



RESEARCH ARTICLE

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

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ABSTRACT

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 during April to September 2018. Three serological diagnostic methods have been used; Osebold agglutination test (OAT), serum tube agglutination test (SAT) and Sabin-Feldman dye test (SFDT). From all serum samples that 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 sex and age of the camels. Risk factors of abortion were related with 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 of 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. Its live in worldwide places especially in middle east areas including Iraq (Bagheri Nejad *et al.*, 2020). The zoonotic diseases of animal origin may be 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 increasing in the prevalence of zoonotic diseases that come from camels, therefore, 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*) has an ability to live in the hot and arid environmental conditions due to its special physiological system, consequently, transferring and wandering these animals in different areas may be increase the probability for infections and effect of the public health (Shabbir *et al.*, 2020; Alatabi *et al.*, 2020). Many agents were causing diseases for animals or humans due to consumption of animal products or contact with infected animals. Among a major pathogens

that have been as food-borne pathogens 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 an important etiological agents to cause listeriosis, brucellosis and toxoplasmosis respectively in human populations (Dhama *et al.*, 2015; Iacobucci *et al.*, 2019; Njenga *et al.*, 2020).

Listeria monocytogenes is a gram positive, facultative, intracellular bacterium which has protrude as one of most significant food-borne pathogens and occurs in set of food items like dairy products and milk ruminants even seafoods. The bacterium causes human listeriosis with foodborne appearances and distribution of pathogenic serotypes amongst the *L. monocytogenes*. Serotypes are 1/2a, 1/2b and 4b have been found in food stuffs and 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 foodborne pathogens which has been attracted in new years by ingestion of animal products, directly or indirectly by consumption unpasteurized raw milk or/and cheese and contact with raw blood (Dhama *et al.*, 2015; Njenga *et al.*, 2020; Shabbir *et al.*, 2020). Camel infect by two types of brucella spp. are *Brucella melitensis* and *Brucella abortus*. Camels are commonly infected with *Brucella spp.* organisms, particularly when they in handle with infective smallholder (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* by tissue cysts among intermediate hosts (without definitive hosts) as well by oocysts among definitive host 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 were several information's about transmission of *T. gondii* by infected animal's milk (Dubey, 2016; Iacobucci *et al.*, 2019). Camels are characterized to 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 consider as significant sources of all of the pathogens described above. The aim of the this study was to study the sero-prevalence 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 have been collected from camels from Kirkuk city in northern Iraq. Native breed camels aged between (5 months, 22 year) in this area are used as a source of meat and milk in Kirkuk regions. In this area, animals (cattle, sheep and horses are raised in one stable) are commonly grown. For this reason, many diseases are easily spread among animals' species. About 10 ml blood samples were obtained from jugular vein of 76 local camels (6 males, 70 female) and transported to the parasitological laboratory of medical laboratory department/Technical college Kirkuk. The blood samples 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 were 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* 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 First step, the

whole cell antigens were prepared from *Staphylococcus aureus* (ATCC 29213) strains by the Osebold technique. In the second step, *Listeria* antigens was prepared from *L. monocytogenes* 1/2a, 1/2b, 4b, 4c and 4d strains were 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 titre 1:100 were considered positive.

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

Seventy 76 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 was consider 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 examined for toxoplasmosis by SFDT utilizing vital antigen and methylene-blue dye. 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* or *B. abortus* or *T. gondii* and also detected single infection was 29/42(69.04%) by one of the previous agents or mix infections 13/42(30.95%) by more than one agents. Table 1; Fig. 1.

The pattern about distribution of examined groups regarding to age anti *L. monocytogenes* O antibody seropositivity occur in age <5months was 1(20%), 4(28.6%) occur in age 1-2 years, while in more than 2 year was 10(19.7%) camels have anti *L. monocytogenes* O antibody seropositivity. Serum tube agglutination test seropositivity mostly occur in about 6(10.5%) camels within age group more than 2 year and lowest in age<5months was negative. The positive Serum Sabin-Feldman Dye Test is mostly occurred in age groups 1-2 year and more than 2 year in about 2(14.3%) and 17(29.8%) respectively. The occurrence of seropositivity were non-significant according to the age group Table 2; Fig. 2.

Among gender, overall infections were 70(92.1%) in females. Anti-*L. monocytogenes* O antibody, tube agglutination test, Sabin-Feldman Dye Test were 15 (21.4%), 7(10%) and 19(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.

