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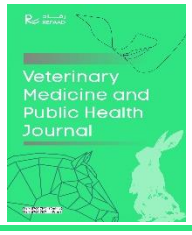

Seroprevalence and Risk Factors of Human Brucellosis and Toxoplasmosis in Ghazni Province of Afghanistan

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Abstract: Brucellosis and toxoplasmosis are considered neglected zoonotic diseases with a worldwide distribution, which are endemic in Afghanistan. A cross-sectional study was carried out from May 24, 2021, to Feb 20, 2022 to determine the seroprevalence and risk factors associated with human brucellosis and toxoplasmosis in suspected patients admitted to the public and private laboratories of Ghazni province of Afghanistan. To detect *Brucella* IgM. a semi-quantitative agglutination test was used, while for the detection of *Toxoplasma* IgM and IgG, a chromatographic immunoassay was applied. Seroprevalence of brucellosis and toxoplasmosis were 32.1% (76/237) and 19.6% (18/92) in tested patients, respectively. Whereas, only in five cases dual infections were found. The majority of brucellosis positive cases were among the age group >45 years old (57.1%; $p<0.05$), in district residents (43.5%; $p>0.05$), and among those with abortion (30.6%; $p<0.05$) and history of positive brucellosis cases (50.0%; $p<0.05$) in their family members. High seropositive cases of toxoplasmosis were observed in males (50.0%; $p>0.05$), 21-45 years old people (20.8%; $p>0.05$), those who owned dogs and cats ($p>0.05$), among those with the history of abortion cases in their animals (41.7%; $p<0.05$), and positive toxoplasmosis cases in their family members (66.7.0%; $p\leq0.05$). The odds of being seropositive for brucellosis was higher in those that had history of abortion (OR: 4.3; 95%CI: 1.3-4.2) and brucellosis cases (OR: 7.1; 95%CI: 1.7-28.9) among their family members. Furthermore, the odds of being seropositive for toxoplasmosis was 13.2 times more (95%CI: 2.1, 82.2; $p<0.05$) in those which had history of abortion cases in their animals. The high seroprevalence could be attributed to the study population being drawn from suspected cases rather than the general population. To assess the cross-connection of infections in human and animal populations, it would be more beneficial to study a large number of human and animal samples concurrently.

Keywords*: brucellosis; toxoplasmosis; seroprevalence; risk factors; Ghazni province of Afghanistan.*

1. Introduction

Brucellosis is one of the most important neglected zoonotic diseases in the world, with 500,000 new cases every year (O'Callaghan, 2020). It is caused by gram negative bacteria of the genus *Brucella* (Pappas et al., 2006, 2005, Seleem et al., 2010; Samadi, 2022). Although the genus includes several species, six well-defined species which affects animals are as follow: *Brucella abortus* (*B. abortus)* in cattle, *B. melitensis* in goat and sheep, *B. ovis* in sheep, *B. suis* in pig, *B. canis* in dog and *B. neotomae* in desert wood rat (Adrian et al., 2014; Głowacka et al., 2018; Berhanu and Pal, 2020). Although the majority of *Brucella* species are host-specific, cross-species infections are widespread (except *B. ovis*). Abortion, infertility, sperm abnormality, orchitis, epididymitis and arthritis are the most common signs of brucellosis in infected animals. The infectious agent is primarily excreted by infected animals through their milk, aborted fetuses, placentas, and associated secretions. The main routes of *Brucella* transmission from infected animals to humans are unpasteurized milk and undercooked meat, but direct and indirect contact with aborted fetuses, placentas, and discharges can also transmit the bacteria to humans (Gowacka et al., 2018; Berhanu and Pal., 2020).

Meanwhile, toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* (*T. gondii*) which is one of the most widespread and neglected zoonotic diseases worldwide (Foroutan et al., 2018). Although, felids serve as definitive hosts for *T. gondii*, the parasite infects most warm-blooded mammal species including sheep, cattle, pigs, deer, raccoons, chickens and humans (Dubey et al., 2005; Wana et al., 2020; Abas et al., 2019). *T. gondii* cause both clinical and subclinical diseases in infected cats, while in other species (sheep, goats and cattle) clinical toxoplasmosis is rare, but abortion and still births are common clinical manifestation in domestic animals (Foroutan et al., 2018). Cats with clinical toxoplasmosis are characterized by anorexia, depression, pneumonia and sudden death (Mose et al., 2020; Dubey and Jones, 2008). The infected cats are the main source which shed the oocytes in the environment, facilitating the infection of other mammals. Whereas other mammals and humans are considered dead-end hosts and do not shed the oocytes. The main routes of disease transmission among animals are ingestion of infectious oocytes with contaminated water and vegetables, whereas disease transmission to humans is facilitated by consumption of undercooked meat of infected animals, and cat feces' contaminated water and vegetables. Although the transmission of disease from human to human has not been documented, transplacental/congenital transmission has been reported in both humans and animals (Robert-Gangenux and Darde, 2012; Dubey and Jones, 2008; Mose et al., 2020). Most cases of toxoplasmosis in humans are subclinical, except in fetuses, newborns, people with secondary illnesses, and immunologically compromised people with HIV, where *T. gondii* causes encephalitis, chorioretinitis, congenital infection, and neonatal mortality (Mose et al., 2020).

