, or the genitalia of an infected animal shortly after it has aborted or delivered (Walker, 1999). It can also be transmitted through inhalation, conjunctival and genital routes (Radostits *et al*., 2006; Corbel, 2006).

2.1.4 Pathogenesis

Following exposure, the bacteria penetrate intact mucosal surfaces (Radostits *et al.*, 2006). After penetration, the organisms are phagocytized by macrophages in an effort by the host to eliminate the organism (Walker, 1999; Radostits *et al.*, 2006). However, *Brucella* is able to survive and replicate inside the phagocytic cells by inhibiting phagolysosome fusion, blocking bactericidal action of phagocytes, and suppressing the myeloperoxidase H_2O_2 halide system (Walker, 1999). After the initial invasion of the body, localization occurs primarily in the regional lymph nodes. Then they proliferate, spread homogenously and localize in the reticuloendothelial system and reproductive tract (Radostits *et al.*, 2006; Wernery, 2016). The destination of the invading bacteria is mainly determined by cellular defenses of the host chiefly macrophage and T-lymphocytes though specific antibody also plays apart (Radostits *et al.*, 2007). Gross inflammatory lesions may be found in the predilection sites in the uterus, udder, testicles, lymph nodes, joint capsules and bursa, and placenta (Bekele, 2004). The probable possibilities for abortion in animals may be due to placentitis, the direct effect of endotoxins, or inflammatory response in foetal tissue (Walker, 1999; Radostits *et al.*, 2006; Wernery, 2016).

2.1.5 Clinical Findings

Human brucellosis has a broader spectrum, presenting difficulties in diagnostic because it imitates other diseases, for example, malaria, typhoid, joint diseases, and other conditions causing pyrexia (Andriopoulos *et al.*, 2007; Kunda *et al.*, 2007). The disease shows continued intermittent or irregular fever (hence the name “undulant fever”), headache, weakness, profuse sweating, chills, arthralgia, depression, weight loss, hepatomegaly, and splenomegaly, and generalized aching. Cases of arthritis, spondylitis, osteomyelitis, epididymitis, orchitis, and in severe cases neurobrucellosis, liver abscesses, and endocarditis with infection of the aortic valves and other multiple valves with *Brucella* have been reported in human (María *et al.*, 2007).

In animals, brucellosis could be suspected in any herd with history of abortion during the last stage of pregnancy, infertility, orchitis, epididymitis, stillbirths, neonatal mortality, and hygroma (Radostits *et al.*, 2006). The main clinical sign in the first stage of the disease is abortion; however, other signs , including uterine infections, placentitis, foetal death and mummification, delayed maturity, infertility, arthritis and reduced milk production may be observed (Radostits *et al.*, 2006; Radostits *et al.*, 2007; Gwida *et al.*, 2012). The clinical manifestation of human brucellosis is varied and nonspecific (Corbel, 2006). It can be acute febrile illness, fatigue, malaise, weight loss, sweats, headache, arthralgia, and back pain, and may progress to a chronically debilitating disease with severe complications (WHO, 1986; Corbel, 2006).

2.1.6 Diagnosis

Diagnostic tests are applied with different objectives: confirmatory diagnosis, screening or prevalence studies, certification, and, in countries where brucellosis is eradicated, surveillance to avoid the reintroduction of brucellosis through the importation of infected animals or animal products (Robinson, 2003). The diagnostic methods include direct tests, involving isolation of organism or DNA detection by polymerase chain reaction (PCR)-based methods and indirect tests, which are applied either *in vitro* (mainly to milk or blood) or *in vivo* (allergic test). The diagnosis of human brucellosis is usually based on the isolation of *Brucella* spp. from blood, tissue specimens, body

fluids, and bone marrow, on the serological tests for the detection of anti-*Brucella* spp. antibodies and on the molecular methods for the detection of *Brucella* spp. DNA (Sakran *et al.*, 2006).

