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Brucellosis Prevalence and Serologic Profile of Male One-Humped Camels Reared in Somaliland and Eastern Ethiopia for Meat Production

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Abstract: Egypt imports camels from east Africa to compensate for the gap in meat production. Some imported camels are kept for breeding as scattered populations countrywide without being ear-tagged. The seroprevalence of brucellosis among camels in the source countries is an indicator for potential transboundary brucellosis being introduced to Egypt through untested camels. Brucella antibody profile was studied in male one-humped camels from Somaliland and Eastern Ethiopia quarantined in Berbera for intended export to the Arabian Gulf States for meat consumption. A total of 3,200 camels were screened for brucellosis by the modified Rose-Bengal plate test, where 103 failed the test. The apparent prevalence was 2.9375% and the estimated true prevalence was 0.445%. These samples were further tested using the buffered acidified plate antigen (BAPA) test, brucellosis card test (BCT), microplate agglutination test with and without EDTA and mercaptoethanol, rivanol-precipitation plate agglutination test (Riv.T) and competitive ELISA (C-ELISA). Complement fixation was used as a gold standard in lieu of bacteriologic examination. Statistical analyses including analysis of variance followed by post hoc test, receiver operating characteristics curves, kappa agreement and diagnostic performance metrics, *viz*. sensitivity, specificity, false positive/ negative rates, positive/ negative predictive values, likelihood ratios of positive and negative results and diagnostic odds ratio were used. Results were fully discussed. It was concluded that the serologic profile had a dominating IgG_1 subclass of antibodies due to active infection. BAPA and modified BCT performed really well as binary screening markers. The performance indicators suggested Riv.T as an excellent candidate for simple and rapid disease confirmation. C-ELISA needs further adaptation to boost its performance in camels.

Key words: Brucella · Prevalence · Egypt · Sensitivity · Specificity · Likelihood Ratio

INTRODUCTION

Growing interest in camels is the outcome of the fact that they bear up harsh environmental conditions of heat, dryness and lack of pasture and yet remain productive. The spread of brucellosis among camels can silently jeopardize their reproduction, where the disease is less symptomatic as compared to cattle [1] given that the only frequent clinical sign of animal brucellosis is abortion [2]. Other reproductive manifestations in camels include dystocia, early embryonic death and infertility [3]. Camel brucellosis has a special zoonotic impact due to frequent human contact with infected animals and the consumption of milk without heat treatment to keep its natural taste or its assumed curative value.

There are more than 24 million one-humped camels in the world including 80% in Africa with the highest population in Somalia (7 million) and Sudan (4.25 million) as reported by Al-Juboori and Baker [3]. In east Africa, the adjacent countries Sudan, Somalia, Ethiopia, Djibouti and Kenya together contain 84% of African camels and over half (60.1%) the world's camel population, where camels are mainly bred for slaughter [4]. Camel population in Egypt has always been underestimated as 95,000 in 1978 [5] and 120,000 in 2005 [6] sporadically distributed in rural areas.

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Camel population is growing as Egypt imports large numbers of camels from east African countries to compensate for the gap in meat production. Imported from Somaliland and Ethiopia, camels are shipped from Djibouti through the Red Sea either to the port of Safaga for Upper Egypt or to the port of Suez for Lower Egypt and Sinai. The final destination of imported camels is either markets like Birqash camel market in Imbaba, Giza, or abattoirs for slaughter. Still, some camels are kept for breeding as scattered populations countrywide that are not ear-tagged and hence, they are rarely enrolled in lab testing for diseases.

Camels imported from Sudan walk in convoys either one of two ways. Camels from west Sudan walk the "Forty-road" starting from El-Fasher, North Darfur State, to Dongola quarantine, heading north alongside the River Nile to Argine on the southern Egyptian border, crossing the border to Abu Simbel quarantine and finally to Daraw camel market in Aswan. Camels from east Sudan walk from Kassala, Kassala State heading north to Halayeb and Shalateen. Some camels are smuggled to Egypt through the desert. These imported mostly untested camels constitute a likely breakthrough to the microbiological integrity of in-contact animals and humans.

