

Original Research Article

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Epidemiological Study on Toxoplasmosis of Human and Animals at Dhamar Governorate, Yemen

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ABSTRACT

This cross sectional study was carried out in Dhamar governorate from December 2015 to November, 2016 with main objective to determine the seroprevalence of toxoplasmosis in humans and animals. A total of 323 individuals and 323 animals (241 sheep, 69 cattle and 13 goats) from different districts of Dhamar governorate were selected and tested using ELISA and PCR techniques for detection antibodies against *Toxoplasma gondii* in human and animals. The results revealed that, the overall seroprevalence of toxoplasmosis in human was 20.43%. The seroprevalence rates of IgG, IgM and both IgG +IgM were 13.62%, 4.33% and 2.48% respectively. The higher rate of infection was recorded (29.50 %) in age group of 11- 20years old; whereas, the lower rate (11.11%) was in age group of less than 10 years old. Females were more susceptible to infection (23.19 %) compared to males (2.02 %). The higher seroprevalence rate was recorded in summer (28.41%); whereas, the lower rate in spring (13.15%). The higher prevalence of rate was recorded in Utoma district (32.00%); whereas, lower rate in Alhada district (3.85%). Statistically, significant differences (P<0.05) were observed between seroprevalence rate and age group, sex; while none with season and area factors. In animals, the results of ELISA revealed that, the overall seroprevalence of toxoplasmosis was 23.84 percent, the seroprevalence of IgG, IgM and both IgG +IgM were 15.5%, 1.5% and 6.8% respectively. Out of 241 sheep, 69 cattle and 13 goats tested, 21.16%, 31.88%, 30.77% respectively were seropositive for toxoplasmosis. The infection rate of toxoplasmosis was high in age group of 5 years old and above (33.33%); whereas, low (21.55%) in age group of two years. The seroprevalence rate in males was (28.28%) higher compared to females (21.88 %). The higher seroprevalence rate was recorded in spring season (33.33%); whereas, the lower rate in winter (18.02.67%). The higher of seroprevalence was recorded in Utoma (35.71%); whereas, the lower rate in Mifa`a ans (10.715%) and none in Jabal ashariq. Statistically, significant differences (P<0.05) were observed between seroprevalence rate and animal species, season, area factors; while, none with other factors studied. In the present study, all ELISA seropositive samples of human and animals were retested by PCR as confirmatory tool. Results of PCR revealed that, out of 66 and 77 samples of human and animals tested 30 (45.45%) and 18 (23.38%) were found positive respectively for toxoplasmosis. The results of the present study revealed that toxoplasmosis is prevalent in human and animals in study area. Combinations of serological and molecular techniques are recommended for accurate diagnosis of Toxoplasmosis.

Keywords

Animals, ELISA, Human, PCR, Prevalence, Toxoplasmosis, Dhamar, Yemen

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Introduction

Toxoplasmosis is one of the most worldwide parasitic zoonoses caused by the protozoan *Toxoplasma gondii* (Muqbil and Alqubatii, 2014; Zhou *et al.*, 2018). It is a major public health concern for human and domestic animals (Montoya *et al.*, 2009; Gebremedhin *et al.*, 2013; Luo *et al.*, 2017; Tonouhewa *et al.*, 2017). Domestic cats and wild felids play a crucial role in the epidemiology of toxoplasmosis as the definitive hosts, through the shedding of millions of oocysts when infected (Dubey, 2010; Gebremedhin *et al.*, 2013). Human beings and other warm-blooded animals become infected primarily by ingesting food or water contaminated with sporulated oocysts or by ingesting meat that contain tissue cysts of *T. gondii* (Hamzavi *et al.*, 2007; Dubey and Jones, 2008; Gasior *et al.*, 2013; Smit, *et al.*, 2017; Sroka *et al.*, 2018).

