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Seroprevalence of Brucellosis in Cow by using iELISA, Complement Fixation Test and Rose Bengal Plate Test with Comparison between Tests in Babylon Governorate

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Abstract: ***Investigation was conducted on (90) serum samples, (47) samples were collected from aborted cows and (43) samples from non-aborted cows from Babylon governorate, there were 7, 14.89% cases infected with Brucellaabortus by iELISA test while 10, 21.27% and 9, 19.14% cases infected with Brucellaabortus by Complement fixation test and rose Bengal plate test respectively from infected cow, there were 6, 13.95%; 9, 20.93% and 9, 20.93% cases infected with Brucellaabortus by using iELISA, Complement fixation test and rose Bengal plate test respectively from non-infected cow.***

Keywords: Brucellosis, iELISA, CFT, Rose Bengal plate test, Babylon Governorate.

1. Introduction

Abortion can be defined as an expulsion from the uterus of a dead or a living fetus before it reaches a viable age at any stage of gestation [1].

There are many infectious and non-infectious causes have been investigated and found frequently leading to expulsion of many fetuses without being seen, especially in animals which were not closely observed, Bacterial, Viral, Reckittsial, Mycotic and Protozoal agents are the main infectious causes of abortion responsible for the major economic losses in different countries all over the world.

In Iraq, Brucellosis was reported as early as 1938 (Al-Zahawi, 1938), and since that date studies have been done particularly during the last 20 years, concerning Brucellosis in man and animals (Beattie *et al.,* 1939; Karim*et al.,* 1979; Al-Adhami and Jawad, 1982).

However, Brucellosis received only little attention. Epidemiological studies were needed to cover its role in abortion among animals in different parts of country.

2. Materials And Methods

The animals of experiment were collected randomly from deferent location from Babylon governorates. The animal's age ranged from 2-5.5 years old that live in rural condition, the history of animals showed abortion of some animals, some animals show reproductive disorder as metritis, endometritis and retained placenta but some animals showed no any signs (apparently healthy), all animals of experiment were non-vaccinated against Brucellosis, The non-aborted animals was in direct contact with aborted animals. Blood (10ml) was collected from jugular vein of aborted and non-aborted animals after antiseptic the site by Ethanol. The collected blood put in evacuated tubes to complete clotting, then put in a cold container and transferred to a laboratory, the samples placed in a refrigerator at (4-8º), at the next day the serum was separated from clot by using centrifuge at 3000r/min for 10 min. the samples were placed in plastic tube (screw tube) and marked by number and stored at (-18º).The information about animals includes the case history, physiological state, health state, age of animal, breed were documented in a special note book for this purpose. The rose Bengal plate test done according to (Morgan *et al.,* 1978); Enzyme linked immunosorbent assay (ELISA) done according to (Gillesspie and Tomoney, 1981; MacMillan *et al.,* 1990); Complement fixation test (CFT) for done according to (Alton *et al.,* 1988).

3. Results and Discussion

Rose Bengal plate test (RBPT)
A total of 90 samples collected from aborted and non-aborted cows were examined by RBPT for Brucellosis 9, 19.14% of 47 samples of aborted cows showed positive results, the remaining 38 samples considered negative, while out of 43 non-aborted cows 9, 20.93% of 43 samples were positive, the remaining 34 samples considered negative. However, the total percentage of positive results (aborted and non-aborted) was 19, 20% cases (tables 1).

Enzyme linked immunosorbent assay (ELISA)
A total of 90 samples collected from aborted and non-aborted cows were examined by iELISA test, 7, 14.89% samples of aborted cows showed positive results, , while the non-aborted cows showed 6, 13.95%, However, the total percentage of positive results (aborted and non-aborted) was 13, 14.44% cases (table 1).

Complement fixation test for Brucellosis
A total of 90 samples collected from aborted and non-aborted cows were examined by CFT, 10, 21.27% of 47 samples of aborted cows showed a titer of 1/5 and over, while the non-aborted cows showed 9, 20.93% samples infected with brucellosis. However, the total percentage of positive results (aborted and non-aborted) was 19, 21.11% cases (Table 1).

Table 1: Detection of Brucella antibodies by using indirect ELISA, complement fixation test and rose Bengal plate test

Test	*State of animal*	*No. of sera*	*Brucella positive*	
			No.	*%*
RBPT	Aborted	47	9	19.14
	Non-aborted	43	9	20.93
Total		90	18	20
ELISA	Aborted	47	7	14.89
	Non-aborted	43	6	13.95
Total		90	13	14.44
CFT	Aborted	47	10	21.27
	Non-aborted	43	9	20.93
Total		90	19	21.11