Cheaper than Sudanese camels, Somali and Ethiopian camels are more preferable and more common in Egypt. Consequently, the seroprevalence of brucellosis among Somali and Ethiopian camels represents an indicator for potential transboundary brucellosis being introduced to Egypt through untested camels. To make matters worse, camels kept for breeding may not only include males but also females, which act as biological bombs during abortion or normal parturition [7] and subsequent lactation, where Sayour [8] isolated *Brucella melitensis* biovar 1 from milk of a she-camel in Giza, Egypt.

This work aimed at investigating the brucellosis prevalence among male one-humped camels reared in Somaliland and eastern Ethiopia for meat production as well as their serologic profile as revealed by buffered acidified plate antigen (BAPA), brucellosis card (BCT), rivanol precipitation plate agglutination (Riv.T), warm microtiter, complement fixation (CFT), microplate agglutination (MAT), EDTA modified MAT (MAT-EDTA), MAT with mercaptoethanol (MAT-ME) and competitive ELISA (C-ELISA) tests.

MATERIALS AND METHODS

Animals: A total of 3,200 male Somali one-humped camels 1.5 to 3 years old were ear tagged and bled for serologic examination with neither brucellosis signs nor history of vaccination against brucellosis. These animals were quarantined in Berbera, Somaliland for intended export to the Arabian Gulf States for meat consumption. These camels belonged to three localities, *viz*. Burao (Burco) and Shiikh in Togdheer region and Berbera in Woqooyi region, Somaliland (Northern Somalia) in addition to some camels from the Somali region of eastern Ethiopia during the second half of 2012. All camel sera were tested using the modified brucellosis card (Rose-Bengal) test of Blasco *et al.* [9] known to be almost as sensitive as the buffered acidified plate antigen (BAPA) test. All the modified BCT positive samples (n = 103) in addition to 10 negative sera were taken to Egypt for further serologic testing in the Animal Health Research Institute, Brucellosis Research Department. It is noteworthy that camel pastoralists in areas where camel sera were collected rely entirely on open grazing for feeding their animals together with other animal species including cattle, sheep and goats.

Serologic Tests

Buffered Acidified Plate Antigen (BAPA) and Brucellosis Card (BCT) Tests: Antigens for the BAPA and BCT 8% were produced by the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt. BCT antigen with 3% packed cell volume was prepared in the Department of Brucellosis Research, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt. The BAPA was performed according to the OIE Terrestrial Manual [10]. The BCT was implemented as described by Alton *et al.* [11]. Although BAPA and BCT are qualitative tests, their results were recorded as scores of 1+ to 4+ according to the degree of agglutination for the sake of comparison with other quantitative tests.

Rivanol-Precipitation Plate Agglutination (Riv.T) Test: Antigen for the Riv.T was prepared, standardized and verified in the Department of Brucellosis Research, AHRI, Dokki, Giza, Egypt, according to Alton *et al.* [11]. A complete agglutination at 1/25 or higher was positive, an incomplete 1/25 was doubtful and no agglutination at any dilution was negative.

Microplate Agglutination (MAT), MAT-EDTA and MAT-Mercaptoethanol (MAT-ME) Tests: Antigens for such tests were produced by the VSVRI, Abbassia, Cairo, Egypt. The MAT was done in one tenth the volume of the old British tube method [12] still adopted in Egypt to reduce false positive reactions and hence, over

condemnation of animals. A titer of ≥ 2/40 (≥ 80 IU/ml of the OIEISS) was considered positive. Based on MAT, two modifications, *viz*. MAT-EDTA and MAT-ME were carried out [11, 12]. An MAT titer range of 2/20 to 1/40 (40 ≤ range < 80 IU/ml) was suspicious. A titer < 2/20 (< 40 IU/ml) was negative. As for the MAT-EDTA and MAT-ME, the diagnostic threshold was half that of MAT.

Complement Fixation Test (CFT): Antigen for the CFT was imported from NVSL/DBL, USDA, USA. The antigen was used at a working dilution of 1/500 in the test proper according to the manufacturer's instructions.

Complement and hemolysin were prepared and preserved according to Alton *et al.* [11] and were titrated according to the American CFT method described in Hennager (current version) (H. E. Stowell, personal communication, November 15, 2010), NVSL/DBL, USDA, USA [13]. Sheep RBCs were collected on Alsever's solution from an adult healthy ram serologically negative to brucellosis. These were standardized to 2% suspensions in veronal buffer saline.