T. gondii has a worldwide distribution in human populations infecting up to one third of the global population and a wide range of other mammalian and avian species (Sukthana, 2006; Gharavi *et al.*, 2018). Toxoplasmosis is a major public health problem, with a high socioeconomic impact in terms of human suffering including the cost of caring for sick, mentally retarded and blind children (Roberts *et al.*, 1994; Mahdi *et al.*, 2008; Zhou *et al.*, 2018). The parasite is an extremely successful pathogen, responsible for significant morbidity and mortality, especially in congenitally infected and immunocompromised individuals (Tenter *et al.*, 2000; Luft and Remington, 1992; Elsheikha, 2008; Mahdi *et al.*, 2008). In animals, toxoplasmosis leads to great economic losses in food animals by causing embryonic death, fetal death, abortion and infertility (West, 2002; Tonouhewa *et al.*, 2017). The risk of infection can be reduced by proper food preparation. Meats thoroughly cooked, avoid cross

contamination of foods and utensils with oocysts of cats (Cook *et al.*, 2000).

The diagnosis of *Toxoplasma gondii* infection is conventionally made by the direct demonstration or isolation of the parasite from biopsy or autopsy material (Jula *et al.*, 2013), but such techniques are unsuitable for use in large-scale surveys. Therefore, recourse has been made to immunoserological tests for specific host antibody, and a variety of tests such as PCR has been used (El_Ghany *et al.*, 2012; Jula *et al.*, 2013; Tonouhewa *et al.*, 2017).

An increasing prevalence of toxoplasmosis in humans and animals has been drawn the attention of the researchers and workers to address toxoplasmosis as serious public health problem and many studies have been conducted in different geographical regions of the world and Yemen (Nimri *et al.*, 2004; Sharif *et al.*, 2006; Hamzavi *et al.*, 2007; Saleh *et al.*, 2010; Shahiduzzaman *et al.*, 2011; Subasinghe *et al.*, 2011; Tasawar *et al.*, 2011; Gebremedhin *et al.*, 2013; El-Madawy and Metawea, 2013; Kadle, 2014; Saif *et al.*, 2014; Ahmad *et al.*, 2015; Bakre, 2016; Tonouhewa *et al.*, 2017; Luo *et al.*, 2017; Gharavi *et al.*, 2018). However, the information on toxoplasmosis of human and food animals are limited at Dhamar governorate, Yemen. Therefore, the present study was designed to investigate the toxoplasmosis prevalence and associated risk factors in human and animals at Dhamar Governorate, Yemen.

Materials and Methods

Study areas

The cross sectional study was conducted in different districts of Dhamar governorate, namely, Alhada, Jahran, Anis, Jabal Alshaq, Mifa'a ans, Manar, Wusab, Utoma and

Dhamar city during the period from December, 2015 to November, 2016. Geographically, Dhamar is located at 14° 58'N latitude, 44° 43'E longitude and at an altitude of 2425 meter above sea level. The average rainfall, temperature and relative humidity is 40.5mm, 17.3°C and 57.8% respectively during the year of 2016.

Study population and size of sample

The study subjects were individuals attending to public and private hospitals for routine examination and farmers from different districts of Dhamar governorate. Animal population investigated were sheep, goats and cattle from various district areas and central slaughterhouses of Dhamar governorate. A total of 323 individuals and 323 animal heads were selected by systematic random sampling method. The sample size was calculated according to Thrusfield (2007) technique considering 30 % expected prevalence and 95% confidence interval with a 5% desired absolute precision using the following formula: $N = \frac{(Z)^2 P(1-P)}{d^2}$, where, (p) expected prevalence and (Z) 95% confidence interval (Z= 1.96) and (d) a 5% desired absolute precision.

Collection of samples

In humans

Blood samples were obtained from individuals/subjects referred to public and private hospitals for clinical and routine examinations. A 5 ml venous blood sample was collected from each screened subject. Serum was separated from half of each sample and kept at -20 °C until used, while the other half of the sample was placed in a sterilized EDTA tube and stored at -80 °C for amplification by PCR. Information related to subject i. e age, sex, area, season were recorded and maintained confidentiality. The blood samples were then brought to the

Veterinary parasitology laboratory, Faculty of Agriculture and Veterinary Medicine, Thamar University, Dhamar for processing and testing.

