EUROPEAN ACADEMIC RESEARCH
Vol. V, Issue 10/ January 2018

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Comparative evaluation of serological tests for detection of *Brucella* infection in Somali camels

AHMED A. H. KADLE[1]
International Committee of the Red Cross, Mogadishu, Somalia
ABDALLA M. IBRAHIM
Abrar Research and Training Centre, Abrar University
Mogadishu, Somalia
SHADIA A. MOHAMED
College of Veterinary Medicine, University of Bahri
Khartoum, Sudan
Faculty of Veterinary Medicine, University of West Kordofan
Kordofan, Sudan
ABDULKARIM A. YUSUF
Abrar Research and Training Centre, Abrar University
Mogadishu, Somalia

Abstract:

The comparative performance of serological methods for diagnosing brucellosis in camels under Somali context has not been evaluated. Thus, the objective of the present study was to compare serological tests (Rose Bengal Plate Test [RBPT], modified RBPT [mRBPT], micro-Serum Agglutination Test [mSAT] and competitive Enzyme Linked Immunosorbent Assay [cELISA]) for detection of Brucella antibodies in camel sera collected from camel herds reared in Mogadishu, Somalia. The seropositivity was 1.7% by RBPT, 3.9% by mRBPT, 3.9% by mSAT and 3.9% by cELISA. The last three tests showed excellent agreement whereas RBPT indicated moderate agreement. Moreover, the mRBPT performed well in the sensitivity compared to the standard RBPT. The standard RBPT is often reported to have a high sensitivity and recommended to use as a screening test

[1] Corresponding author: kadle010@gmail.com

for brucellosis control programs. However, in the current study, mRBPT achieved incomparable sensitivity than standard RBPT and equal sensitivity mSAT and cELISA. Therefore, the authors recommend to use modified-RBPT to screen Brucella infection in camels from Somalia for export and control purposes and confirm the positive animals with competitive ELISA.

Key words: Camel, *Brucella*, ELISA, mRBPT, mSAT, Somalia.

INTRODUCTION

Brucellosis is an infectious bacterial disease of worldwide importance in domestic ruminants. The causative bacteria are transmitted to humans through contact with infected animals or by consumption of contaminated animal products [1, 2]. The disease has significant animal health, public health and international trade consequences [3]. Diagnostic tests are applied with different objectives: confirmatory diagnosis, screening or prevalence studies, certification, and, in countries where brucellosis is eradicated, surveillance in order to avoid the reintroduction of brucellosis through importation of infected animals or animal products [4]. Bacteriological isolation and identification of *Brucella* species is the gold standard for diagnosis of brucellosis [3, 4]. Appropriate facilities are needed to isolate and identify all suspect *Brucella* species. This led the technique more complicated, time consuming and may risk the human health through laboratory exposure [1, 5, 6]. Hence, serological tests are crucial for laboratory diagnosis of brucellosis since most of control and eradication programs rely on these methods [1, 3, 4, 7]. However, cross-reactions between *Brucella* species and other gram negative bacteria such as *Yersinia enterocolitica* O:9, *Francisella tularensis, Escherichia coli* O:157, *Escherichia hermannii*, Salmonella serotypes group

N, *Vibrio cholerae* and *Stenotrophomonas maltophilia* are the major problems in the serological diagnosis of the disease [1, 8]. The first sero-diagnostic test for brucellosis was developed by Wright and Smith in 1897 using a simple tube agglutination test [9]. Subsequently, numerous other tests have been developed to increase test accuracy [3]. These tests include: RBPT, modified RBPT, SAT, Antiglobulin (Coomb's) test, Milk Ring Test (MRT), Complement Fixation Test (CFT) and ELISA [3, 7, 10, 11].

Serological tests must have high sensitivity to ensure that all true serological reactors are detected [2, 12]. However, Specificity of the tests should be considered. [13]. Diagnostic tests must therefore demonstrate a high level of specificity and yet maintain an effective sensitivity [2]. Although none of the serological tests are validated for detection of Brucella antibodies in camel sera, a combination of different serological brucellosis tests can increase diagnostic accuracy in camels [1, 3]. Therefore, our objective was to compare different serological tests for detection of Brucella antibodies in camel sera and to suggest a reliable and low cost test for screening brucellosis in camels in Somalia towards better control strategy in the next future.

MATERIALS AND METHODS

Study Area:

The study was conducted in Banadir region of Somalia. The region itself is coextensive with the Mogadishu, the capital city of Somalia.

Sample collection:

The present study of camel brucellosis was conducted from 2015 to 2017 in Mogadishu, Somalia. One hundred and eighty camels (*Camelus dromadarius*) of both sexes, mixed ages and from

different districts were sampled. The camel population in the study area has never been vaccinated against brucellosis. Blood samples were collected and transferred to Abrar Research and Training Centre (ARTC) laboratory, allowed to clot at room temperature and the sera were stored in aliquots at -20^{o}C until tested.

