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Diagnostic Study of Canine *brucella* Infection of Dogs in Mosul, Iraq, Using Indirect ELISA Testing

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Abstract

Canine brucellosis, a zoonosis that affects both sexes' fertility and is characterized by endometritis, placentitis, miscarriage, and/or stillbirth in females and epididymitis and orchitis in males, is mostly caused by *Brucella canis*. This study assessed the prevalence of antibodies to *Brucella canis* across various dog breeds, sexes, and ages in Mosul, Iraq. A total of 92 blood samples were analyzed using indirect ELISA tests. Results indicated that eleven tested canines were positive for IgG antibodies with an infection rate of 11.9%, and six tested positive for IgM antibodies with an infection rate of 6.5%. No significant differences were observed based on sex and age, but local dogs showed a higher IgG infection rate (18.6%) compared to K9 dogs (11.6%). German Shepherds had a notably higher IgG rate (12%). A significant age-related difference was noted, with local dogs aged 3-4 years having an IgG rate of 28.5%. This study indicated that antibodies to *Brucella canis* are present in the Mosul local community and in the K9 dog population. Therefore, preventive measures should be implemented to eliminate hazardous infectious agents.

Key words: *Brucella canis,* Dogs, Indirect ELISA, Mosul City-Iraq.

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Introduction

There are thirteen species described in the genus *Brucella spp*., each having unique host preferences and pathogenicity profiles. The genus was first identified in Malta in 1860 and isolated from a pure culture in 1887 (1). Human beings can contract zoonotic infections from *B. melitensis, B. abortus, B. suis, and B. canis*. Zoonotic transmission is a serious concern because the bacterium can infect multiple vertebrate hosts, although strains from marine animals and other species seldom cause illness in humans (2). Genome sequencing has revealed significant similarity between species despite host-range differences, complicating accurate species recognition (3-5). These gram-negative coccobacilli are tiny, non-motile, and do not generate spores. They can live both inside and outside host cells. Within 48–72 hours at 37°C, they show as colonies on suitable culture media. Small, convex, shiny, "as dust" colonies result from overnight incubation. Like other Brucella species*, B. canis* has a high affinity for the lymphoreticular and reproductive systems. (7,8). Usually, infection affects the spleen, milk glands, and vaginal organs (9). The infection is widespread, with a significant seroprevalence in cities with large numbers of roaming dogs and in rural areas of the Southeast. Intact dogs, particularly those in unsupervised or commercial breeding environments, serve as substantial reservoirs for *B. canis* infections (5). Canine brucellosis outbreaks have become more common in countries such as Sweden, Italy, Hungary, Colombia, and the United States and are frequently associated with the worldwide trade of breeding dogs. (7,10-13). Purebred breeds grown in extensive commercial breeding operations are particularly susceptible to the illness. (5, 14). Because some sick dogs do not have obvious clinical signs, diagnosis is much more challenging. For both men and women, infertility may be the only symptom, making history-based diagnosis erroneous (15-17). The symptoms could include infertility, abortions, and stillbirths, as well as significant spinal pain (discospondylitis), stiffness, and lameness. On the other hand, many infections are either persistent or asymptomatic. Although a laboratory diagnosis is essential, not one test yields conclusive data. Because of this, laboratory testing is crucial, and bacterial isolation is the highest standard, even though it requires third-level biosafety settings and is often sensitive (18). Although PCR is fast and precise, it may miss infections brought on by sporadic bacteremia (19,20). Common serological tests for screening and diagnosis include the Rapid Slide Agglutination Test (RSAT), Enzyme-Linked Immunosorbent Assay (ELISA), 2-Mercaptoethanol (2ME) test, and Agar Gel Immunodiffusion (AGID), although each has limitations such as cross reactivity and false negatives. Combining culture, PCR, and serology will yield a trustworthy diagnosis (5,15,18). Diagnostic imaging procedures, including CT (Computed Tomography) and MRI (Magnetic Resonance Imaging), ultrasonography, and radiotherapy, can assist in identifying systemic involvements such as discospondylitis and splenomegaly (21). This study collects existing data on canine brucellosis to clarify infection rates across different regions and dog populations, identify relevant risk factors, and evaluate diagnostic tools such as ELISA IgG and IgM. IgM, the first responder, provides temporary protection during early, acute infections by producing a big pentamer. The majority of serum antibodies are made up of IgG, a smaller, monomeric antibody that may cross the placenta, survives for years, and appears later in life for long-term immunity.