Results were interpreted as positive at a titer of 1/5 (25% fixation) or higher (=20 ICFTU/ml of the OIEISS).

Competitive ELISA: Commercial multispecies competitive ELISA kit (COMPELISA 400), batch No. C39, produced by the Veterinary Laboratories Agency (now Animal Health and Veterinary Laboratories Agency), New Haw, Addlestone, Surrey KT15 3NB, UK, was purchased via Lillidale Diagnostics, UK and Pharmachem International, Egypt. This kit uses *Brucella melitensis* lipopolysaccharide antigen.

The kit was validated according to the kit instructions, the validation guidelines of the OIE [14] and ISO/IEC 17025:2005 [15] and Crowther [16]. The test was performed according to the kit instructions. The positive cutoff point was calculated as 0.540 nm. Additionally, the percent inhibition (PI) was also calculated from the formula:

PI = 100 – [(Mean OD samples×100)/ (Mean OD Conjugate control)]

Statistical Analyses: All the following analyses were performed using IBM® SPSS® Statistics, Version 21, IBM Corporation, 2012, under the environment of Windows® 8.1, Microsoft Corporation.

One-Way Analysis of Variance (ANOVA): The titers of MAT, MAT-EDTA, MAT-ME, Riv.T and CFT were converted to IU/ml of serum. The OD readings of C-ELISA were expressed as PI. The scores of BAPA and BCT versions were recorded as scores of 0 to 4 according to the degree of reaction. These raw data were statistically tested for the normal distribution using SPSS different parameters. Unfortunately, the assumption of normal distribution of data was rejected as indicated by the SPSS' Shapiro-Wilk test at p value < 0.05. Data were then transformed using log base 10 + 1 to follow normal distribution. A one-way ANOVA with post hoc test using the least significant difference (LSD) were used to study the statistical significant differences in the means of serologic tests as an independent factor and their effect on the results (the dependent factor).

Kappa (κ) Agreement and Relative Sensitivity/ Specificity: The kappa (κ) agreement of agglutination tests with CFT was used to assess the matching of results at $p < 0.05$. Relative sensitivity/ specificity pairs were also calculated. Suspicious MAT results were included with negative results when calculating the κ agreement.

Estimation of the True Prevalence: It was estimated according to Rogan and Gladen [17] from the following equation.

True prevalence = apparent prevalence + combined specificity of BAPA and C-ELISA - 1 / combined sensitivity of BAPA and C-ELISA + combined specificity of BAPA and C-ELISA - 1

Performance Indicators of Serologic Tests: This included the calculation of FPR (false positive rate), FNR (false negative rate), PPV (positive predictive value), NPV (negative predictive value), LR+ (likelihood ratio of a positive test), LR- (likelihood ratio of a negative result) and DOR (diagnostic odds ratio). These were calculated according to McGee [18], Loong [19] and Macaskill *et al*. [20].

Receiver Operating Characteristics (ROC) Curves: Considering the CFT as the serologic gold standard, ROC curves expressing the sensitivity (true positive rate) versus the false positive rate were plotted for all serologic tests. Data were obtained from ROC curves including the area under the curve (AUC) representing accuracy, the best positive cutoff points and the equivalent true positive/ false positive rates according to Hanley and McNeil [21].

RESULTS AND DISCUSSION

Serum immunoglobulins of camels include mainly IgM, IgG_1, IgG_2 and IgG_3 [22]. IgG_1 can fix complement, while IgG_2 and IgG_3, devoid of the light chains and the CH(1) domain [23], neither crosslink efficiently with different antigens, nor fix complement, despite the presence of the C1q binding site that might be hindered by the proximity of the variable domains [24].

The seroconversion pattern of camels induced by exposure to *Brucella* species has not been fully studied. Unlike the case with bovines [10] and small ruminants [25], there has been no reference *Brucella* international standard serum specific for camels. The OIE Terrestrial Manual [10] recommended the use of bovine serologic tests for the diagnosis of brucellosis in camels provided that validation of every test carried out. To date, these serologic tests have not been utterly validated in camels. Proper validation dictates referring to a gold standard of adequate sera from confirmed culture positive and known free animals [26]. Bacteriologic examination for *Brucella*, as the only definitive gold standard for brucellosis [10], was unfortunately not performed in this investigation due to the inaccessibility of samples.

