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RESEARCH ARTICLE

Seroprevalence of Camels Listeriosis, Brucellosis and Toxoplasmosis from Kirkuk Province-Iraq

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A B S T R A C T

This study was conducted to diagnose the seroprevalence of Listeriosis, Brucellosis, and Toxoplasmosis in camels from Kirkuk province in Iraq. Seventy-six serum samples were randomly collected and analyzed from camels from April to September 2018. Three serological diagnostic methods were used; Osebold agglutination test (OAT), Serum tube agglutination test (SAT) and Sabin-Feldman dye test (SFDT). From all serum samples that were collected, 42(55.26%) showed infections with Listeria, Brucella, and Toxoplasma as following 15(19.7%), 7(9.2%), and 20(26.3%) respectively. Additionally, the study revealed a highly significant relationship between aborted camels and their infection with Brucellosis and Toxoplasmosis (58.3%). In contrast, infections with these pathogens have no significant relationship with the sex and age of the camels. Risk factors of abortion were related to the high prevalence of Brucellosis and Toxoplasmosis in camels' herds. Furthermore, this study found out that the camels in Kirkuk city might be the source of these infectious agents and disseminated the diseases to the human through their contaminated dairy products and meat.

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INTRODUCTION

Camels have been considered as one of the most important food sources for human populations. They are present in worldwide places especially in middle east areas including Iraq (Bagheri Nejad *et al*., 2020). The zoonotic diseases of animal origin may reach 60% among them 75% of serious infectious diseases. Furthermore, camel zoonotic pathogens can be one of the important burdens in the Middle East or all camel-rearing areas. Due to the increase in the prevalence of zoonotic diseases that come from camels, constant observation and screening programs are necessary to inhibit the outbreak of zoonotic diseases in humans from camels (Mohammadpour *et al*., 2020). In general, camel (*Camelus dromedarius*) can live in hot and arid environmental conditions due to its special physiological system, consequently, transferring and wandering these animals in different areas may increase the probability of infections and effect on public health (Alatabi *et al*., 2020; Shabbir *et al*., 2020). Many agents cause diseases for animals or humans due to the consumption of animal products or contact with infected animals. Among major pathogens that have been as food-borne and water-borne pathogens often associated with camel meat milk and their products including *Listeria monocytogenes*, *Brucella abortus* and *Toxoplasma gondii*. These organisms are also important etiological agents to cause listeriosis, brucellosis and toxoplasmosis in human populations (Dhama *et al*., 2015; Iacobucci *et al*., 2019; Njenga *et al*., 2020).

Listeria monocytogenes is a gram-positive, facultative, intracellular bacterium that has protrude as one of the most significant food-borne pathogens and occurs in a set of food items like dairy products and milk ruminants even seafood. The bacterium causes human listeriosis with foodborne appearances and distribution of pathogenic serotypes amongst the *L. monocytogenes*. Serotypes, 1/2a, 1/2b and 4b, have been found in foodstuffs and the environment (Mashak *et al*., 2021). Usually, foodstuff embroiled as a source of the organism involves salads, fermented meats and raw meats as beef, pork, lamb, poultry and fishes (Yehia *et al*., 2016; Chemweno *et al*., 2019; Yehia *et al*., 2020; Mashak *et al*., 2021) and other sources in a wide range of domestic and wild animals and also some birds (Faramarzpour *et al*., 2017).