Material and Methods

Animal and sample collections

This study comprised 92 dogs of both sexes and diverse ages (<1-2 years, 3-4 years, and >years old), breeds, and management techniques collected through various districts of Mosul city. These dogs were clinically suspected of being infected with canine brucellosis, depending on clinical signs. Between September 2025 and February 2026, every single dog was securely attached, and 5 mL of venous blood was aseptically drawn from the cephalic vein into a well-labeled plain vacutainer tube with a sterile needle. Blood samples were allowed to coagulate before centrifugation at 3000 rpm for at least 5 minutes. The sera were then decanted into new, clearly labeled sample containers. Before performing the IELISA IgG and IgM on serum samples, they were frozen at -20°C (22, 23).

Indirect ELISA test IgG and IgM Kits

A commercial indirect ELISA application (Sun Long/China, Catalog Number: SL0087Ca for IgM and SL0192Ca for IgG) was used to analyze each serum sample. Both tests are authorized to detect IgG and IgM antibodies to *Brucella canis* in dogs. The tests are intended to screen for specific IgG and IgM antibodies to B. canis in canine serum or plasma, and they provide a qualitative evaluation of Brucella IgG and IgM in these samples, as well as in culture media or other biological fluids, using solid-phase technology (24,25).

Statistical analysis

SPSS Version 22 (Inc., Chicago, USA) was used to analyze the data in this study, employing the chi-square ($\chi 2$) test. When the P value was less than 0.05, the data were considered statistically significant.

Results

The study's results, based on the examination of 92 serum samples, showed that the infection rates for canine brucellosis were 11.9% and 6.5% using the enzyme-linked immunosorbent assay (ELISA) for IgG and IgM, respectively (Table 1).

Table (1): Infection rate of canine brucellosis in dogs using indirect ELISA technique

No. of Serum samples	Type of ELISA test	No. of Positive samples	Infection rate %
92	Indirect ELISA IgG	11	11.9
92	Indirect ELISA IgM	6	6.5

The study found no significant difference in infection rates of canine brucellosis among females (14.6%) and males (9.8%) in the ELISA (IgG) test and females (12.1%) and males (1.9%) in the ELISA (IgM) test. Further, the study also examined infection rates across several age groups of dogs, with the highest rate (18.9%) observed in dogs aged 3-4 years; however, there were no significant differences between age categories (Table 2).

Table (2): Infection rate of canine brucellosis in dogs according to sex and age using Indirect ELISA techniques

Factors		No. of samples test	No. of samples tested	Indirect ELISA IgG (11)		Indirect ELISA IgM (6	
				No. of Positive samples	Percentage%	No. of Positive samples	Percentage%
Sex	Females	41	6	14.6[a]		5	12.1[a]
	Males	51	5	9.8[a]		1	1.9[a]
Age	<1-2 year	25	2	8[a]		1	4[a]
	3- 4 years	37	7	18.9[a]		5	13.5[a]
	> 4 years	30	2	6.6[a]		0	0[a]

Vertical letter differences (a, b, c) indicate significant differences in values below P<0.05.

The study found a significant difference (P≤0.05) in infection rates between animal breeds (Local and K9 dogs), with a higher rate in Local dogs (18.6%) than in K9 dogs (11.6%). Using the enzyme-linked immunosorbent assay (ELISA IgG) and ELISA IgM Table (3).