2.1.7 Treatment

The essential element in the treatment of human brucellosis is the administration of effective antibiotics for an adequate length of time (Corbel, 2006). The treatment should consist of a combination of drugs with at least one drug having good penetration into macrophages and can act in the acidic intracellular environment cells and should be prolonged (Mantur *et al.*, 2007)**.** Treatment failure and relapses of treated cases were reported in human brucellosis (Corbel, 2006). A combination of doxycycline (200 mg/day) with either streptomycin (15 mg/Kg body weight intramuscularly) for two to three weeks or rifampicin (600-900 mg/day) for six weeks is recommended by the WHO for the treatment of human brucellosis (WHO, 1986). Childhood brucellosis can be successfully treated with a combination of two drugs: doxycycline 4 mg/Kg/day and rifampicin 10 mg/Kg/day orally for six weeks (Mantur *et al*., 2004). Several chemotherapeutic agents have been tried in the treatment of animal brucellosis, but the results have not been entirely satisfactory (Corbel, 2006). A limited number of studies have shown rapid reductions in the incidence of brucellosis after treatment (Radwan *et al*., 1992, 1993, 1995). However, the use of antibiotics is forbidden in many countries because of the doubt related to the infective status of the treated animals and antibiotic resistance (Wernery, 2016).

2.1.8 Control and Prevention

Directly or indirectly, human brucellosis is linked with infected animals or their products. The control of human brucellosis depends on minimizing/controlling disease in animals and reducing animal to human transmission (Zinsstag *et al*., 2007; Rubach *et al*., 2013). The most frequent route of human infection is the consumption of no pasteurized dairy products from *Brucella* infected animals. So, pasteurization of milk will reduce *Brucella* transmission to humans (Rubach *et al.*, 2013). If pasteurization is not available, boiling or heating milk at 80–85°C (176–185° F) for several minutes will also kill the bacteria (Corbel, 2006). Exposed people can reduce the risk through personal

hygiene measures and adaption of safe working practices including use of protective clothing, disinfection of protective clothing, and disinfection of potentially infected utensils and premises.

2.2 TOXOPLASOMOSIS

2.2.1 History

T. gondii was first recognized in an African rodent (*Ctenodactylus -gundi*) by Nicolle and Manceeaux (1908). The first human case was recorded in the same year in a young (20 years) person in Panama (Darling, 1908). However, the first case of congenital toxoplasmosis was discovered in a child (3 months old) suffering from hydrocephalus (Janku, 1923). There was only a single species causing human infection (toxoplasmosis) and capable of infecting a wide range of hosts (Wolf and Cowan, 1937). The importance of animal toxoplasmosis was first reported in New Zealand (Hartley *et al.*, 1954; Hartley and Marshall, 1957). In the late 960s cat was incriminated as the animal that could shed the environmentally stable form (oocyst) of the parasite in their faeces (Hutchison, 1965) which led to consider cats as the definitive host of the parasite (Hutchison *et al.*, 1970) and the sporulated oocyst as the major source of infection for animals and humans (Soulsby, 1986; Urquhart *et al.*, 1996; Dubey, 2004; Taylor *et al.*, 2007).

2.2.2 Transmission

T. gondii can be transmitted through ingestion of food, feedstuff, and water contaminated with sporulated oocyst or/and tachyzoites and bradyzoites (Tenter *et al.*, 2000; Dubey, 2004; Weiss and Kim, 2007; Taylor *et al.*, 2007; Tenter, 2009). Tachyzoites of *T. gondii* have been detected in body fluids, including saliva, sputum, urine, tears, semen, and milk of several intermediate hosts, such as sheep, goats, cows, and she-camel (Tenter *et al.*, 2000; Tenter, 2009; Manal 2003; Fusco, 2007). Consumption of unpasteurized goat's milk has been associated with acquired clinical toxoplasmosis in humans (Tenter *et al.*, 2000; Tenter, 2009). Though intake of milk or milk by-products containing tachyzoites may cause the infection in animals and humans (Dubey and Beattie, 1988; Ataseven *et al*., 2006). Tachyzoites can penetrate the mucus membrane; thereby gain access to the circulatory and lymphatic system of the host