In animals

Blood samples were collected from animals. Approximately a 5 ml of whole blood sample was collected from each animal by venipuncture from the jugular vein using disposable plain vacutainer tubes and needles. Animal species include sheep, cattle, and goats. The blood samples were kept in two tubes, one with anticoagulant for PCR analysis; while another with none for serological testing. The blood samples were allowed to clot and then centrifuged to separate the serum. The serum was collected into 1.5 ml Eppendorf tubes and brought to the Veterinary parasitology laboratory, Faculty of Agriculture and Veterinary Medicine, Thamar University, Dhamar for processing and testing. All samples were labeled properly with necessary information.

Testing of samples (Sera)

The entire sera of human and animals were screened for *Toxoplasma gondii* infection with serological technique (ELISA) and then, the sero-positive samples of serological technique were retested by molecular technique (PCR) as confirmatory tool.

Serological technique

In laboratory, All the collected sera samples of human and animals were tested for the presence of IgG, IgM and both antibodies against *T. gondii* by indirect enzyme linked immunosorbent assay (ELISA) following the protocol of the manufacturers.

ELISA

The samples were testing by ELISA (Dia. pro Toxo-IgM kit, Milano, Italy) according to the

manufacturer's instructions and guidance of Gondim *et al.*, (1999) and Al-Harthi *et al.*, (2006).

Molecular technique

Extraction of DNA from whole blood

DNA was extracted from whole blood for both human and animals using a commercial purification system (AccuPrep® Genomic DNA Extraction Kit, Bioneer, Korea) according to manufacturer instructions.

Nested PCR Assay (Simplex Polymerase Chain Reaction)

Conventional PCR was used on all DNA samples to amplify a fragment of DNA *Toxoplasma gondii*. The specific primers for used for amplification of the sequence of *T. gondii* DNA were: forward ITS1-F 5`ACA CGT CCT TAT TCT TTA TTA ACC A3` and revers primers ITS1-R 5`ATC CCA ACA GAG ACA CGA ATT 3` as described by (Rahumatullah *et al.*, 2012).

These primer amplify the 234bp from ITS1 gene of *Toxoplasma gondii* were obtained from AccuPower ® HotStart PCR PreMix (bioneer Korea). The PCR reaction was contain 1µl of each primer, 1µl of DNA and 18µl ddH₂O until the total volume of mixture becomes 20 µl.

Thermal PCR conditions consisted 94°C for five minutes, followed by 35 cycles at 94°C for 45 seconds, 57°C for 45 seconds and 72°C for 45 and a final extension at 72°C for 5 minutes. A 10 µL aliquot of the amplified product was analyzed on 1% agarose gel and stained with ethidium bromide. Every PCR run included positive and negative controls. After electrophoresis, generated bands were screened and digitally photographed under UV light.

Ethical consideration

Verbal consent from all subjects involved in this study was obtained after provided them detailed explanation on objective of the study. Subjects also were assured that the obtained information should be kept confidentially and used only for research purposes.

Statistical analysis

The data obtained from this study were entered in Microsoft excel spreadsheet (Microsoft Corporation). All statistical analyses were performed using the statistical package for the social sciences SPSS (version 17). The frequencies and percentages were presented and Chi square test was used to analyze the *T. gondii* seroprevalence in respect of species, age, season, gender, and area. The differences were considered to be statistically significant when the P value was less than 0.05.

Results and Discussion

Results of human study

Out of 323 sera samples of human examined, 66(20.43%) were found seropositive for toxoplasmosis according to ELIZA results. In addition, Out of 66 samples seropositive for toxoplasmosis, 44(13.62%), 14(4.33%) and 8(2.48%) were seropositive for IgG, IgM and both IgG and gM respectively (Table 1). There were significant differences (P<0.05) among seroprevalence rate of antitoxoplasma antibodies in human.