To compensate for the absence of a gold standard direly needed for validation of serologic tests, the CFT, as the best test currently available [10] achieving maximum balance of sensitivity and specificity [27], was used as the reference comparator with expected minor misclassification of the true disease status. Another candidate comparator, the C-ELISA, was excluded for being mainly standardized for cattle and small ruminants according to kit instructions. Still, it can be used theoretically for other species including camels, where the conjugate combines the monoclonal competing with *Brucella* antibodies for LPS epitopes.

To ensure best results from the CFT as a comparator, the problem of frequent anticomplementary activity in camel sera [28] especially common with cold fixation [11] had to be solved. A number of 15 out of 103 reactor camel sera (14.56%) revealed no hemolysis in the anticomplementary control wells. This anticomplementary problem was effectively overcome either by treatment of sera with 5% bovine serum albumen (BSA) prior to inactivation [11] at 62°C or by increasing the heat inactivation time of sera to 60 minutes. Complete disappearance of anticomplementary activity was achieved in both conditions, with appreciable but occasional serum titer reduction in the latter situation. CFT-BSA was therefore preferred. The CFT revealed no clear prozones at any titer despite the fact that warm fixation, usually notorious for common occurrence of prozones [27], was adopted. On the other hand, 10 out of the 103 (9.71%) reactor camel sera revealed prozones in the first one (1/10) or two (1/20) dilutions of MAT with titers ranging from 4/40 to 4/160.

CFT titers were significantly higher than agglutination titers as revealed by MAT, MAT-EDTA, MAT-ME and Riv.T to the extent that a very high CFT titer of 4/5120 corresponding to 35840 IU/ml of serum was detected in a positive camel with an MAT titer of 3/640 equivalent to 1488 IU/ml. The maximum agglutination titer obtained in this study was 4/640 analogous to 1696 IU/ml. Musa and Shigiti [29] and Omar *et al.* [30] reported higher maximum agglutination titers equivalent to 1969 and 3282 IU/ml of serum respectively in positive camels. The fact that CFT titers were generally too much higher than agglutination titers and that MAT but not CFT revealed some prozones in the current work indicate the dominance of the complement fixing IgG_1 [24] characteristic of active longstanding *Brucella* infection [27], especially when knowing that the non-complement fixing IgG_2 and IgG_3 comprise 75% of camel serum IgG [23].

The apparent prevalence was calculated (Table 1) as the percentage of positive cases to both the modified BCT and the CFT in all the 3200 camel sera. Being 2.9375% in male camels, the current prevalence is an indication of a much higher prevalence among their female counterparts, where the prevalence of brucellosis is usually higher in female animals [31]. The current prevalence is close to the 3.9% and 3.1% estimated by Ghanem *et al*. [32] using the Rose-Bengal and indirect ELISA respectively to examine 1246 camel sera from 42 sporadic small scale camel herds in three main districts of camel-rearing regions of Somaliland (Awdal, Waqoyi Galbed and Togdheer) in the period from July to November, 2008. Based on the combined sensitivity of BAPA and C-ELISA (94.5%) and the corresponding combined specificity (80%) taking the CFT as a reference standard, the true prevalence in the current work was estimated to be 0.445% according to Rogan and Gladen [17].

The 113 camels including 103 modified-BCT positives and 10 negatives were examined with all immunoassays (Table 1). The highest number of 103 test positives among the 113 camels was revealed by the binary BAPA and the lowest number of 57 by the ordinal MAT. This huge difference reflects very poor MAT sensitivity due to its indecisive nature [33], especially when compared with its enhanced version using EDTA. Other tests detected varying numbers of positive camels from 73 (MAT-ME) to 97 (BCT 3%) with CFT-BSA giving 95 positive cases.

