# EVALUATION OF SEROLOGICAL TESTS FOR DIAGNOSIS OF BRUCELLOSIS IN CAMELS FOR EXPORT AND CONTROL PURPOSES IN THE SUDAN

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## المستخلص

انتشار البروسيلا في الجمال في السودان عالي والمرض يؤثر على إنتاجيتها ومن أكبر العوائق لتصدير الإبل إلى شبه الجزيرة العربية. صممت هذه الدراسة للمقارنة بين اختبارات الروزبنقال الصحني, والروز بنقال الصحنى المعدل و الإليزا التنافسية فى فحص البروسيلا فى 492 عينة مصل إبل من ولاية كسلا لأغراض السيطرة على المرض والتصدير. تم فحص عدد 383(77.8%) من جملة العينات بالاختبارات الثلاثة و109(22.2%) باختباري الروز بنقال الصحني, والروز بنقال الصحنى المعدل و 463(94%) عينة باختبار التراص الأنبوبي, باستخدام مستضد البروسيلا المجهضة و مستضد اليرسينيا إنتيروكوليتكاO:9 كل عينة بالمستضدين على حدة, وبعد الحضانة فى درجة 37م لمدة 24 ساعة تم اختبارالطباقى كلٍ بنفس الاختبار باستخدام المستضد غير المتجانس لتقصى الأجسام المضادة اليرسينيا إنتيروكوليتكاO:9.اتفقت نتائج اختبارات الروز بنقال و الروز بنقال المعدل و الإليزا التنافسية في نتائج.28.5% عينة موجبة, و30% سالبة و3.9% سالبة كاذبة و 36.3% موجبة كاذبة و 1% سالبة لاختبار الروز بنقال و موجبة للاختبارين الآخرين. العينات التى تم فحصها باختبارى الروز بنقال, اتفقت نتائجها فى15,6% عينة سالبة ,و 66,1% موجبة و اختلفت فى نتائج 18,4% عينة سالبة لاختبار الروز بنقال وموجبة لاختبار الروز بنقال المعدل. وضح من اختبار ال 463 عينة أن 2,6% منها مشكوك فى أنها تحتوى على أجسام مضادة لليرسينيا إنتيروكوليتكا O:9 أوصت الدراسة باستخدام اختبار الروز بنقال المعدل لمسوحات الإبل للبروسيلا لأغراض السيطرة والتصدير وتأكيد النتائج الموجبة باختبار تأكيدى لأغراض السيطرة, وإعادة اختبار الحيوانات السالبة للاختبار بعد شهرين للتأكد من أن الإبل ليست سالبة كاذبة.

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## Abstract

Prevalence of brucellosis in camels in the Sudan is high and the disease affects their productivity and is a main constraint to their export to the Arabian Peninsula. This study was designed to compare the Rose Bengal Plate Test (RBPT), the modified m(RBPT) and competitive ELISA (c ELISA) for examination of 492 camel serum samples from Kassala State for brucellosis for export and control purposes. Of the total, 383(77.8%) samples were examined with the three tests, and 109(22.2%) with the RBPT and mRBPT and 463 samples were examined with the Serum Agglutination Test (SAT) , each with both *Brucella abortus* ( *B. abortus*) and *Yersinia enterocolitica* 0:9 ( *Y. enterocolitica* 0:9) SAT antigens. After incubation at 37ºC for 24 hours and reading the SATs , the supernatants of each were tested for agglutination with the heterologus antigen for detection of *Y. enterocolitica* O:9 antibodies. The results of RBPT, mRBPT and cELISA agreed in 28.5% of the positive, 30.3% of the negative and disagreed in 3.9% of false negative and 36.3% of false positive samples; and 1% of the samples were negative with the RBPT, positive with the mRBPT and cELISA. Of the samples examined with the RBPTs, the results agreed in 15.6% of the negative, 66.1% of the positive and disagreed in 18.4% of the negative with the RBPT and positive with the mRBPT. Of the 463 samples, 2.6% were suspected to contain *Y. enterocolitica* 0:9 antibodies.