The effects of risk factors age, sex, season and area on distribution of toxoplasmosis in human were investigated and the results were presented in Table 2. The results revealed that, the higher seroprevalence rate (29.50%) was recorded in age group of 11-20 year; whereas, the lower rate (11.11%) in age group of 10≤

years old. The seroprevalence rate of toxoplasmosis in males was 2.02 %; whereas, the rate in female was 23.19%. The higher seroprevalence rate was recorded in summer season (28.41%); whereas, the lower rate in spring season (13.15%). The higher of seroprevalence of toxoplasmosis was recorded in Utoma district (32.00%); whereas, the lower rate in Mifa`a ans district (15.00%). Statistically, significant differences (P<0.05) were observed between seroprevalence rate and age group, sex; while none with season and area factors. The results of the correlation analysis revealed that, the association between seroprevalence and metrological such as temperature (r = -0.327, P< 0.01), rainfall(r = -0.357, P< 0.01) and humidity (r =-0.201, P< 0.01).

Results of animals study

A total of 323 animals were examined, 77(23.84%) animals were found infected with toxoplasmosis according to ELIZA technique results. In addition, Out of 77 seropositive samples of animals for toxoplasmosis, 50 (15.5%), 5(1.5%) and 22(6.8%) were seropositive for IgG, IgM and IgG +IgM respectively (Table 3). There are significant differences (P<0.05) among Seroprevalence of antitoxoplasma antibodies in animals.

The association between the seroprevalence of toxoplasmosis in animals and risk factors

also was investigated in this study and the results are presented in Table 4. As shown, the seroprevalence rates recorded in Sheep, cattle and goats were 21.16%.31.88 and 30.77% respectively. The higher seroprevalence rate was recorded (33.33%) in age group of 5 year and above; While, the lower (21.55%) in animals group of two years old. The seroprevalence of toxoplasmosis in males (28.28%) were higher compared to females (21.88 %). The higher seroprevalence rate was recorded in spring season (33.33%) and the lower rate in winter (18.02%).

The higher seroprevalence rate of toxoplasmosis in animals was recorded in Utoma district (35.71%); and the lower rate in Mifa`a ans (10.715%) and none in Jabal asharg district. Significant differences (P<0.05) were observed between the seroprevalence rate and animal species, season, area factors; while none in with other factors investigated.

In the present study, all ELISA seropositive samples of human and animals were retested by PCR as confirmatory technique tool. Results of PCR revealed that, Out of 66 and 77 samples, 30(45.45%) and 18(23.38%) were found positive for toxoplasmosis in human and animals respectively (Table 5 and Fig. 1). Significant differences were (P<0.05) observed between the prevalence rates of human and animals.

Table.1 Seroprevalence of anti *Toxoplasma gondii* IgM and IgG in human at Dhamar governorate

Test	No. Samples examined	No. of positive	Seropositivity%	P valu
ELIZA IgG	323	44	13.62	0.000
ELIZA IgM	323	14	4.33	
ELIZA IgG & IgM	323	8	2.48	

Table.2 Risk factors influencing distribution of toxoplasmosis in human

Risk factor	No. of subjects examined	No. of subjects infected	Seropositivity Prevalence %	P value	
Age	10≤ yrs	9	11.11	0.007	
	11-20 yrs	78	29.50		
	21-30 yrs	104	11.33		
	31-40	85	20.71		
	41-50 yrs	38	21.94		
	above 50	9	0.00		
Sex	Male	263	61	23.19	0.006
	Female	60	2	2.02	
Season	spring	66	13	13.15	0.068
	Summer	88	25	28.41	
	Autumn	101	13	17.58	
	winter	68	15	22.06	
Area	Alhada	53	2	3.85	0.564
	Jahran	58	13	22.41	
	anis	19	4	21.05	
	Jabal alshaq	15	1	6.67	
	Mifa`a ans	20	3	15.00	
	Manar	29	6	20.69	
	Wusab	28	7	25.00	
	Utoma	25	8	32.00	
	Dhamar city	77	18	23.38	
	Total	323	62	50.00	

Table.3 Seropositive of anti toxoplasmosis IgG and IgM in animals based on ELISA tests