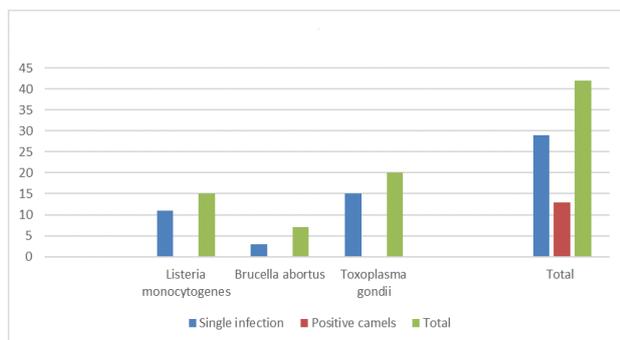


Fig. 1: Distribution of Single and Mix-infections in camels.

Table 1: Single and Mix-infections in camels

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

Aborted camels were recorded overall infection was 12(17.1%), 3(25.0%) for anti-*L. monocytogenes* O antibody and 7(58.3%) for both tube agglutination Test and Sabin-Feldman Dye Test, highly significance with aborted camels in contrast with nonaborted Table 4. Fig. 4.

DISCUSSION

Listeriosis, Brucellosis and Toxoplasmosis are diseases that are zoonotic possibly in camels (Ibrahim *et al.*, 2016; Mohammadpour *et al.*, 2020). There are 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 found 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 was agreement with other studies or similar results in raw meat samples for *L. monocytogenes* was detected (16%) of contamination in Riyadh, Saudi Arabia (Yehia *et al.*, 2020), for camel brucellosis in Al-Najaf province, Iraq, was (6.97%) using Rose Bengal test (Alatabi *et al.*, 2020), and for toxoplasmosis was (13.3%) using latex Agglutination Test (LAT) in West Kordofan and Blue Nile states, Sudan (Abdelbaset *et al.*, 2020). The single infection has been detected *L. monocytogenes* or *B.*

abortus or *T. gondii* was 29/42(69.04%) and 13/42 (30.95%) in mix infections. Table 1; Fig. 1.

Globally, Listeriosis is zoonotic disease of human and domestic animals. *L. monocytogenes* has been involved as cause of food borne outbreaks and establish in 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 different study 40.9% of 132 examined cattle were seropositive on cattle in Adana, Turkey, using the Osebold approach and serological assays showed the presence *L. monocytogenes*, *T.gondii* infections were higher than *Brucella* spp. seropositivity (Yücel *et al.*, 2014). In Iraq as a whole and particularly in Kirkuk region, again in our speculating there is no documented studies, revealing the sero-prevalence of listeriosis in farm animals rather than camels, therefore this is first study that detect the incidence of this bacteria in camels in Iraq and the result were 15(19.7%) from 76 sample. Sometimes varying in infection rates this explain some factors are related with contaminated animal products or exposing to different contamination factors.

Serological tests may act 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 the probable transmission of infection to man (Yücel *et al.*, 2014; Ibrahim *et al.*, 2016; Mohammadpour *et al.*, 2020; Alatabi *et al.*, 2020; Shabbir *et al.*, 2020; Bagheri Nejad *et al.*, 2020). The main risk factors for camel was abortion have been significant effect for Brucellosis and Toxoplasmosis was 7(58.3%) in the current study. Among seronegative camels (Dadar and Alamian 2020) have been isolated *Brucella melitensis* from seronegative in camels (*Camelus dromedarius*) with mild agreements between RBPT (Rose Bengal plate test), SAT serum tube agglutination test and 2-ME (mercaptoethanol test) in results, in addition to complementary role of PCR diagnosis for a best in seronegative camels or chronic stage.

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

| Age group (year) | No. of examined camels | anti- <i>L.monocytogenes</i> O antibody | | anti- <i>B.abortus</i> | | anti- <i>T.gondii</i> | |
|----------------------|---|---|-----------|------------------------|-----------|-----------------------|-----------|
| | | +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- <i>L. monocytogenes</i> O antibody | | anti- <i>B.abortus</i> | | anti- <i>T.gondii</i> | |
|----------------------|--|--|-----------|------------------------|-----------|-----------------------|-----------|
| | | +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. | | | | | | |