(Johnson, 1997). Tissue cyst (bradyzoites) contained in meat, meat products or offal (visceral organs) is an important source of infection for cats (the final host), other carnivores and humans. About 50% of human toxoplasmosis cases were related to foodborne infection (Slifko *et al*., 2000; Tenter *et al*., 2000) during consumption of raw or undercooked meat or other edible parts of meat-producing animals. The risk of acquiring *T. gondii* infection via food varies greatly with cultural and feeding habits in different human societies. Frequent consumption of raw or undercooked meat has been associated with seropositivity for *T. gondii*. The consumption of undercooked lamb was a stronger risk factor (Baril *et al*., 1999) in some part of Europe. Consumption of beef had also been identified as a risk factor in some European countries (Cook *et al*., 2000). Blood transfusion and organs implantation (Schaffner, 2001) also have a role in human Toxoplasmosis. Limited and accidental infections by needles or splashing infective material into the eyes during laboratory work or postmortem examination of infected hosts are also considered as means of transmission (Marquardt and Demaree, 1985). In congenital toxoplasmosis, the transmission occurs through the placenta (Dubey, 1996; Sukthana, 2006). In immunosuppressed women, reactivation of an infection acquired before pregnancy can also lead to congenital toxoplasmosis (Minkoff et al., 1997). Transmission of *T. gondii* through semen was also reported in experimentally infected rams (Lopes *et al*., 2009), buck (Dubey and Sharma, 1980), swine (Moura *et al*., 2007), bulls (Scarpelli *et al*., 2009) and male dogs (Arantes *et al*., 2009). Contaminated soil, water, and food (Aramini *et al*., 1999) with oocyst of *T. gondii* were also considered as a source of infection. Oocyst can also be transmitted mechanically by flies and earthworms and dog (Lindsay *et al*., 1997).

2.2.3 Pathogenesis

Following oocyst ingestion, excystation takes place in the gut releasing sporozoites. These can actively invade the gut cells and multiply to produce tachyzoites. Four days post-infection (d.p.i), tachyzoites may be spread throughout the host body through blood circulation, causing parasitaemia, which may last 12 d.p.i (Dubey and Sharma, 1980). Antibodies start to appear at this time, and the innate and adaptive immune responses are activated to limit tachyzoites multiplication (Lopez et al., 2009; Innes and Vermeulen 2006; Innes and Wastling, 1995). Darcy *et al.* (1988) stated that

antibodies against *T. gondii* play an important role in the immune response of the host, and their presence is an indication of both an exposure to infection and a vital diagnostic aid. In pregnant mammals, the parasite (tachyzoites) circulating in the blood is established in the placenta before invading foetus (Buxton and Finlayson, 1986). The parasite makes the placenta and the foetus susceptible to other pathogens (Yildiz *et al.*, 2009). The inflammatory reactions induced in the placenta by *T. gondii* stimulate the synthesis of prostaglandin F, which decreases progesterone level and subsequent lead to abortion (Engeland *et al.*, 1996).

2.2.4 Clinical Findings

Human toxoplasmosis is usually benign, but may occasionally lead to severe or lethal damages when combined with depressed immunological status, including people with HIV/AIDS or those undergoing cancer therapies (Tenter *et al.*, 2000), organs transplantation, or when transmitted to foetus during pregnancy (Bout *et al*., 2002; Qublan *et al*., 2002). *Toxoplasma gondii* infection during pregnancy can result in abortion or congenital disabilities. The most severe signs appear when the transmission occur in the first trimester (Remington *et al*., 2006). These signs in foetus include spontaneous abortion, stillbirth, and a live infant with classic signs of congenital toxoplasmosis such as hydrocephalus or microcephalus, cerebral calcification, and retinochorioditis or infant who fails to thrive or has CNS involvement. In addition, apparently normal infant who develops retinochorioditis, learning disability and blindness later during his life may be a consequence of vertical transmission (Guerina *et al*., 1994; McLeod *et al*., 2006; Remington *et al*., 2006). Various mild symptoms, which lymphadenopathy is the most significant, may be observed (Remington *et al*., 2006). Severe signs, such as encephalitis, shock, myocarditis, or hepatitis as well as pulmonary and multivisceral involvement may occur, but they are very rare in immunocompetent humans (Demare *et al.*, 2007). In people suffering from acquired immune deficiency syndrome (AIDS) and in other immunocompromised states, reactivation of chronic toxoplasmosis results in severe cell destruction, often leading to severe morbidity and mortality.

In animals, there is a wide spectrum of clinical signs of the diseases associated with *T. gondii* infection, which is dependent on: the host species, the immune status of

the host, and the virulence of the particular strain of *Toxoplasma* (Innes 2010). Cattle and equines are more resistant to clinical *T. gondii* infection than sheep (Munday, 1978; Dubey *et al*., 1999; Dubey *et al*., 2003). In the Sudan, diarrhoea and lymph nodes enlargement were reported as clinical signs of calf-camel delivered by experimentally infected she-camels (Ishag *et al.*, 2006). They also reported different histopathological lesions from the organs of these calf-camels, particularly, haemorrhages, infiltration of lymphoid cells, congestion and focal necrosis. *Toxoplasma* tachyzoites and cysts were detected in the brain of suckling calf-camels and mice inoculated with milk of three experimentally infected she-camels (Manal, 2003). Congenital toxoplasmosis can cause abortion and neonatal death of calf-camel (Hagemoser *et al*., 1990; Ishag *et al*., 2006; Manal and Majid, 2008).

