



Serological and Molecular Surveillance of *Toxoplasma gondii* of Camels in Taif, Saudi Arabia

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TOXOPLASMA *gondii* causes an important zoonotic parasitic disease that is widespread throughout the world. However, recorded data on camel toxoplasmosis are scarce in different areas in Taif governorate of Saudi Arabia. Camels are the main source of milk and meat in different countries, especially the Gulf countries, and due to this contact, they can transmit toxoplasmosis to humans. Therefore, a toxoplasmosis seroprevalence study was conducted on 65 female camels from three different areas (area 1, 2, and 3) using enzyme-linked immunosorbent assay [ELISA] (Toxo-IgG). Data were confirmed by the polymerase chain reaction (PCR) technique. This study reports a prevalence rate of Toxoplasmosis in the examined samples 7/65 (10.8%) and 13/65 (20%) using Toxo-IgG and PCR, respectively. According to positive Toxo-PCR, samples were categorized into two groups: the lowest infection rate was found in the older aged group (>6 years) 8/42 (19.1%). While the highest infection rate was recorded in the younger group (≤6 years) 5/23 (21.7%). In addition, the percentage of the total IgG in camels' sera were the lowest in samples of area 1 and the highest in samples of area 3 that could be related to any previous or recent infection. Statistically, there was no relationship between age and infection rate.

Keywords: *Toxoplasma gondii*, ELISA, Camels, PCR, Taif, Saudi Arabia.

Introduction

In Saudi Arabia, ruminants are considered a source of milk, meat, and hides, and they have economic importance. *Camelus dromedaries* (dromedary camel) is a multi-purpose animal bred for meat, milk, and transportation. It also plays a vital role in wealth and social prestige, as it is considered a financial reserve for pastoralists [1].

Protozoa are single-celled eukaryotes that are parasitic (*Toxoplasma*), free-living (Amoeba), or free-parasitic amoebae (*Acanthamoeba*), and they feed on organic matter such as organic tissues, debris, or other microorganisms. One of the most important zoonotic parasitic diseases is toxoplasmosis, transmitted to humans from

mammals and birds. This disease is caused by *Toxoplasma gondii*, which was discovered by Nicolle & Manceaux [2], which infects many peoples from the world population [3,4]. *Toxoplasma* belongs to the phylum Apicomplexa. Toxoplasmosis in animals, including humans, is transmitted by tachyzoites to the fetus through the placenta or by consuming drinks and foods contaminated with tachyzoites and bradyzoites, such as cheese, unpasteurized milk, unwashed fruits and vegetables, or undercooked meat [5]. Toxoplasmosis infection could lead to different impacts such as blindness, mental retardation in infected children, and abortions of pregnant women.

Camels are infected with many different diseases, including toxoplasmosis, and camels acquire *Toxoplasma* infection by swallowing the sporulated oocysts that wild felids or cats shed in the environment. The prevalence of *Toxoplasma* infection in camels in the world depends mainly on the location [6]. In Saudi Arabia, few reports of *Toxoplasma* antibodies have been published in camels and neighboring countries [7-9]. On the other hand, Aljumaah [9] recorded a prevalence of *T. gondii* antibodies much higher than 22%, while previous studies reported anti-toxoplasma antibodies in the range between 4.2% and 17.4%.

There are different ways to diagnose toxoplasmosis, such as serological detection (latex agglutination test and enzyme-linked immunosorbent assay [ELISA]) of IgG and IgM, bioassays, cell line culture, and molecular techniques [10]. Detection of IgG antibodies in the blood samples of a sick camel means the presence of a previous infection. In contrast, IgM implies that the patient is in the acute stage. Serological tests are considered one of the most preliminary tests for detecting *Toxoplasma*. At the same time, PCR is the best test for diagnosing parasite infection for its specificity, sensitivity, and accuracy better than other diagnostic methods [11].

Due to the lack of information in Saudi Arabia about the existence percentage of toxoplasmosis in camels, this study was conducted to fill this information gap using indirect ELISA and PCR assays in camels present in the Taif governorate of Saudi Arabia.

