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Research article

Seroprevalence of brucellosis and toxoplasmosis in camels of Wasit Province, Iraq

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Abstract

Background: toxoplasmosis and brucellosis are zoonotic diseases, more added a major public health is worldwide because have high distribution in livestock. Which affects social and economic development in developing countries. Objectives: The aim of this study research was to determine the occurrence of the seroprevalence toxoplasmosis and brucellosis in camels in Waist provinces of Iraq from November 2016 to April 2017. Materials and Methods: Overall (237) blood samples collected of animals randomly were from both sex in different herds of animals and diagnosis by A Latex agglutination test (LAT), Rose Bengal Plate Test (RBPT) and indirect (ELISA). Results: An overall prevalence of T. gondii infestation were recorded, the positive sample with LAT test was 76 (32.06%) from all sample, the results of ELISA was shown in different groups 24.14% (7), 30.55% (11), 26.67% (8), 20% (8), 20% (15), 25.9% (7) from group1 to 6 respectively, while in age groups ELISA results was appeared 10.71% (6), 64.29% (36), 25% (14) respectively, the seroprevalence in females 50 (89.18 %) and positive in males 6 (10.72%). While brucellosis RBPT 51 samples positive and 186 sample negative, among 51 positive by RBPT confirmed by ELISA 39 sample positive and 198 negative, This positive sample divided in to 6 livestock groups, from 1 to 6 groups (5) 17.25%, (4) 11.10%, (8) 20%, (7) 17.5%, (10) 13.4%, (5) 18.5%, respectively with final percentage 16.29% while the negative result percentage 83.71%, high seroprevalence was recorded in moderate age (24) 10.12 % and the older than 10 years age (9) 3.79% while the less percentage in group 1 under 5 years of age (9) 2.53 %, while the seroprevalence recorded higher percentage in females (1) 97.43 % and less recorded in male (1) 2.57%. The two tests was used ELISA 56 positive samples (23.62%) and 181(76.28%) negative samples. However, 76 (32.06%) positive by LAT test and 161(67.94%) negative. Statically in (P>0.05)no significant was obtained in infection between groups of camels in this study and between the sex in all camels groups. Conclusions: high seroprevalence in studied camels indicated the importance of these animals as the main source of human infection. The widespread infection of other livestock. Clinical signs alone are not sufficient for diagnosis. Difficulties can arise in chronic camel infection.

Keywords: Brucellosis, Camels, Serology, Toxoplasmosis.

Introduction

All domestic animals and man infected by Brucellosis including camels, it consider serious zoonotic disease. It is more important as one of the major world problem for public health (1). In Africa and Asia was recorded Brucellosis in camel spreading from different countries of its (2). Infected camels' brucellosis that able to transmission to

persons include exposed group (herdsmen, dairymen, veterinary clinicians, butcher men) because direct transmission from animal with high risk of being worker of husbandry, this hazard acquired of special worry for public health (3). *B. abortus and B. melitensis* more common species infected camels, can causing a chronic disease with survive and

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persist in infected cells may be throughout of lifetime (3, 5). Animals in livestock (Cattle, goat, sheep, camels) consider the source and may be infected and transmit brucellosis to human especially pastoralists in endemic areas of infection (6).It is human health hazard in worldwide because zoonotic disease and major cause of heavy economic recognized poses dangerous losses livestock industry (7). The main sources of infection exhaustion of contaminated foods by bacteria. (8) The common pathogens causing disease in the susceptible animals in the same or in other livestock affecting animal species (9). The few clinical signs was appeared in camel brucellosis, so can difficult in diagnosis comprised of disease provokes in clinically course of infected The most conditions cattle (10).of brucellosis fetal death and retention of placenta due to placentitis, uterine infections, mummification, delayed maturity and infertility in female's orchitis, epididymitis in males, it also caused arthritis and hygroma (5, 11, 12). Camels take up infection via contaminated feed or water inter through the alimentary tract or via contaminated dust or droplets through the respiratory system or via semen through the genital system (13). The infection spread among camels and other farm animals via direct contact with uterine secretions, fetuses, blood and placenta, while in human via consumption, milk and milk products or contaminated raw animal products it's the main sources of infection (14, 17) other rout of transmission occurs via skin penetration or via conjunctiva or inhalation and udder contamination during milking. Congenital infection that happens during parturition (1). The toxoplasmosis worldwide diseases of mammals and birds including human, which infects nucleated cells (18) all types of domestic livestock (camels' sheep goats, and wildlife. companion animals), the oocysts felids disseminate into the environment consider of sources of infection (19). Losses of fetus due to abortion responsible for toxoplasmosis infection the important economic effected.

