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PROTOZOOLOGY - ORIGINAL PAPER

Seroprevalence of *Toxoplasma gondii* in one-humped camels (*Camelus dromedarius*) of Thal and Cholistan deserts, Punjab, Pakistan

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Abstract

Toxoplasma (T.) gondii is an important zoonotic protozoan infecting humans and a wide range of animals. In this study, we determine the seroprevalence and risk factors associated with the seroprevalence of *T. gondii* in one-humped camels (*Camelus dromedarius*) in Pakistan. Camels are still an important mean of transportation in some desert areas in Pakistan. In addition, they are the main source of meat and milk for people in those regions; therefore, they have the potential to transmit *T. gondii* to humans. In order to estimate the seroprevalence of *T. gondii*, a total of 897 sera samples were collected from camels in the Thal ($n = 359$) and Cholistan ($n = 440$) deserts, along with other districts of Chakwal ($n = 44$) and Faisalabad ($n = 54$) Punjab, Pakistan, through convenient and snowball sampling techniques. These samples were then analyzed by an indirect enzyme-linked immune-sorbent assay (ELISA) for the presence of *T. gondii*-specific antibodies, using purified recombinant micronemal protein 3 (MIC3) as an antibody-catching antigen. Our results showed an overall seroprevalence of *T. gondii* as 40.1% (Thal = 45%; Cholistan = 35.9%; other districts = 33.7%). Risk factor analysis suggested that infection rate was higher in older animals (70.6%). In addition, female camels carried frequent infection (48.8%) than males (22.4%). What's more, female animals having abortion history showed even higher infection rate (75%) compared to pregnant (68.4%) and non-pregnant (42.4%) animals. Our results reported high seroprevelance of *T. gondii* in camels in Pakistan which provided important information with respect to public health and disease controls.

Keywords Seroepidemiology · *Toxoplasma gondii* · Camel (*Camelus dromedaries*) · Indirect ELISA · Pakistan

Introduction

Pakistan, with a prediction of 1.1 million camels (*Camelus dromedarius*) stands among the chief camel raising countries (Pakistan economic survey 2016–2017). Camel population is roughly dispersed over the country with peak population in Balochistan (41%), Punjab (22%), Sindh (30%), and Kyber Pakhtoon Khwah (KPK) (7%) (Pakistan livestock census 2006). They are dispersed mainly in four separate ecologic zones of Pakistan as sandy deserts, coastal mangroves, mountainous areas, and irrigated plains. Camel gained economic importance being meat, milk and wool producer, moreover, as a transportation means, or social and religious means (Raziq 2009).

Anatomical, physiological, and behavioral characteristics of camel help it to well adapt the desert environmental conditions. Thick skin coat act as an insulator against the heat from sand and become lighter in color during hot summer to avoid sunburn. Pedestal (under the sternum) help to raise the body against hot surface and allow air to pass under body (Simenew 2014). Hairy skin coat also help to protect the body against harsh environmental conditions. Sweat directly evaporates from the skin surface, which help in heat vaporization, body's

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energy conservation and skin cooling (Kataria et al. 2001a). Long slit-like closeable nostrils protect against sand in the air (Butler and William 2005). The upper lip is quite sensitive, extensible and split which assist in prehension whereas their mouths have thick leathery lining, allowing them to chew thorny plants (Simenew et al. 2013). Their eyes are quite prominent and large to see long distances. And long eyelashes and third eyelid help protect their eyes against blowing sand (Soliman 2015; Hussein 2016). Large body size and height is also an advantage because larger masses get heated slowly (Cain et al. 2006). Long and slender legs are helpful for a long walk in the sand. Pad like hoof provide compact support and hoof do not sink in loose sand (Simenew 2014).

