COMPARISON BETWEEN COMPETITIVE ELISA AND ROSE-BENGAL TESTS IN DETECTION OF BRUCELLA ANTIBODIES IN BUFFALO SERA IN MOSUL CITY, IRAQ

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ABSTRACT

The present study was conducted to compare C-ELISA and Rose-Bengal tests in detection of brucella antibodies in buffalo sera in Mosul city, using 132 adult females of indigenous buffaloes from May 2007 to May 2008. Seroprevalence was 50.8% using C-ELISA, and 28.8% when RBT have been used. The data obtained were analyzed statistically to identify the agreement between C-ELISA and RBT using Kappa value. Kappa index was (0.353), which indicates less agreement between the two tests. There were false-negative results of RBT in 36 samples out of 67 samples positive to C-ELISA, and 7 samples recorded as false-positive with RBT out of 65 samples negative to C-ELISA.

INTRODUCTION

Brucellosis in humans and animals is known to be a worldwide problem and still remains a major public health hazard and of great economic importance (1). Buffalo infected with *Brucella abortus* (2). Unequivocal diagnosis can be made only by the isolation and identification of *Brucella* organism from abortion materials (foetal stomach contents and cotyledons), milk and vaginal discharges (3, 4), but it is not always practical and possible, and bacterial culture results are often negative for infected animals (4, 5, 6). Therefore; it is often necessary to resort to serological tests to identify the specific antibodies in the presence of *Brucella* antigens (4, 5). Serological tests can be divided broadly into two groups: screening tests such as Rose-Bengal Test (RBT), and confirmatory tests such as competitive ELISA. RBT have been used in the identification of prevalence of brucellosis in buffalo in Mosul city and the results were 6.3% (7) and 10.95% (8). In spite of that RBT is the most sensitive and rapid screening test (1), false-positive results may produced due to *B. abortus* S19 vaccination or exposure to gram-negative bacteria has lipopolysaccharide (LPS) O-chains similar to those of brucellae, which include *Vibrio cholerae* O1, *E. coli* O:157, *Salmonella* group N (O:30) and *Yersinia enterocolitica* O:9 (1, 4, 9). Also, false-negative results are observed during early incubation of disease and immediately after abortion (10). Therefore; researchers tend to use more accurate tests, (11, 12) indicate that C-ELISA has ability to overcome reactions of remenant antibodies of vaccination or cross reactions of brucella with other bacteria, especially when C-ELISA use specific monoclonal antibody (13). In addition, many researchers indicate that C-ELISA is a valuable tool as a single assay with higher accuracy than the conventional tests and could be applied replacing them in serological diagnosis of brucellosis and recommended as screening test under eradication programs (10, 15, 15, 16, 17). The present study conducted for comparison between C-ELISA and Rose-Bengal tests in detection of brucella antibodies in buffalo sera in Mosul city.

MATERIALS AND METHODS

Sera samples from 132 adult females of indigenous buffaloes from different areas in Mosul city was tested from May 2007 to May 2008 using commercial kits for C-ELISA (Svanova,

Sweden) and RBT (Gokhan, Turkish). The prevalence was determined through several methods of calculation: results of both C-ELISA and RBT separately, denotes only those samples which are positive to both tests "series testing denotation", and denotes all samples which are positive to one or both tests "parallel testing denotation" (18, 19).

Data were statistically analyzed with a computer program from the Statistical Package for the Social Sciences to identify the agreement between C-ELISA and RBT using Kappa value, when Kappa=1 indicates perfect agreement, whereas Kappa=0 indicates that there is no agreement (18, 20). False-positive and false-negative results of RBT were indicated on the base that C-ELISA has a sensitivity 100% and specificity 99.7% (10).

RESULTS

The seroprevalence of Brucellosis in buffalo in Mosul is summarized (Table 1). It was 50.8% using C-ELISA, and 28.8% when RBT have been used. Series testing denotation indicated that the seroprevalence was 23.5%, whereas parallel testing denotation 55.3%.

Table (1) Seroprevalence of brucellosis in buffalo (132 adult females) in Mosul city using C-ELISA and RBT

Test	No. of positive	Percentage
C-ELISA (alone)	67	50.8 %
RBT (alone)	38	28.8 %
Series testing denotation*	31	23.5 %
Parallel testing denotation**	73	55.3 %

* Positive samples to both tests,   ** Positive to one or both tests

In (Table 2), the agreement index between C-ELISA and RBT is shown using the first as a gold standard. There were little agreement between two tests (Kappa value was 0.353). False-negative results of RBT marked in 36 samples out of 67 samples positive to C-ELISA. Also, there were 7 samples recorded as false-positive with RBT out of 65 samples negative to C-ELISA.