Materials and Methods

Study area and sample collection

In the present study, three different areas were selected to collect camel's blood samples in Taif region, Makkah Province, Saudi Arabia. Most of the camels found in Taif were freely grazed in those three areas with latitude and longitude coordinates as referred in Fig. 1: Area 1 (21°5668571N, 40°7019237E), Area 2 (21°25732N, 40°50762E), and Area 3 (21°2606711N 40°510672E). Veterinarians introduced data and samples according to the ministerial recommendation letter. Blood samples and data of 65 females (age ranges from 3-12 years) were collected from December 2020 to February 2021. Six samples were collected from area 1, twenty-eight samples from area 2, and thirty-one samples from area 3. Blood samples (5 ml, from a jugular vein) from each camel were collected in two different vacuum tubes with and without anticoagulant for further PCR and ELISA evaluations, respectively.

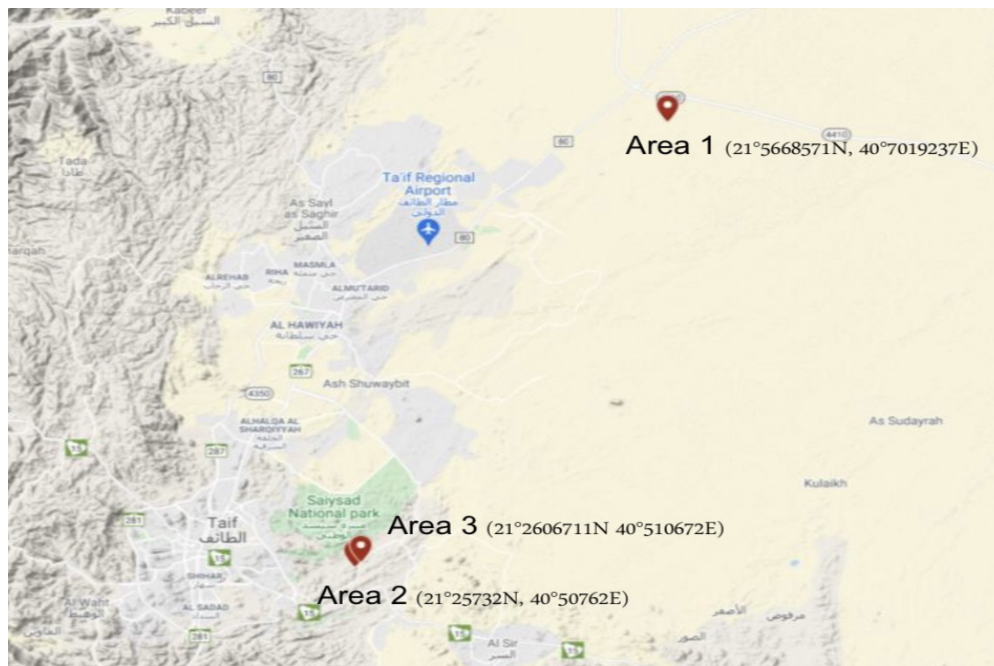


Fig. 1. A map of the Taif region showing all the areas from which samples were collected. Latitude and longitude coordinates of the sampling areas 1, 2 and 3 are pinned (with red marker icons) on the map.

Serological evaluation

Blood samples (about 2 ml without anticoagulant) were centrifuged at 2500 xg for 20 minutes, and in new tubes, sera were collected for serological assay. Thus, two different serological assessments have been done in the present study, total camels' IgG and then *Toxoplasma gondii* IgG evaluation [12].

Total camel immunoglobulin G (IgG)/ *Toxoplasma gondii* IgG

Two separate ELISA kits were used to evaluate total IgG and the other specific for Toxo-IgG with nearly the same procedures, except in their standards and calculation of results. In total IgG, the standard was diluted into six serial dilutions 27 µg/ml, 18 µg/ml, 12 µg/ml, 6 µg/ml, 3 µg/ml, and 1.5 µg/ml, respectively. Those serial dilutions with their optical density (OD) at 450 nm will be used to plot the standard concentration curve for camels' samples IgG assessment. While in Toxo-IgG, standards were present as positive and negative standards (included in the kit), and samples will be evaluated according to the cut-off critical value.

