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ORIGINAL ARTICLE

Brucella abortus Detection by Indirect Competitive ELISA and Rose-bengal tests in Stray Dogs of Some Rural Areas in Wasit, Al-Qadisiyah and Dhi-Qar Provinces - Iraq

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ABSTRACT

Objective: The aims of this study were to detect the presence of specific antibodies against *B. abortus* in stray dogs of some rural areas in Wasit, Al-Qadisiyah and Dhi-Qar provinces by using two serological tests (Rose-Bengal and indirect competitive ELISA) for first time in Iraq, as well as to compare between both used tests according to the diagnostic performance and to the studied provinces, and providing an additional data about the disease in studied regions.
Methods: Two effective serological tests (rose-bengal test and indirect competitive ELISA) were applied to detect an existence of specific IgG antibodies against *B. abortus* in some rural areas of three Iraqi provinces (Wasit, Al-Qadisiyah and Dhi-Qar).
Results: The study reported (28.89%) and (39.26%) as a total infection rate by rose-bengal and ELISA test, respectively. According to studied areas, the seropositive infection rates by rose-bengal and ELISA tests in (Wasit, Al-Qadisiyah and Dhi-Qar provinces) were (31.25% and 45.83%), (38.71% and 54.84%); and (21.43% and 25%), respectively. The significant differences were reported between and within every examined province at level ($P<0.05$).
Conclusion: This study was pioneered in detection of IgG antibodies against *B. abortus* in Iraqi stray dogs through transaction with rose-bengal test and an indirect competitive ELISA that consider as the test of a gold standard. As well as, although the study supplied more accurate outcomes, it's encouraged to extent in search in other areas and for reliance on other reliable and advanced methods in diagnosis.

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INTRODUCTION

Brucellosis is a global zoono-bacterial infectious disease afflicting a large scale of domesticated and wildlife animals as well as humans [1, 2]. Brucellosis is caused by a member of genus *Brucella* that have the ability for replication and persistence in the host cells is, directly, related with their ability to cause a persistent infection and to inhibition the immunity [3, 4]. Although, *B. canis* is the main pathological agent of canine brucellosis, *B. abortus* was incriminated in occurrence of the disease in dogs [5]. *B. abortus* that firstly isolated from cattle in 1897 by the Danish veterinarian Bernard Bang, could afflicts many species of animals in all continents resulting in an economic losses and public health hazards concerned with the reproductive and non-reproductive problems [6]. These bacteria are more widely extent throughout the world than other species of *Brucella* but it's less pathogenic for both animals and humans [7]. In male dogs, *B. abortus* might cause epididymitis and orchitis, while in females could cause an endometritis, placentitis and abortions. The non-reproductive lesions are less common in dogs and involved inflammations in eyes, axial and appendicular skeleton; lymphadenopathy and splenomegaly [8, 9]. The naturally infection of dogs with infected cattle was reported and demonstrated through

dog-to-dog, cattle-to-dog, dog-to-cattle, and dog-to-human [10]. Also, the transmission of infection can be occurred, generally, by ingestion through genital-oral or by mucosal contact with infected material (vaginal discharges, semen, fluids, tissues that related to birth or abortion) and even via broken skin [11, 12, 13].

The clinical features for *B. abortus* infection are not pathognomonic, but the case history is of superior importance during the diagnosis. Hence, the eventual diagnosis of *B. abortus* infection in dogs can be established through the isolation the causative bacteria, but the method is expensive, overdue and unpractical to work in control schemes [10, 12, 14, 15]. Currently, the specific *B. abortus* antibodies can be detected in the infected animals by many serological techniques that can be categorized as screening tests such as the rose-bengal, controlling such as the milk-ring, and confirmative such as the enzyme-linked immunosorbent assay [6, 16]. As well as, the chosen of any method should be appropriate for usage in the examined species or examined areas [17]. WHO/OIE intensified on applying more than one serological test through all epidemiological studies to overcome all their limitations, particularly, when it used for inspection the infection in both individual and herds/flocks [18]. In Iraq, only one study was related to the prevalence of *B. abortus* in stray dogs through using the rose-bengal test [19].

MATERIALS AND METHDS

Samples Collection

A totally of 135 adult stray dogs were included for the present study, which selected randomly from the examined rural agriculture areas in Wasit 48 dogs, Al-Qadisiyah 31dogs and Dhi-Qar 56 dogs provinces, during the period from 18 January to 22 May 2016. From each one, about 5 ml of blood sample was collected under aseptic condition by using a disposable syringe. Then, the blood samples were placed into glassy tubes that without anticoagulants and centrifuged at 1000 rpm for five minutes to obtain the serums. The serum sample of each tested dog was transferred into 1 ml microtubes and stored at -20°C [20].

Serological Techniques

In this study, two commercially serological techniques were used to detect the positive infected cases with specific antibodies against *B. abortus* included:

The rose-bengal test (Institute-Pourquie / France) that based on the agglutination on a plate in a buffered acidified medium (pH 3.6).