Test	No. of animals examined	No. of animals infected	seroprevalence	P value
ELISA IgG	323	50	15.5	0.000
ELISA IgM	323	5.0	1.50	
ELISe IgG & IgM	323	22	6.80	

Table.4 Risk factors influencing distribution of toxoplasmosis in animals in Dhamar and governorate

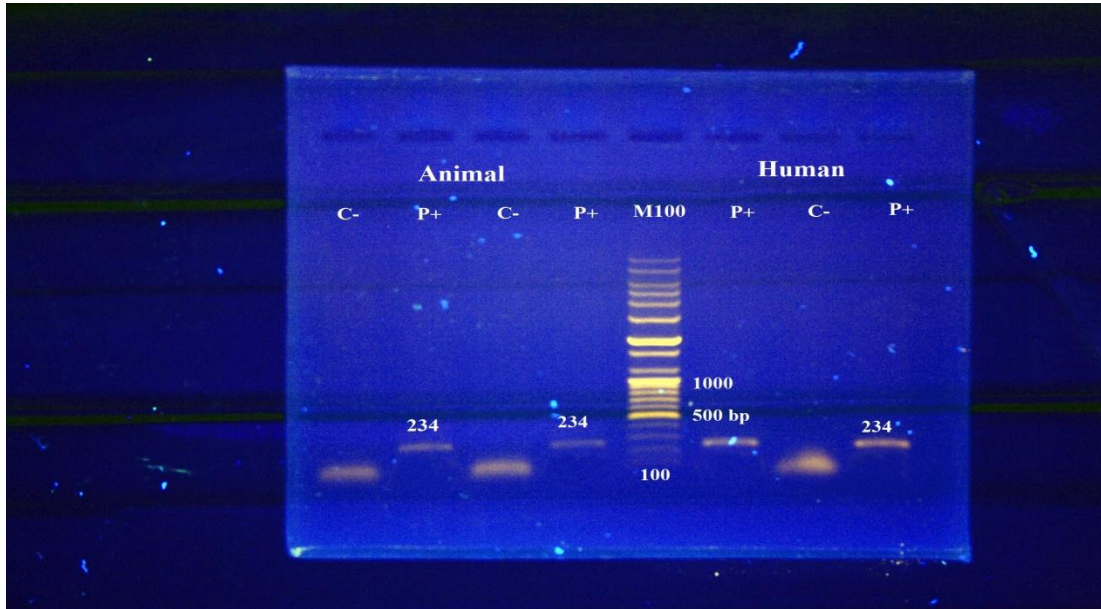
Variables		No. of animals examined	No. animals infected	Sero-prevalence	P value
Animal species					
	Cattle	69	22	31.88	0.015
	Sheep	241	51	21.16	
	Goats	13	4	30.77	
Age group					
	One Year	97	24	24.74	0.861
	2yrs	116	25	21.55	
	32yrs	66	15	22.73	
	42yrs	35	10	28.57	
	5yrs & abov	9	3	33.33	
Sex					
	Female	224	49	21.88	0.158
	Male	99	28	28.28	
season					
	Spring	78	26	33.33	0.031
	Summer	66	14	21.21	
	Autum	68	17	25.00	
	Winter	111	20	18.02	
Areas					
	Alhada	44	10	22.73	0.001
	Jahran	31	10	32.26	
	Aris	26	8	30.77	
	Jabal Alshaq	38	0	0.00	
	Mifa'a ans	28	3	10.71	
	Manar	38	5	13.16	
	W usab	27	9	33.33	
	Utoma	28	10	35.71	
	Dhanar City	63	22	34.92	
	Total	323	77	23.84	

Seroprevalence rate was calculate from total number of samples examined for each variable

Table.5 Prevalence of toxoplasmosis in human and animals using molecular tool (PCR) as confirmatory test for ELIZA positive samples

Subject	No. of ELIZA seropositi samples	No. of Positive for PCR amplified samples	positivity ra	P val
Human	66	33	45.45	0.0
Animal	77	18	23.37	