Camel is blessed with water conservation mechanics hence it can go without drinking water for 10–15 days under mean temperature of 30–35 °C (Kataria et al. 2001b). Its digestive tract is specialized for water conservation in a way to reabsorb water even in the end part of intestine (Breulmann et al. 2007), foregut stores water which is up to 20% of total body's mass and used during the days of water scarcity, and rumen also plays role during dehydration and rehydration to prevent body from hemolytic and osmotic tissue shock conditions (Cain et al. 2006). Camel kidneys have loop of henle six times longer than cattle's and well-developed medulla (Ouajd and Kamel 2009) and can conserve water by increasing the urine osmolarity and the tubular reabsorption of water (Kataria et al. 2001a). Camel's blood plays a principal role in adaption to dehydration and high heat (Ouajd and Kamel 2009) by keeping its volume and hemoglobin function constant (Willmer et al. 2006). An additional feature of blood is the longer life span (90–120 days) of erythrocytes which can extend to 150 days during a period of chronic dehydration to conserve the water and energy level of body (Ouajd and Kamel 2009). Thermoregulation is also an important mechanism to get a better adaption to harsh desert climatic conditions. Under dehydration and high heat load, the body temperature of camel can increase up to 6–7 °C than normal and this stored heat is dissipated at night (by radiation) without any water loss (Grigg et al. 2009). The biphasic air flow pattern also limits the water usage (Gaughan 2011) and cools down the arterial blood before sending it to the brain (Ouajd and Kamel 2009). Dromedary camel has ability to resist hunger and thrust by mobilization of stored fatty acids (Dereje and Ud'en 2005). Dehydrated camels reduce water loss by maintaining a high glycemia (Ouajd and Kamel 2009). As concerned with nitrogen recycling, it increases during the period of dehydration (Souilem and Djegham 1994).

Under severe dehydration and heating climatic conditions, camel makes some behavioral changes for energy and water conservation. As for feeding behavior is concerned, camel choose a few leaves from each plant and prefer feeding early in the morning, late evening, at night, or when the sky is cloudy. Camel has ability to drink large amount of water at once to compensate previous water loss. Camel avoids sitting under the sun if possible; otherwise, it keep its face towards the sun and present the least possible body and keep on moving in line with the sun, and raises its sternum to allow air circulation (Ouajd and Kamel 2009). Breeding season is typically timed to ensure that parturition occurs at a favorable time of year to maximize offspring survival (Cain et al. 2006).

Toxoplasma (T.) gondii is an apicomplexan obligate intracellular parasitic protozoan having three life cycle stages named sporozoites, tachyzoites, and bradyzoites (Dubey 1998). The family Felidae, especially domesticated cats, serves as a definitive host and environmentally resistant oocysts are passed to environment from their feces. These oocysts are transmitted to intermediate host by ingestion and transform in to tachyzoites, whereas, livestock, marine mammals, humans, and birds (Dubey 2010a, b) act as intermediate host of *T. gondii*. Bradyzoites are primarily found in the form of a cyst in muscles and brain of intermediate host. Tachyzoite is an actively replicating life stage and transmit infection if injected to host. *T. gondii* complete its asexual reproduction (schizogony) and sexual reproduction (gametogony) in the epithelial cells of small intestine of cat, leading to the production of unsporulated oocysts. These unsporulated oocysts are shed in feces and under favorable conditions change into infectious sporulated oocysts. Infection in the intermediate and definitive host occurs upon ingestion of oocysts, tachyzoites, or bradyzoites; however, the prepatent period could be variable according to the life stage ingested. *T. gondii* follows three routes to spread and produce infection: (a) horizontally from the environment through oral ingestion, (b) horizontally through ingestion undercooked meat, vegetables which contain of tissue cysts and offal of intermediate host, and (c) vertically through transplacental transmission. Additionally, tachyzoites may also transmit through milk (Dubey 1991).

T. gondii is one of the most common zoonotic parasites worldwide. Disease in humans caused by *T. gondii* was studied in late 1930s in an 11-months old infant (Janků 1923). Since then, it has been estimated that up to one third of world human population has been exposed to the parasite (Dubey 1998) which makes it one of the major public health problems (Assmar et al. 1997; Montoya and Liesenfeld 2004; Dubey 2010a, b). *T. gondii* is of great importance in food safety, animal husbandry, and zoonotic significance (Xiao et al. 2010; Dubey 1999) in causing toxoplasmosis to its hosts (Smith 1995) and responsible for great economic losses in the form of abortion, still birth, and neonatal losses (Chen and Tan 2009).

Like other livestock, *T. gondii* can also transfer to camel through ingestion or by inhalation of sporulated oocysts from air, which is shed by cats or wild felids in the environment

(Elamin et al. 1992). Using different methods, the prevalence of *T. gondii* in camels varies widely among different areas of the world (Shaapan and Kahlil 2008) ranging from 3.12% in Iran (Dehkordi et al. 2013) to 90.9% in Turkey (Utuk et al. 2012). High prevalence of *T. gondii* in camel could be due to poor husbandry practices, like the presence of stray cats (pre-exposed to *T. gondii*) with camel, disposal of wastes (including cat feces, slaughter byproducts and offal, and food leftovers having potential to contaminate) near water channels and vegetation, movement of pre-exposed camel to the healthy ones and handling the aborted animal in close vicinity to healthy animals. These practices can be helpful in easy spread of *T. gondii* in the environment and healthy animals.