Table 1. **The interpretation of Rose-Bengal results**

Result	Interpretation
No agglutination	Absence of antibodies
Agglutination (Even slight)	Presence of antibodies

The indirect competitive ELISA test (Svanova Biotech AB / Sweden) that based on a solid phase competitive ELISA. In this procedure, the samples together with a mouse monoclonal antibody (mAb) specific for an epitope on the o-polysaccharide portion of the S-LPS antigen, are exposed to *B. abortus* smooth lipopolysaccaride (S-LPS) coated wells on microtiter plates. If *Brucella* antibodies are present in the test sample they will bind to the antigens in the well and block these antigenic sites. If *Brucella* antibodies are absent in the sample, these sites will remain free and the mAb which was added together with the sample will bind to these free antigenic sites. After an incubation period the unbound materials are removed by rinsing and a goat anti- mouse horseradish peroxidase (HRP) conjugated IgG is added to the plate. The HRP conjugate will bind to the specific (mAb) in absence of *Brucella* antibodies in the sample. Unbound materials are removed by rinsing prior to the addition of the substrate. Subsequently a blue color develops which is due to the conversion of the substrate by the conjugate. The negative result was indicated by the development of a blue color. The reaction was stopped by addition of stop solution; the color changes to yellow. The results were read by an ELISA microplate reader (BioTek, USA) at a wavelength of 405 nm. The optical density (OD) of the ELISA was read on an automatic plate reader and the Percent Positivity values (PP) of the test samples were calculated by the following formula:

$$PI = \frac{(OD\ sample\ or\ control\ x100)}{OD\ conjugate\ control\ Cc}$$

Table 2. **The interpretation of indirect ELISA results**

Group Results	Interpretation	
Control	OD Cc	0.57-2
	PI Positive control	80-100
	PI Weak Positive control	30-70
	PI Negative control	-10 - 15
Samples	Negative	< 30%
	Positive	≥ 30 %

Statistical Assay

All data results were tabled by a computerized Microsoft office excel program (2013) and analyzed by using the IBM SPSS program (v.23). Chi-square and t-test were used and the differences considered significant at level of ($P<0.05$) [21].

RESULTS

Physiological and Biochemical parameters

In Table 3 that dealt with the total seroprevalence of *B. abortus* in 135 tested stray dogs, the results were revealed on 39 (28.89%) and 53 (39.26%) seropositive cases by rose-Bengal and indirect competitive ELISA tests, respectively. The significant difference was reported between the positive results by both serological tests at a level ($P<0.05$).

Table 3.**The total seroprevalence of *B. abortus* in 135 tested dogs, according to diagnostic tests**

Test	Positive Dogs		Negative Dogs	
	No.	%	No.	%
Rose-Bengal	39	28.89 b	96	71.11
ELISA	53	39.26 a	82	60.74

The difference in small letters, vertically, referred to a significant difference at level $P< 0.05$.

In related to studied provinces, the results of Figure1 that concerned with the seroprevalence of *B. abortus* in 135 tested stray dogs, showed that the positive cases by rose-bengal test were 15/48 (31.25%), 21/31 (38.71%) and 12/56 (21.43%) in Wasit, Al-Qadisiyah and Dhi-Qar provinces, respectively. Whilst in Figure 2, the results were showed that the positive dogs by indirect competitive ELISA test were 22/48 (45.83%) in Wasit, 17/31 (54.84%) in Al-Qadisiyah and 14/56 (25%) in Dhi-Qar provinces. According to Figures 1 and 2, the significant difference was reported between the positive results of the examined provinces according to each test, at level (*P<0.05*).

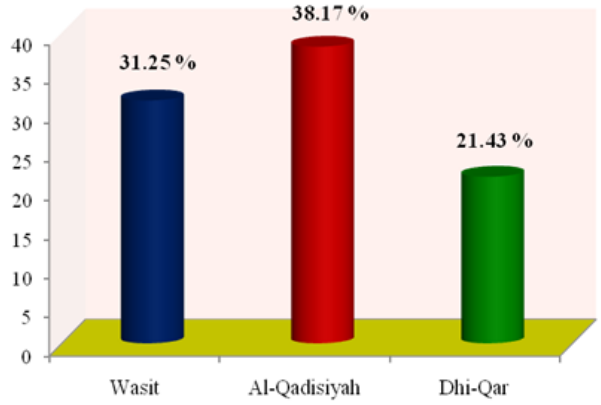

Fig.1 **Positive dogs by rose-bengal test**

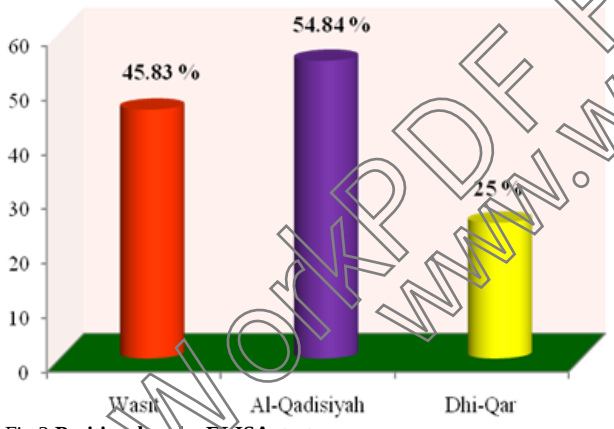

Fig.2 **Positive dogs by ELISA test**

In Table 6 that dealt with the seropositive prevalence of *B. abortus* between tested provinces, the results were as follow:

In Wasit province, the seropositive rate of 48 tested dogs were 15 (31.25%) and 22 (45.83%) by Rose-Bengal and ELISA tests, respectively.