Table 1: Apparent and true disease prevalence, test outcome type and number of positives as depicted by immunoassays for the diagnosis of brucellosis in male one-humped camels

Immunoassay	Test outcome	Number of tested camels	Number of test positives	Apparent prevalence[1]	Estimated true prevalence[5]
BCT modified	Binary[2]	3200	103	2.9375%	0.445%
BAPA	Binary	113	103		
BCT 8%	Binary	113	94		
BCT 3%	Binary	113	97		
MAT	Ordinal[3]	113	57		
MAT-EDTA	Ordinal	113	82		
MAT-ME	Ordinal	113	73		
Riv.T	Ordinal	113	75		
CFT-BSA	Ordinal	113	95		
C-ELISA	Continuous[4]	113	87		

1 = the number of cases positive to both the modified BCT and CFT divided by the total number of camels tested, 2 = qualitative readings of single serum dilution as yes or no, 3 = serum titration (semi-quantitative reading of multiple dilutions), 4 = quantitative readings of single serum dilution, 5 = true prevalence = apparent prevalence + combined specificity of BAPA and C-ELISA - 1 / combined sensitivity of BAPA and C-ELISA + combined specificity of BAPA and C-ELISA - 1

Table 2: Analysis of variance (ANOVA) of the effect of different serologic tests on the results of male one-humped camels

	Sum of squares	df	Mean square	F	Significance
Between groups	503.518	9	55.946	133.863	0.000
Within groups	464.746	1112	0.418		
Total	968.264	1121			

df = degree of freedom

Although it can, at least theoretically, detect all antibody isotypes that compete with the monoclonal, C-ELISA identified 87 infected animals out of probably more infected ones despite the presence of IgG_1 in 95 CFT positive camels. This could result from low avidity antibodies that weakly competed with the monoclonal. Such antibodies might have been elicited in response to related Gram negative bacteria like *Yersinia enterocolitica* serovar O9 and *Yersinia pestis* known to be harbored in camels [34] and to cross react with *Brucella* lipopolysaccharide [11].

Analysis of variance (ANOVA) indicated statistically significant influence by the type of immunoassay on the serologic results of camels at $p < 0.05$ (Table 2). To further detect significant variation among serologic test results, a post hoc test using the least significant difference was carried out (Table 3). There was insignificant variation among the results of qualitative screen markers, *viz*. BAPA and all BCT versions. The quantitative supplemental markers revealed slight significant variation between MAT and MAT-ME and significant difference between MAT-ME and MAT-EDTA, but not between MAT-EDTA and MAT. The semi-quantitative confirmatory marker Riv.T uniquely varied from all other markers due its distinctive mechanism of IgM elimination from the agglutination reaction allowing only IgG to participate [11]. Likewise, the standard quantitative confirmatory marker CFT differed significantly from all other immunoassays as a result of its dependency on complement fixation for detection of antibodies especially IgG_1 [27]. C-ELISA, the quantitative confirmatory marker, significantly varied from all but the supplemental markers despite their discrete nature. C-ELISA should have varied from MAT formats, but this probably happened because they detected comparable numbers of infected camels.

Taking the CFT-BSA as the reference comparator, the following performance indicators were calculated for immunoassays (Table 4). These included the kappa (κ) agreement, relative sensitivity, relative specificity, false positive rate, false negative rate, positive predictive value, negative predictive value, likelihood ratio of positive and negative results and diagnostic odds ratio. For comparative matching of the index assays with the comparator, kappa (κ) agreement was calculated. Landis and Koch [35] characterized κ values < 0 as indicating no agreement and 0- 0.20 as slight, 0.21- 0.40 as fair, 0.41- 0.60 as moderate, 0.61- 0.80 as substantial and 0.81- 1 as almost perfect agreement. The C-ELISA (0.629) and Riv.T (0.622) agreed substantially with CFT followed by the BAPA (0.597), modified BCT (0.597) and MAT-EDTA (0.515). Sensitivity eagerly required for screening purposes was best achieved by both the BAPA and the modified BCT (98.9%), while specificity needed for confirmatory testing was best achieved by the Riv.T (88.9%), but, by

Table 3: Post hoc test using LSD to reveal significant variations among serologic test results