2.2.5 Diagnosis

Because of the lack of specific clinical manifestations during acute infection, *T gondii* is mainly associated with a laboratory diagnosis. Diagnosis of *T. gondii* in animals and humans must be made either by the organism demonstration or the antibodies against it (Taylor *et al*., 2007). The most effective diagnostic tools include: serological tests, bioassay, histopathology, immunohistochemistry, and molecular techniques as well as tissue impression smear. The diagnosis of congenital toxoplasmosis can be performed by identifying the agent using histological slides and the polymerase chain reaction (PCR) with aborted fetuses and placentas (Pereira-Bueno *et al*., 2004). Pregnant women at risk of toxoplasmosis infection are required to undergo monthly serological testing (Baril *et al*., 1999).

2.2.6 Treatment

Treatment of toxoplasmosis typically includes combinations of two antimicrobials, most often inhibitors of dihydrofolate reductase (DHFR) (pyrimethamine and trimethoprim) and dihydropteroate synthetase (sulfonamides, such as sulfadiazine, sulfamethoxazole, and sulfadoxine), which block folic acid synthesis. Pyrimethamine, a key DHFR inhibitor, appears to be the most effective drug against *T. gondii* and is the basis for effective treatment. These include pyrimethamine-sulfadiazine (pyr-sulf), the gold standard against which other regimens are measured, and pyrimethamine

combined with clindamycin, atovaquone, clarithromycin, or azithromycin (Dunay *et al.,* 2018).

2.2.7 Control and Prevention

Food and water should be kept away from cat's faeces and any contaminative environment (Dubey, 1991). Education of farmers on the principles of the route of infection and measures that reduce the prevalence of clinical cases as well as vaccination will reduce animal and human toxoplasmosis (Buxton *et al*., 2007; Ogendi *et al*., 2013). Heating of meat to 67ºC or higher is considered sufficient to immediately kill tissue cysts (Dubey, 2000).

2.3 HUMAN TOXOPLASMOSIS AND BRUCELLOSIS SITUATION IN SOMALIA

Regardless of its public importance, studies on human brucellosis in Somalia are insufficient. Hussein *et al.* (1978) investigated 353 human serum samples using Serum Agulutination Test (SAT) and found 0.6% seropositive, while Wiegand and Marx (1983) recorded no Brucella infection out of 11 human blood sera tested. *Brucella melitensis* was isolated from the cerebrospinal fluid sample of a patient from Somalia admitted to the Royal Hallamshire Hospital in United Kingdom (Wheat *et al.* 1995). Human brucellosis in the country needs more attention and research.

Regardless of the deleterious impact of primary infection of toxoplasmosis during pregnancy, as well as the reactivation of the disease in immunocompromised patients, few studies have been published from Somali people with a prevalence ranging from 10 to 61.2% in the general human population (Kagan and Cahill, 1968; Zardi *et al.* 1980; Hinda *et al.* 1988) and 0% in pregnant women (Hinda *et al.* 1988).

In Somalia, there are little or no medical and veterinary efforts to maximize toxoplasmosis and brucellosis detection rates in humans and animals in the country, thus preventing recognition of any association between zoonotic diseases and immunocompromised patients, including pregnant women. Therefore, there is a need to promote an inter-sectoral collaboration among veterinary, medical, and public health professionals at the federal and country-level in a one-health approach.for better and sustainable integrated health development and implementing effective control strategies against these zoonotic diseases.

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3 RATIONALE AND OBJECTIVES

3.1 RATIONALE

Somalia is a tropical developing country; climatic and living conditions favour many pathogens, including zoonotic pathogens. Despite the public and economic importance of toxoplasmosis and brucellosis, few data are available in Somali people (Hussein *et al.* 1978; Zardi *et al.* 1980; Hinda *et al.* 1988) and their animals (Andreani *et al.* 1982; Amina 1987; Ghanem *et al.,* 2009; Kadle *et al.* 2017). In addition, there are little or no concerted medical and veterinary efforts to maximize toxoplasmosis and brucellosis detection rates. Therefore, there is a need to generate information about the epidemiological situation of *T. gondii* and *Brucella* spp. in pregnant women in Mogadishu, Somalia.