Serological tests have been a reliable tool to identify exposure to *T. gondii* in humans as well as animals. Among serodiagnostic methods, ELISA (enzyme-linked immunesorbent assay) is preferred as it could be semi-automated. Indirect ELISA method has been known to be highly sensitive, flexible, and cost-effective (Shanmugham et al. 2010). Some of *T. gondii* recombinant proteins expressed in *Escherichia* (*E.*) *coli*, bind to *T. gondii*-specific antibodies and afterwards used for *T. gondii* antibodies detection during serological diagnostic studies (Pietkiewicz et al. 2004). Among all other proteins of *T. gondii*, microneme protein 3 (MIC3) is a major adhesive protein, capable of binding to host as well as parasite cell surface (Garcia-Reguet et al. 2000). Considering this property, MIC3 could be used as an antibody to detect the *T. gondii* infection.

Very less research has been conducted on the seroprevalence of *T. gondii* in camel in Pakistan. So far, only one report from Cholistan is available from Chaudhary et al. (2014), whereas, no study on camel toxoplasmosis has been conducted in Thal desert. In this study, using indirect ELISA, we tried to investigate the seroepidemiology of *T. gondii* in camel populations in the Thal and Cholistan deserts and additionally from the districts of Chakwal and Faisalabad in Punjab, Pakistan. Results obtained through this study will provide valuable information about seroprevalence of *T. gondii* in camels of above said areas. The role of identified risk factors in the transmission of *T. gondii* is also discussed. This study will also be helpful to highlight this issue at national level and turn the attention of stakeholders to such an important but neglected disease.

Materials and methods

Study area

Pakistan's second largest province Punjab covers an area of 205,344 km^2. Land of this province is blessed with five rivers and two deserts. Cholistan (Rohi) desert with an area of 26,300 km^2 is the second largest desert in Pakistan with annual mean temperature ranging between 20 °C to 40 °C and mean annual rainfall varying from less than 100 mm in the west to 200 mm in the east. In this desert, rain is the only source of fresh water. Absence of permanent water reservoirs is compensated by collection of rainwater in artificial ponds. During rainy season, the rainwater is collected in natural depressions or manmade ponds, locally called "tobas." According to an estimate compiled by the Pakistan Council Research in Water Resources, Regional Office, Bahawalpur (PCRWR), there are more than 1500 tobas in the Cholistan area (Akram et al. 1986). According to Pakistan livestock census (2006) total livestock population of this desert is 1.56 million including camel (0.080 million), cholistani cattle (0.667 million), sheep (0.450 million), and goat (0.350 million). Important felids of Cholistan are Indian caracal (*Felis* (*F.*) *caracal*), jungle cat (*F. chaus*), and desert cat (*F. libyca*) (Sial 1989) (Fig. 1).

Thal desert has its vast expansion between the Jhelum and Sindh rivers, with a total length of 190 miles from north to south, and a maximum breadth of 70 miles and minimum breadth 20 miles. Geographically, it resembles the deserts of Cholistan and Thar. Camel breeds available in Cholistan and Thal are locally named as Bagri, Barela and Marecha while Parahri (mountainous) breed is specific to Chakwal. Figure 2 explains the desert areas of Pakistan investigated in this study.

Selection of animals

Convenient and snowball sampling techniques were used for selection of study animals (Thrusfield 2007). Sampling was performed from July to August of 2016 in Cholistan (Bahawalnagar and Bahawalpur districts) and Thal (Bhakkar, D.G. Khan, Khushab, Mianwali, Muzaffargarh and Rajan Pur). Additionally, convenient sampling was also performed from district Chakwal and Faisalabad. Chakwal is mainly a hilly area located in the northern Punjab while Faisalabad is a plain area in central Punjab zone (Fig. 2).