It is recommended to screen camels for brucellosis for export and control purposes with the mRBPT, confirm the positive cases with a confirmatory test for control and reexamine the negative cases after two months with the test for to make sure that there are no false negative camels.


**Key words:** Camels, Brucellosis, Kassala State, *Yersinia enterocolitica* O:9 Antibodies

## Introduction

Brucellosis is a serious contagious disease of animals which is transmissible to man. The disease causes substantial economical losses in livestock and a severe or chronic debilitating disease in severe humans that needs long periods of therapy with a combination of antibiotics (Whatmore,2009).

The disease is caused by 10 species of the genus *Brucella* each with a preferred host or hosts, of which *Brucella abortus* (*B. abortus*) *B. melitensis* and *B. suis* infect many secondary hosts including livestock, wildlife and humans (Nicoletti, 1980; Whatmore, 2009). Camels are susceptible to brucellosis and their infection depends on the *Brucella* species in other animals in their habitats (Gwida *et al.,* 2011). In the Sudan, the prevalence of the disease in camels is increasing. Abu Damir *et al*.(1984) reported a prevalence of 2%, 3% and 7.5% of the disease in camels in central, western and eastern Sudan respectively. While Omer *et al*.( 2010) reported 37.55% prevalence in eastern Sudan. The disease in camels is the main constraint to their exportation to the Arabian Peninsula for breeding purposes, and every year many consignments are rejected because of detection of brucellosis despite their screening prior to shipment with the RBPT.

The aims of this study were to compare the RBPT, the mRBPT and cELISA used for examination of camel serum samples from Kassala State for brucellosis for evaluation for export and control purposes and for the possibility of detection of *Y. enterocolitica* O: 9 SAT antibodies in the camels.

## Materials and Methods

**Samples for the study:**

A total of 492 camel blood for serum samples were collected from camels not vaccinated against brucellosis and were of both sexes and different age groups in Kassala State, eastern Sudan.

**Serological tests:**

The samples were examined with the standardized RBPT and SAT antigens and cELISA kits

supplied by the Veterinary Laboratories Agency UK (VLA,UK); and *Y. enterocolitica* O:9 SAT antigen prepared and standardized locally using the OIE standard antiserum (Fatah el Rahman, 2010). Of the total samples 383(77.8%) were screened with the RBPT and mRBPT and confirmed with the c ELISA (OIE, 2004) while 109 (22.6%) were tested with the RBPT and mRBPT as no cELISA kits were available. The mRBPT was described by Blasco *et al*. (1994). The samples tested with the RBPTs were interpreted positive when the RBPT and the mRBPT were positive or when the later was positive (Blasco *et al*., 1994)

**Agglutination and cross agglutination tests:**

A total of 463 samples from the 492 were examined with the SAT and cross examined with the *B. abortus* and *Y. enterocolitica* O:9 SAT antigens as described by Meltzer *et al*.(2007). In their procedures, each sample was tested with both *B. abortus* and *Y. enterocolitica* O:9 SAT antigens (OIE, 2004) each separately, incubated at 37ºC for 24 h ,read for agglutination and the supernatants of each were placed in a similar series of tubes and cross examined with the heterologus antigens. Any sample in which the titre with the *Y. enterocolitica* O : 9 antigen was higher than that of the *B. abortus*, and similarly when the titres of the cross agglutinations of *Y. enterocolitica* O : 9 antigen with the supernatants of *B. abortus* were at least two fold higher than those of *B. abortus* antigen with the supernatants of *Y. enterocolitica* O: 9 were suspected to contain *Y. enterocolitica* O:9 antibodies. (Fig. 1)

## Results

Results of the serological tests of the 492 samples are presented in Table 1. As shown in the table of the 383 samples examined with the RBPT, mRBPT and cELISA, the tests results agreed in 109 (28.5%) of the positive samples and 116 (30.3%) of the negative samples,15 (3.9% ) of the samples were

false negative, 139 ( 36.3%) were false positive and 4(1%) were negative with the RBPT, but positive with both mRBPT and cELISA.