Reproductive failure of infection a high risk to public health (20). All mammalian can become infected with toxoplasmosis by taking cat- oocysts that contaminated water, soil, food or ingesting raw undercooked meat cysts. **Toxoplasmosis** containing tissue causes congenital disease include abortion and more common parasitic zoonosis (21, 23). The accidental ingestion of oocysts, the intermediate hosts get the infected through shed cats oocysts. Transmission occur by transplacental in case of camels' goat and sheep (24). When Toxoplasma gondii infection individual the immune response complex and compartmented, heterogeneity in genetic background causes the individual variation severity of diseases (25). Central nervous system CNS and placenta is a specific immune response, in addition, ability to reach in different tissues and each tissue closet has its own. An additional some strains Toxoplasma causes recurrent virulence the degree of complexity due to the possibility (26). Subclinical infection vast majority of infections in livestock camels, sheep, and goats. Generally, non-specific clinical signs are present, and may have a period of respiratory disorder, anorexia, fever and diarrhea (27). Camels play an impotent role in economic sources through provision of milk, meat and leather industry (28, 29).

Materials and Methods Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: **383**

Study area:

The present area of study in the eastern region of Wasit province Iraq, this area contain the natural pastures that enhances the grazing of camels (*Camelus dromedaries*), in the area between (32° 29′ 38.86″N 45° 48′ 51.7″E. Almost the animals grazing in rural areas and contacts with different animals livestock. Information from examined camel was collected including its location, health status, sex, age, and herd size, no vaccination

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history of selected camels females and male against brucellosis

Blood samples:

Two hundred and thirty seven blood samples (209 females &28 males) the sample collection from November 2016 to April -2017, were collected from puncture of jugular vein from each animal, the collected in tubes without anti-coagulant and give a specific cods of each sample, at 3000 r.p.m. for 10 minutes samples were centrifuged. Serum were separated and pickup in (1.5 ml) Eppendorf tubes, samples stored deep freezing for further analysis. All these samples collected randomly from different herds' animals, the range age (1-15years).

Serological examination

Latex agglutination test:

TOXO-Latex ® Spain Latex agglutination test (SPINRER EACT, S. A. Ctra. Santa Coloma) was used to screen the serum. Polystyrene latex particles in the reagent was coated with soluble T. gondii antigen. Antigen-antibody reaction enhance visual observe particles. The serum and the antigens were mixed on the entire circle of the plate with a stirrer over it's, for 4-6 minutes the plate, the reading was recoded immediately after end time, visible agglutination result positive, while the plate without agglutination in the serum no reaction (negative). The test procedure depend on manufacturer's protocol.

Rose Bengal Plate Test (RBPT):

All sera samples collected were initially screened by Rose Bengal plate test RBPT antigen (Institute Pourquer, 3409 Montpellier Cedex 5, France. Sera samples were kept in

Results

Seroprevalence of *T gondii* by LAT and ELISA test:

In this study collected two hander thirty seven blood samples, this samples collected randomly from six herds camels in Waist province from different localities The data of the collection sample from 6 different herd 237 sample, 207 females and 28 males and refrigerator at 4 C° before testing. Sera and antigen were left at room temperature for half an hour before the test to maintain to room temperature. Briefly, 40µl from serum mixed with 40µl from RBPT reagent and the mixture were rotating for 4 minutes. The positive result was recorded when clear agglutination appeared (30).

Indirect ELISA for Brucellosis:

On the other hand, the indirect ELISA (ID. VET. Innovative diagnostics, France) was also used for the diagnosis of IgG anti-Toxoplasma antibodies with anti-*T. gondii* ELISA. Depend on the manufacturer's instructions antibody titer were estimate by following on the set at the laboratory. The commercial kit of ELISA was used. The reading of results in instrument depend on 450 nm Optical densities (OD). The test procedure was carried out as per the manufacturer's protocol

Competitive (c-ELISA) for Toxoplasmosis:

The confirming of infection by used Competitive (c-ELISA): The positive samples with RBPT were further confirmed by EUROIMMUN Anti-Brucella ELISA Camel (IgG) Anti-Brucella ELISA Camel (IgG), was also used for the evaluation of IgG antibodies with ELISA set. Depend on the manufacturer's instructions antibody levels were evaluated by following on the set at the laboratory.

Analysis of data:

Analyze the data was used to Social sciences (SPSS) version 12.0 All data were using computer and statistically significant a *p*-value less than 0.05.

three groups of age 1 to 5 years (31) 25.74% and moderate age (140) 48.95% and old age (66) 27.85%,

Brucella test:

A total of 76 positive by latex from sex locals herd in study confirmed by ELISA from 56 (23.62%) positive from 76 positive by LAT, this result follows: 24.14% (7), 30.55% (11),

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26.67% (8), 20% (8), 20% (15), 25.9% (7) group1 to group 6 respectively according table (1).