Brucella abortus is one of the most important foodbor ne pathogens which has been attracted in new years by ing estion of animal products, directly or indirectly by consumption of unpasteurized raw milk or/and cheese and contact with raw blood (Dhama *et al*., 2015; Akhtar *et al*., 2019; Njenga *et al*., 2020; Shabbir *et al*., 2020). Camels infected by two types of Brucella spp. are *Brucella melitensis* and *Brucella abortus*. Camels are commonly infected with *Brucella spp.* organisms, particularly when they are handled with infective smallholders (pastoralists and mixed farmers) and livestock and screened for IgG antibodies (Njenga *et al*., 2020). *Toxoplasma gondii* is the only member of genus Toxoplasma that is pathogenic to mammals and avian species. The major transmission of *T. gondii* is by tissue cysts among intermediate hosts (without definitive hosts) as well as by oocysts among definitive hosts without intermediate hosts. Animal toxoplasmosis can infect by ingesting water or food contaminated with infective oocysts and trans-placental diffusion of protozoans to the fetus. Also, there was some information about the transmission of *T. gondii* by infected animal's milk (Dubey, 2016; Iacobucci *et al*., 2019). Camels are characterized by endurance in high dryness and altitude possibility to turn into the little resources of the desert into meat and milk (Mohammadpour *et al*., 2020). Overall, meat and milk are considered significant sources of all of the pathogens described above. The current research work aimed to study the seroprevalence of the *L. monocytogenes*, *B. abortus* and *T. gondii* in camel in Kirkuk province of Iraq.

MATERIALS AND METHODS

Area of study and animals: Between April and September 2018, samples of blood were collected from camels from Kirkuk city in northern Iraq. About 10 ml blood samples were obtained from the jugular vein of 76 local camels (6 males, 70 females) and transported to the parasitological laboratory of the medical laboratory department/Technical college Kirkuk. The blood samples were centrifuged at a temperature of 4°C for about 10 minutes (4000 RPM of centrifugation), then serum was removed and deposited at -20°C until testing was performed in the Laboratory of Refik Saydam National Hygiene Research Center, Communicable Diseases Research Department. The Statistical Package of data was used to analyze the differences in prevalence among age, genders groups, and abortion animals by using the T-test.

Serological assays

Listeriosis; Osebold agglutination test (OAT): A titration test was performed in accordance with the method characterized by Osebold *et al.* (1965) to find antibodies to *L. monocytogenes* (Osebold *et al*., 1965; Yücel *et al*., 2014). The antigen used in this study was made in the laboratories of the Refik saydam national hygiene center, Department of communicable diseases research, and in three steps, the assay was accomplished. In the First step, the whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains by the Osebold technique. In the second step, Listeria antigens were prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c and 4d strains and combined in the same suspension. In the last step, an agglutination test was performed after the absorption of sera samples with *S. aureus* antigen. Samples with a titre1:100 were considered positive.

Brucellosis; serum tube agglutination test (SAT): To identify Brucella infection, serum samples were diluted from 1/10 to 1/80 and examined using the Rose-Bengal agglutination test, and positive sera were confirmed positive for Brucella antigens by Serum tube agglutination test (SAT) with serum provided by Refik Saydam Hygiene center Antigen-Antiserum Production and Research Laboratory (RSHM).

Seventy-six serum products were mixed on a white enamel plate with an antigen quantity. For 4 min at room temperature, the mixture was softly shaken. The mixtures that form agglutination were considered positive without considered negative and probability of result again to decision positive or negative. In SAT, serum samples were diluted at 1:10, 1: 20, 1:40 and 1: 80 with *B. abortus* antigen. The results were assessed at 37°C in 24-48 hours after incubation (Fatima *et al*., 2016).

Toxoplasmosis; Sabin-Feldman Dye Test (SFDT): In this test; the serum samples were examined for toxoplasmosis by SFDT utilizing vital antigen and methylene-blue dying. The Sera specimens were inactivated at 56°C for 30 minutes, then tested for anti *T. gondii* antibodies with fourfold dilutions (1:16, 1:64, 1:256 and 1:1024). An antibody titer of 1/16 or over was accepted to be positive (Sabin and Feldman, 1948; Yücesan *et al*., 2019).

RESULTS

Generally, the camels were infected with *L. monocytogenes, B. abortus* or *T. gondii;* a single infection was detected in 29/42 (69.04%) by one of the previous reagents, and mixed infections in 13/42 (30.95%) using more than one reagent (Table 1; Fig. 1).

The distribution of anti *L. monocytogenes*`O antibody seropositivity varied according to the age of camels: its presence at age <5 months was 1/5 (20%), 4/14 (28.6%) were positive at age 1-2 years, while among camels over 2 years 10/51 (19.7%) were positive. Serum tube agglutination test seropositivity was found in 6/57 (10.5%) of camels of age group more than 2 years and was negative in age <5 months.