Serologic test (I)	Serologic test (J)	Mean difference (I-J)	Standard error	Significance (p value)
MAT	BAPA	1.35683	0.08601	0.000
	BCT 8%	1.44398	0.08601	0.000
	BCT 3%	1.41522	0.08601	0.000
	BCT modified	1.31507	0.08601	0.000
	MAT-ME	0.16909	0.08601	0.050
	Riv.T	0.43984	0.08601	0.000
MAT-EDTA	BAPA	1.37735	0.08601	0.000
	BCT 8%	1.46450	0.08601	0.000
	BCT 3%	1.43573	0.08601	0.000
	BCT modified	1.33559	0.08601	0.000
	MAT-ME	0.18961	0.08601	0.028
	Riv.T	0.46035	0.08601	0.000
MAT-ME	BAPA	1.18774	0.08601	0.000
	BCT 8%	1.27488	0.08601	0.000
	BCT 3%	1.24612	0.08601	0.000
	BCT modified	1.14598	0.08601	0.000
	Riv.T	0.27074	0.08601	0.002
Riv.T	BAPA	0.91700	0.08601	0.000
	BCT 8%	1.00414	0.08601	0.000
	BCT 3%	0.97538	0.08601	0.000
	BCT modified	0.87523	0.08601	0.000
CFT-BSA	BAPA	1.62327	0.08601	0.000
	BCT 8%	1.71041	0.08601	0.000
	BCT 3%	1.68165	0.08601	0.000
	BCT modified	1.58150	0.08601	0.000
	MAT	0.26643	0.08601	0.002
	MAT-EDTA	0.24592	0.08601	0.004
	MAT-ME	0.43553	0.08601	0.000
	Riv.T	0.70627	0.08601	0.000
	C-ELISA	0.30947	0.08763	0.000
C-ELISA	BAPA	1.31380	0.08763	0.000
	BCT 8%	1.40094	0.08763	0.000
	BCT 3%	1.37218	0.08763	0.000
	BCT modified	1.27203	0.08763	0.000
	Riv.T	0.39680	0.08763	0.000

Negative mean differences (I-J) were excluded to avoid repetition.

Only significant variations at the 0.05 level are revealed in the table

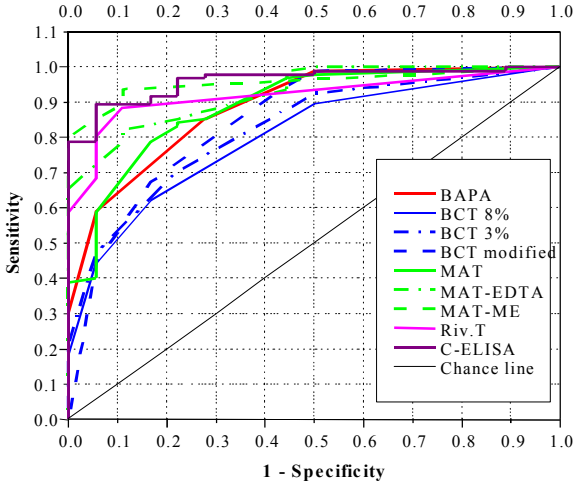

Fig. 1: Performance characteristics of immunoassays expressed as receiver operating characteristics (ROC) curves in male one-humped camels

Table 4: Diagnostic performance metrics of serologic tests taking CFT-BSA as a comparator in male one-humped camels

		CFT-BSA results											
Serologic Index immunoassays		-	+	Agreement (κ value)	Relative Se %	Relative Sp %	FPR %	FNR %	PPV %	NPV %	LR+	LR-	DOR
BAPA	-	9	1	0.597 ± 0.113	98.9	50	50	1.1	91.3	90	1.978	0.022	94
	+	9	94										
BCT 8%	-	9	10	0.386 ± 0.115	89.5	50	50	10.5	90.4	47.4	1.790	0.210	8.5
	+	9	85										
BCT 3%	-	9	7	0.446 ± 0.116	92.6	50	50	7.4	90.7	56.3	1.852	0.148	12.6
	+	9	88										
BCT modified	-	9	1	0.597 ± 0.113	98.9	50	50	1.1	91.3	90	1.978	0.022	94
	+	9	94										
MAT	-	12	38	0.155 ± 0.076	60	66.7	33.3	40	90.5	24	1.802	0.600	3
	+	6	57										
MAT-EDTA	-	14	14	0.515 ± 0.097	85.3	77.8	22.2	14.7	95.3	50	3.842	0.189	20.3
	+	4	81										
MAT-ME	-	13	16	0.444 ± 0.099	83.2	72.2	27.8	16.8	94	44.8	2.993	0.233	12.8
	+	5	79										
Riv.T	-	16	12	0.622 ± 0.090	87.4	88.9	11.1	12.6	97.6	57.1	7.874	0.142	55.3
	+	2	83										
C-ELISA	-	15	10	0.629 ± 0.093	89.5	83.3	16.7	10.5	96.6	60	5.359	0.126	42.5
	+	3	85										