Of the samples examined with RBPT and mRBPT, the results of the tests agreed in72 (66.1%) of the positive, 15(6%) of the negative and disagreed in 20(18.4) of the RBPT negative and mRBPT positive. The results of agglutination and cross agglutination of the 463 sera with the RBPT, mRBPT ,SAT *B. abortus* and *Y. enterocolitica* O : 9 SAT antigens showed that 12 (2.6%) were suspected to contain *Y. enterocolitica* O:9 antibodies ( Table 2).

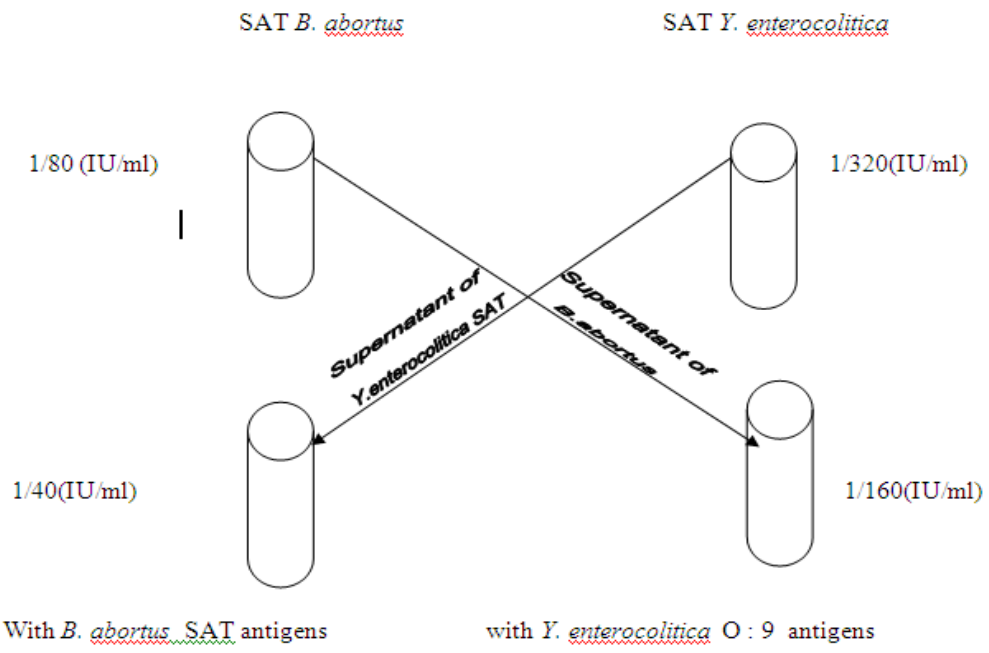


**Fig. 1:** Sample suspected to be positive for *Y. enterocolitica* O:9 antibodies

**Table 1:** The serological tests results.

| RBPT | mRBPT | cELISA | NO. +ve |
|---|---|---|---|
| +ve | +ve | +ve | 109 (28.5%) |
| +ve | +ve | NT | 72 (66.1%) |
| -ve | +ve | +ve | 004 (1.04%) |
| -ve | +ve | NT | 20 (18.4%) |
| -ve | -ve | +ve | 015 (30.3%) |
| -ve | -ve | -ve | 116 (23.6%) |
| -ve | -ve | NT | 17 (15.6%) |
| +ve | +ve | -ve | 139 (38.3%) |
| Total + ve | | | 220 (44.7%) |
| Total – ve | | | 272 (55.3%) |