Table (3): Infection rate of canine brucellosis in dogs according to dogs breed by

Indirect ELISA techniques

dogs breed	No. of samples test	Indirect ELISA IgG (11)		Indirect ELISA IgM (6	
		No. of Positive samples	Percentage%	No. of Positive samples	Percentage%
Local dogs	43	8	18.6[a]	5	11.6[a]
K9 dogs	49	3	6.1[a]	1	2[b]

Vertical letter differences (a, b) indicate significant differences in values below P<0.05.

The study identified a significant difference (P≤0.05) in infection rates based on species K9, with a higher infection rate in German Shepherds (12%) in the ELISA test (IgG), but not any difference in the IgM. Table (4).

Table (4): Infection rate of canine brucellosis according to K9 breed dogs using Indirect ELISA techniques.

Breed of K9dogs	No. of samples test	Indirect ELISA IgG (3) No. of Positive samples	Indirect ELISA IgG (3) Percentage%	Indirect ELISA IgM (1) No. of Positive samples	Indirect ELISA IgM (1) Percentage%
Malinois	24	0	0 [a]	0	0[a]
German Shepherds	25	3	12[b]	1	4[a]

Vertical letter differences (a, b) indicate significant differences in values below P<0.05.

Discussion

Canine brucellosis is often diagnosed utilizing clinical laboratory findings, sperm inspection, serological testing, bacterial isolation, and isolated genetic detection (26). In this paper, we report the infection rate of *B. canis* detection in Mosul City, Iraq, using ELISA (IgG and IgM). The infection rates in the current study were 11.9% for chronic infection and 6.5% for acute infection. Alfattli (2016) (27) found that 12.76% of the dogs in Al-Qadisiyah, Iraq, had an infection in earlier research using ELISA. The variations in the distribution of canine brucellosis by region within a country are caused by factors such as breeding practices (kennels versus stray dogs), testing limitations (missed subclinical cases), and specimen handling (type and amount of samples), which result in discrepancies in reported prevalence, particularly with B (28).Further worldwide research has shown that different experimental methods show different rates of canine brucellosis infection in dogs (29). Instances comprise Turkey; which has a rate of 18% (30), the North-Central Nigeria, which has a rate of 31.4% (23); in Jordan, which has a rate of 8.3% by Rapid Slide Agglutination Test (RSAT) (31); in India, which has a rate of 6.8% (32); in the USA, which has a rate of 4.6% (33), Depending on a variety of factors, such as ecology, management techniques, and diagnostic efficacy, the rate of brucella species in dogs might vary from continent to continent (34). Furthermore, the study showed canine brucellosis infection rates in females (14.6%) and males (9.8%) in the ELISA (IgG) test and in females (12.1%) and males (1.9%) in the ELISA (IgM) test, but did not show a significant difference in infection rates. This investigation is in good agreement with previously reported data that, in dogs, both sexes can become infected, but intact bitches are the most significant potential source for environmental contamination through the shedding of bacteria after an abortion and, therefore, important to outbreak dynamics (14).

The study also examined infection rates across age groups, with the highest rate (18.9%) in dogs aged 3-4 years; however, there were no significant differences among age groups. This study is consistent with other research (14) and disagrees with (26), whereas a crucial study by Gwaltney *et al.* (36) found no significant relationship between age and seropositivity in these imported canines. This contrasts sharply with the age-related pattern in endemic pet populations,

emphasizing the importance of vertical transmission and kennel exposure in this group. In adult K9s, the risk remains high if they came from or worked in endemic areas without robust testing standards, particularly due to the potential for exposure to infectious agents that are prevalent in those regions.Imported adult dogs may have been exposed before their arrival (Xiang et al., 2025, 37). Variations in research could be attributable to a variety of plausible reasons. These include sample bias (the study might have used a sample with distinct transmission dynamics), diagnostic and sampling procedures, and true epidemiological diversity. The study also found that local dogs were more likely to contract canine brucellosis than police dogs(k9). This study complements the previous one (24). High risk, but variable prevalence. The study also found brucellosis infection rates in police dogs (K9), with German Shepherds having the highest rate (12%) compared with Malinois, which had no infections.