Se = sensitivity, Sp = specificity, FPR = false positive rate (probability that the test is positive if the disease is not present), FNR = false negative rate (probability that the test is negative if the disease is present), PPV = positive predictive value (probability that the disease is present if the test result is positive), NPV = negative predictive value (probability that the disease is absent if the test result is negative), LR+ = likelihood ratio of a positive test (the probability of an animal that has the disease testing positive divided by the probability of an animal that does not have the disease testing positive), LR- = likelihood ratio of a negative result (the probability of an animal that has the disease testing negative divided by the probability of an animal that does not have the disease testing negative), DOR = diagnostic odds ratio (summarizes the diagnostic accuracy of the test as a single number that describes how many times higher the odds are of obtaining a test positive result in a diseased rather than a non-diseased animal).

Table 5: Data obtained from ROC curves of immunoassays including accuracy (AUC), the best positive cutoff and parallel sensitivity/ false positive rate, regarding CFT-BSA as the gold standard

Immunoassay	Accuracy (AUC ± standard error)	The best cutoff *	Parallel sensitivity (%)	Parallel false positive rate (%)
BAPA	0.887 ± 0.041	1 < score < 2*	85.3*	27.8*
		slightest agglutination	98.9	50.0
BCT 8%	0.803 ± 0.051	1 < score < 2*	62.1*	16.7*
		slightest agglutination	89.5	50.0
BCT 3%	0.826 ± 0.049	1 < score < 2*	70.5*	22.2*
		slightest agglutination	92.6	50.0
BCT modified	0.851 ± 0.053	1 < score < 2*	94.7*	44.4*
		slightest agglutination	98.9	50.0
MAT	0.891 ± 0.041	66.5 IU/ml*	58.9*	5.60*
		49.75 IU/ml*	78.9*	16.7*
		36.75 IU/ml	84.2	22.2
MAT-EDTA	0.927 ± 0.027	60 IU/ml*	65.3*	0*
		43.25 IU/ml	82.1	11.1
MAT-ME	0.955 ± 0.019	33.25 IU/ml*	80.0*	0*
		23.25 IU/ml	85.3	5.60
		8.38 IU/ml*	93.7*	11.1*
Riv.T	0.920 ± 0.028	46.5 IU/ml*	58.9*	0*
		23.25 IU/ml	80.0	5.60
		10 IU/ml*	88.4*	11.1*
C-ELISA	0.961 ± 0.018	68.89 PI	78.9	0
		45.11 PI	89.5	5.60

PI: Percent inhibition.

* Cutoff point, parallel sensitivity and parallel false positive rate were not preferred.

no means, dominating the specificity of C-ELISA, where Riv.T specificity may be an inverse reflection of low sensitivity. Among the supplemental assays, MAT-EDTA achieved the best sensitivity/ specificity balance. Positive and negative predictive values depend upon disease prevalence, which is unlikely to be consistent among studies [20]. Among camels that had a positive BAPA or BCT, the probability of disease was relatively lower requiring further confirmation, while those of MAT-EDTA, Riv. T and C-ELISA were higher.