Table (2) Comparison of C-ELISA and RBT results and measuring the agreement between the two tests

C-ELISA		RBT	
		Positive	Negative
Positive	**67**	31	36 *
Negative	**65**	7 **	58
Total	**132**	**38**	**94**
Kappa value 0.353			
* False-negative result to RBT.			
** False-positive result to RBT.			

DISCUSSION

Present study was conducted for comparison between C-ELISA and Rose-Bengal tests in detection of brucella antibodies in buffalo sera in Mosul city. RBT has been used in the identification of prevalence of brucellosis in buffalo in Mosul city and the results were 6.3% (7) and 10.95% (8). The results of present study revealed elevation in seroprevalence of brucella antibodies in buffalo sera in Mosul comparing with previous years (7, 8), it were 28.8 % and 50.8 % using RBT and C-ELISA respectively. This elevation may be due to absence of integrable control program on the disease. Furthermore, the free movement of animals from area to other assist in spreading the disease from endemic to free areas.

Present study revealed that seroprevalence of brucellosis in Mosul City was 23.5% using series testing denotation (positive samples in both C-ELISA and RBT). This percentage was less than that of C-ELISA alone (50.8%), and less than that of RBT alone (28.8%). Series testing denotation increase the specificity of combined tests and used in situations where it is too difficult or costly to assess the true heath status (18). Seroprevalence was 55.3% by using parallel testing denotation (positive samples on one or each tests). It is more than that of C-ELISA alone, and more than that of RBT alone. Parallel testing increases the sensitivity of combined tests; because there is no bypassing of to any positive results to any tests used (19). Both series and parallel testing may be useful protocols for providing an orderly transition from the use of conventional tests to primary binding tests by diagnostic laboratories (19).

Statistical analysis revealed that Kappa index was (0.353), which indicates less agreement between the two tests. The little agreement between C-ELISA and RBT in present study may be due to false-negative and false-positive results of RBT, which may give false-negative results due to recent infection (5). False-positive results of RBT may yield because of the cross reaction resulting from exposure of the animals to gram-negative bacteria with LPS O-chains similar to those of brucellae, especially *Y. enterocolitica* O:9 (1, 5, 9).

C-ELISA was developed as more sensitive and specific alternative to conventional test such as RBT, which is unable to distinguish between *B. abortus* strain 19 vaccinated animals and naturally infected animals (19). Vaccination induces antibody thought to be of lower affinity due to a short exposure time to the antigen because it is eliminated by the immune system. Alternatively, antibody produced in response to natural infection is of higher affinity because the antigen is not removed as quickly by the immune system, therefore, persist for much longer period (21). Thus, C-ELISA was developed to overcome this problem. It is capable of distinguishing vaccinated animals or animals infected with cross-reacting organisms from naturally infected animals, thereby reducing the number of false-positive reactions (19).

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مقارنة بين اختباري الإليزا التنافسي ووردية البنكال في الكشف عن أضداد البروسيلا في مصول الجاموس في مدينة الموصل، العراق

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الخلاصة

أجريت الدراسة الحالية للمقارنة بين اختباري الإليزا التنافسي ووردية البنكال في الكشف عن أضداد البروسيلا في مصول الجاموس في مدينة الموصل باستعمال 132 حيوانا من الجاموس المحلي للفترة من أيار 2007 إلى أيار 2008. بلغت نسبة تواجد أضداد البروسيلا 50.8% باستخدام اختبار الإليزا التنافسي، و 28.8% عند استخدام اختبار وردية البنكال. حُللت البيانات التي تم الحصول عليها إحصائيا لمعرفة مدى التوافق بين الاختباري وذلك من خلال إيجاد قيمة Kappa. بلغت قيمة Kappa (0.353) والتي تعني وجود توافق قليل بين الاختبارين. أظهر اختبار وردية البنكال نتائج سالبة كاذبة في 36 عينة من أصل 65 عينة موجبة باختبار الإليزا التنافسي، ونتائج موجبة كاذبة في 7 عينات من أصل 65 عينة سالبة باختبار الإليزا التنافسي.

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