Despite brucellosis has been eradicated in some developed countries, it remains a severe concern in developing countries such as Afghanistan. Brucellosis has been endemic in Afghanistan for a long time, with a considerable number of cases reported from human and animal populations throughout the country (Kozojed et al., 1976; Mosawi et al., 2019) and in the neighboring countries (Khamesipour et al., 2014; Foroutan et al., 2018). Meanwhile, many reports indicate the presence of Toxoplasmosis in human and animal populations in Afghanistan (Hamid, 2007; Ndu et al., 2015; Kozojed et al., 1976).

In the present study, we sought to determine the seroprevalence of human brucellosis and toxoplasmosis, and associated risk factors in the patients admitted to the public and private laboratories of Ghazni province of Afghanistan.

2. Materials and Methods

2.1. Study Area

The present study was carried out from May 24, 2021, to Feb 20, 2022 in Ghazni province of Afghanistan. Ghazni province is located in southeastern Afghanistan which has 18 districts with a population density of 63 per km^2 and with a total area of 22460.5 km^2. The province's overall population is 1,362,504, with a nearly equal gender distribution, however 95 percent of the population lives in rural areas (NSIA, 2021). Agriculture and animal husbandry are the most common occupations of the people in Ghazni province (https://en.wikipedia.org/wiki/Ghazni Province). According to an old data (2003), 447,792 domestic animals, including 84,795 cattle, 246,083 sheep, 76,266 goats, 721 camels, 39,087 donkeys, and 840 horses, as well as 328,741 backyard poultry (chicken, duct, and turkey) were present in Ghanzi province, accounting for 2.1% of all domestic animals and 2.5% of all poultry in Afghanistan (FAO, 2006). During the data collection, four labs (central public health facility and three private labs) consented to cooperate in the survey.

2.2. Study design and data collection method

A structured questionnaire and the results of an agglutination test were used to collect the essential data for this cross-sectional investigation. All laboratories received the questionnaire in the form of a registration book. The data about patients' age, sex, occupation, residence (district), animal ownership, abortion cases in the family and their animals, past brucellosis and toxoplasmosis cases in the patients or their family members, their practices with aborted fetuses, and history of brucellosis vaccine administration in their animals were gathered.

2.3. Laboratory tests

The physicians referred the suspected patients to the diagnostic labs. Five mL of blood was collected from each patient, and serum was separated by centrifugation at 112 g for 5-10 minutes. The semi-quantitative agglutination test for *Brucella* IgM and the chromatographic immunoassay for *Toxoplasma* IgM/IgG were carried out, and the results were interpreted according to the manufacturer's instructions.

Brucella reagent containing suspensions of killed and stained bacteria was used to detect *Brucella* IgM antibodies in human serum (*Brucella* reagent, DAILAB, Neudorf, Austria). This reagent detects *B.* abortus, *B. melitensis*, and *B.* suis. Fifty µl of serum and 50 µl of control reagents were placed in separate test fields of a reusable slide for each patient. Following that, 1 drop of reagent was added to both the sample and control reagents. After mixing with a mixing stick, the slide was placed on a mechanical rotator or manually rotated for one minute. Finally, within 1 minute of being removed from the rotator, the slide was examined macroscopically for the presence or absence of clumps. The presence of clumps in slide field was considered as *Brucella* positive case.

For the detection of IgM or IgG of *Toxoplasma* in patients referred to the diagnostic laboratories, ToRCH rapid test cassettes were used (Safecare Biotech, USA). The ToRCH rapid taste is a combo chromatographic immunoassay which can detect the IgM and IgG of *T. gondii*, Rubella virus, Cytomegalovirus and Herpes simplex virus type 1 and 2. According to the manufacturer's guidelines, one drop of blood serum was added to the test cassette and subsequently 2 drops of buffer were added on it. After 15 minutes, the test result was judged based on the appearance of lines in each column. The presence of line in IgM, IgG or in both columns were referred to Toxoplasmosis positive cases.