The positive Serum Sabin-Feldman Dye Test has mostly occurred in age groups 1-2 years and more than 2 years in about 2(14.3%) and 17(29.8%) respectively. The occurrence of seropositivity was non-significant according to the age group (Table 2; Fig. 2).

Considering gender, overall infections were 70/76 (92.1%) in females. Positive results for anti-*L. monocytogenes* `O antibody, tube agglutination test for Brucella, Sabin-Feldman Dye Test for Toxoplasma were 15/70 (21.4%), 7/70 (10%) and 19/70 (27.1%) respectively.

The total infection in male was 6(7.9%) and was 1(16.7%) for Sabin-Feldman Dye Test in male and negative for other. None significant for genders at $P<0.05$, (Table.3; Fig. 3).

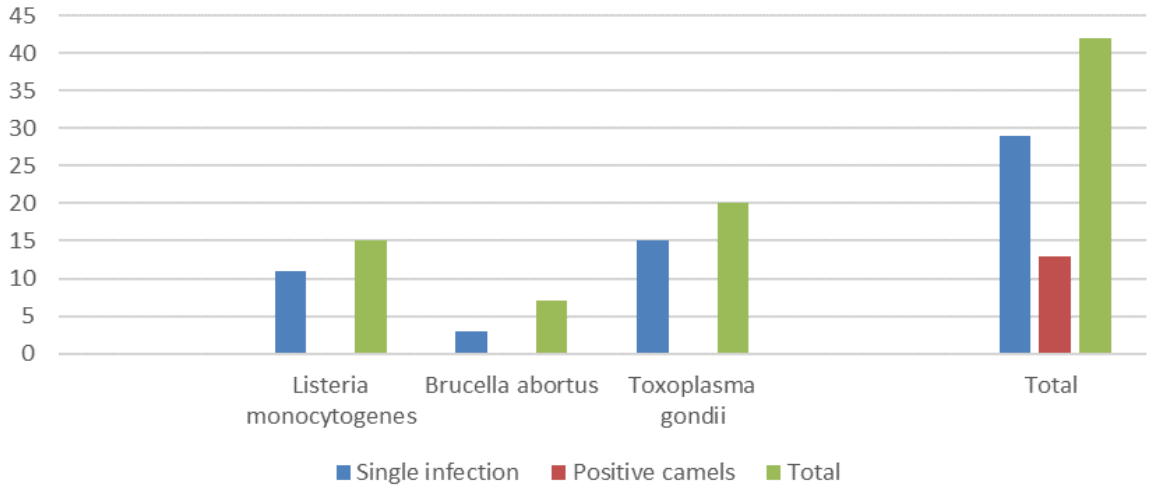

Fig. 1: Distribution of Single and Mix-infections in camels.

Table 1: Single and Mix-infections in camels

Infectious agent	Single infection	Positive camels mix infected	Total
Listeria monocytogenes	11	1(*Listeria+Brucella+Toxoplasma*)	15
Brucella abortus	3	2(*Brucella+Toxoplasma*)	7
Toxoplasma gondii	15	2(*Listeria+Toxoplasma*)	20
		1(*Listeria+Brucella*)	
Total	29	13	42

Overall infection in aborted camels was 12/70 (17.1%), with 3/12 (25.0%) and 7/12 (58.3%) seropositive for anti-*L. monocytogenes*`O antibody, for both tube agglutination Test and Sabin-Feldman Dye Test, respectively; these results were highly significant with aborted camels in contrast with non-aborted (Table 4; Fig. 4).