In Al-Qadisiyah province, the seropositive rate of 31 tested dogs were 12 (38.71%) and 17 (54.84%) by Rose-Bengal and ELISA tests, respectively.

In Dhi-Qar province, the seropositive rate of 56 tested dogs were 12 (21.43%) and 14 (25%) by Rose-Bengal and ELISA tests, respectively.

While the results of both tests were compatible in detection of infection in the examined provinces, but ELISA test was more efficient in revealing the infected dogs. Statistically, significant differences (*P<0.05*) were reported between the tested provinces and within each province by both tests.

Table 6. **According to examined provinces, seropositive prevalence of *B. abortus* by rose-bengal test and indirect competitive ELISA test in 135 tested dogs**

Province	Positives of rose - Bengal		Positives of ELISA	
	No.	%	No.	%
Wasit (48)	15	B 31.25 b	22	A 45.83 b
Al-Qadisiyah (31)	12	B 38.71 a	17	A 54.84 a
Dhi-Qar (56)	12	B 21.43 c	14	A 25 c
Total (135)	39	28.89	53	39.26

The difference in small letters, vertically, and large letters, horizontally, referred to a significant difference at level *P< 0.05*.

DISCUSSION

In Iraq, *B. abortus* regarded as one of the more, increasingly, important endemic pathogens since first detected in cattle and recorded in 1937 [22, 23]. The present study reported a high rate of *B. abortus* infection in dogs by both tests, particularly, with the indirect-competitive ELISA test. As well as, it's noted the paramount rate of dog's contagion in all examined provinces, especially, in Al-Qadisiyah and Wasit provinces. Although, *B. abortus* afflicted mainly cattle, but it can be transmitted amongst species after contact with infected cattle or other reservoirs because of the fact that say "All *Brucella* species are not host specific" [24]. Experimentally, dogs could be infected and spread the causative pathogens that capable to infecting a cattle, if placed in close contact. Naturally, no confirmatory studies demonstrated the transmission of pathogen from dogs to cattle and no epidemiological surveys affirmed that dogs be able to play a role in spreading of infection for other animals [1, 25]. *B. abortus* might still exist in the field in case of absence the control and eradication schedules [5]. In Iraq, the extent of *B. abortus* amongst animals and humans may be explained by the attention's weakness through the medicinal specialists or governmental laboratories [26]. Also, one of the most important cause for increasing brucellosis in Iraq, was related to using of multi-original vaccines, especially in last decades, which different in its strain's virulence and levels of immunity that provided by each one [23].

The simplest, cheapest and effectiveness of rose-bengal test, were beyond the widespread usage of test and semi-full dependence on it [17]. Ruiz-Mesa *et al.*, (2005) demonstrated that, although, the test was very best in detection of *Brucella* if not exposed to these bacteria previously, but it had low sensitivity in endemic areas or in frequently exposed animals to causative bacteria [27]. In general, the test was counted to be less efficient than other serological test in detection of infection and in differentiate between the IgG and IgM antibodies[28, 29, 30]. For perfect, precise and compatible purpose, the indirect

competitive ELISA test had been assumed to be a gold standard method in detection of *B. abortus* infection. Chachra *et al.,* (2009) reported that the method was showed a high efficient during the screening studies, if it used alone or incorporation with other test [31]. Also, irrespectively on the period or frequent exposure, the competitive ELISA test reported more sensitivity and specificity in detection of specific antibodies, if IgG or IgM disregard on the time of infection [32]. Whilst, Yohannes *et al.* (2012) showed that no solitary method was typical in detection of infection and the diseased history was required to decrease the diagnostic faults, Saleem and Eman, (2012) proposed that the extra-screening is wanted [16, 19].

The significant differences in seroprevalence of *B. abortus*, which recorded by both diagnostic techniques in examined provinces were in agreement with previous studies had been done involved (cattle, sheep, goat, buffaloes, camels, dogs and human) in different regions of Iraq [19, 23, 33, 34, 35, 36]. In Iraq, most herds or fields have not, fully, applicable controlled by the veterinary authorities, due to social or political relevance affairs and/or for practical considerations, as well as, due to the seasonality motion or transmigration of herds amongst various areas of Iraqi provinces or to neighbouring countries as a result of inactive, vacillating and undecided agriculture or farming schemes [23, 37].

CONCLUSION

The present study had the ability for estimating IgG antibodies against *B. abortus* stray dogs of some rural areas in three provinces, effectively, by application two modified and advanced techniques (rose-bengal test and an indirect competitive ELISA).

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