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Seroprevalence of toxoplasmosis among one-hampered camels (*Camelus dromedarius*) in AL Butana plain, Sudan

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Abstract

Sudan is the second largest camel populated country in the world. Samples were collected at Great Butana (part of five states) from four sites: two camel markets: Tamboul and Wadnimir (Gezira state) and two Small-scale livestock production (semi-intensive management): Showak (Gadarif state), Girba (Kasala state). Six hundred camels of different breed, gender and age were examined during the three seasons to determine the prevalence of toxoplasmosis. Hematological method by latex agglutination test was used to examine the venous blood samples. The overall prevalence [180 infected animals (30%)] was higher in the two camels' market additionally, high prevalence was reported in old animals group. Seasonality has no significant variation however, higher prevalence incidence was in autumn. Gender's effect reported high prevalence in females than males. It could be concluded that such results can aid in updating such disease epidemiology. However, further studies are required for better and continual evaluation of such disease occurrence and management.

Keywords: toxoplasma, camels, Butana, Sudan

Introduction

Sudan is the second largest camel populated country in the world with a camel population over 3.3 million camels owned by pastoralists (FAO, 2015) [15]. In Sudan, Camels belt extends between latitudes 12–16 N (Eisa and Mustafa, 2011) [9]. Beside camel's products (e.g. milk and meat), camels play a central role in the livelihood of pastoralists by determining the owners' wealth and social status (FAO, 2002) [12]. However, camels suffer from various common parasitic diseases that have public health and economic importance including toxoplasmosis (Parsani *et al.*, 2008; Tabassam *et al.*, 2019; Alireza *et al.*, 2019) [25, 31, 3]. Toxoplasmosis is a widespread zoonotic parasitic disease worldwide (Zemene *et al.*, (2012) [36]; Gebremedhin *et al.*, (2013) [14]; Walle *et al.*, (2013) [34]; Utuk *et al.*, 2012 [33]; Dehkordi *et al.*, 2013) [7]. Infected animals may exhibit calcification of the heart, obscured vision, and difficulty in walking in addition to its implication in increasing the susceptibility of the intermediate hosts to predation. Furthermore, its ability to maintain a benign coexistence with its host, high infection rate with its worldwide distribution and hence, causes considerable economic losses to animals' industry. Therefore, all such features allowed it to be widely regarded as one of the most successful parasites on earth (Tonouhewa *et al.*, 2017 and references therein) [32]. Moreover, camels could acquire *T. gondii* infection through ingestion or inhalation of sporulated oocysts shed by cats or wild felids. However, humans, particularly in pastoral and rural communities, could become infected via drinking raw, unpasteurized milk (Dehkordi *et al.*, 2013) [7], ingesting raw or undercooked meat (Hilali *et al.*, 1995; Muksin *et al.*, 2011) [16, 23] and by passage of tachyzoites through the placenta to the fetus (Petersen, 2007) [26]. With regard to the Clinical manifestations in infected humans with the organism, they varied from congenital infection and abortion to fetal encephalitis and eye disease (Kula J. and J. Adem, 2016) [21].

Several reports revealed worldwide spread prevalence of toxoplasmosis among camels in as in Africa: in Ethiopia (Muksin *et al.*, 2011) [23], in Egypt (Elsheikha *et al.*, 2009) [11]; in Asia, such as, in Saudi Arabia (*Al-Anazi*, 2012) [2], in China (Wang *et al.*, 2012) [35]; in Europe such as in Italy (Villari *et al.*, 2009); in South America as in Brazil (Ragoz *et al.*, 2009) [27] and in United States of America (Dubey and Jones, 2008) [8]; overall, the seroprevalence of human toxoplasmosis in Nigeria is estimated at 32% (Ohiolei and Isaac, 2016) [24]. In Sudan, few reports were revealed from literature survey indicated the Seroprevalence of *Toxoplasma gondii* in farm animals such as camels, cattle, and sheep (Abbas *et al.*, 1987; Bronstein and Musa, 1987; Khalil and Elrayah, 2011) [1, 4, 20]. At Butana area, it was demonstrated that the presence of developmental stages of *Toxoplasma gondii* in camel meat and isolated *Toxoplasma* oocysts from kittens fed cameline meat (Elamin *et al.* (1992); [10] Ishag (2006) [17]; Medani and Mohamed, 2016) [22]. Such reported *Toxoplasma* prevalence among Sudanese camels deserves high concern into its public health impact especially among nomads who consume raw cameline milk and liver and into its economic impact. Because of camels' social, ecological and economic importance in Sudan, the present study however, attempted to diagnose *T. gondii* in naturally infected camels in Great Butana (three states), Sudan as step towards better public health management.