Questionnaire-based surveillance

A questionnaire containing opened- and closed-ended questions was designed about the determinants associated with the host, agent, and environment (Fig. 1). The questionnaire was refined through formal (undertaken on a small random sample of the population on which the full survey will be conducted) and informal (by colleagues who can detect defects in the questionnaire design) testing procedures (Thrusfield 2007). Comparison of the area-wise prevalence was made to find out geographical associations. The animals of the study area were divided into three groups based on their age ranges: (i) ≤ 3 years, (ii) 4–6 years, and (iii) ≥7 years. Gender (male and female) based grouping was done to know the difference of *T. gondii* prevalence. Reproductive state of female camels was also

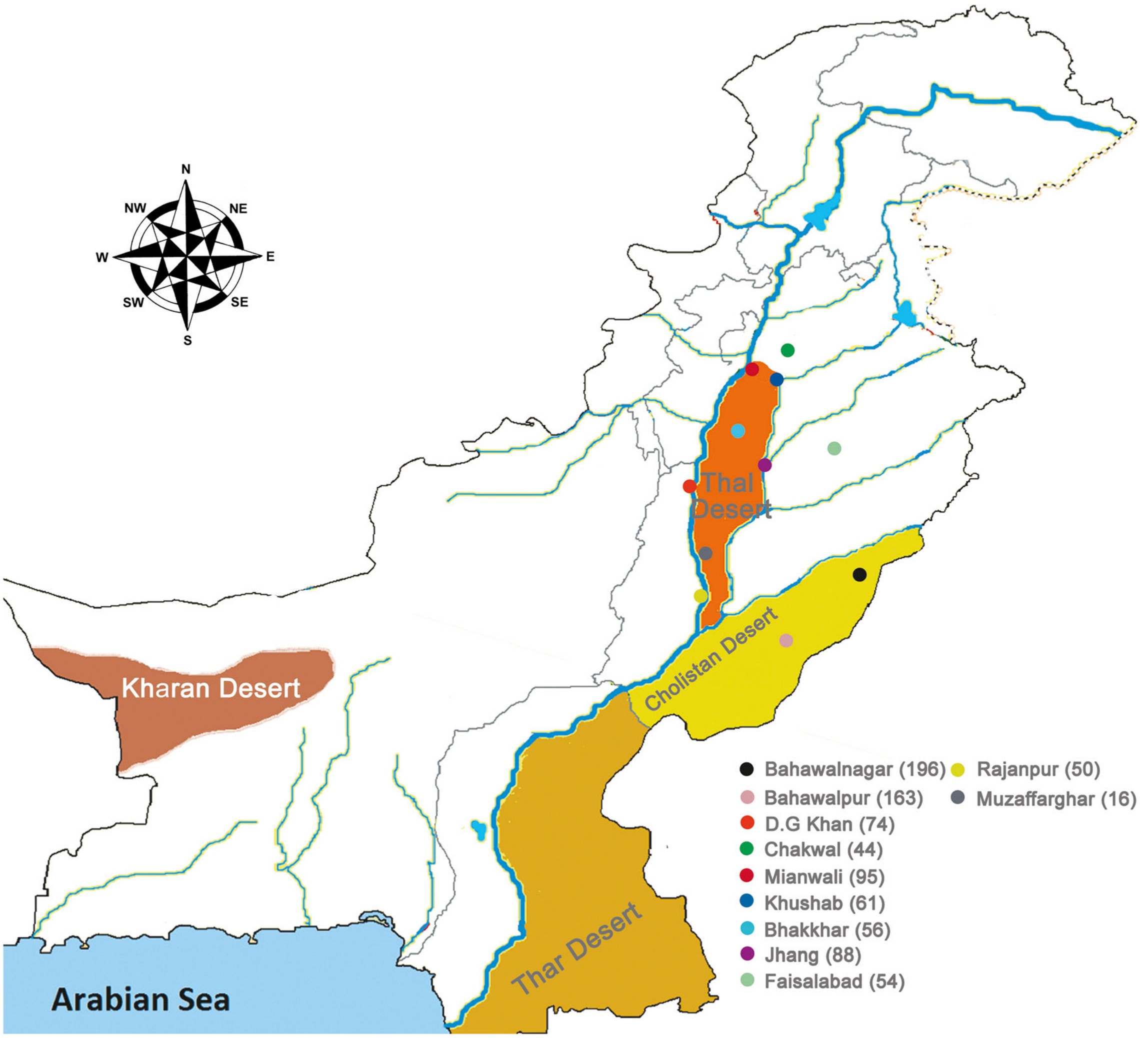

Fig. 1 Map of Pakistan showing all desert areas. Areas of sampling are highlighted

recorded to compare non-pregnant, pregnant, and camels that had abortion. Effect of breed, rearing systems (nomadic/non-nomadic), and purpose of keeping camels (milk, meat, and draught) was also included in the study.