DISCUSSION

Listeriosis, Brucellosis and Toxoplasmosis are diseases that are zoonotic in camels (Ibrahim *et al*., 2016; Mohammadpour *et al*., 2020). Three important zoonotic pathogens were recorded among desert camels including *L. monocytogenes*, *B. abortus* and *T. gondii* which belong to bacterial and parasitic pathogens, respectively (Mohammadpour *et al*., 2020). The present study using conventional non-DNA-based diagnostic methods showed that the prevalence of *L. monocytogenes*, *B. abortus* and *T. gondii* were 19.7% by OAT, 9.2% by SAT and 26.3% by SFDT respectively. The results of the study are in agreement with other studies; similar results were found for raw meat samples where *L. monocytogenes* was detected in 16% of samples in Riyadh, Saudi Arabia (Yehia *et al*., 2020), for camel brucellosis in Al-Najaf province, Iraq, with 6.97% of samples using Rose Bengal test (Alatabi *et al*., 2020), and for toxoplasmosis in West Kordofan and the Blue Nile states, Sudan, with 13.3% of samples positive using latex Agglutination Test (LAT) (Abdelbaset *et al*., 2020). Using latex Agglutination Test (LAT) in West Kordofan and the Blue Nile states, Sudan (Abdelbaset *et al*., 2020), Single infection with either *L. monocytogenes*, *B. abortus* or *T. gondii* was detected in 29/42 (69.04%), and mixed infections in 13/42 (30.95%) (Table 1; Fig. 1).

Globally, Listeriosis is a zoonotic disease of humans and domestic animals. *L. monocytogenes* has been involved as a cause of foodborne outbreaks and establish in the environment, human and healthy animals and most infections are subclinical. The organism is so resistant to dryness and might remain viable in dry soil and feces for up to 2 years (Dhama *et al*., 2015; Yehia *et al*., 2016).

In farm herds, the *L. monocytogenes* in different kinds of meats in fresh camel was 12/24(50%) and in frozen camel was 6/24(25%) in Tehran province, Iran (Mashak *et al*., 2015). In a different study, 40.9% of 132 examined cattle were seropositive on cattle in Adana, Turkey, using the Osebold approach and serological assays showed that the presence of *L. monocytogenes* and *T. gondii* infections were higher than Brucella spp. seropositivity (Yücel *et al*., 2014). In Iraq as a whole and particularly in the Kirkuk region, there is no documented study, revealing the sero-prevalence of listeriosis in farm animals rather than camels, therefore this is the first study that detected the incidence of this bacteria in camels in Iraq and the results were 15(19.7%) from 76 samples. Sometimes variation in infection rates is explained by factors related to contamination with animal products or being exposed to different contamination factors.

Serological tests may act as a fundamental tool for the diagnosis of camel brucellosis; but concerns arise in the scientific population considering the direct alteration from livestock without sufficient validation (Serhan *et al*., 2019). In farm animals, the *Brucella* infection is considered a major problem in most world countries, thus, the initial detection of *Brucella* infection in a herd is a prerequisite for the successful control and eradication of one of the major problems considered to be a predisposing agent leading to infertility along with the probable transmission of infection to man (Yücel *et al*., 2014; Ibrahim *et al*., 2016; Alatabi *et al*., 2020; Bagheri Nejad *et al*., 2020; Mohammadpour *et al*., 2020; Shabbir *et al*., 2020). The risk factors for camel abortion include significant effects of Brucellosis and Toxoplasmosis in the current study. Dadar and Alamian (2020) have isolated *Brucella melitensis* from seronegative camels (*Camelus dromedarius*) with mild agreements between RBPT (Rose Bengal plate test), SAT serum tube agglutination test and 2-ME (mercaptoethanol test) results, in addition to complementary role of PCR diagnosis for the best detection of seronegative camels or chronic stage.