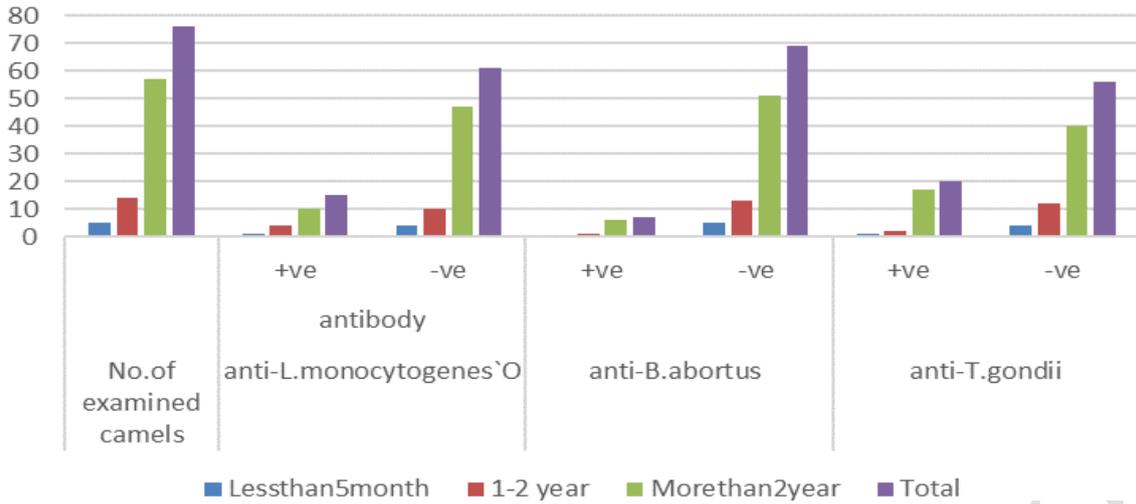


Fig. 2: Relation of *L.monocytogenes*, *B.abortus*, *T.gondii* according to ages.

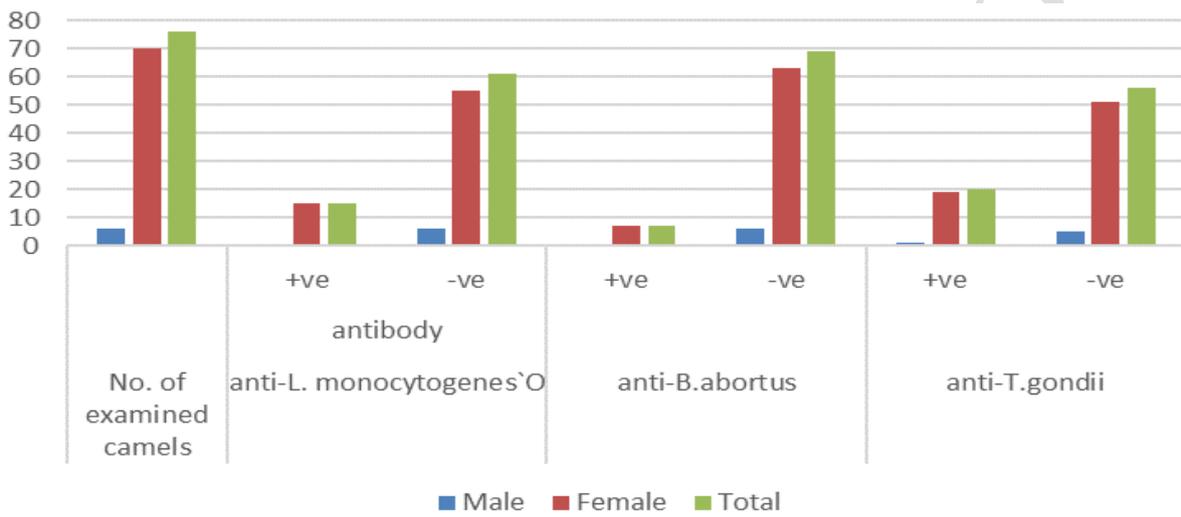


Fig. 3: Relation of *L. monocytogenes*, *B. abortus*, *T. gondii* according to genders.

