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Studies on ruminant brucellosis in El Salam canal area, Egypt

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Abstract

The current study is aimed to provide some epidemiological data about brucellosis and its impact among ruminants in the area around El-Salam canal, North Sinai. In addition to determination of the sensitivity, specificity; positive & negative predictive values and diagnostic efficacy of Brucella Antibody Test Kits as a rapid field assay for the diagnosis of brucellosis. Our results revealed that, the overall prevalence rate of brucellosis was 7.1% (12/168) and 10.1% (17/168) using ELISA and Brucella Antibody Test Kits respectively. Out of 168 serum samples; ٩ were seropositive and 148 were sero-negative for brucellosis by both ELISA and Brucella Antibody Test Kits. Another 3 and 8 serum samples were only positive with ELISA and Brucella Antibody Test Kits respectively, revealing 75% sensitivity, 94.8% specificity, 47% positive predictive value, 53.5% negative predictive value and 93.4% diagnostic efficacy for this test. Brucellosis was found to be one of the causes of abortion in the study area as 61.9% (13/21) of aborted animals were seropositive for brucellosis. While to a little extent, it can be considered one of infertility causes as 4.7% (7/147) of infertile animals were seropositive for brucellosis. On the other hand, there were 38% (8/21) and 95.2% (140/147) of aborted and infertile animals respectively were sero-negative for brucellosis revealing that, there were another causes of abortion and infertility in the area of study rather than brucellosis and there was a necessity for their further investigations in order to improve the fertility and productivity of the animals in this targeted area.

Keywords: Brucellosis, Ruminant, El Salam Canal, ELISA.

1. Introduction

Brucellosis is a worldwide bacterial zoonotic disease that is cause heavy economic losses to the livestock industry and poses serious human health hazard [1]. The species that infect livestock are *B. melitensis* which found mainly in sheep and goats; *B. abortus*, found mostly in cattle; *B. suis*, reported principally from pigs; and *B. ovis*, found mainly in sheep[2]. It has been found that, a higher percentage of camel brucellosis reactors were in that contact with other farm animals than that kept in closed farms [3] and [4]. In the natural hosts, the disease is generally represented by abortion, reduced fertility and in ruminants, lowered milk production [5]. Diagnosis of brucellosis by the bacteriological methods may not always be practicable, therefore, the diagnosis is often depends on the serological means [6]. For an effective disease control, detailed information about the diagnosis of the pathogen in a specific area is of great importance. For that reason, serological surveillances are required to understand its epidemiological patterns but no single ideal test can catch all infected animals [7]. Therefore serological diagnosis is mainly depending upon the use of more than one serological assay for the confirmation of *Brucella* diagnosis.

2. Materials and Methods

2.1 Area of study

El Salam canal is that canal transport the Nile water into Sinai and extended through different localities in North Sinai. The area around this canal and its branches is newly reclaimed and used for different agricultural and animal production activities through which local animals of North Sinai and newly introduced animals from old Delta governorates lives closely and sharing the same environmental conditions.

2.2 Samples

A total of 168 serum samples were collected from 30 cows, 15 buffaloes, 50 ewes, 50 she goats and 23 she dromedary camels with a history of abortion and infertility. All of animals are sharing the same environmental conditions however they were belonged to different owners. Sera were tested with ELISA and Brucella Antibody Test Kits for the detection of Brucella antibodies.

2.3 Brucella antibody test kits

The test was applied using (B. brucella, Gs. Brucella and Camel Brucella) antibody test kits (BIONOTE, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea) which are used for rapid qualitative detection of *Brucella* antibodies in different species according to the kit used depending on a rapid antigen antibody immunochromatographic assay in which the formation of 2 purple color bands in the test and control bands within 20 minutes is considered a positive result. The test was performed as described by the enclosed pamphlet.

2.4 ELISA

The assay was done using COMPELISA 160 &400 competitive ELISA kit (APHA scientific, aphascientific@apha.gsi.gov.uk) which is a competitive ELISA kit for detection of antibodies against *Brucellosis* in serum samples of multi species animals. The lack of color development indicates positive result. Cut-off was calculated as

60% of the mean of the Optical Density (OD) of the 4 conjugates control wells. Any test sample giving OD equal to or below this value should be regarded as being positive.

2.5 Sensitivity and specificity

The following definitions were used to calculate the corresponding diagnostic parameters according to Jacobson, Thrusfield and Noordhuizen et al., [8-10] with the using of ELISA as a standard reference test.