Blood collection and sera isolation

After proper restraining of the animals, 5 mL of blood was collected from jugular vein using sterile needle syringes. Soon after blood collection, samples were shifted to gel-clot activator tubes (Improvacuter, China). Sera were obtained after centrifugation (3000 rpm for 10 min) and stored at −80 °C until further processing (Tuck et al. 2009).

Preparation of MIC3 proteins

Recombinant MIC3 protein was purified using previously described protocol by Jiang et al. (2008). Briefly, the bacteria (*E. coli*) which expressed MIC3 protein were harvested after induction with isopropyl-β-*D*-thiogalactopyranoside (IPTG) for 4 h. The cells were resuspended in phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Triton X-100, 0.1% lysozyme, 2% deoxycholic acid sodium, and lysed by ultrasonication in an ice bath. The lysate was centrifuged at 15000*g* for 15 min, and the resulting pellet was dissolved in buffer A (50 mM Tris–HCl, pH 8.0, 0.5 mM EDTA, 0.5 mM NaCl, 5% glycerine, 0.5 mM DTT) containing 20% sarkosyl (SKL). After incubation at room temperature for 2 h and centrifugation, the supernatant was treated with 1.0 mM oxidized glutathione (GSSG), 2.0 mM-reduced glutathione (GSH) and 0.2% polyethylene glycol 4000 for 1 h at room temperature. Later, it was dialyzed in PBS (pH 7.4) at 4 °C for 2 days followed by purification by glutathione sepharose 4B column according to the manufacturer's instructions (Amersham Pharmacia Biotech, USA).

Fig. 2 Questionnaire designed for information collection about camel, *T. gondii* and associated determinants

Annexure

Title of project:

Date of Survey: ______________________ Performa #: _________________

Name of Owner: ____

Location/Address: ____

Breed:

Bagri	Barela	Mareecha	Pahari

Age:

≤3 Years	3-6 Years	≥7 Years

Sex:

Male	Female

Reproductive Status:

Non-Pregnant	Pregnant	Aborted

Rearing System:

Non-Nomadic	Nomadic

Purpose:

Draught	Draught and Meat	Milking

Development of an in-house indirect ELISA using MIC3as an antibody-catching antigen

The optimal concentration of the coating antigen and the serum, conjugate dilution were determined by checkerboard titration assay using known positive and negative sera. As a result, the concentration showing the highest discrimination value between positive and negative sera was considered to be optimal. After optimization, the ELISA was carried out using standard conditions. Briefly, 96-well microplates were coated with 100 μL of coating buffer and MIC3 protein to a final concentration of 3.4 μg/mL and incubated overnight at 4 °C. The following day, the wells were washed (three times) with PBS-T for 3 min each time and blocked with 150 μl of blocking buffer for 45 min at 37 °C. After another rounds of washing, 150 μl of sera diluted at 1:100 was added to the wells and incubated at 37 °C for 45 min. At the end of the incubation time, the wells were washed again and 100 μl of HRP-conjugated protein A (diluted 1:6000) was added for 30 min at 37 °C, followed by final three-time wash. The immunoenzymatic color reaction was developed by adding 100 μL of TMB substrate and the plate was further incubated at 37 °C for 10 min. Finally, the reaction was stopped by adding 50 μl of $2MH_2SO_4$ and the optical density (OD) at 630 nm was then measured by using SpectraMax M Series Multi-Mode Microplate Readers (USA). The cutoff value was established as the average OD value. Sera were considered negative or positive when their optical density are less or more than adjusted cutoff value respectively (Ferrandiz et al. 2004; Selseleh et al. 2012).

Statistical analyses

SPSS 18.0 (SPSS for Windows Ver. 18) software was used for data analyses. For determining, the prevalence of parasite percentage descriptive statistics was applied. Logistic regression analysis was applied to find out the effect of relationship among variables. Odds ratio at 95% confidence level was used for the determinants in influencing the epidemiology of parasites. Chi-squared test was performed to know the relationship of prevalence with dependent variables and differences were considered significant at a P value of < 0.05 (Schork and Remington 2010).