2.4. Statistically analysis

Statistical analysis was performed using SPSS software (IBM, version 20, USA). Chi-square test was used to identify the association between test results and other predictor variables. P-values were set at ≤0.05 to find the statistically significant association between risk factors and patients' infection with brucellosis or toxoplasmosis. Logistic regression was applied as a final model to calculate the odds ratios of each independent variables with the test results.

3. Results

3.1. Demographic information of the patients

During the study period, 252 samples were referred and analyzed for brucellosis and toxoplasmosis, where 116 samples (46%) being tested in public health hospital laboratory. The patients' average age was 31.2 years old (range= 12-80 years old), with the majority (n =212, 84.13%) were in the most active and working age groups (21-40 years), but only 7.54 percent and 8.33 percent in the lower (20 years) and upper (41 years) age groups, respectively. Based on the sex distribution, 83.7% of the participants were females, while the rest were males. Moreover, three-fifths of the patients (64.4%) came from the province's center, 16.7% came solely from the Andar and Qarabagh districts, and 5.6% came from adjacent provinces such as Wardak, Logar, Paktia and Paktika.

3.2. Husbandry practice and companion animals' ownership among the patients

From the total of the participants whom responded, 45.3% raise cows, and 41.6% raise sheep and goats. Meanwhile, 69.2% of respondents had cats, while 31.9% had dogs in their homes. These practices were different in the province's center and districts, with 76.7% of districts' inhabitants keeping cows compared to only 33.0% of province center residents. Almost the same proportions were observed in sheep and goats raising in districts (75.6%) and the center (28.0%) of the province, respectively. Meanwhile, dog ownership was lower in the center (20.2%) than districts (61.5%), however cat ownership was somehow prevalent among both, the center (63.8%) and districts (82.5%) residents of Ghazni province.

3.3. History of brucellosis and toxoplasmosis cases

In the last three years (2018– 2020), 36.6% of the respondents (n=101) had abortion cases in their family members. Meanwhile, 14.4% (n=17/118) reported brucellosis cases in their family members in the last three years while 11.5% (n=14/122) reported toxoplasmosis cases in their family members. In addition, 20.4% of research participants reported abortion cases in their animals in the previous three years while only 2% of respondents vaccinated their ruminants against brucellosis.

3.4. Seroprevalence of brucellosis and toxoplasmosis

Seroprevalence of brucellosis and toxoplasmosis were 32.1% (76/237) and 19.6% (18/92), respectively. Only five tested cases had dual infections. The majority of brucellosis positive cases were among females (33.7%; $p>0.05$) and in the age group >45 years old (57.1%; $p<0.05$), in district residents (43.5%; $p>0.05$), and among those with abortion (30.6%; $p<0.05$) and history of positive brucellosis cases (50.0%; $p<0.05$) in family members in the previous three years (table 1). In the last three years, high seropositive cases of toxoplasmosis were observed in males (50.0%; $p>0.05$), 21-40 years age group (20.8%; $p>0.05$), those which owned dogs and cats ($p>0.05$), and those with abortion cases in their animals (41.7%; $p<0.05$) and positive toxoplasmosis cases in family members (66.7.0%; $p\leq0.05$) (table 1).

3.5. Logistic regression results

Two separate binary logistic regression models for brucellosis and toxoplasmosis were carried out to

assess the impact of specific factors on the likelihood of *Brucella* and *Toxoplasma* infections on tested patients. In the case of brucellosis, the model contained two independent variables (abortion and brucellosis cases in the family members in the last three years). The full model containing both predictors was statistically significant, (2, N = 252) = 13.454.63, p <0.05 (Omnibus test for model coefficients), indicating that the model was able to distinguish between seropositive and seronegative patients. Hosmer and Lemeshow test also indicated that the model was good fit (chi-square, 0.486, p = 0.784) and both independent variables had good statistically significant contribution to the model (table 2). Regarding toxoplasmosis, the model contained just one independent variable (abortion cases in their animals in the last three years) which was statistically significant, (1, N = 252) = 8.717, p <0.05 (Omnibus test for model coefficients), that indicate that the model was good fit (table 2).

As shown in table 2, the odds of a patient being seropositive for brucellosis was 4.3 times more in those that had cases of abortion in their family members in the last three years (95%CI: 1.3, 14.2; p<0.05), controlling for the second variable in the model. At the same time, patients which had history of brucellosis cases in their family members were more likely to be seropositive for brucellosis (OR: 7.1; 95%CI: 1.7, 28.9; p<0.05). Furthermore, the odds of being seropositive for toxoplasmosis was 13.2 times more (95%CI: 2.1, 82.2; p<0.05) in those which had abortion cases in their animals in the last three years (table 2).