Diagnostic sensitivity: The proportion of known infected individuals that test positive in an assay. Infected individuals that test negative are considered as false negatives (fn).

Diagnostic specificity: The proportion of uninfected reference individuals that test negative in the assay. Uninfected reference individuals that test positive are regarded as false positives (fp).

True positive (tp): Positive samples by both assays.

True negative (tn): Negative samples by both assays.

Sensitivity = tp x 100/ (tp + fn).

Specificity = tn x 100 / (tn + fp).

Diagnostic efficacy = (tn +tp) x 100 / (tp +fp + tn + fn).

Prevalence: is the ratio between the number of diseased animals at a particular point of time and the total number of animals.

Positive predictive value: is the proportion of test positive individuals that actually have the disease.

$$PPV = \frac{Prevalence \times Sensitivity}{Prevalence \times Sensitivity + (1 - Prevalence)(1 - Specificity)} \times 100$$

Negative predictive value: is the proportion of test negative individuals that are actually healthy.

$$NPV = \frac{Specificity \times (1 - Prevalence)}{(1 - Sensitivity) \times Prevalence + Specificity \times (1 - Prevalence)} \times 100$$

3. Results and discussion

Depending upon the sensitivity and specificity of the serological methods in the epidemiological studies about brucellosis, they can be classified as a screening (rapid, easy, inexpensive and highly sensitive) and confirmatory test that should be more specific to decrease the false positive reactors. Therefore, in the current study ELISA was used as a standard reference serological assay to evaluate the diagnostic efficacy and the other epidemiological parameters of another rapid field test (Brucella Antibody Test Kits "BATK") for the diagnosis of brucellosis in this study area. The results in table (1) showed that, the overall prevalence rate of brucellosis was 7.1% (12/168) and 10.1% (17/168) using ELISA and Brucella Antibody Test Kits respectively. Out of 30 cows, 15 buffaloes, 50 sheep, 50 goat and 23 camel serum samples, *Brucella* antibodies were detected in 13.3% (4/30) & 16.6% (5/30), 0% (0/15) & 6.6% (1/15), 6% (3/50) & 4% (2/50), 8% (4/50) & 12% (6/50) and 4.3% (1/23) & 13 % (3/23) using ELISA and Brucella Antibody Test Kits respectively. These results go in hand with that of Maymona et al., [11] as they recorded (7.2% and 8.3%) a total prevalence rate of sheep, goat and camel using competitive ELISA and RBPT respectively .The difference between the obtained results by the both tests may be attributed to the immunological responses for brucellosis which are sometimes irregular and or delayed according to the animal species and individual variations, moreover Brucella antibodies do not function equally well in all assays[12] and so there is a necessity for the use of more than one test for the diagnosis of brucellosis[13].

Concerning the sensitivity and specificity using ELISA as a reference standard confirmatory test and as demonstrated in table (2), it was found that, out of 12 seropositive samples by ELISA, 9 were seropositive using Brucella Antibody Test Kits "BATK" (true positive), while the other 3 serum samples were BATK negative (false negative). On the other hand, from 156 sero-negative samples by ELISA, 8 were sero-positive using BATK (false positive), while the other 148 serum samples were BATK negative (true negative). Our results revealed that, Brucella

Antibody Test Kits as a rapid field assay had 75% sensitivity, 94.8% specificity, 47% positive predictive value, 53.5% negative predictive value and 93.4% diagnostic efficacy in the diagnosis of brucellosis. The eight false positive samples may be attributed to cross reactivity with other Gram-negative bacteria having similar antigenic characters such as *Yersinia enterocolitica (O:9),* Group *N Salmonella(O:30), Echerichia coli (O:157 and O:116), Pseudomonas maltophilia and Vibrio cholerae* as mentioned by Adone and Pasquali [7]. In contrast, the presence of three false negative samples may be due to low antibody titer that cannot be detected by the rapid test or due to choronocity

Table (1) Prevalence of brucellosis among different animals using ELISA and Brucella Antibody Test Kits.

Animal		**ELISA positive**		**Brucella Antibody Test Kits positive**	
Species	**Number examined**	**Number**	**%**	**Number**	**%**
Cow	30	4	13.3	5	16.6
Buffalo	15	٠	٠	1	6.6
Sheep	50	3	6	2	4
Goat	50	4	8	6	12
Camel	23	1	4.34	3	13.04
Total	168	12	7.1	17	10.1

Table (2) Comparative results between ELISA (as a standard reference test) and Brucella Antibody Test Kits (BATK) for the diagnosis of brucellosis in ruminants.