Results

Over a period of 2 months (from July to August 2016), a total of 897 camel sera samples from selected districts were collected. Of the total camels screened, 360 (40.1%) were found positive for *T. gondii*. District wise, 359 serum samples were collected from district Bahawalpur and Bahawalnagar in Cholistan Desert, 440 serum samples were collected from district Bhakkar, D.G. Khan, Jhang, Khushab, Mianwali, Muzaffargarh, and Rajan Pur in Thal Desert, whereas 98 serum samples were collected from other districts including Chakwal and Faisalabad. Overall, the districts of D.G. Khan (54.1%; 40/74) and Khushab (54.1%; 33/61) showed the highest seropositivity, while district Faisalabad (33.3%; 18/54) showed the lowest seropositivity of *T. gondii*. Camels from Thal Desert found to be high in *T. gondii* (45%; 198/440) than those from Cholistan Desert (35.9%; 129/359) and other areas (33.7%; 33/98). The reason of high prevalence in Thal Desert could be the use of stagnant water as rain water is collected in ground ("tobas" in local dialect), and, thereafter, used in common for human and animals. Wild shrubs and herbs are common fodder for animals in desert areas, which cause indigestion and poor immune status making animals sensitive to acquire *T. gondii* from its surroundings. Animals at the age of ≥ 7 years were at higher risk (70.6%; 180/255) than young animals [(4–6 years 33.1%; 139/420), (≤ 3 years; 18.5%; 41/222)]. Female animals showed higher prevalence (48.8%; 294/602) than male animals (22.4%; 66/295). Our results showed that animals with abortion history showed higher seroprevalence (75.0%; 21/28) than pregnant (68.4%; 78/114) and non-pregnant animals (42.4%; 195/460). No significant differences were observed between Barela (41.2%; 215/522), Marecha (40.6%; 43/106), Bagri (38.7%; 87/225), and Pahari (34.1%; 15/44) breeds. No significant differences were observed between nomadic (39.0%; 166/426) and non-nomadic (41.2%; 194/665) populations (Table 1). Risk factor analysis of *T. gondii* in camel revealed highly significant association ($P < 0.01$) of *T. gondii* with the age of camel. Camel ≥ 7 years showed 77.5% (110/142), 61.8% (55/89), and 62.5% (15/24) prevalence from Thal, Cholistan, and other areas, respectively. Prevalence of *T. gondii* was found to be highly significantly associated ($P < 0.01$) with the sex of camel. Female animals showed 58.1% (158/272), 42.2% (108/256), and 37.8% (28/74) prevalence rate from Thal, Cholistan, and other areas, respectively. Prevalence of *T. gondii* was found to be significantly associated ($P < 0.01$) with the reproductive status of female camel where aborted females showed higher prevalence, i.e., 92.3% (12/13), 50.0% (4/8), and 71.4% (5/7) from Thal, Cholistan, and other areas, respectively. Prevalence of *T. gondii* was found to be non-significantly ($P > 0.05$) associated with the breed of camel, purpose of keeping animal, and the system of keeping animal. Table 2 describes the seroprevalence of *T. gondii* in camels by explanatory variables as detected by indirect ELISA using chi-squared test where area, age, and sex are significantly associated.

Table 1 Data presenting the number of samples collected from each district with overall determinant wise positive and negative percentages for *T. gondii*

Determinants		Total screened	Positive count	Prevalence (%)
Area	Cholistan	359	129	35.9
	Thal	440	198	45.0
	Other	98	33	33.7
Age (years)	≤ 3	222	41	18.5
	4–6	420	139	33.1
	≥ 7	255	180	70.6
Sex	Male	295	66	22.4
	Female	602	294	48.8
Reproductive status	Non-pregnant	460	195	42.4
	Pregnant	114	78	68.4
	Aborted	28	21	75.0
Breed	Bagri	225	87	38.7
	Barela	522	215	41.2
	Marecha	106	43	40.6
	Pahari	44	15	34.1
Purpose	Draught	234	89	38.0
	Draught and meat	141	56	39.7
	Milking	522	215	41.2
Animal keeping	Nomadic	426	166	39.0
	Non-nomadic	471	194	41.2

Associated risk factors analyses for *T. gondii* seropositive and seronegative camels by an indirect ELISA were compared using univariable logistic regression analysis. For the statistical analysis, the first level of every independent variable was used as reference category. Results depict that the area, age, and sex are significantly associated with *T. gondii* prevalence ($P < 0.05$) whereas breed, purpose, and keeping method are non-significantly associated. Multivariable logistic regression analysis was measured for variables including the area, age, sex, breed, purpose of keeping camel, and method of raising camel. The analysis showed a significant association between *T. gondii* seropositivity and study area, age, sex, and method of raising camels. The results of the logistic regression analysis are presented in Table 3.