Briefly, the procedure of both kits was the same. According to the Sunlong Biotech® instruction manual (China), the steps have been followed, 50 µl of diluted serum (1:5/1:4 in a dilution buffer for total IgG/Toxo-IgG, respectively) were loaded to micro-ELISA wells that are coated with a specific antigen to total IgG/Toxo-IgG. Gently the plate was shaken at 37°C and then incubated for 30 minutes; with a washing solution, the plates were washed five times. In each well, a reagent of Horseradish Peroxidase conjugate was added, incubated, and then washed. Next, for color development, chromogen solutions were added, set at 37°C (15 min), and finally, to terminate the reaction 50 µl of the stop solution was added to each well.

Both plates were read at 450 nm to report OD of samples. The concentration of total camel IgG in samples was determined by plotting the sample's OD on the Y-axis of the standard concentration curve multiplied by the dilution factor. The standard concentration curve was drawn by plotting known concentrations of camel IgG standard (included in the kit) and its corresponding OD on the log scale (x-axis) and the log scale (y-axis), respectively. At the same time, Toxo-IgG were detected by comparing samples' readings with their cut-off values. The

critical value (cut-off) was calculated as the average value of negative control + 0.15.

DNA extraction

0.5 ml of camel's blood (with EDTA as anticoagulant) was mixed with 0.5 ml of low salt buffer (100mM Tris-HCl, pH 7.4, 250mM sucrose, 10mM EDTA), incubated at room temperature for 20 minutes, and then centrifuged for 10 min at 4000 xg. The supernatant was decanted, and then 480µl of high salt buffer TKM2 (Tris HCl 10 mM pH 7.6, 10 mM KCl, 10 mM MgCl₂, 0.4 M NaCl and 2mM EDTA), 75µl 10% SDS, and 10µl proteinase k enzyme (10 mg/ml) were added to the pellet and incubated overnight at room temperature. NaCl (6M) was added for protein precipitation, centrifuged, and the supernatant was collected in a new microtube. DNA was precipitated from supernatant by cold ethanol addition, centrifuged, dried, and finally dissolved in autoclaved Milli-Q water [13].

Polymerase chain reaction PCR

Locus of *B1* gene was targeted by forward, 5'- GGAAGTGCATCCGTTTCATGAG-3', and reverse primers, 5'-TCTTTAAAG CGTTC GTGGTC-3', to detect *T. gondii* [14]. The PCR reaction was set up with initial denaturation at 94° (5 mins), 30 cycles of denaturation at 94° (30 s), primer annealing at 50° (30 s), and then primer extension at 72° (30 s). Final extension at 72° (10 mins) was necessary done for complete amplification (Programmable Thermal Cycler, PTC-100TM thermal cycler, Model 96; MJ Research, Inc., Watertown, MA, USA). PCR products were separated on agarose gel (1%, stained with ethidium bromide) and then visualized under gel documentation system (Bio-Rad, USA) [15].

Statistical analysis

The results were analyzed with Chi-square test, using SPSS version 23. Significance was reported at $p < 0.05$.

Results

Serology

Over three months (from December 2020 to November 2021), a total of 65 samples of female camel serum were collected from three different regions from Taif, Saudi Arabia (Fig. 1).

Female camels were divided according to age into ≤ 6 years and more than 6 years old. The concentration of Immunoglobulin G

(IgG) in the serum of camel females in Taif Governorate, Kingdom of Saudi Arabia, as follows: Females less than 6 years old recorded 7/65 (10.8%) samples at low concentration of IgG (1.5-3 µg/ml), while females older than 6 years recorded 12/65 (18.5%) samples at the same concentration. In the median (Med) concentration (6-12 µg/ml), 11/65 (16.9%) were recorded in the samples less than six years old, while 18/65 (27.7%) samples were recorded in the older camels. While in the highest concentration (18-27 µg/ml), five out of 65 (7.7%) females were recorded in the younger age, while 12/65 (18.5%) camels were recorded in the older samples (Table 1). The previous results indicated that the younger camels had stronger immunity than the older aged female camels. The highest percentage of IgG was at the older aged (>6 years) camel samples that are infected with toxoplasmosis (n=3, 23.1%), while the lowest percentage of IgG was in camels less than six years old (0%).