Materials and Methods

Study area

The current study was conducted in Al-Butana plain, a semiarid clay region covers most of Kassala, Gadaref and parts of Gezira, Khartoum and River Nile States in Sudan. It lies between Latitude 13 40' and 17 50' North and Longitude 32 40' and 36 00' East. The region is bound by the Main River Nile on its northwestern border, the Blue Nile on its southwestern edge, the Atbara River in the northeast and by the railway connecting Kassala and Sennar on the south. The area is composed of mountainous ranges intersecting the plain to the western and southern borders. It is crossed by many seasonal rivers namely, Atbara, Seitite, BaSalam, Gash and Rahad Rivers. Small temporary seasonal valleys do run through these plains during the rainy season. The rainfall in Butana region is highly variable from one year to the other. It ranges between 600 mm/year in the southeast to less than 100mm/year in the northwest. As always in the semiarid regions, rainfall is the most important climatic factor in Butana because people and their livestock depend on this factor which supports the growth of the vegetation for their animals. The annual mean temperature ranges from 32 °C during the day to 16 °C at night in January (winter) and from 46 °C during the day to 27 °C at night in the rest of the year. Two vegetation zones are existing in the area, namely semi-desert *Acacia* shrub and short grasslands of the North Central Sudan and secondly, the low woodland savannah of Central Sudan. The vegetation of Butana is constantly changing as a result of annual rainfall, accidental fire outbreaks and expansion of agriculture and grazing (Saint-Martin *et al.*, 1992).

Camel population

Camel population in Sudan was estimated to be 4.623 million heads (Ministry of Animal Resources and Fisheries 2011). In Butana plain, pastoralists depend solely on Camels breeding, an activity that, contribute significantly their livelihood. Pastoralists and agro-pastoralists living in this fragile

environments of the deserts breed different ecotypes of camels. Three camel management systems were identified for breeding of one humped camel in this region namely, Traditional nomadic system (Shuiep *et al* 2008) [30]; Transhumance or semi-nomadic system (Eisa and Mustafa 2011) [9] and Sedentary or semi-sedentary system (Shuiep and El Zubeir, 2012) [29].

Study design and sample collection

The study adopted a cross-sectional herd base survey, in which 600 animals were examined from four locations namely Elgirba, Tamboul, Elshowak and Wadnimir. Blood samples taken from each animal, under story conditions after camels restrain, by jugular venipuncture into a plain vacuonator (Becton – Dickinson, France). The blood samples were left to clot at 37 °C and sera were decanted into plastic tubes. Separated sera were stored at – 20 °C until used. Structure and test questionnaire were used to collect data from examined animals. Variables such as camels' breed and age and location, physiological status, and husbandry system were recorded for each animal. Seropositivity of test camels for Toxoplasma infection was assessed using Latex Agglutination Test (LATEX). According to manufacture instruction, serum samples and Toxoplasma antigen (Spin react, S.A. /S.A.U., Ctra. Santa Coloma, Spain) were kept one hour at room temperature before beginning of the test. A total of 50 µl of each serum to be tested was placed on a LAT plate. Then, the vial of antigen was shaken gently and 25 µl of antigen was added. The antigens and the serum were mixed on the plate with a stirrer and spread over the entire circle. The plate then, was rotated manually for 4 minutes and the reading was taken immediately. Any agglutination was considered as positive, whereas no reaction (negative) was indicated as the absence of Toxoplasma antibody in the sera.

Statistical analysis

The study variables were pre-coded for entry into Excel and then exported to the Statistical Package for the Social Sciences software (SPSS version 20) for analysis. Descriptive analyzes is an initial step used to describe the data sets in terms of frequency and proportion for the categorical variables of the study using SPSS analysis package. Considering its relevance to this study the analytical statistical tool, chi-square test was applied. The chi-squared test has applied to screen for an association between antibody seropositive results and the several risk factors included in the study.