3.2 HYPOTHESIS

- Toxoplasmosis and Brucellosis are prevalent in pregnant women in Somalia.

3.3 OBJECTIVES

3.3.1 General Objective

- Contribute to the diseases management in Somalia by availing recent epidemiological data on human toxoplasmosis and brucellosis.

3.3.2 Specific Objectives

- Evaluate the seroprevalence of *Toxoplasma gondii* and *Brucella* spp. in pregnant women in Mogadishu, Somalia.
- Determine factors associated with exposure/infection by *T. gondii* in pregnant women in Somalia.

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4 MANUSCRIPT: PREVALENCE OF ANT-*TOXOPLASMA GONDII* AND ANTI-*BRUCELLA* SPP. IN PREGNANT WOMEN FROM MOGADISHU, SOMALIA

Manuscript submitted to Frontiers in Reproductive Health, section Reproductive Epidemiology Journal.

4.1 ABSTRACT

Toxoplasmosis and brucellosis are zoonotic diseases of worldwide distribution. They both cause abortion and infertility in human and animals. Limited data are available about these pathogens in Somali people and their animals. Hence, this study has evaluated the prevalence of anti-*Toxoplasma gondii* and anti-*Brucella* spp. antibodies in pregnant women in Mogadishu, Somalia. Serum samples from 307 pregnant women from Mogadishu, Somalia were tested for anti-*T. gondii* antibodies by Latex Agglutination Test (LAT) and anti-*Brucella* spp. antibodies by Rose Bengal Plate Test (RBPT) and a commercial competitive-ELISA (cELISA). A total of 119/307 (38.76%) pregnant women had a prior history of abortion. A total of 159/307 (51.79%; 95% CI: 46.2–57.35%) pregnant women were seroreactive for *T. gondii* by LAT at different stages of pregnancy. For *Brucella* spp., when RBPT and cELISA were combined four/307 (1.30%; 95% CI: 0.36 – 3.30%) pregnant women were seroreactive to anti-*Brucella* spp. antibodies, being 2/307 (0.65%; 95% CI: 0.18 – 2.34%) by RBPT and 3/307 (0.98%; 95% CI: 0.33 – 2.83%) by cELISA. Two women were seroreactive for both agents. A high seropositivity to *T. gondii* and low seropositivity to *Brucella* spp. have been found in pregnant women from Mogadishu, Somalia. Considering the high number of abortions in the country associated to the fact that awareness on other zoonotic abortifacient pathogens in Somalis is very low, further studies should be conducted to evaluate the potential causes of abortions.

Keywords: Foodborne pathogens, Toxoplasmosis, Brucellosis, Abortion, One Health, Somalia.

4.2 INTRODUCTION

Toxoplasmosis, caused by *Toxoplasma gondii,* and brucellosis, caused by *Brucella* spp., are important zoonotic diseases with worldwide occurrence [1-3]. These zoonotic pathogens may be transmitted from animals to human beings and lead to negative health consequences such as abortion and complete sterility [4].

Brucellosis in humans is commonly caused by *Brucella melitensis* and/or *Brucella abortus*, and is characterized by inflammation of the genitals and fetal membranes, abortions, sterility and lesions in the lymphatic system and joints [4,5]. Endocarditis and neurological outcomes including motor deficits, cranial nerve deficits, sciatica, confusion and/or psychological disturbances, meningitis and seizures are severe clinical presentations of the disease [6]. Spontaneous miscarriage and intrauterine fetal death during the first trimesters have also been reported among pregnant women [5,7]. The most common causes of human infection were linked to consumption of unpasteurized dairy products and labour conditions (e.g., veterinarians, slaughterhouse workers, and animal breeders) [4,8].