The concentration of the total IgG in the first region was 6/65 (9.2%) in total three concentrations (low, med, and high), while in the second region 28/65 (43.1%) in all concentrations, finally the highest percentage was recorded in the third region 31/65 (47.7%) (Table 2). The highest percentage of IgG was found in samples collected from area 3 (3, 23%) that are infected with toxoplasma. On the other hand, the lowest percentage of IgG was found in samples related to area 1 (0%).

Of the total 65 female camels examined, no *T. gondii* infections were found in area 1, while infected camels were found in area 2 and 3. In general, the lowest IgG seropositivity was recorded in area 2 (3/28; 10.7%), while the highest IgG seropositivity was found in region 3 (4/31; 12.9%) (Table 3).

The highest seropositivity (4/23; 17.4%) was recorded in the younger camels, while it was the lowest seropositivity (3/42; 7.2%) in older camels. These results show that older female camels were less susceptible to infection with the parasite, while younger camels were more susceptible to infection with *T. gondii*. The seropositivity of the older camels may be due to a previous infection with which formed antibodies that made it more resistant to toxoplasmosis (Table 4). There was not relationship between the age of female camels and infection rate.

PCR

Molecular detection using PCR: Serum-positive or negative samples using a Toxo-IgG test were subjected to confirmation using PCR targeting B1 gene fragments of *T. gondii*. *Toxoplasma gondii* DNA was not detected in region 1 of the serum-negative samples confirmed by the Toxo-IgG test. At the same time, the DNA of 5 samples was detected for *Toxoplasma gondii* in region 2. On the other hand, the DNA of 8 samples for *Toxoplasma gondii* was detected in region 3 (Table 3). These results show that PCR was more specific, sensitive, and accurate than the ELISA test.

TABLE 1. Data representing the total IgG level, and Toxo-IgG (+/-) of female camels' blood samples according to age.

Female camels' age	Age ≤ 6			Age ≥ 6			Total no. (%)
	Low (1.5-3 µg/ml) number (n.o) (%)	Med (6-12 µg/ml) no. (%)	High (18-27 µg/ml) no. (%)	Low (1.5-3 µg/ml) no. (%)	Med (6-12 µg/ml) no. (%)	High (18-27 µg/ml) no. (%)	
Toxo-Positive (+)	3 (23.1)	2 (15.4)	0 (0)	3 (23.1)	2 (15.4)	3 (23.1)	13 (20.0)
Toxo-Negative (-)	4 (7.7)	9 (17.3)	5 (9.6)	9 (17.3)	16 (30.7)	9 (17.3)	52 (80.0)
Total	7 (10.8)	11 (16.9)	5 (7.7)	12 (18.5)	18 (27.7)	12 (18.5)	
	Total= 23 (35.3%)			Total = 42 (64.6%)			65

The chi-square statistic is 4.82. The p-value is 0.437, therefore results are not statistically significant at p<0.05. Low= samples ≤0.9 OD, Med = 0.9 >samples ≥1.8 OD, High= more than 1.8 OD at 450nm.

TABLE 2. Data representing the total IgG level, and Toxo-IgG (+/-) of female camels' blood samples according to sampling areas.

TABLE 2. Data representing the total IgG level, and Toxo-IgG (+/-) of female camels' blood samples according to sampling areas.