Results and Discussion

Nevertheless Sudan is the second in camel production worldwide however, little is known about camels' productivity and health problems despite such social, ecological and economic importance. The goal of this study is to evaluate the recent prevalence of *T. gondii* in camel's blood in Great Butana, Sudan.

Out of 600 camels sera collected from 4 locations (Tamboul, Wadnimir, Showak and Girba) and tested by toxo_latex agglutination for Toxoplasma antibodies, 180 were positive giving an overall prevalence of 30%. (Fig.1). the infection rate recorded for different locations is depicted in Table (1). No significant difference ($p>0.05$) was observed for locations, the prevalence of toxoplasma antibodies was 31.3% in Tamboul, followed by Wadnimir (30%), Showak (29%) and Girba (29%). Table (2) through (4) illustrated, the seroprevalence of seropositive for *Toxoplasma* according to

variable concerning study animals, namely sex, age, and season. Chi-square was done to assess between seropositive *Toxoplasma* and the factors. Results showed that gender has no significant effect on *Toxoplasma gondii* infection. Higher infestation was reported in female than male (Value=0.525). ($p>0.05$). To assess the effect of age on *Toxoplasma gondii* infection, examined camels were categorized into five age groups: 1-3 years; 4-6 years; 7-9 years; 10-12 years and >13 years old. The results (Table 3) show that the older animals (>10 years) recorded significant higher infection rate ($p<0.01$) when compared to other groups. As shown from Table (4), seasonality has no significant effect ($p>0.05$) on *Toxoplasma gondii* infection among examined camels. Higher incidence rate was reported in autumn 69(34.5%) followed by winter 56(28. %) and then summer 55(27.5%).

In Butana, as always in the semiarid regions, rainfall is the most important climatic factor that enhances the growth of the vegetation for the livestock. Thirty percent seropositive camels might indicate naturally *T. gondii* infection circulating in Butana. Such infection could be recently acquired or recuperated from previously *T. gondii* infection. In the same region, seroprevalence has been reported in camels, sheep and goats (Elamin *et al.*, 1992; Khalil and Elrayah, 2011) [10, 20]. Therefore, variations in such prevalence could be due to several factors such as the samples size, differences in sensitivity of the diagnostic methods, differences in the density of cats and wild felids and in climatic conditions beside the differences in studies' dates.

Notwithstanding, the prevalence of *T. gondii* infection in the present results assumed that such animals can easily be contaminated with tachyzoites of *T. gondii*. However, camel species can also shed tachyzoites from their milks. Therefore, such findings are important in terms of public health. For instance, in such rural areas of Sudan, consumption of camel raw milk and uncooked camel's liver is common. Additionally, the higher prevalence of *T. gondii* in autumn (rainy season and more vegetation) could mean that the levels of contaminated milk consumption could be higher compared with summer and winter for instance. Furthermore, *T. gondii* transmission by blood-sucking arthropods, such as ticks should not be excluded. Ten tick species were collected from such camels (data in process for publication). It seems, however, as the could-be ticks role in transmission of the disease to their hosts influences by the higher temperature and relative humidity that provide appropriate conditions for the higher prevalence of *T. gondii*. Therefore, it would be worth recommending that it is essential performing detailed inspection for the presence and ways of infection of *Toxoplasma* in such region. Moreover, camel is not one of the primary hosts of *T. gondii*, however, the high prevalence of this parasite in camel species could be due to the close contact of these animals with infected primary hosts such as cats (Zhu *et al.*, 2012). In fact, in such area, camels are found on pastures associated with other animals such as sheep, goats, cats and dogs. High seroprevalences were observed in wild animals and small ruminants in many areas of the world (Dubey and Jones 2008; Tonouhewa *et al.*, 2017) [8, 32]. Therefore, the influence on acquiring toxoplasmosis in such camels could increase due to the contamination of the field with oocysts from such domestic and stray animals. Studies stated that camels themselves can shed *T. gondii* into their milk and can be easily transferred to humans. Therefore, drinking raw milk and eating undercooked meat could be risk factors that had strongest influence in pressure of infection with *T. gondii* (Cook *et al.*, 2000; Boyer *et al.*, 2005; Ishag *et*

al., 2006; FAO-OMS, 2015, Tabassam *et al.*, 2019; Alireza *et al.*, 2019) [17, 13, 31, 3]. Bearing in mind that Butana is semi-arid region, the high prevalence scored in the present study was expected. That is because, shortage of raining this year favors camel migration in search of feed and browsing feeding habit. Therefore, the availability of such high viable *T. gondii* cysts in camels meat ready for human consumption could augment the potential of toxoplasmosis transmission to humans via consumption of raw milk and/or undercooked camel meat such as camel's liver that commonly consumed uncooked by pastoralists in Butana.