4. Discussion

Brucellosis and toxoplasmosis, both of which have serious health and socioeconomic consequences, are considered neglected zoonotic diseases with a worldwide distribution (Denk et al., 2022; Firoozi Jahantigh et al., 2020; Schurig et al., 2021). Given the importance of ruminants as the primary source of income for the majority of Afghans (Samadi and Hailat, 2019), abortion and stillbirths in ruminants caused by *Brucella* and *Toxoplasma* infections could be devastating for the country's farmers and rural communities. Furthermore, abortion and painful systematic disease in *Brucella* patients (Samadi et al., 2010) and severe *Toxoplasma* infection in high-risk groups such as pregnant women and immune-compromised individuals (Foroutan et al., 2018), are both considered life-threatening health conditions for humans, particularly in developing societies including Afghanistan (Mosawi et al., 2019; Akbarian et al., 2015).

Although brucellosis and toxoplasmosis cases have been reported from human and animal populations in Afghanistan on a regular base (Foroutan et al., 2018; Mosawi et al., 2019; Akbarian et al., 2015; Kozojed et al., 1976; Corbel, 1997; Pappas et al., 2006; Aronson, 2008; FAO, 2012; Cárdenas et al., 2019; Piroozi et al., 2019; Samadi, 2022), this was the first study which determined the seroprevalence of these two neglected zoonotic diseases in humans in Ghazni province of Afghanistan.

Based on the previous reports of brucellosis in human and animal populations in Afghanistan, such a high seroprevalence (32.1%) in laboratory admitted people in Ghazni province was expected. Akbarian et al. (2015) reported 24.5% seropositive *Brucella* cases in households and their animals in a study in Herat province, in the west part of Afghanistan. Another study found 20.5% of human brucellosis seroprevalence in Bamyan Province (FAO, 2012).

Millions of doses of Rev.1 and S19 Brucella vaccines have been administered to small ruminants and cattle in Afghanistan over the last decade, respectively (Samadi, 2022). Both vaccines are live attenuated, causing abortion in vaccinated animals and systematic disease in human, and immunized animals can shed the vaccine strains, causing brucellosis in humans (Wallac et al., 2008; Samadi et al., 2010ab; O'Callaghan, 2020). Since there is no any post vaccination evaluation study performed in Afghanistan, continuous uncontrolled vaccination campaigns using these live attenuated vaccines in food animals could be on the factors which are causing infection in human and animals.

Although there were no statistically significant differences in brucellosis seroprevalence based on the patients' sex, age, residence (urban or rural), and animal ownership (p>0.05), patients with a history of abortion and positive cases in their family members were more likely to be seroreactive than others (p<0.05). Despite the fact that infected humans do not act as sources of infection for other humans or animals (with the exception of a few cases of vertical transmission) (Seleem et al., 2010), these two factors could indicate continuous contact of the same household members with other infectious sources such as infected animals and their contaminated products, which are the main sources of infection for humans (Pal, 2018; Dadar et al., 2019). Since brucellosis is regarded as a disease of poverty (O'Callaghan, 2020; Samadi and Hailat, 2010), the disease's continued uncontrolled occurrence in animal and human populations would be disastrous for Afghanistan, where more than 90 percent of the population already lives in poverty.

As with brucellosis, a high number of toxoplasmosis seropositive cases were observed in males, ruminant raisers, dog and cat keepers, and those with a history of toxoplasmosis cases in their family members (p>0.05), but a history of abortion cases in their animals was a statistically significant risk factor for becoming seropositive. It is obvious that aborted fetuses and other related materials from *T. gondii*-infected animals could contain a large number of three infectious stages of *T. gondii*, particularly tachyzoites, which can facilitate transmission of infection to the cats (Denk et al., 2022). Accordingly, abortion cases in the animals, which are mostly kept in close contact with household members, particularly in rural areas of Afghanistan, could prolong the infectious cycle in intermediate and definitive hosts.