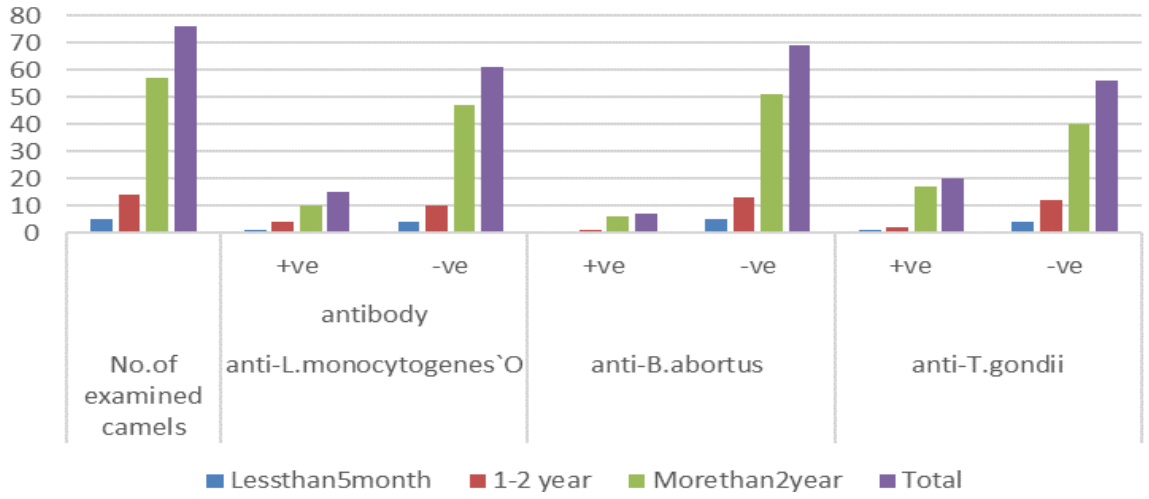

Fig. 2: Relation of *L. monocytogenes, B. abortus, T. gondii* according to ages.

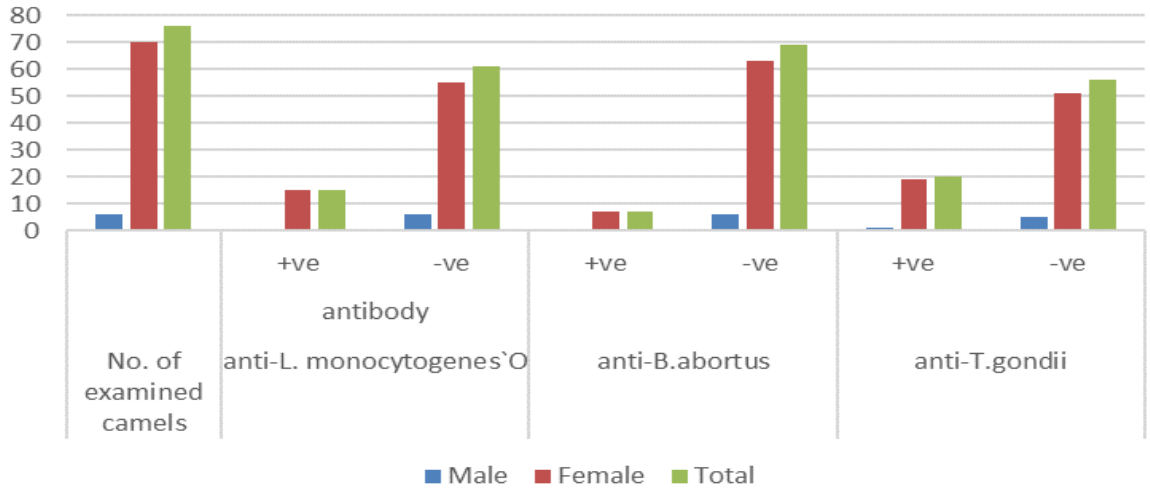

Fig. 3: Relation of *L. monocytogenes*, *B. abortus*, *T. gondii* according to genders.