**Key :** NT = not tested; -ve = negative with the test; +ve = positive with the test

**Table 2:** Samples suspected to contain *Y. enterocolitica* O : 9 antibodies

| RBPT | mRBPT | SAT B | SAT Y | Y/SAT B | B/SAT Y | Result |
|---|---|---|---|---|---|---|
| + | +++ | 1/40 | 1/320 | NT | NT | Y |
| + | ++ | 1/40 | 1/80 | 1/20 | -ve | Y |
| + | ++ | 1/40 | 1/80 | 1/20 | -ve | Y |
| + | ++ | -ve | 1/80 | 1/40 | -ve | Y |
| + | ++ | 1/20 | 1/80 | 1/20 | -ve | Y |
| ++ | ++ | 1/80 | 1/320 | 1/40 | 1/10 | Y |
| - | - | -ve | 1/40 | 1/320 | 1/10 | Y |
| +++ | ++++ | 1/160 | 1/320 | 1/160 | 1/10 | Y |
| +++ | ++++ | 1/640 | 1/10240 | 1/640 | 1/40 | Y |
| +++ | +++ | 1/160 | 1/320 | 1/40 | 1/10 | Y |
| +++ | +++ | 1/320 | 1/1280 | 1/160 | 1/80 | Y |
| +++ | +++ | 1/640 | | 1/160 | 1/80 | Y |

**Key:**

+ to ++++ = Degree of agglutination from 25% to 100% ; -ve = Negative agglutination1/10--1/10240 = +ve titres at serial 10 fold dilutions; SAT B = SAT with *B. abortus* antigen SAT Y = SAT with *Y. enterocolitica* O:9 antigen; Y/SAT = Agglutination of the supernatant of SAT with *B. abortus* antigen, with *Y. enterocolitica* O:9 antigen; B/SAT = Agglutination of the supernatant of SAT with *Y. enterocolitica* antigen with *B. abortus* antigen; Y = positive for *Y. enterocolitica* O:9 antibodies.

## Discussion

Camels destined for export in the Sudan, are screened for brucellosis with the RBPT. The importing countries reexamine the animals with the same test upon arrival and reject the consignment if the total positive cases exceeded 2%. The RBPT is a sensitive test and is recommended by the OIE (2004) for screening animals for export and control measures. But, the test can result in false positive serological reactions (FPSR) in cases of vaccination with *B. abortus* S19 vaccine or in cases of presence of antibodies due to cross reacting organisms. In few cases false negative reactions with the RBPT could occur. As a result, RBPT results are confirmed with the CFT and ELISA tests (OIE, 2004), or with mRBPT if other confirmatory tests are not available (Blasco, 1994). In some reports (Diaz-Aparicio *et al*.,1994) the sensitivity of the mRBPT was reported to be 100%.. In this study, the RBPT resulted in more 139(36.3%) false positive serological reactions and fewer 15 (3.9%) false negative serological reactions ( OIE, 2004). Since the camels examined were not vaccinated with any *Brucella* vaccine, the false positive serological reactions could be due to cross reacting organisms, the most important of which is *Y. enterocolitica* 0: 9 (Nielsen *et al*., 2006). The cross agglutination tests results (Table 2 ) supported the speculation of occurrence of *Y.enterocolitica* O: 9 in the state which could be the reason of the continuous rise of prevalence of the disease in camels from 12% in 2004, 15% in 2005, 37.5% in 2007( Omer *et al*.,2010) and 44.7% in this study and also could be responsible for some FPSRs. But, the cross agglutination tests and the *Y. enterocolitica* O:9 antigen need standardization and the organism must be isolated from camels in the state to confirm its prevalence . However, the high 36.3% FPSRs could also be due to the fact that the cELISA is less sensitive (68.8%) than the RBPT (70.7%) in camels (Gwida *et al*., 2011). The mRBPT detected more confirmed positive samples than the RBPT and is recommended for screening camels for brucellosis for export. Diaz-Aparicio *et al*.(1994) found that the use of mRBPT did not result in false negative results in goats and recommended its use for optimal sensitivity. The mRBPT is also recommended for screening camels for control purposes but, the positive results should be confirmed with the CFT or cELISA, and the

negatives animals reexamined after 2-3 months (Jungersen *et al*.,2005) to avoid false serological reactions.

The occurrence of cross reacting organisms especially *Y. enterocolitica* O:9 should be investigated by cultural procedures.

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