Test		**ELISA**		**Total**
		+ve	**-ve**	
BATK	**+ve**	9	8	17
	-ve	3	148	151
Total		12	156	168

The obtained results of moderate sensitivity and high specificity of the Brucella Antibody Test Kits assay revealing that, it can be used in the *brucella* diagnosis in high prevalent and endemic areas in agreement with Adone and Pasquali [7] who concluded that, the different characteristics of the examined herds and the serological assays are important points in the control and diagnosis of brucellosis. As when the prevalence of infection is high, a test with acceptable sensitivity and high specificity is preferred in order to detect the majority of diseased animals and decrease the false positive reactors and the vice verse is true with the examination a free or a low prevalent herds in order to minimize the false negative reactors. As well as this test has several practical advantages making it the method of choice when testing animals from nomadic and other migratory populations [14]. Relatively similar findings were recorded by Elshemey and Abd-Elrahman [15] who recorded 94.44 % sensitivity and 100 % specificity suggesting that, it is a simple and rapid method that provides accurate detection of antibodies to *B. abortus* in cattle. Therefore, it can be practically used in the epidemiological surveillances of brucellosis. On the contrary, lower results were obtained by EL- Eragi et al., [16] who recorded 59.2% sensitivity and 80 % specificity.

Concerning to animal species and the relation between different tests used as shown in table (3), it was found that, the same results by the both assays (true positive + true negative) were obtained in 90% (27/30), 93.3% (14/15), 98% (49/50), 92% (46/50) and 91.3% (21/23) of cows, buffaloes, sheep, goat and camel respectively. This meaning that there was a good correlation between the both tests and the *brucella* diagnosis in sheep, buffalo, goat, camel and cow respectively. For buffalo, there was only one positive sample for brucellosis which was detected by BATK. For sheep there was not any sample BATK positive and ELISA negative while the opposite was true in camel as there was not any sample BATK negative and ELISA positive.

Table (3) The relation between the results of ELISA and Brucella Antibody Test Kits (BATK) and the examined animal species.

Animal		**ELISA +ve**		**ELISA –ve**	
Species	**Number examined**	**BATK +ve**	**BATK –ve**	**BATK +ve**	**BATK –ve**
Cow	30	3	1	2	24
Buffalo	15	0	0	1	14
Sheep	50	2	1	0	47
Goat	50	3	1	3	43
Camel	23	1	0	2	20
Total	168	9	3	8	148

As mentioned in demonstrated table (4), the serological examination of 12.5% (21/168) recently aborted animals revealed that, 9.5% (2/21), 19% (4/21) and 33.3% (7/21) were serologically positive with ELISA, BATK and ELISA&BTAK respectively while 38% (8/21) of the recently aborted animals were sero-negative with the both techniques. On the other hand, the serological examination of the 87.5% (147/168) infertile animals revealed that, 0.6% (1/147), 2.7% (4/147) and 1.3% (2/147) were serologically positive with ELISA, BATK and ELISA&BTAK respectively while 95.2% (140/147) of the infertile animals were sero-negative with the both techniques. As brucellosis is a herd problem, the diagnosis of one or more infected animal is sufficient to confirm the infection in this animal population under these conditions and suggesting that other serologically false negative animals may be found incubating the disease.

Table (4) The relation between the case history of examined animals and the seropositivity of the different diagnostic assays.

Serological results	Case history & Clinical manifestations of examined animals				Total
	Recently aborted		Infertile		
	Number	%	Number	%	
ELISA +ve	2	9.5	1	0.6	3
BATK +ve	4	19	4	2.7	8
ELISA and BATK +ve	7	33.3	2	1.3	9
ELISA and BATK –ve	8	38	140	95.2	148
Total	21	12.5	147	87.5	168

4. Conclusion

Brucellosis was one of the causes of abortion in the study area as a total of 61.9% (13/21) of aborted animals were seropositive for brucellosis. While to a little extent, it can be considered one of infertility causes as 4.7% (7/147) of infertile animals were seropositive for brucellosis. On the other hand, a total of 38% (8/21) and 95.2% (140/147) of aborted and infertile animals respectively were serologically negative for brucellosis revealing that, there were another causes of abortion and infertility in the area of study rather than brucellosis and there was a necessity for their further investigations in order to improve the fertility and productivity of the animals in this area.

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