Figure.1 PCR amplification of the ITS gene of *Toxoplasma gondii* genome. In right side result of human, Lane 1. Marker, Mol. wt. marker (100 bp ladder), lane 2 positive samples, Lane 3, Negative control, Lane 4, positive sample. Left side, results of animal, lane 1, Negative control, Lane 2 positive samples, Lane 3, Negative control, Lane 4, positive control, Lane 5 Marker



Seroprevalence of *T. gondii* infection in human and animals has been used as an indicator of the endemicity of the parasite (Jones *et al.*, 2001; Xiao *et al.*, 2010). The epidemiology of toxoplasmosis in human in many countries has been investigated, but at Dhamar Yemen has not been clear. In this study, results revealed the overall seroprevalence of toxoplasmosis in human in Dhamar governorate was 20.43% which was generally lower than that reported in some governorates of Yemen (Qotinah, 2009; Al-Nahari, and Al-Tamimi, 2010; Saleh *et al.*, 2010; Saif *et al.*, 2014; Muqbil and Alqubatii, 2014) and other countries of the world (AlKalaby *et al.*, 2002; Chaudhary *et al.*, 2006; Madi *et al.*, 2008; Hassanain *et al.*, 2013; Gasiar *et al.*, 2013; Almasian *et al.*, 2014; Gharavi *et al.*, 2018) the prevalence rate reported by above workers was ranged from 29.8%- 83.1%. However, our result was higher than seroprevalence rate (12.3%) reported by Xiao *et al.*, (2010) in China. The contrary between the results of current study

and findings of above workers could be attributed to difference in environmental conditions, immune status of the individuals, socio-cultural differences, habit of consuming meat, abundance of definitive as well as intermediate as suggested by Yamaoka and Konishi (1993) and Xiao *et al.*, (2010). In this study the results revealed out 66 samples positive for toxoplasmosis, 44(13.62%), 14(4.33%) and 8(2.48%) were seropositive for IgG, IgM and IgG7 and gM. These results are lower than findings of other workers in Yemen (Al-Nahari, and Al-Tamimi, 2010; Saif *et al.*, 2014) and different part of the world (Mahdi *et al.*, 2008; in Qatar; Xiao1 *et al.*, 2010 in China; Hassanain *et al.*, 2013 in Egypt), who reported the seroprevalence rates of IgG, IgM and both IgG and IgM between (11.88-60%), (1.5-19.6%) and (3.5-22.6%) respectively. The discrepancy between the results of this study and findings of above may be due to the size samples, human errors, efficiency of equipment used and immunological status of patients.

In the present study, the higher seroprevalence rate of toxoplasmosis was recorded in age groups of 10-20 years' old and lower rate in age group of less than 10 years old. These results are in consistent with findings of Madi *et al.*, (2008) in Qatar, Saleh *et al.*, (2010); Muqbil and Alqubatii (2014) in Yemen, who studies the effect of age in distribution seroprevalence rate of toxoplasmosis in humans. However; it's lower rate than findings of Mohamad *et al.*, (2013). The reason behind, the higher infection rate in age group of 10-20 years old and above might be due to that elder individuals are exposed to infection more than younger. Furthermore, Dupouy-Camet *et al.*, (1993) and Remington *et al.*, (2001) suggested that seroprevalence of *Toxoplasma* is well known to increase with age.

The results of the current study displayed that, the seroprevalence of toxoplasmosis was higher in female compared to male. These results are in agreement with findings with others workers (Xiao *et al.*, 2010 in china; Saleh *et al.*, 2010 and Muqbil and Alqubatii, 2014 in Yemen), who studied the relationship between the seroprevalence of toxoplasmosis and gender factor in human. The higher infection rate in females could be attributed to the females are more associated to animals either in the home or in field including cats the final host of the toxoplasmosis, thus may be facilitating the transmission of infectious diseases to them. Moreover, in Yemen, traditionally, women undertaking the care of animals including feeding, breeding, milking of animals etc either in field or home (Al-Nahari and al-Tamimi, 2010).