Serological diagnosis:

Rose Bengal Plate Test: The antigen was received from Central Veterinary Research Laboratory (CVRL), Sudan and was performed in the laboratory of Abrar Research and Training Centre according to OIE guidance [3].
Modified Rose Bengal Plate Test: This test is similar to the standard RBPT but only vary from the volume of serum (75 µl) and this was done according to Blasco *et al*., [14].
Micro-Serum Agglutination Test: This test was done in the CVRL, Sudan and it is performed in microplates according to Alton *et al.,* [11].
Competitive Enzyme Linked Immunosorbent Assay: This test also performed in CVRL, Sudan according manufacture's guidance (*Brucella*-Ab cELISA, Svanova Biotech, Uppsala, Sweden).

Data analysis:
The data collected through laboratory results were stored into Excel database and analysed using SPSS 20.0 software package (SPSS Inc, Chicago, Illinois, USA). Kappa analysis was used to evaluate the degree of the agreement between serological tests used in this study.

RESULTS

The overall serological prevalence among tested camels was 1.7% by RBPT, 3.9% by mRBPT, 3.9% by mSAT and 3.9% by cELISA. However, 8 of samples were seropositive for at least one of the tests used in this study. Therefore, the true prevalence of camel Brucellosis in the present study area was 4.4%.

The results of the four serological tests agreed in 3 (1.7%) of positive samples. 4 (2.2%) samples were negative with the standard RBPT but positive with mRBPT, mSAT and cELISA.

In the comparison between standard RBPT and modified RBPT, 4 samples tested negative by RBPT but positive by mRBPT (Table 1). The sensitivity of the RBPT was found to be 43% with a 95% confidence interval (CI) of 0.1–0.82, and the specificity was 100% (CI = 0.98–1.00) when compared with mRBPT, and the agreement between tests was calculated as 0.596 using Kappa analysis. Nonetheless, the apparent sensitivity of mRBPT, mSAT and cELISA were identical (86%) and higher than the standard RBPT (43%). Hence, the Kappa value (0.841) showed excellent agreement between the first three tests and moderate agreement (0.589) in the standard RBPT. Moreover, all these diagnostic tests detected persistently 3 samples (1.7%) of the tested camel sera. As delineated in table (1), the modified RBPT demonstrated reliable results similar to the cELISA in a relatively short time for detecting Brucella antibodies in camels reared in Somalia.

Table (1): Comparison of RBPT, mRBPT, mSAT and cELISA

Brucella serological tests	**Standard RBPT**		mRBPT		mSAT		cELISA	
	+ve	–ve	+ve	–ve	+ve	–ve	+ve	–ve
Tests results	3 (1.7%)	177 (98.3%)	7 (3.9%)	173 (96.1%)	7 (10.1%)	62 (89.9%)	7 (10.1%)	62 (89.9%)
Number of animals tested	180		180		69		69	

DISCUSSION

Control and eradication programmes of brucellosis in animals and humans depend on the reliability of the tests used for the detection and identification of the Brucella organism [6]. Previous brucellosis serological studies in Somalia indicated that the camel brucellosis is an endemic disease in the country [15 – 18]. Although animal brucellosis is an endemic disease in Somalia, the epidemiology of the disease in human and animals is not well studied in the country. In addition, the risk of getting the disease from uncooked milk is the major cause of human health hazard.

In this study, four serological tests were evaluated for detection of brucellosis in camels. The results of these tests revealed that 1.7%, 3.9%, 3.9% and 3.9% of the camel sera investigated were positive by RBPT, mRBPT, mSAT and cELISA respectively. Comparable diagnostic sensitivity results were obtained with the mRBPT, mSAT and cELISA. However, the mRBPT detected more positive cases than the standard RBPT. This result is in disagreement with that previously reported by Omer *et al.*, (2010) [19] that recorded no difference between the standard and modified RBPT in terms of sensitivity in camel sera. However, these authors stated that mRBPT facilitates the reading of agglutination reactions and hence recommended as an alternative screening test for brucellosis in camels [19]. Hosein *et al.,* (2016) [20] also suggested the use of mRBPT for screening camel sera for brucellosis. The standard RBPT is often reported to have a high sensitivity and recommended to use as a screening test for brucellosis control programs [3, 6]. However, in the current study, the RBPT achieve very low sensitivity in comparison to mRBPT. Our findings will add to the little information on the application of mRBPT for detection of Brucella infection in camel serum [19, 20].

In the present study, cELISA results obtained high sensitivity and this in incompatible with the results revealed by Gwida *et al.,* (2011) [6]. However, our study aligns with those reported by Omer *et al.,* (2010), Hosein *et al.,* (2016) and Maymona *et al.,* (2013) [19 – 21].

It is concluded that a combination of the modified RBPT as a screening test and the competitive ELISA as a confirmatory test would be an appropriate choice for those working on export and control measures of brucellosis in camels.

ACKNOWLEDGEMENT

The authors would like to acknowledge Abrar University, Mogadishu, Somalia for providing a project grant to conduct this study and their overall guidance during the research work. Our gratitude also goes to Dr. Mihad Fathelrahman and Dr. Maha Khojaly from Central Veterinary Research Laboratory, Khartoum, Sudan. We also like to thank camel owners for their teamwork during the sample collection.

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