Discussion

In spite of an active member of the food producing family of livestock, for a long time camel remained the most neglected animal in the field of scientific research (Njiru 1993) and

Table 2 Seroprevalence of *T. gondii* in camels by explanatory variables as detected by indirect ELISA

Variable	Categories	No. tested	No. positive	Positive (%)	Chi-square	*P* value
Area	Cholistan	359	129	35.93	6.722	**0.010**
	Thal	440	198	45.00		
Age (years)	≤3 years	205	40	19.51	135.818	**0.000**
	4–6 years	363	122	33.61		
	7+ years	231	165	71.43		
Sex	Female	528	266	50.38	57.533	**0.000**
	Male	271	61	22.51		
Breed	Bagri	209	82	39.23	0.364	0.834
	Barela	487	203	41.68		
	Marecha	103	42	40.78		
Purpose	Draught	174	69	39.66	0.297	0.862
	Draught and meat	138	55	39.86		
	Milking	487	203	41.68		
Animal keeping	Nomadic	426	166	38.97	1.449	0.229
	Non-nomadic	373	161	43.16		

P values of statistically significant variables were highlighted in bold

national development strategies. An important reason for neglect seems that it is predominantly found in arid, semi-arid and tropical areas, where deprived nutrition and poor husbandry practices are the major shortcomings (Sohail 1983). *T. gondii* is one of the major parasites in camel showing asymptomatic to serious clinical manifestation where consequences are severe in immune-compromised individuals (Cantos et al. 2000).

In this report, for the first time, we investigated the seroprevalence of *T. gondii* and association of risk factors in Thal and Cholistan deserts additionally including the districts of Chakwal and Faisalabad. An estimated overall seroprevalence of 40.1% was detected by using MIC3-based indirect ELISA. Thal and Cholistan deserts are highly populated and famous for camel population whereas district Chakwal is a hilly area blessed with the ample population of hilly camel. Two deserts harbor nomadic and non-nomadic populations and pastoral and settled farming systems, helping in easy comparisons. Districts of Faisalabad and Chakwal were included in the study plan due to the ease of access to these areas. High seroprevalence in the current study might be a function of collective effect of the age of camels (Tenter 2009) related to the absence of regular culling programs.

Table 3 Seroprevalence of *T. gondii* infection in camels as detected by indirect ELISA

Variable	Categories	No. of positive (%)	Uni-variable OR (95% CI)	*P* value	Multi-variable OR (95% CI)	*P* value
Area	Cholistan	129/359 (35.93)	1		1	
	Thal	198/440 (45.00)	1.46 (1.096–1.942)	**0.010**	2.90 (1.559–5.378)	**0.001**
Age (years)	≤3 years	40/205 (19.51)	1		1	
	4–6 years	122/363 (33.61)	2.09 (1.388–3.141)	**0.000**	2.10 (1.370–3.203)	**0.001**
	7+ years	165/231 (71.43)	10.3 (6.588–16.14)	**0.000**	10.1 (6.252–16.19)	**0.000**
Sex	Female	266/528 (50.38)	1		1	
	Male	61/271 (22.51)	0.29 (0.205–0.399)	**0.000**	0.35 (0.239–0.499)	**0.000**
Breed	Bagri	82/209 (39.23)	1		1	
	Barela	203/487 (41.68)	1.107 (0.795–1.541)	0.547	1.00 (0.618–1.625)	0.994
	Marecha	42/103 (40.78)	1.066 (0.659–1.725)	0.794	0.64 (0.264–1.536)	0.315
Purpose	Draught	69/174 (39.66)	1		1	
	Draught and meat	55/138 (39.86)	1.01 (0.639–1.592)	0.971	1.06 (0.422–2.678)	0.896
	Milking	203/487 (41.68)	1.09 (0.764–1.548)	0.641	–	
Animal keeping	Nomadic	166/426 (38.97)	1		1	
	Non-nomadic	161/373 (43.16)	1.189 (0.897–1.578)	0.229	0.49 (0.271–0.887)	**0.018**