Mosawi et al. (2019) reported a high seroprevalence (48.03%; 95%CI: 43.33–52.75) of toxoplasmosis among pregnant women in Kabul (the capital of Afghanistan), and they found that the residents in non-concrete floor houses (OR: 1.8) and those that use well or river as the sources of drinking water (OR: 1.94) were at high risk of toxoplasmosis ($p<0.05$). It has been suggested that geographical location, climate, and nutritional habits all play important roles in *T. gondii* survival and spread (Foroutan et al., 2018). Oria et al., (2022) described a case of Dandy-Walker syndrome in a 16-year-old female patient in Kabul, which might be caused by *T. gondii* infection (Hamid, 2007; Ndu et al., 2015). Kozojed et al. (1976) reported a high seroprevalence of toxoplasmosis (15.7 - 73.7%) in domestic animals (camels, zebus, goats, sheep, buffalos and cattle). These reports, as well as the current study's findings, indicate that toxoplasmosis occurs frequently in human and animal populations.

Table (1): Seroprevalence of brucellosis and toxoplasmosis based on the other independent variables, in Ghazni province, 2021-2022.

		Brucellosis test results					Toxoplasmosis test results				
Variables		**Positive (%)**	**Negative**	**Total**	**χ^2 value**	***p*-value**	**Positive (%)**	**Negative**	**Total**	**χ^2 value**	***p*-value**
Sex	Male	10 (24.4)x	31	41*	1.34	>0.05	1 (50.0)	1	2	1.2	>0.05
	Female	66 (33.7)	130	196			17 (18.9)	73	90		
Age categories	<20 year	7 (38.9)	11	18	7.42	<0.05	1 (20.0)	4	5	0.65	>0.05
	21-45 year	57 (28.8)	141	198			16 (20.8)	61	77		
	>45 year	12 (57.1)	9	21			1 (10.0)	9	10		
Raising ruminants	Yes	19 (25.39)	56	75	0.11	>0.05	9 (26.5)	25	34	0.66	>0.05
	No	17 (27.9)	44	61			6 (18.2)	27	33		
Dog ownership	Yes	12 (27.3)	32	44	0.029	>0.05	5 (29.4)	12	17	0.75	>0.05
	No	22 (25.9)	63	85			10 (20.0)	40	50		
Cat ownership	Yes	23 (24.2)	72	95	11.11	>0.05	10 (27.8)	26	36	1.30	>0.05
	No	12 (33.3)	24	36			5 (16.1)	26	31		
Residency	Center	32 (31.1)	71	103	2.62	>0.05	8 (16.7)	40	48	0.54	>0.05
	Districts	27 (43.5)	35	62			10 (22.7)	34	44		
Abortion cases among family members in the last 3 years	Yes	11 (30.6)	25	36	4.88	<0.05	5 (20.8)	19	24	2.01	>0.05
	No	8 (12.5)	56	64			2 (7.1%)	26	28		
Abortion cases in the animals in the last 3 years	Yes	3 (15.8)	16	19	0.05	>0.05	5 (41.7)	7	12	10.35	<0.01
	No	14 (17.9)	64	78			2 (5.1)	37	39		
History of disease in the family in the last 3 years	Yes	8 (50.0)	8	16	5.64	<0.05	2 (66.7)	1	3	6.35	≤0.05
	No	22 (22.0)	78	100			8 (12.9)	54	62		

***The differences in total numbers in the whole table are due to missing data**

Table (2): Binary logistic regression model results of predictor variables for the seroprevalence of brucellosis and toxoplasmosis in lab admitted patients in Ghazni province, 2021-2022.

	Variables	**B**	**Standard error**	**Wald**	***df***	***p*-value**	**OR**	**95% CI for OR**	
								Lower	**Upper**
Brucellosis	Abortion cases among family members	1.459	0.609	5.739	1	0.017	4.302	1.304	14.191
	Brucellosis cases among family members	1.963	0.715	7.534	1	0.006	7.121	1.753	28.927
	Constant	-2.560	0.511	25.114	1	0.000	0.077		
Toxoplasmosis	Abortion cases in the animals	2.581	0.933	7.660	1	0.006	13.21	2.124	82.213
	Constant	-2.918	0.726	16.154	1	0.000	0.054		

5. Conclusion

The recurrence of neglected zoonotic diseases such as brucellosis and toxoplasmosis endanger the health of human and animal populations in Afghanistan. The high seroprevalence of these diseases in Ghazni province residents could be attributed to the fact that the study population was drawn from suspected cases of these two diseases rather than being drawn at random settings from the general population. It would be more beneficial to study a large number of human and animal samples concurrently in order to assess the cross-connection of infections in human and animal populations. So, the only method for combating such zoonotic diseases and other health-related conditions with shared animal-human interference is, the One Health integrated approach.

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Compliance with Ethical Standards:

The data for this article was collected from those patients which were referred by the physicians to the diagnostic laboratories of Ghazni province, and this study does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest:

The authors declare that they have no conflict of interests.

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