However, research contradicts this study (31). Police and military working dogs can be considered a high-risk population for B. canis based on the nature of their work (searches/apprehensions that often bring contact with suspects' mucous membranes/body fluids), training conditions (kennels, shared tools), and, in some cases, breeding history (35). Infection rates recorded vary widely depending on the country, import status, and testing methods (34). The achievements of well-managed police dog schools are not by accident but result from a multi-level blanket defence strategy, namely, the selection of dogs from low-risk breeds and through pre-entry testing; avoiding unwanted dogs to produce offspring; high biosecurity measures in place; and a strict policy for testing and elimination, which leads to a decline in the prevalence of illness (38). Finding an outbreak in a military (K9) kennel setting demonstrates how kenneled working dogs, regardless of age, are particularly sensitive to environmental contamination and close contact.

Conclusions

The current study found a relatively acceptable infection rate in both local breed dogs and police dog units (K9), including both acute and chronic cases. These results underscore the importance of increasing routine screening of dogs and public health education for dog owners to reduce risks to both humans and animals. Finally, controlling brucellosis in dogs will help limit zoonotic disease transmission and reduce economic losses associated with poor reproductive performance in dogs in Mosul, Iraq.

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Conflict of Interest

The authors declare no potential conflicts of interest associated with the publication of their work.

Ethical approval

The study was authorized by the Institutional Animal Care and Use Committee of the University of Mosul's College of Veterinary Medicine on August 7, 2025 (UM.VET.2025.029)

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دراسة تشخيصية لعدوى البروسيلا لدى الكلاب في الموصل، العراق، باستخدام اختبار الاليزا غير المباشر

هبة موفق فتحي ,سلام عبد إسماعيل

فرع الطب الباطني والوقائي ، كلية الطب البيطري، جامعة الموصل، الموصل- العراق

الخلاصة

داء البروسيلات الكلبي، مرض مشترك بين الانسان والحيوان يؤثر على خصوبة كلا الجنسين ويتسم بالتهاب بطانة الرحم، والتهاب المشيمة، والإجهاض فضلا عن ولادة اجنة نافقة عند الإناث، والتهاب البربخ والتهاب الخصية عند الذكور، ويحدث في الغالب بسبب جراثيم البروسيلا الكلبية. قامت هذه الدراسة بتقييم معدلات الإصابة بأضداد البروسيلا الكلبية في سلالات مختلفة من الكلاب، من الجنسين، والأعمار المختلفة في مدينة الموصل، العراق، باستخدام 92 عينة دم تم تحليلها بتقنية الاليزا. أشارت النتائج إلى أن 11 كلباً كانت نتائج اختبارها إيجابية لأجسام مضادة من نوع IgG بنسبة 11.9%، وستة كلاب كانت نتائج اختبارها إيجابية لأجسام مضادة من نوع IgM بنسبة 6.5%. لم تُلاحظ فروق ذات دلالة إحصائية بناءً على الجنس أو العمر، ولكن الكلاب المحلية أظهرت معدل إصابة أعلى بأجسام مضادة من نوع IgG (18.6%) مقارنةً بالكلاب البوليسية (11.6%). وسُجّل معدل إصابة أعلى بشكل ملحوظ لدى كلاب الراعي الألماني (12%). ولوحظ فرق ذو دلالة إحصائية مرتبط بالعمر ، حيث بلغ معدل الإصابة بأجسام مضادة من نوع IgG لدى الكلاب المحلية التي تتراوح أعمارها بين 3 و4 سنوات 28.5%. واستنتج من هذه الدراسة عن وجود أجسام مضادة لجراثيم البروسيلا الكلبية في الكلاب المحلية في الموصل و الكلاب البوليسية، مما يستعي اتخاذ إجراءات وقائية اكثر صرامة.للقضاءعلى العوامل المعدية الخطرة.

الكلمات المفتاحية: البروسيلا الكلبية، اختبار الإليزا غير المباشر ،الكلاب, مدينة الموصل، العراق.