Toxoplasma gondii is an important food and waterborne opportunistic pathogen that causes severe disease in immunocompromised individuals including pregnant women which may result in abortion, fetal anomaly, stillbirth, fetal growth restriction, and preterm birth [4,9,10]. Acute phase of the disease during pregnancy also cause congenital toxoplasmosis [11]. Several risk factors have been associated with human toxoplasmosis, particularly cat contact and a history of raw or undercooked meat consumption [4]. Somali people do not usually keep pets, but stray cats sometimes let into the houses and some households perform their house activities on the ground with a possibility of high risk of contamination [12]. Hygienic conditions, socio-economic structure, food and environment can collectively have a

notable influence on the diffusion of *T. gondii* [13]. Moreover, a previous study has reported the presence of *T. gondii* (3.12%) in raw milk of camel from Iran [14].

Somalia is a tropical developing country in which climatic and living conditions favors the dissemination of zoonotic pathogens. Despite public and economic importance of toxoplasmosis and brucellosis, few data are available in Somali people [12,13,16] and their animals [15,17-20]. In addition, there are little or no concerted medical and veterinary efforts to maximize toxoplasmosis and brucellosis detection rates. Therefore, the present study aimed to assess the prevalence of anti-*T. gondii* and anti-*Brucella* spp. antibodies in pregnant women in Mogadishu, Somalia.

4.3 MATERIALS AND METHODS

4.3.1 Ethics statement

This study was approved by the Ethics Committee on Human Research at Abrar University, Somalia (reference number AU/ARTC/EC/04/02/2017). The directors of the involved Health Offices gave their permission to conduct the research in their respective facility. All pregnant women that accepted being part of this study provided a written consent to participate.

4.3.2 STUDY DESIGN

A cross-sectional study design was conducted from August 2017 and November 2018 to determine the prevalence of anti-*T. gondii* and anti-*Brucella* spp. antibodies in pregnant women referred to the Banadir Maternity and Children Hospital or Maternal and Child Health (MCH) clinics in Mogadishu city, Somalia. Facilities were selected based on their specialty in this sector, while the pregnant women were selected on their willingness to cooperate for this study. Participants were informed about the study and a written consent was signed. The age of

pregnant women was stratified into groups of 15-30, 31-40 and >40 years old for statistical analysis. All study participants were interviewed using a questionnaire which included demographics and obstetric information comprising age, gestational age and history of abortion.

4.3.3 Sampling

A total of 307 blood samples including first trimester (gestational age of <14 weeks; n = 44), second trimester (gestational age between 14 and 28 weeks; n = 53) and third trimester (gestational age >28 weeks; n = 210) pregnant women were evaluated. Blood samples (3 mL) were collected by nurses by venipuncture of brachial vein using plain sterile vacutainer tubes and labelled. Samples were kept at room temperature (25 °C) until visible clot formation, and then centrifuged at 1500 × g for 5 min and stored at − 20 °C until laboratory analysis.

4.3.4 Serological detection of anti-*T. gondii* and anti-*Brucella* spp. antibodies

For the detection of anti-*T. gondii* antibodies, serum samples were screened by a commercial latex agglutination test (LAT) (SPINREACT, S.A/S.A.U Ctra, Santa Coloma, Spain), according to the manufacturers' instructions. The positive reactors were then diluted; two-fold dilution, 1:2 up to 1:128. Sera showing titer of ≥1:2 were considered positive for *T*. *gondii*.

For anti-*Brucella* spp. antibodies detection, serum samples were initially screened by the Rose Bengal Plate Test (RBPT) (CVRL, Khartoum, Sudan) and retested by a commercial competitive-ELISA (cELISA) (Svanova Biotech AB, Uppsala, Sweden), according to the manufacturers' instructions. The optical density (OD) was measured using a wavelength of 450 nm, and samples with a percentage of inhibition (% I) ≥30% were considered positive by cELISA. Samples were

considered seropositive for anti-*Brucella* spp. antibodies when the serum tested positive to RBPT and/or cELISA.

4.3.5 Data analysis

Data were compiled and analyzed by Statistical Package for Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA). Either Chi-square or Fisher's exact test was used to assess association of the age, gestational age and history of abortion with seropositivity of anti-*T. gondii* and anti-*Brucella* spp. antibodies. Odds ratio (OR), 95% confidence intervals (95% CI) and *P*-values were calculated, and results were considered significant when $P < 0.05$.

4.4 RESULTS

The majority of pregnant women were found within the age group 15-30 years (85.34%) and two-thirds were presented in the third trimester of gestational age (68.40%). A total of 119/307 (38.76%) pregnant women had a prior history of abortion (Table 1).