4. Discussion

The present study showed that the commonly used conventional serodiagnostic tests for Brucellosis, RBPT may not be absolutely reliable (Kanani, 2007). This test is considered one of important screening and rapid test to investigate Brucellosis in animals, there is some false positive results that may be due to presence of antibodies originated from vaccination, or may be due to infection with other organism such as *Yersinia enterocolitica* (Godfroid*et al*., 2002), and the RBPT give a false negative results in early stage of infection, or immediately after abortion (Radostits *et al.*, 2007). Many samples give a rapid agglutination, the agglutination appear after (30) seconds to (2) minute, that referred to the high titer of antibodies in serum (Soni, 1978). The RBPT results obtained during this study, showed that the test detected 22.30% of aborted animals, 21% of non-aborted animals, compared with the same sera of both groups tested by CFT &ELISA which were 21.80%, 17.60% and 16.14%, 12.50% respectively. The infection rate in aborted animals was higher than that in non-aborted animals that may be due to attenuation in immune system that leads to proliferation of Brucella in the body tissue (Chappel, *et al*., 1978). There are many variations between governorates involved in this study some of which had a high percentage of positive results that may be due to many factors such as housing, environmental conditions, and health sanitation, or may be due to housing of cows near the sheep and goats. The Rose Bengal test was the prescribed test for international trade. In most laboratories this test, in unvaccinated animals, was 100% specific and had high sensitivity; however the sensitivity is not sufficiently high to allow completely accurate detection of infection in an individual animal (Mac-Millan, 1997; Jimenez, 1992). Depending on the results recorded by this study the Rose Bengal Plate Test was considered more reliable due to its high sensitivity in detecting of Brucella antibodies (Chivandi, 2006). These results agree with Al-Farwachi *et al.*, (2009), and converged with Rhaymah *et al.*, (2009), and with Samih*et al.*, (2009). The infection with virulent strains of *Brucellaabortus*, even in the chronic phase, is stimulate predominantly the production of IgG (Elberg, 1973), the CFT for bovine Brucellosis detect both IgG and IgM, the former being the more effective and the complement fixing ability of bovine IgG has been attributed entirely to IgG1 (Curtain, 1971; CHO and Ingram, 1972). Complement fixation test considered one of the most important confirmatory serological tests that used in diagnosis of Brucellosis infection in farm animals, and it has high efficacy in the diagnosis of chronic cases and in differentiation between infected and vaccinated cases (Radostits, *et al.*, 2007; FAO/WHO, 1986; Kulshersth*aet al.*, 1978). The percentage of infection that detected by CFT higher than the percentage of infection that detected by iELISA test, but its percentage converged with Rose Bengal Plate Test. The cause of this difference due to the ability of CFT to detect the presence of Ab in blood of infected animals along the period of infection (Morgan *et al.*, 1978). CFT sensitive to Ab type (IgM) that appears in early stage of infection and to Ab type (IgG) that appears in last stage of infection (Bundle *et al.*, 1987). These results referred to CFT as most sensitive test could be used to detect the disease in serum than iELISA test, while the percentage converged with RBPT. The differences in serological response to three tests may be due to the differences in Ab type (IgG or IgM), or may due to the stage of infection (early or late stage), the titer of Ab, time after abortion, or vaccination (Waghela*et al.*, 1980). These results agree with Auko&Philpott (1972); Salem *et al.*, (1977); Hoise*et al.*, (1985) and Alton (1987). These results indicate that the CFT was the most sensitive test could be used to detect a higher percentage of infected animals (aborted and non-aborted) in compassion with iELISA as a quantative test widely used in the world, However, the RBPT showed a higher percentage of positive reactors among aborted and non-aborted cows and this emphasized the importance of RBPT as a rapid screening test for aborted and non-aborted animals. Rose Bengal plate test negative sera was positive by both CFT and ELISA and this agree with Morgan *et al.*, (1978) that some of Rose Bengal plate test negative sera may not be areal negatives and these should be subjected an accurate diagnostic test like CFT and ELISA and this has been already done in our study that all the collected samples were tested by RBPT, CFT and ELISA simultaneously. The indirect ELISA was shown to be a good test to be used alone or in addition to other serological tests for detection of Brucellosis in farm animals, but for an accurate and fool proof diagnosis of infection suggested to be done in combination with Rose Bengal Plat test and complement fixation test (Jacques *et al*., 1998). ELISA is considered as one of the most important confirmatory serological test in diagnosis of Brucella in farm animals because it had sensitivity (100%) and specificity (99.7%) (Leal, 2005). This test detects the infection in all animals and it detects many cases with high optical density that referred to the high titer of antibodies in the serum that may be due to the samples collected at the time of peak antibodies titer, while there are many samples had low optical density that referred to low titer of antibodies due to that samples were collected after long period after abortion when the antibodies at the lowest level of antibodies (Mantur*et al.*, 2008a). The indirect ELISA (iELISA) is a useful test during an eradication program, after vaccinations has ceased, for screening or as a supplementary test to the complement fixation test Preliminary evaluations of the iELISA test alone, or in combination with the complement fixation test and monoclonal antibodies, indicating some comparative advantages over other serological tests (Radostits*et al.*, 2007). The iELISA has gained wide acceptance for serological diagnosis of bovine Brucellosis because of its ability to detect antibody of all isotopes, unlike the conventional tests (Nielsen, 2002). The iELISA can be useful in conjunction

with the complement fixation test during the latter stages of an eradication program when it is important to reduce the number of false-negative Rose Bengal Test reactions which contribute to the persistence of problem herds. The sensitivity and specificity of indirect ELISA is excellent but it could not distinguish between the antibody response induced by vaccination with *B. abortus* strain 19 and natural infection with the organism (Saravi, 1995).

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