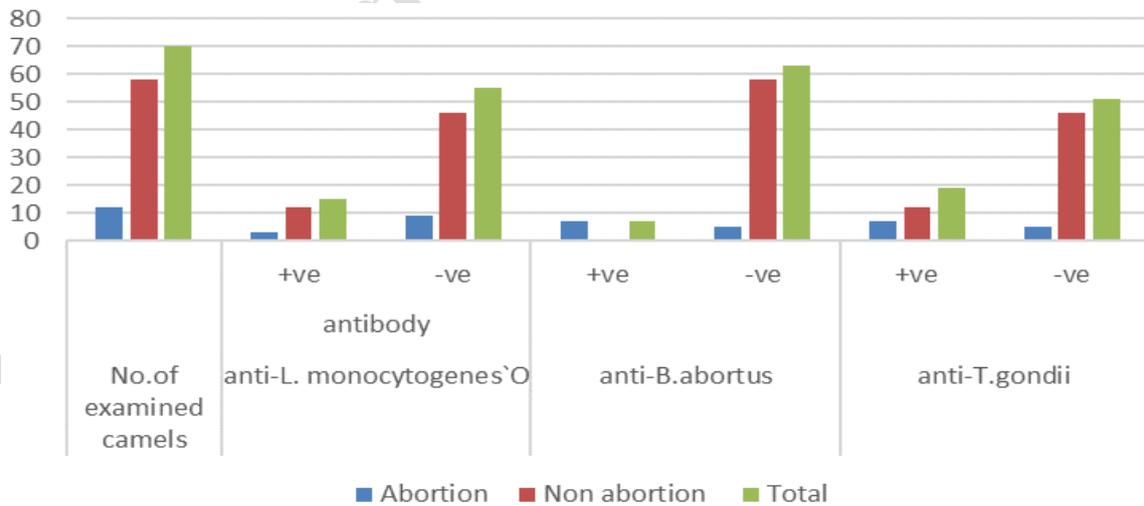


Fig. 4: Relation of infections in aborted and no aborted camels.

Table 4: Seroprevalence infections in aborted and no aborted

| Age group (year) | No. of examined camels | anti- <i>L. monocytogenes`O</i> antibody | | anti- <i>B.abortus</i> | | anti- <i>T.gondii</i> | |
|----------------------|--|--|-----------|------------------------|------------|-----------------------|-----------|
| | | +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 to brucellosis in cattle. Sera were collected from 1822 Bactrian camels, 1155 cattle using Rose Bengal Test which have 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). Aborted camels were more commonly in Borana, Ethiopia, in camels was (23.4%) than cattle (13.8%) and goats (12.4%) (Megersa *et al.*, 2011). In the current study, the sero-prevalence of brucellosis was determined to be 9.2% by using SAT method. The outcomes of current study is slightly higher than (Yawoz *et al.*, 2012) who were reported of 66 camels were tested using the Rose Bengal test in 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, may be SAT test is more precise than RBS test. The occurrence of *B. melitensis* or *B. abortus* in camels was 2.2% and found to be linked to their presence in their livestock reservoir, i.e., small ruminants and cattle, 10.6% and goats was 1.9% (Megersa *et al.*, 2011), and similarly our study; conventional screening test (RBPT) was reported 10/200 (5%) more potential than confirmatory test CELISA 4/200 (2%) and molecular detection rtPCR 3/200 (1.5%) potential risk factors (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 significance ($P < 0.000009$) between aborted 12(17.1%) and 58(82.9%) in non-abortion camels, Table 4; Fig. 4. These results were agreement with results in female camels, which appear higher prevalence of brucellosis in aborted female then non-pregnant and pregnant, this due to the livestock mixed keeping with other showed more at risk than single animals (Fatima *et al.*, 2016).

Toxoplasma gondii is zoonotic protozoan parasite have ability to infect all warm-blooded hosts and birds and cause congenital defects and abortions in humans and animals. Congenital toxoplasmosis is regarded to have the highest global illness burden of any foodborne disease and examined the potential role of milk as a route of *T. gondii* transmission between humans and livestock within Mongolian herders (Iacobucci *et al.*, 2019). The seropositivity rate was 6/45 (13.3%) in Sudan. The seroprevalence of *T. gondii* was highly in livestock refers to their potential role in the transmission of human toxoplasmosis in Sudan and the high spread contamination of *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 sero-prevalence of toxoplasmosis by using different techniques. This study agreement within Saudi Arabia; prevalence of many animals involving camels were investigated and the 68 camel (34.2%) from 199 were positive (Mohammed *et al.*, 2020). The seropositive and identify risk factors of *T. gondii*, were detected in domestic ruminants in East Hararge zone of Oromia region, Ethiopia. The total infection was 302/1360 (22.2%), and in sheep, goats, cattle, and camels was 33.7, 27.6, 10.7 and 14.4%, respectively (Tilahun *et al.*, 2018). However, disagreement with (Yücesan *et al.*, 2019) have been

reported toxoplasma infection in stray cats was 86 (66.6%) of 129 animals in Ankara/Turkey, using SFDT, and indirect effect for human. The variation of the results may be due to the samples size of different studies also, the different methodology used and may be for another factors like the virulence and type of *T. gondii* status and age. Furthermore, these agents take part in the variation of results among these studies and our study. Until now SFDT is not commonly used because of the requirements for the use of live parasites, it still standard for gold 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 furthers studies of these infective agents in camels in another cities in Iraq not only in camels but also in ruminants and felines. Regular checking of camels and detect seropositive and even seronegative can screen by modern methods. Vaccination of uninfected camels.

Authors contribution: MY, SEJ, FA and CB contributed to the conception of the article. MY and SEJ contributed to data analysis and interpretation. FA was prepared and revised the manuscript. All authors declare no conflict of interest.

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