Regarding the effect of season (month variation) on seroprevalence rate of toxoplasmosis in human, the results revealed that, the higher rate of infection was recorded in summer; whereas, lower rate in spring. The higher infection rate in human recorded in

summer may be due to the environmental conditions in summer are suitable for propagation and survival of the causative agent of the infection.

On area basis, the results displayed that, the higher seroprevalence rate of toxoplasmosis was recorded in individuals tested from Utoma district; while, the lower rate from Alhada district. This could be explained in view of Muqbil and Alqubatii (2014) who stated that, educational status of the people, society manner, number of stray cats available in study areas and hygienic conditions are influencing significantly in distribution prevalence of toxoplasmosis among different areas of study,

In animals

The results revealed that an overall seroprevalence of toxoplasmosis in animals was 23.84 % according to ELIZA results. This seroprevalence rate is lower than rate reported by other workers in different regions of world (Moreno *et al.*, 1991, in Spain; Kritsepi, 1992, in Serbia; Klun *et al.*, 2006, in Greece; Santos *et al.*, 2009, in Brazil; Iovu *et al.*, 2009 in Romaia; Shahiduzzaman *et al.*, 2011, in Bangladesh; Rahman, 2012, in Iraq; Hassanian *et al.*, 2013, in Egypt; Kadle, 2014, in Somalia; Ahmed and Taswar, 2015, in Pakistan; Zhou *et al.*, 2018; Tilahun *et al.*, 2018, in Ethiopia). The seroprevalence rates reported by them were ranged between 27.75-92.0%. However, the rate of our study was higher than that seroprevalence rate recorded by Gondum *et al.*, (1999) in Brazil; Song *et al.*, (2011), in Korea; Jula *et al.*, (2013) in Iran; Gąsior *et al.*, (2013) in Poland. The contrary between current results and findings of previous studies may be explained in view of Gąsior *et al.*, (2013) who suggested the differences in sero-prevalence of toxoplasmosis in animals between various countries depend on many factors such as

type of breeding and management practices, zoo-hygienic status, age of examined animals, serological techniques and geographical regions.

In current study, the results revealed that, out of 77 animals positive for toxoplasmosis, 15.5%, 1.5 % and 6.8 % were seropositive for IgG, IgM and both IgG and IgM respectively. These results are lower than findings of Prelezov *et al.*, (2008) in Bulgaria; Zakaria (2011) in Iraq, Shahiduzzaman *et al.*, (2011) in Bangladesh; Jula *et al.*, (2013) in Iran; Gebremedhin *et al.*, (2013) in Ethiopia; who reported the seropositive rates ranged between (18.3-83.8%), (17.9- 82.1%) and for IgG, IgM and both respectively. The detection and presence of IgG and IgM could be explained as high levels of specific IgG antibodies indicate that the individual has been previously infected. However, these antibodies do not distinguish a recent infection from one acquired a long time before. Detection of specific IgM antibodies can help to determine if infection recently occurred; although, these antibodies can persist for months or even years after acute infection (Liesenfeld *et al.*, 2001).

The risk factors such as animal species, age, sex, season and areas were studied and considered potentially influencing in seroprevalence rate of toxoplasmosis in animals. Regarding to animal species investigated. The results revealed that the seroprevalence rate of toxoplasmosis in sheep was 21.16%. These results are in agreement of findings of Savio and Nieto (1995) in Uruguay; whereas, lower than findings of Asgari *et al.*, (2013) in Iran; Klum *et al.*, (2006) in Serbia; Iovu *et al.*, (2009) in Romania; Kadle(2014) in Somalia, who reported the seroprevalence rates ranging between 34%-84.5%. In goats the seroprevalence recorded was 30.77%. These results are in consistent with findings of Shahiduzzaman *et al.*, (2011) in Bangladesh

and lower than rate reported by kadle (2014) in Somalia. However, Its higher than findings of Asari *et al.*, (2013) in Iran; Gondum *et al.*, (1999) in brazil, who recorded the seroprevalence rate as 18.8% and 28.93% respectively. In cattle, the seroprevalence rate was 31.88%. This result is lower than that recorded by Asgari *et al.*, (2013) in Iran and klum *et al.*, (2006) in Serbia, who reports the prevalence as 55% and 76.3 % respectively. However its higher than seroprevalence rate recorded by Shahiduzzaman *et al.*, (2011) in Bangladesh; kadle (2014) in Somalia who, reported the seroprevalence rate ranging between 7.1- 27.85%. The contrary between the seroprevalence rate recorded in present study and findings of above workers in animal species could be attributed to number of animals examined, farming management, hygiene condition, grazing habit, and methods used for detection of specific antibodies against *T. gondii*.