Sampling area	Area 1			Area 2			Area 3			Total no. (%)
	Conc. IgG	Low (1.5-3 µg/ml)	Med (6-12 µg/ml)	High (18-27 µg/ml)	Low (1.5-3 µg/ml)	Med (6-12 µg/ml)	High (18-27 µg/ml)	Low (1.5-3 µg/ml)	Med (6-12 µg/ml)	
Toxo (+/-)	number (n.o) (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)
Toxo-Positive (+)	0	0	0	2 (15.3)	3 (23.0)	0	4 (30.7)	1 (7.7)	3 (23.0)	13 (20.0)
Toxo-Negative (-)	2 (3.8)	3 (5.7)	1 (1.9)	5 (9.6)	14 (26.9)	4 (7.7)	6 (11.5)	8 (15.3)	9 (17.3)	52 (80.0)
Total	2 (3.1)	3 (4.6)	1 (1.5)	7 (10.8)	17 (26.2)	4 (6.2)	10 (15.4)	9 (13.8)	12 (18.5)	
	Total= 6 (9.2%)			Total =28 (43.1%)			Total= 31 (47.7%)			65

The chi-square statistic is 4.82. The p-value is 0.437, therefore results are not statistically significant at $p < 0.05$. Low= samples ≤ 0.9 OD, Med = 0.9 > samples ≥ 1.8 OD, High= more than 1.8 OD at 450nm.

TABLE 3. Data representing the number of camels infected with Toxoplasma and their percentages according to their sampling area.

Areas	Total screened	Positive Toxo-IgG (%)	Positive Toxo-PCR (%)
1*	6	0 (0)	0 (0)
2	28	3 (10.7)	5 (17.9)
3	31	4 (12.9)	8 (25.8)
Total	65	7 (10.8)	13 (20.0)

The chi-square statistic is 0.036. The p-value is 0.848, therefore results are not statistically significant at $p < 0.05$
*Area 1 results were deleted because they were Zero

TABLE 4. Data presenting the age of female camels infected with Toxoplasma

Age (year)	Total screened	Positive Toxo-IgG (%)	Positive Toxo-PCR (%)
≤ 6	23	4 (17.4)	5 (21.7)
More than 6	42	3 (7.2)	8 (19.1)
Total	65	7 (10.8)	13 (20.0)

The Chi-square statistic is 0.641. The p-value is 0.423, therefore results are not statistically significant at $p < 0.05$.

Discussion

The camel has long been the most neglected animal among ruminants in scientific research, despite being an active member of milk and meat production in the Arab countries. Among its main shortcomings that lead to neglect of camels are deprived feeding and poor breeding practices in arid, semi-arid, and tropical regions [16]. This study was conducted on dromedary camel showed the presence of antibodies against several infectious agents that have implications for livestock health and humans. *Toxoplasma gondii* is considered the most important and dangerous parasite that infects camels, causing severe clinical symptoms, and the consequences become severe among individuals with weakened immunity [17].

Humoral immunity in camels consists mainly of antibodies. In the blood and extracellular fluids, there is a major type of antibody, IgG, with a percentage of 75 percent, which allows it to control various infections that affect the camel's body (such as fungi, bacteria, viruses, and protozoa). When IgG binds to any pathogen, it freezes and binds together by agglutination. When IgG sticks to pathogens, it allows immune cells to identify, engulf and eliminate them. The amount of IgG in the blood indicates the presence of previous or current diseases in the camel's body, according to its concentration [18].

The present prevalence rate of *T. gondii* on camels were mentioned in the result, which is near to what was recorded in previous studies of Saudi Arabia and neighboring countries. Hussein [19] have reported that the infection rate in Saudi Arabia was 16%, and it was higher among females than males. This prevalence rate was variant among other regions of Saudi Arabia, such as in Riyadh Province 4.6% prevalence rate [7], however, Alanazi [20] has reported a higher infection rate 23.6% of slaughtered camels also in Riyadh Province. In addition, Mohammed [21] have recorded the infection rate in other regions of Saudi Arabia: such as Hofuf (15.8%), Riyadh (15.8%), Tabuk (39.5%), Jizan (46.2%), and Taif (51.2%). Toxoplasmosis prevalence rate differs in neighboring countries such as in Egypt 17% [22], 28.06% in Iran [23], and 14.56% in Yazd Province of Iran [8]. While few previous studies have recorded a higher infection rate of camels with *Toxoplasma gondii*, such as Utuk [24] detected the infection rate in Nevsehir

Province of Turkey was 90.9%, while Elamin [25] reported the infection rate in Butana plains of Sudan was 67%.