Toxoplasma test:

In addition, 51 positive by RBPT samples of this study and showed 186 negative, among 51 samples positive by RBPT confirmed by ELISA 39 sample positive and 198 negative, This positive sample divided in to 6 livestock groups, from 1 to 6 groups (5) 17.25%, (4) 11.10%, (8) 20%, (7) 17.5%, (10) 13.4%, (5) 18.5%, respectively with final percentage 16.29%, (table 1) while the negative result percentage 83.71%, table (1).

Seroprevalence depend Age:

In brucella test out of 237 samples tested classified depend on age groups follows: (1-5) years 72, (5-10) years 36, (more than 10)years 24, ELISA results of test in camels age groups 10.71% (6), 64.29% (36), 25% (14) respectively. The seroprevalence among the age there was no significant differences in table (2). In toxoplasma test High seroprevalence was recorded in moderate age (24) 10.12 % and the older than 10 years age (9) 3.79% while the less percentage in group 1 under 5 years of age (9) 2.53 %, (table 2). In

brucella From out of 237samples 207 females and 28 males, the seroprevalence recorded in was also higher in females (1) 97.43 % than male (1) 2.57% (Table 3). In toxoplasma test the positive females 50 (89.18 %) and positive in males 6 (10.72%), the seroprevalence between females and males were significantly different. (Table 3).

Comparison (LAT) & ELISA test and RBPT and ELISA test:

The specificity and sensitivity of test used in serological diagnosis of brucella, RBPT with 51(21.52%) and negative results 186 (78.48%) while the results of ELISA 39 (16.45%) was positive and 198 (83.55%) appeared negative. (Table 4). The samples 237 collected 76 (32.06%) sera were positive and 161(67.94%) negative to LAT detection of antibodies (IgG) depended the protocol of the manufacturer while Indirect (ELISA) 56 positive samples (23.62%) and 181(76.28%) negative samples tested antibodies by the indirect ELISA kit protocol. (Table 4). The sensitivity, specificity for both test was calculated as P = 0.015 with significant differences.

Table (1): The seropositive both brucellosis and toxoplasmosis in different herd groups

Farm 1/6	Less 5 y	5- 10 y	More 10 y	Total	Brucella ve+.	percentage	Toxo ve+	Percentage
F 1	3	22	4	29	5	17.25%	7	24.14%
F 2	9	19	8	36	4	11.10%	11	30.55%
F 3	3	17	10	30	8	20%	8	26.67%
F 4	5	22	13	40	7	17.5%	8	20%
F 5	8	40	27	75	10	13.4	15	20%
F 6	3	20	4	27	5	18.5%	7	25.9%
	31	140	66	237	39		56	
							Chi- 2.02	P= 0.846

Table (2): The seropositive both brucellosis and toxoplasmosis in different age groups

table (2). The scropositive both brucehosis and toxopiasmosis in univerent age groups							
No (237)	1-to 5 y	5-10 y	More 10 y	All			
Brucella ve+	6	24	9	39			
% ve+	15.39%	61.54%	23.02%				
Brucella ne+	25	116	57	198			
% ne+	12.63%	58.59%	28.78%				
Toxo ve+	6	36	14	56			
% ve+	10.71%	64.29%	25%				
Toxo ne+	25	104	52	181			
% ne+	13.81%	57.46%	28.73%				
	31	140	66	237			
			Chi=1.48	P = 0.961			



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Table (3): The seropositive both brucellosis and toxoplasmosis depend on sex in different herd groups

No (237)	1-to 5 y	5-10 y	More 10 y	All	Percentage
Brucella ve+	6	24	9	39	
Male (28)	/	1	/	1	2.57%
Female (209)	6	23	9	38	97.43 %
Toxo ve+	6	36	14	56	
Male (28)	1	3	2	6	10.72
Female (209)	5	30	12	50	89.28
				Chi=1.97	P = 0.997

Table (4):Sensitivity and specificity of the seropositive both brucellosis and toxoplasmosis in different herd groups

Test	RBBT test		ELISA		Sensitivity - Specificity	
	Ve+	Ne+	Ve+	Ne+	ar ar and a grant	
Brucellosis	51	186	39	198		
%	21.52	78.48	16.45	83.55		
Test	LTEX ve+	Ne+	ELISA ve+	Ne+		
Toxoplasmosis	76	161	56	181	P = 0.015	
%	32.06	67.94	23.62	76.38	Chi=10.53	