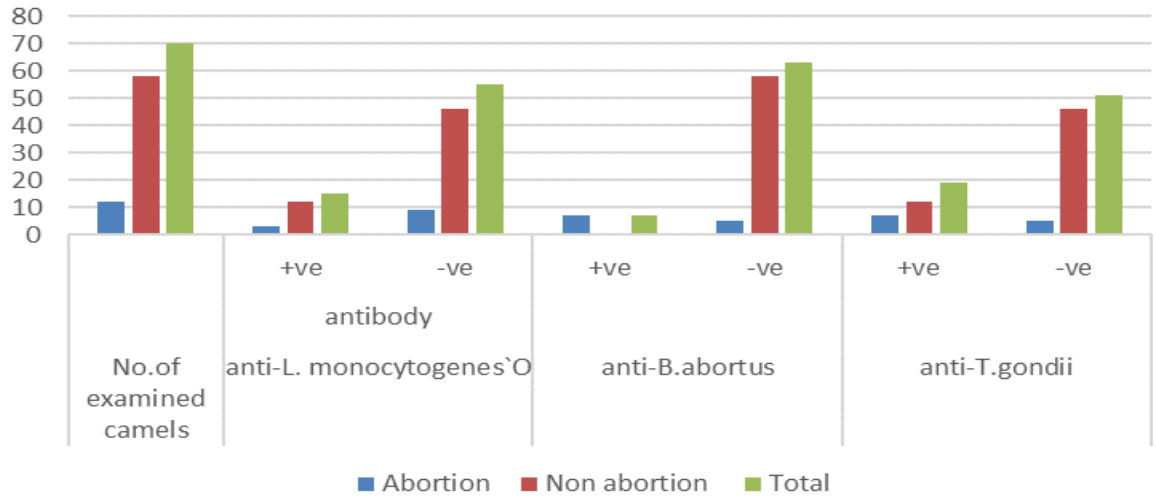

Fig. 4: Relation of infections in aborted and no aborted camels.

Table 2: Seroprevalence of *L. monocytogenes, B. abortus, T. gondii* according to ages

Age group (year)	No. of examined camels	anti-*L.monocytogenes*`O antibody		anti-*B.abortus*		anti-*T. gondii*	
		+ve	-ve	+ve	-ve	+ve	-ve
Lessthan5month	5(6.6%)	1(20%)	4(80%)	0(0.00%)	5(100%)	1(20%)	4(80%)
1-2 year	14(18.4%)	4(28.6%)	10(71.4%)	1(7.1%)	13(92.9%)	2(14.3%)	12(85.7%)
Morethan2year	57(75%)	10(17.5%)	47(82.5%)	6(10.5%)	51(89.5%)	17(29.8%)	40(70.2%)
Total	76(100%)	15(19.7%)	61(80.3%)	7(9.2%)	69(90.8%)	20(26.3%)	56(73.7%)
Statistical analysis	P-value of T-test=0.87; non-significance.						

Table 3: Seroprevalence of *L. monocytogenes*, *B. abortus*, *T. gondii* according to genders

Sex group (year)	No. of examined camels	anti-*L. monocytogenes*`O antibody		anti-*B. abortus*		anti-*T. gondii*	
		+ve	-ve	+ve	-ve	+ve	-ve
Male	6(7.9%)	0(0.00%)	6(100%)	0(0.00%)	6(100%)	1(16.7%)	5(83.3%)
Female	70(92.1%)	15(21.4%)	55(78.6%)	7(10.0%)	63(90.0%)	19(27.1%)	51(72.9%)
Total	76(100%)	15(19.7%)	61(80.3%)	7(9.2%)	69(90.8%)	20(26.3%)	56(73.7%)
Statistical analysis	P-value of T-test=0.5; non-significance.						

Table 4: Seroprevalence infections in aborted and no aborted

Age group (year)	No. of examined camels	anti-*L. monocytogenes*`O antibody		anti-*B.abortus*		anti-*T.gondii*	
		+ve	-ve	+ve	-ve	+ve	-ve
Abortion	12(17.1%)	3(25.0%)	9(75%)	7(58.3%)	5(41.7%)	7(58.3%)	5(41.7%)
Non abortion	58(82.9%)	12(20.7%)	46(79.3%)	0(0.00%)	58(100.0%)	12(20.7%)	46(79.3%)
Total	70(100%)	15(21.4%)	55(78.6%)	7(10.0%)	63(90.0%)	19(27.1%)	51(72.9%)
Statistical analysis	P-value of T-test=0.000009; highly significance.						