Apparently, in such epidemiological study, it would be valuable should the risk factors for acquiring and transmission of *T. gondii* being more concerned of (Zhu *et al.*, 2019). For instance, results showed that sex, herd size and production system had no significant association with *T. gondii* seropositivity of the camels while location, age, history of abortion, presence of domestic cats and presence of wild felids were found to be significantly associated with *T. gondii* infection hence, independent predictors of *T. gondii* seropositivity (Gebremedhin *et al.*, 2014) [15]. Additionally, isolation of such parasites from camels' tissues and the limited knowledge of camel's owners about toxoplasmosis could suggest the need for prevention measures and pastoralists education. Toxoplasmosis, a global zoonotic disease, can infect humans and wide range of hosts. It therefore, causes severe public health problems and has great veterinary and economic importance worldwide. Ismail *et al.*, (2016) proved the possibility of developing an improving preventive and treatment program to reduce the overall severity of such disease. Furthermore, to unravel the impact of camel toxoplasmosis deserves further investigation. It is therefore, reasonable to consider risk factors for occurrence of *T. gondii* and should have priority in future investigations. Generally, as future work, it would be pleasant should such investigation performed with more accurate, specific, sensitive, inexpensive, rapid and safe diagnostic methods and consider better management measures. Risks of such diseases especially among labors, family members' and consumers of raw and undercooked meat in addition to managing such risks of disease transmission should be evaluated precisely to mitigate such risks.

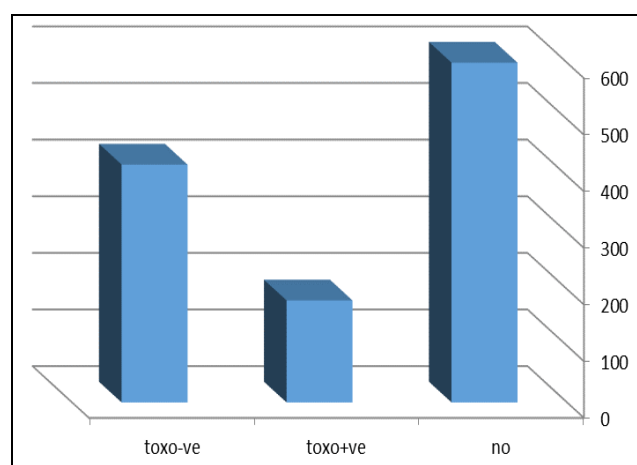


Fig 1: Overall prevalence of Toxoplasmosis in Butana area in camels during 2014-2015.

Table 1: Prevalence of *T. gondii* infection among examined camels according to location

Location	No. positive	No. negative	Total
Tamboul	47(31.3%)	103(68.6%)	150
Wadnimir	45(30%)	105(70%)	150
Showak	44(29.3%)	106(70.7%)	150
Gerba	44(29.3%)	106(70.7%)	150
Total	180	420	600

Table 2: Prevalence of *Toxoplasma gondii* infection among examined camels according to sex

Sex	No.+ve	No.-ve	Total
Male	30(27.5%)	79 (72.4%)	109
Female	150(30.5%)	341(69.4%)	491
Total	180	420	600

Table 3: Prevalence of *Toxoplasma gondii* among examined camels according age group

Age group	No.+ve	No.-ve	Total
1(1-3)	21(26%)	59(73.7%)	80
2(4-6)	35(22%)	124(77.9%)	159
3(7-9)	64(30.5%)	146(69.5%)	210
4(10-12)	39(38.6%)	63(61%)	102
5(>13)	21(42.8%)	28(57%)	49
Total	180	420	600

Table 4: Prevalence of *Toxoplasma gondii* among examined camels according to season

Season	No. positive	No. negative	Total
Summer	55(27.5%)	145(72.5%)	200
Autumn	69(34.5%)	131(65.5%)	200
Winter	56(28%)	144(72%)	200
Total	180	420	600

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