P values of statistically significant variables were highlighted in bold

Furthermore, the movement of camels to other areas in search of food and water, the insufficient veterinary facility, inadequate attention by government, local change in land ownership and increased farming which impose cat keeping to control rodents, might have also contributed to the high prevalence. Recently, a relatively lower seroprevalence (10%) of *T. gondii* in camels was reported from the district of Bahawalpur (Chaudhary et al. 2014). The present seroprevalence (40.1%) is closer to those of Gebremedhin et al. (2014), i.e., 40.49% using indirect ELISA and Manal and Maijd (2008), i.e., 46–54.2% from Sudan. As compared to the present finding, much higher seroprevalence has been reported from Turkey (90.9%) (Utuk et al. 2012) and Sudan (67%) (Elamin et al. 1992). Lower seroprevalence was recorded from Egypt (17.4–31.4%) (Shaapan and Khalil 2008), Iran 3.12% (Dehkordi et al. 2013), Sudan (20%) (Khalil and Elrayah 2011), Saudi Arabia 6.5%, 13.1% and 16% by Al-Anazi (2011), Hussein et al. (1988) and Al-Anazi (2012), respectively and in United Arab Emirates (22.4%) (Abu-Zeid 2002).

D.G. Khan and Khushab are located in Thal area where there is poor availability of food and water. Use of stagnant water for animals could be a cause of higher prevalence in both areas, whereas district Faisalabad is a plain area with an ample amount of fresh water and fresh fodder. Healthy camels are mostly transported to district Faisalabad for sacrificial purposes which could be a cause of low prevalence in this district. Camels take water from water bodies resulting from rainfall which are vernacularly named as “tobas.” The same water is used for human consumption and animal use as well. Such contaminated water may be a source of high prevalence in Thal Desert. Only one report from Bahawalpur (Cholistan) was found where Chaudhary et al. (2014) reported only 10% prevalence of *T. gondii* among camel population.

The high seroprevalence in older camels than young camels might be due to lower immune status and higher likelihood of exposure of older camels to any one of the risk factors to acquire *T. gondii* (Randall et al. 2000). This finding is similar with the studies conducted by Gebremedhin et al. (2014) in Ethiopia and in Saudi Arabia by Elamin et al. (1992) and Hussein et al. (1988), respectively, who reported a higher seroprevalence in adult than young camels. But current findings do not match the findings of Chaudhary et al. (2014) and Elamin et al. (1992) who reported very less seroprevalence in older camels than young ones.

According to the results of the current study, female animals were found to be at higher risk than males. Kamani et al. (2010) found no association between the prevalence of *T. gondii* and sex of camel. However, studies in Brazil (Lopes et al. 2010), China (Wang et al. 2011), Ghana (van der Puije et al. 2000), and Pakistan (Ramzan et al. 2009) revealed higher prevalence of *T. gondii* in female than in male sheep. According to Kittas et al. (1984), innate immune responses are greater in males. According to this study, female animals with the history of abortion were at higher risk with prevalence percentage followed by pregnant and non-pregnant. Result of this factor was in association with the results of Chaudhary et al. (2014). No significant impact of breed was observed on the prevalence of *T. gondii*. Likewise, no significant impact of animal rearing system (nomadic/non-nomadic) and purpose of keeping on the prevalence percentage of *T. gondii* were observed during this study.

The current data confirmed the prevalence of *T. gondii* in camels in the Thal and Cholistan deserts and in other areas (including Chakwal and Faisalabad) as well. The prevalence of *T. gondii* infection in animals as well as in human is often linked with infection in pets. Little consideration has been given to domestic pet despite their intimate contact with animals and their feed. This study was performed on limited basis due to lack of funds, the rest of camel-populated areas should also be screened for *T. gondii*.

Conclusions and future prospects

The results of this study address the new insights into the epidemiology of *T. gondii* in Thal and Cholistan deserts including the areas of Chakwal and Faisalabad Punjab, Pakistan. Besides infecting almost all animal populations, *T. gondii* is one of the major public health concerns. *T. gondii* has been observed to be capable of infecting almost all warm blood animals including human. This unique quality makes it one of the most successful parasites on land. Females suffering from *T. gondii* ends up with abortion in acute cases. Schizophrenia and ocular abnormalities has also been observed in human. Strain and type of *T. gondii* along with the housing management of diseased and non-diseased animals, management of pregnant and aborted animals, the feeding and water management practices, medication and/vaccination schedule may influence the transmission of *T. gondii*. Therefore, attention should be given to such factors so as to control the zoonosis related to *T. gondii*. The farmers should be educated through extension programs related to factors influencing the transmission dynamics of *T. gondii* in order to control the disease in endemic areas in Pakistan.

Thus, this research will be helpful for the researchers to design strategies for screening of rest of the animal population as well as human population must also be screened at national level. Based on the results of this study *T. gondii* control programs must be launched by the government at national level to save the at risk camel population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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