In most of the studies conducted on camels by the prevalence of antibodies to *Toxoplasma gondii*, it was found that old camels had a higher rate of infection than younger camels [19,26], which is contrary to this study, as younger camels had the highest infection rate. Calves are also affected by the infection, but it was not certain whether they became infected as a result of harmless consumption of sacs originating from members of the Felidae family or as a result of vertical transmission from mothers because several studies have shown transmission of *Toxoplasma gondii* through milk [27-29]. Transmission can occur through contaminated water or animal feed. The definitive host of *Toxoplasma gondii* was common in the areas where camels were sampled. While cats may contaminate camel feed and infected oocysts may remain viable until the camels eat their feed. At the same time, some camel owners complained about the abortion of some animals for no apparent reason [30].

There are many methods for detecting *T. gondii* antibodies in camels, which depend on the level of infection, availability of the final hosts, and the test used in the examination. Most of the previous studies depend on indirect fluorescent antibody test (IFAT), modified agglutination test (MAT), latex agglutination test (LAT), and enzyme-linked immunosorbent assay (ELISA) in serological surveys dealing with *T. gondii* for the detection of antibodies [7,21,31]. The previous three studies revealed that the use of ELISA gave a high rate of antibody detection in animals. While Andreotti [32], when testing sheep serum samples, found that the specificity of ELISA reached 98.3% compared to that of IFAT%. In this study, the specificity of ELISA reached to 20% compared to that 10% of PCR.

Molecular techniques are more specific, sensitive, and accurate than serological techniques that detect antibodies produced against *T. gondii* because molecular techniques detect the parasite's DNA. The results revealed that PCR confirmed all seropositive samples and detect other positive samples that were seronegative in the ELISA test. Many studies confirmed these results [28,29,33].

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Conflict of interest

The author confirms that there are no conflicts of interest.

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المراقبة المصلية والجزيئية لداء التوكسوبلازما جوندي في الإبل بالطائف بالمملكة العربية السعودية

جميله المالكي

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تسبب التوكسوبلازما جوندي مرضًا طفيليًا حيوانيًا هامًا ينتشر في جميع أنحاء العالم. ومع ذلك، فإن البيانات المسجلة عن داء التوكسوبلازما في الإبل شحيحة في مناطق مختلفة في محافظة الطائف بالمملكة العربية السعودية. تعتبر الإبل المصدر الرئيسي للحليب واللحوم في مختلف البلدان، وخاصة دول الخليج، وبسبب هذا الاتصال بين الجمال والبشر، فمن الممكن أن ينقل داء التوكسوبلازما إلى البشر. لذلك، أجريت دراسة الانتشار المصلي لداء التوكسوبلازما على ٦٥ عينة من إناث الجمال باستخدام مقايضة المناعي المرتبط بالإنزيم [Toxo-IgG] (ELISA). تم تأكيد البيانات بواسطة تقنية تفاعل البلمرة المتسلسل (PCR). تشير هذه الدراسة إلى معدل إنتشار داء التوكسوبلازما في العينات التي تم فحصها (٦٥/١٣، ٢٠٪). تم تصنيف نتائج تفاعل البوليميراز المتسلسل إلى مجموعتين: أقل معدل إصابة في المجموعة الأكبر سنًا كان (٤٢/٨، ١٩،١٪). في حين كانت أعلى نسبة إصابة في المجموعة الأصغر (٢٣/٥، ٢١،٧٪). بينما كانت نسبة إجمالي IgG في جسم الإبل هي الأقل في المنطقة ١ والأعلى في المنطقة ٣. إحصائياً، لم تكن هناك علاقة بين العمر ومعدل الإصابة.