A total of 161/307 (52.44%; 95% CI: 46.85–57.99%) pregnant women were seroreactive for at least one pathogen. Anti-*T. gondii* antibodies were detected in 159/307 (51.79%; 95% CI: 46.2–57.35%) pregnant women by LAT. The antibody titers to *T. gondii* positive sera were 5 (3.14%), 55 (34.59%), 50 (31.45%), 26 (16.35%), 16 (10.06%), 7 (4.40%) and 0 (0%) by dilution of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128 respectively. Most of pregnant women (34.59%) had antibody titer of 1:4 while higher antibody titers, 1:64, were detected in seven pregnant women serum sample.

Anti-*Brucella* spp. antibodies were detected in two out of 307 (0.65%; 95% CI: 0.18 – 2.34%) and three out of 307 (0.98%; 95% CI: 0.33 – 2.83%) pregnant women

by RBPT and cELISA, respectively. Only one out of 307 (0.33%; 95% CI: 0.06 – 1.82%) pregnant woman was seroreactive for *Brucella* spp. by both methods. All pregnant women seroreactive for *Brucella* spp. were in the third trimester of gestational age and had a history of abortion (Table 1).

Two out of 307 (0.65%; 95% CI: 0.18 – 2.34%) pregnant women were seropositive for both *T. gondii* and *Brucella* spp. The seroprevalence of *Brucella* spp. and *T. gondii* for each variable evaluated is summarized in Table 1.

4.5 DISCUSSION

Toxoplasmosis and brucellosis are zoonotic diseases that may lead to negative health consequences worldwide [1-4]. Hence, determining the prevalence of these pathogens among pregnant women is paramount for prophylactic measures towards the susceptible women and reducing the adverse health events towards the seropositive women [21]. Since the Somali civil war of the 1990s, no studies have been conducted on the prevalence and importance of toxoplasmosis and brucellosis in human beings from Somalia, a livestock dependent country in East Africa with a population of around 12.3 million [22]. Previous studies conducted in the 1980s in the country have showed toxoplasmosis prevalence ranging from 10 to 61.2% in the general human population [12, 13, 23] and 0% in pregnant women [12], while prevalence of brucellosis ranged between 0 – 0.6% [16, 24].

Herein, the overall seroprevalence of brucellosis in pregnant women was low (1.30%), in agreement with the prevalence of brucellosis in animals (1.7%) from Somalia [15]. This may be due to the traditional prevention of diseases in livestock farms through culling of animals with proven reproductive problems. A strong association between human and animal seropositivity of brucellosis has been reported in a linked study in Kenya [25]. However, the public health associated risk

factors of zoonotic pathogens like prevailing tradition of unheated milk consumption and handling of aborted materials and reproductive excretions with bare hands are commonly practiced in Somalia [15], and this should be considered as a potential risk factor for brucellosis and other abortifacients zoonotic pathogens. Moreover, in the present study, all pregnant women seroreactive for *Brucella* spp. were in the third trimester of gestational age and had a prior history of abortion. However, pregnancy complications associated with brucellosis in the country needs further investigation.

The current finding on the prevalence of toxoplasmosis (51.79%) is slightly higher than the previous reports of 43.6% [13] and 40% [12] in Mogadishu, Somalia. However, the present study is similar with previous reports from Kisumu, Kenya (52%) [26], but lower than the findings from Arba Minch, Ethiopia (79.3%) [27]. The high seroprevalence of toxoplasmosis found herein may be related to risk factors described previously for the same location [12]. Moreover, the differences in the prevalence of toxoplasmosis between studies may also be due to the antigen used (recombinant protein vs crude antigens).

In the present study, the seroreactivity rates to toxoplasmosis were higher with the age, in agreement with previous studies [4, 27]. This may be explained by the longer period of exposure to risk factors [28]. Moreover, association between seroreactivity to *T. gondii* and history of abortion was not found ($p = 0.579$). Although the cause of abortion is multi-factorial, previous studies have associated spontaneous abortion in pregnant women and seroreactivity to *T. gondii* [2, 29].