The BAPA and modified BCT offered high NPV of 90% emphasizing their high screening ability, as the probability of disease freedom was very high excluding negative cases from further confirmatory testing. The positive and negative likelihood ratios respectively quantify the change in the certainty of the diagnosis conferred by test results [20]. LRs > 1 argue for the disease diagnosis and the bigger the number, the more convincingly the disease is suggested, while LRs between 0 and 1 argue against the disease and the closer the number to 0, the less likely the disease [18]. Riv.T and C-ELISA significantly argued for brucellosis diagnosis by LRs+ of 7.874 and 5.359 respectively, while BAPA and modified BCT argued against brucellosis diagnosis by LR- value of 0.022, being the closest to zero. This confirms the fitness of such tests for their purpose. The diagnostic odds ratio summarizes the diagnostic accuracy of the index test as a single number that describes how many times higher the odds are of obtaining a test positive result in a diseased rather than a non-diseased animal [20]. The highest DOR measures were provided by BAPA (94), modified BCT (94), Riv.T (55.3) and C-ELISA (42.5). The MAT gave poor DOR.

ROC curves were plotted depending on CFT results (Figure 1) to select the best diagnostic thresholds with corresponding relative sensitivity and false positive rate (Table 5). Sensitivity, specificity, FPR and FNR are assay bound and prevalence independent [19]. The relationship between each of the pairs of sensitivity/ specificity, sensitivity/ FNR and specificity/ FPR is negative. To fit the purpose of screening, specificity is usually sacrificed for the sake of sensitivity. The BAPA and the modified BCT each achieved a high sensitivity of 98.9%. For the purpose of quantitative determination of agglutinating titer by MAT and its EDTA and ME versions, the diagnostic threshold of ≥ 60 IU/ml recommended by Elbauomy *et al.* [33] proved to achieve the least possible false positive rate (5.6% or lower). To seek better sensitivity (around 80%), however, a lower cutoff point of ≥ 40 IU/mL can still be safely used in camels without substantial elevation of the false positive rate. The Riv.T interpreted at 1/25 as positive achieved good balance between sensitivity (80%) and specificity (94.4%). The C-ELISA proved an excellent specific test on the expense of sensitivity when interpreted as positive at the recommended threshold of 70% inhibition. The test needs further standardization/ adaptation in camels for even better results. The current findings exceeded the expectations of Fowler [36] and Sutmoller [37] in terms of 90 to 95% sensitivity and 90% specificity for bovine testing procedures in camelids.

The closer the ROC curve to the vertical axis, the better the test performance [38]. ROCs of the binary screening markers were the farthest from the Y axis with the BAPA being the best, followed by the ordinal markers with MAT-ME being the best and finally the continuous marker, C-ELISA (Figure 1 and Table 5). This was reflected as an assay accuracy by estimation of the area under each of the ROC curves (AUCs). The AUC measures how well the test separates the positive from negatives without reference to a particular decision threshold, where AUCs of 0.9-1, 0.8-0.9, 0.7-0.8 and 0.6-0.7 indicate excellent, good, fair and poor test respectively, while an AUC of 0.5-0.6 designates an invalid test [21]. It should be noted that the diagnostic accuracy is a population specific parameter, as it depends upon the specific biological characteristics of the study population. The good accuracy values of the binomial classifiers in descending order were 0.887, 0.851, 0.826 and 0.803 for the BAPA, modified BCT, BCT 3% and BCT 8% respectively. The superior BAPA accuracy makes it the best candidate for screen testing of camels. The excellent accuracy values of the ordinal markers MAT-ME (0.955), MAT-EDTA (0.927) and Riv.T (0.920) make each a reliable semi-quantitative supplemental test for camels taking into consideration that Riv.T is also a rapid test. The highest AUC of 0.961 was achieved by the continuous confirmatory marker C-ELISA.

CONCLUSION

Under conditions of this study, it is concluded that the apparent prevalence was 2.9375% and the estimated true prevalence was 0.445%. The serologic profile had a dominating IgG_1 subclass of antibodies characteristic of active infection with CFT titers reaching 35840 ICFTU/ml way higher than agglutination titers of 1696 IU/ml. BAPA and modified BCT performed really well as binary screening markers. Likewise, the performance indicators suggested Riv.T as an excellent candidate for simple and rapid disease confirmation. C-ELISA needs further standardization/ adaptation to boost its performance in camels. Proper validation of serologic tests in camels may require an international reference *Brucella* standard anti-camel serum. The current Egyptian quarantine regulations need an amendment to include male animals imported for slaughtering in brucellosis serologic testing, where it is hard to prevent their potential mingling with other animals or even their smuggling for breeding purposes.

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