In present study, the higher seroprevalence rate of infection was recorded in elder animal groups; whereas, the lower rate in younger animal groups. These results are in line with findings Savio and Nieto (1995) in Uruguay; Oncel and Vural (2006) in Turkey; Hassanain *et al.*, (2013) in Egypt; Elfahal *et al.*, (2013) in Sudan who observed that elder animals are more susceptible to toxoplasmosis infection compared to younger animals. The higher seroprevalence rate infection in elder animal groups could be attributed to that aged animals have longer exposure time to oocysts of *T. gondii* compared to younger, higher likelihood on ingestion of oocysts by elder animals and immunological factors. Moreover, Gebremedhin *et al.*, (2013) suggested that the rate of infection is increasing with age. The results revealed that the seroprevalence rate was higher in male compared to females. These results are in agreement with findings of Asgari *et al.*, (2013) in Iran. Moreover; the impact of gender/sex on the frequency of toxoplasmosis

has been previously studied by many researchers (Oncel and Vural, 2006, in Turkey; Rahman, 2012, in Iraq; Jula *et al.*, 2013, in Iran; Elfahal *et al.*, 2013, in Sudan; Gebremedhin *et al.*, 2013, in Ethiopia) who observed the prevalence of toxoplasmosis was high in females compared to males. The differences between the results of current study and finding of above workers could be explained in view of Gebremedhin *et al.*, (2013) who suggested the females are retained in the farm for longer periods for breeding purpose than males this may increase the probability of infection; whereas, Few males are retained for mating while the majority are culled and sold for cash purpose. In addition, the hormonal difference in relation to stress of lactation and pregnancy leading to immunosuppressant may also increase susceptibility to toxoplasmosis in females.

Results of season wise revealed that, the higher of seroprevalence of infection was recorded in summer which coinciding with rain season; whereas, the lower rate in winter. These results are in consistent with findings of Oncel and Vural (2006) who studied the effect of seasons on distribution of seroprevalence rate of toxoplasmosis in sheep in Turkey. The higher seroprevalence rate during summer could be attributed to the longer viability of *T. gondii* oocysts in warm and humid environments which is suitable for propagation of parasite as suggested by Puije *et al.*, (2000).

PCR has been used as confirmatory tool for serological tests results (Nimri *et al.*, 2004; Hassanain *et al.*, 2013; AL-Dujaily and Amir, 2014; Hashoosh and Majeed, 2014; Sroka *et al.*, 2018). In the present study, all ELISA seropositive samples of human and animals were retested by PCR technique. The results of PCR revealed that, out of 66 and 77 samples of ELIZA, 45.45 % and 23.3% were positive for toxoplasmosis. This result could

be explained in view of El-Madawy and Metawea (2013) who stated that, Although serological testing is considered the main diagnostic technique for toxoplasmosis, it has some limitations because of the false positive results which have been reported by many investigators, in addition, to the false negative results that may happen during the active phase of *T. gondii*. The need for more sensitive techniques as PCR assay is essential to demonstrate the actual infection in human and animal, to obtain more data on prevalence, frequencies and phases of the parasite. Moreover, Hassanain *et al.*, (2014) also, suggested that, the presence of toxoplasma-specific antibodies in an insufficient criterion for identifying toxoplasma infection.

The results of this study, based on serological and molecular assays, confirmed our expectation that toxoplasmosis is indeed prevalent in Dhamar governorate. Combinations of serological and molecular techniques are recommended for accurate diagnosis of toxoplasmosis.

Competing interests

The authors declare that they have no competing interests.

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