Camel herd seropositivity was more closely associated with brucellosis in cattle. Sera were collected from 1822 Bactrian camels, 1155 cattle using the Rose Bengal Test which has a history of abortion and milk samples for bacteriological culture. The total infection in camels was 2.3%, with isolation of *Brucella abortus* from cattle and camels (Bayasgalan *et al*., 2018). In Borana, Ethiopia, abortion was more common in camels (23.4%) than in cattle (13.8%) or goats (12.4%) (Megersa *et al*., 2011). In the current study, the seroprevalence of brucellosis was determined to be 9.2% by using the SAT method. The incidence in the current study was slightly higher than Yawoz *et al*. (2012) reported for 2/66 (3.03%) camels tested using the Rose Bengal test in the South of Kirkuk city, Iraq. Our results are in contrast with this survey because by using SAT, 7 (9.3%) from 76 camels were positive; it may be that the SAT test is more precise than the RBS test. The occurrence of *B. melitensis* or *B. abortus* in camels was 2.2% and found to be linked to their detection in the livestock reservoir, i.e., small ruminants and cattle in 10.6% and goats in 1.9% (Megersa *et al*., 2011). Similarly in our study, the conventional screening test (RBPT) reported 10/200 (5%) with more potential infections than the confirmatory CELISA test 4/200 (2%) and molecular detection rtPCR test 3/200 (1.5%), with potential risk factors including rearing system, season, and orchitis history or abortion. In the current study there is no significant effect between ages (Table 2; Fig. 2, and sex, Table 3; Fig. 3), while highly significant differences were found (P<0.000009) between aborted and non-aborted camels with 12 (17.1%) and 58 (82.9%) respectively (Table 4; Fig. 4). These results were in agreement with results in female camels, which show a higher prevalence of brucellosis in aborted females than non-pregnant and pregnant; this is due to keeping livestock mixed with other animals more at risk than single animals (Fatima *et al*., 2016).

Toxoplasma gondii is a zoonotic protozoan parasite, having the ability to infect all warm-blooded hosts and birds and causes congenital defects and abortions in humans and animals. Congenital toxoplasmosis is regarded to have the highest global illness burden of any foodborne disease. The potential role of milk as a route of *T. gondii* transmission between humans and livestock within Mongolian herders was investigated (Iacobucci *et al*., 2019). The seropositivity rate was 6/45 (13.3%) in Sudan. This high seroprevalence of *T. gondii* in livestock shows their potential role in the transmission of human toxoplasmosis in Sudan and the widespread contamination with Toxoplasma oocysts in the rural environment (Abdelbaset *et al*., 2020).

Toxoplasma sp., infection was 26.4% by SFDT in the current study. Different studies have detected the seroprevalence of toxoplasmosis by using different techniques. This study is in agreement with results from Saudi Arabia; prevalence in many animals including camels were investigated and 68/199 camels (34.2%) were positive (Mohammed *et al*., 2020). The seropositive and identify risk factors of *T. gondii*, were detected in domestic ruminants in the East Hararghe zone of Oromia region, Ethiopia. The total infection was 302/1360 (22.2%), and in sheep, goats, cattle, and camels were 33.7, 27.6, 10.7 and 14.4%, respectively (Tilahun *et al*., 2018). Yücesan *et al*. (2019) reported toxoplasma infection in stray cats was 86 (66.6%) of 129 animals in Ankara/Turkey, using SFDT, and indirect effects for humans. Also in human toxoplasmosis, Shani *et al*. (2012) recorded positive seroprevalence as a significant increase in the level of IgG, IgM, C3, and C4 in sera of infected women in comparison to the healthy control group by single radial immune diffusion test in Basrah, Iraq. While there is a significant decrease in the mean number of CD^+ (T-helper cells) in sera of infected women compared to a healthy one, and this may be attributed to immune suppression mechanisms (Shani *et al*., 2010). The variation of the results may be due to the sample sizes of different studies, the different methodology used, and may be other factors like the virulence and type of *T. gondii* status and age. Furthermore, these agents may contribute to the variation of results among these studies and our study. Until now SFDT was not commonly used because of the requirements for the use of live parasites, though it is still the gold standard for detection in many hosts (Dubey, 2016), so we have used this standard method for this reason.

Conclusions: This study confirmed the presence of *Listeria* sp., *Brucella* sp. and *Toxoplasma* sp. infections in Kirkuk Province, Iraq. It is recommended to carry on further studies of these infective agents in camels in other cities in Iraq, not only in camels but also in ruminants and felines. Regular checking of camels can detect infection in seropositive animals and modern methods can detect infections even in seronegative animals. Vaccination of uninfected camels can protect against infection and abortion.

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