Discussion

Brucellosis in livestock animal's cause's massive economic, losses includes premature birth, death of fetal, abortion, decreased milk production, infertility and transmission to other animal, adding the zoonotic effected of the disease in camels to human (31). The most infected of brucellosis in camels can be the cross transmission between camels and other species sharing their habitat on the husbandry system (6) (32), during calving or abortion occurs contamination together animals when poor management directly related to brucellosis rate infection (2).In this study the brucellosis positive sample in 6 livestock groups, from 1 to 6 groups (5) 17.25%, (4) 11.10%, (8) 20%, (7) 17.5%, (10) 13.4%, (5) 18.5%, respectively with final percentage 16.29% while, the negative result percentage 83.71%, Therefore, this results agreement with true seroprevalence brucellosis of camel in Jordan in the south province with used the RBPT and CFT is 15.8%. (33) This survey of brucellosis in Al-Hodeida in Yemen confirmed the presence of Brucella spp. the prevalence rate showed a significant in camels with (11%) (34). Results of serological test in Al-Mudawwara location of brucella in camels in Saudi Arabia, positive cases (17%) were recorded (35).While some study recoded percentage of prevalence of infection one of this study in eastern Sudan reported 16.5-32.3% from the 948 camels in different herds, while in seven herds examined with 416 camels in western Sudan prevalence rate found a 23.3% (36). The reported of higher prevalence of brucellosis (23.8%) from camel kept mixed with ruminant species in western Sudan (6). However, seroprevalence was high relatively of infection in camel recorded in Sudan (37). Another study recoded low percentage of infection, in Abu Dhabi Emirate the prevalence of brucellosis of camels that confirmed by c-ELISA (4.4%), (38) while in Egypt 7.3% (39). The study of brucellosis in Eastern Ethiopia revealed 2.43% of camel brucellosis (40). To study brucellosis in 3413 camels raised in areas of Sudan 72 (7.3%) out of 993 males and in 196 (8.1%) out of 2420 females (41). This seroprevalence is same the previous reports of 2.8% in Ethiopia (42), while 1.8% Southern Ethiopia (43) and from Eritrea Ethiopia, with recorded 3.1% (44) and some study in the Somalia was reveled 0.3 to 1.9% (45), and in other study was recorded 3.1% (46). In this study LAT test was recorded 76 (32.06%) while in ELISA test showed 56 (23.62%). Some different study of Toxoplasmosis prevalence in Iraq refers to widespread infection in camels, with different infection

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rate in 1998 in study of (47) 6.04%, while 16.35% (48) in 2006 and 20.34% recorded by (49) in 2012. ELISA test showed that 15 (16.4 %), Using LAT, out of 360 serum samples 91 (25.2%) (50).On other different study, the toxoplasmosis incidence compared with our results, the prevalence in Sudan 20% (51), and in Turkey 90.9% by using (52), another study in Egypt recorded 30.7% (53). More study in Saudi Arabia rate of 13.6% have been detected in 2012 (54). seropositivity While the reported toxoplasmosis in camel with percent of 6.5 %. (55). Furthermore 4% infection rate with Toxoplasmosis were registered in Iran in 2006 (56). Iran (14.57%) by using LAT (57). The different condition of animals included environmental factors husbandry system, and management practices reveal the different percentage of infection (58). The ingestion with contamination food and water or inhalation of oocysts that are detected by cats in the environment, it is conceivable exposure that the longer an animal lives, the greater the chance Toxoplasma infection by animals (59). Using LAT and ELISA assays in determine the incidence of toxoplasmosis antibody in infected animals serum, when detection of IgM seroprevalence sever case who able to transmission the oocyst to another farm animals, reflects the risk among animals with a recent infection, as in the contagious stage of the infection the animals, tachyzoites appeared in all fluids of body including milk (60). In brucellosis max herded of camels with different herded of cattle, sheep and goats lead to a close directed contact between infected susceptible animals in a herd lead to promote the spread of infection with possible source

of infection. The share of the same watering source points and same pastures may be increase higher incidence of the brucellosis in camels (6) (41). the stray dogs and foxes may spread the infection by deliver the aborted material on the pasture on the wide area of the pasture (41). The detection nonspecific antibodies by LAT for T.gondii (cross-reacted with other microorganism) was used ELISA to test definitive diagnosis of positive samples with specific detection of only IgG antibodies or IgM. (61) For all studies LAT is less sensitive than ELISA, while LAT can be used for epidemiological studies (62). Using different serological tests, the variation between the results obtained depended the serological test specificity and sensitivity. ELISA, low coast, quantitative, sensitivity, but requires standardization of the antigen used (63).

Conclusion:

The present study showed seroprevalence of brucellosis and toxoplasmosis in camel a moderate percentage of the incidence of infection association with herd size, a widely extended grazing, and situation vaccination, susceptibility to infection by virulence strain and delay diagnosis of bad control infection, however, and management to abortion and stillbirth. Although seroprevalence of camel brucellosis increased with the susceptibility of animals watering points in the river, and the seropositive animals may serve as foci of spreading of infection, with increased public health risk. The commonly used serological test because difficulties in diagnosis after that finally can be conformance by molecular and bacteriological cultures.

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