Previous studies have reported the majority of human beings infected by *T. gondii* after birth are asymptomatic, however, some may develop a mild disease or in rare cases, a more severe systemic illness [2]. Nonetheless, transplacental transmission of *T. gondii* occurs if women acquire primary infection during pregnancy [30]. However, the risk of vertical transmission to the fetus increases from the first

trimester (10-24%) to the third trimester (60-90%), but the potential of congenital defect on fetus is more serious with earlier infections [27, 31]. In the present study, we found higher rates of seroreactivity to *T. gondii* in the first and second trimesters of gestational age, indicating more serious effects on the fetus if fetal transmission is developed. Unfortunately, n**o follow-up was conducted to trace congenital transmission of toxoplasmosis and health consequences of seroreactive pregnant women.** Screening for *T. gondii* infection during pregnancy is not regularly performed at all maternity hospitals and clinics in Somalia, and most facilities neglect this screening (data not shown). Prompt diagnosis and treatment is essential for the prevention of a possible vertical transmission of *T. gondii* and may minimize the fetal sequelae [21].

4.6 CONCLUSION

A high seropositivity to *T. gondii* and low seropositivity to *Brucella* spp. have been found in pregnant women from Mogadishu, Somalia. Considering the high number of abortions in the country associated to the fact that awareness on other zoonotic abortifacient pathogens in Somalis is very low, further studies should be conducted to evaluate the potential causes of abortions.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

AAY, AAHK, AMI and MAHK designed the study. AAY, AAHK, MAHK and AMY collected the data. AAY, AAHK and MAHK curated the data. AAY, AAHK, AMI, MAHK, AMY, MK, JLG and RFCV carried out the methodology. AAY, AAHK and

RFCV performed the data analysis. AAY, AAHK, JLG and RFCV drafted the manuscript. All authors edited and approved the final manuscript.

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Table 1: Prevalence of anti-*Toxoplasma gondi*i and anti-*Brucella* spp. antibodies in pregnant women from Mogadishu, Somalia.

Variable		LAT(For anti-*Toxoplasma gondii)*				RBPT(for Brucellosis)		cELISA(for Brucellosis)	
		+/*n*	Prevalence (%) (95% CI)	*P*-value	OR (95% CI)	+/*n*	Prevalence (%) (95% CI)	+/*n*	Prevalence (%) (95% CI)
Age	15-30*	132/26	50.38 (44.37-56.39)			1/262	0.38 (0.00-2.35)	2/262	0.76 (0.03-2.9)

		2							
	31-40	25/42	59.52 (44.47-72.98)	0.272 (χ^2 = 1.2)	1.4 (0.7-2.8)	1/42	2.38 (0.00-13.44)	1/42	2.38 (0.00-13.44)
	>40	2/3	66.67 (20.25-94.37)	0.575 (χ^2 = 0.3)	2 (0.2-22)	0/3	0.00	0/3	0.00
Gestational age	1st trimester	24/44	54.55 (40.06-68.3)	0.507 (χ^2 = 0.4)	1.2 (0.6-2.4)	0/44	0.00	0/44	0.00
	2nd trimester	32/53	60.38 (46.92-72.43)	0.140 (χ^2 = 2.2)	1.6 (0.9-2.9)	0/53	0.00	0/53	0.00
	3rd trimester*	103/210	49.05 (42.36-55.76)			2/210	0.95 (0.03-3.59)	3/210	1.43 (0.29-4.25)
History of Abortion	Yes	64/119	53.78 (44.85-62.48)	0.579 (χ^2 = 0.3)	1.1 (0.7-1.8)	2/119	1.68 (0.08-6.2)	3/119	2.52 (0.52-7.29)
	No*	95/188	50.53 (43.45-57.6)			0/188	0.00	0/188	0.00

Abbreviations: * reference, +, number of positive samples; *n*, number of samples; 95% CI, 95% confidence interval; OR, odds ratio; LAT, latex agglutination test; RBPT. Rose Bengal plate test; cELISA; Competitive Enzyme linked immunosorbent assay

6 GENERAL CONCLUSIONS

The present study has shown that toxoplasmosis is prevalent in pregnant women from Somalia. Moreover, low seroreactivity to *Brucella* spp. among pregnant women in Mogadishu, Somalia was also recorded. Since the awareness on these zoonotic pathogens in Somali people is incredibly low, the people in this country are at great public health risk. Additionally, there are little or no medical and veterinary efforts to maximize toxoplasmosis and brucellosis detection rates in humans and animals in the country, thus preventing recognition of any association between zoonotic diseases and immunocompromised patients, including pregnant women, Hepatitis and HIV patients. Therefore, there is a need to promote the One Health concept among multi-sectoral professionals for better and sustainable integrated health development and implementing effective control strategies against these zoonotic diseases.

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