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EVALUATION OF LOCALLY PREPARED ROSE BENGAL PLATE AND BUFFERED ACIDIFIED PLATE ANTIGENS OF BRUCELLA ABORTUS.

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SUMMARY: The rose bengal plate and the buffered acidified plate antigens locally prepared from *Brucella abortus* S 99 and 1119/3 gave similar results when compared with the same imported antigens. The agreement between both tests and the standard tube agglutination test was discussed.

The large scale production of such antigens in Egypt will save hard currency and greatly increase the efficiency of the brucellosis control programmes proposed.

INTRODUCTION

In the last decade, large number of foreign breeds of cattle were imported in Egypt in order to improve beef and milk production. Parallel to the increased importation there was an increase in the incidence of brucellosis. Therefore, the veterinary authorities considered the control of such disease as a first priority.

The control of brucellosis depends on proper diagnosis. The tube agglutination test has been considered as the standard method of diagnosis since 1934 (Pietz and Cowart, 1980) however, non-specific agglutination were

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demonstrated in cattle sera by Hess and Roepke (1951). Such defect in this important test has initiated many investigators to develop new antigens such as the acid plate antigen (Roepke et al., 1957) and the card test antigen (Wilson et al., 1965) which detect almost only the specific agglutinins. Such antigens are therefore widely used in many countries of the world. In Egypt, these antigens are imported, therefore, the aim of the present work was to prepare the rose bengal and buffered acidified plat antigens and to compare them with the imported ones with the ultimate goal of large scale production.

MATERIAL AND METHODS

Strains:

* *Brucella abortus* strain 99 was obtained from Weybridge, England.

** *Brucella abortus* strain 1119/3 was obtained from Ames Iowa, USA.

Imported antigens:

Both rose bengal plate test (RBPT) antigen and buffered acidified plate antigen (BAPA) were imported from Ames Iowa, USA.

Preparation of antigens:

Both antigens were prepared according to the method described by Alton et al., (1975) using the above mentioned strains and the recommended chemicals and equipments.

The locally propared and imported antigens were tested for their efficiency using 110 cattle sera and 15 sheep sera already tested by the Department of Brucella, Animal Health Research Institute, Dokki, Giza.

All sera were first tested by the standard tube agglutination test (SAT) and the results were interpreted according to the Food and Agriculture Organization (FAO), Central Veterinary Laboratories, Weybridge, England (CVL) and United States Department of Agriculture (USDA), then by the rose bengal plate and buffered acidified plate tests. Comparison between the different antigens and different tests was done.

RESULTS

1. Cattle sera:

a- Standard tube agglutination test:

Table (1) shows the results of the standard tube agglutination test. Such results varied according to the way of interpretation. Using the FAO interpretation (with S 99 antigen), it was possible to classify 67 sample as positive, 20 as suspicious and 23 as negative, while the CVL interpretion (using S 99 antigen) revealed 80 positive, 18 suspicious and 12 negative. On the other hand, the use of USDA interpretation (with S 111 9/3 antigen) resulted in 73 positive, 20 suspicious and 17 negative.

b- Comparison of locally prepared and imported rose bengal plate and buffered acidified plate test antigens:

As shown in Table 2, there was a complete agreement between the results of locally prepared and imported antigens.

c- Relation between RBPT, BAPA and standard agglutination test (SAT):

From Tables (3 and 4) it is clear that there was a good correlation between the result of the three tests in

Table (1): Results of the standard tube agglutination test on 100 cattle sera by S 99 (FAO and CVL interpretation) and 1119/3 (USDA interpretation).

FAO			CVL			USDA		
I.U* Content	Interp-retation	No. of cases	I.U content	Interp-retation	No. of cases	I.U. content	Interp-retation	No. of cases
>106	positive +	67	>61.5	positive +	80	>100	positive +	73
53-106	suspicious ±	20	31.5 61.5	suspicious ±	18	50-100	suspicious ±	20
< 53	negative -	23	< 31.5	nrgative -	12	< 50	negative -	17

* I.U. = International unit.

Table (2): Results of locally prepared and imported RBPT and BAPA on 110 cattle sera.

Reaction	RBPT		BAPA	
	local	imported	local	imported
Positive (+)	76	76	78	78
Suspicious (±)	11	11	12	12
Negative (-)	23	23	20	20

N.B. The results are the same for both S 99 and S 1119/3.

animals having high and low I.U. content (positive and negative SAT). However, almost one half of the samples showing suspicious SAT reaction was positive in both RBPT and BAPA. Also it is observed that there is one serum, of the animals classified as positive, gave negative reaction in both RBPT. On the other hand, one sample that showed low I.U. content in SAT was positive in the other two tests in addition to 1 and 4 negative samples in SAT were suspicious RBPT and BAPA respectively.

Statisically, the harmonic coefficient (R) in case of S 99 (Table 3) was found to 0.74 and 0.70 in RBPT and BAPA respectively, while in case of S 1119/3. it was 0.74 for RBPT and 0.73 for BAPA (Table 4).

Table (5) shows the agreement between all the tests used in the group of animals classified as positive. It is obvious that there is a very good agreement between RBPT, BAPA, in CVL and USDA interpretations of SAT. The FAO interpretation possesses also a good agreement, but to a lesser extent, with RBPT and BAPA.

Table (3): Reactions of RBPT and BAPA antigen, prepared from S 99, in relation to I.U. detected by SAT (USDA interpretation).

SAT (FAO)		No. of cases	Reactions of RBPT			Reactions of BAPA		
			positive	suspicious	negative	positive	suspecious	negative
I.U.	High 106	67	66	-	1	66	-	1
	Moderate 53.106	20	9	10	1	11	8	1
	Low	23	1	1	21	1	4	18
Harmonic Coefficient			0.74				0.70	

Table (4) : Reactions of RBPT and BAPA antigen prepared from S 1119/3, in relation to I.U. detected by SAT (USDA interpretation).

SAT(USDA)		No. of cases	Reactions of RBPT			Reactions of RBPT		
			positive	suspicious	negative	positive	suspicious	negative
I.U.	High 100	73	72	-	1	72	-	1
	Moderate 50-100	20	3	11	6	5	11	4
	Low	17	1	-	16	1	1	15
Harmonic coefficient				0.74			0.73	

Concerning the animals identified as suspicious (Table 6) the best agreement was found to be between RBPT and BAPA.

In animals classified as negative there was a good agreement between RBPT and BAPA. The FAO interpretation showed a complete agreement with the RBPT and the CVL interpretation was the lowest agreement. On the other nand, the USDA interpretation showed a good agreement.

Table (5): Precentage of gareement between RBPT, BAPA and SAT in animals classified as positive.

		RBPT	BAPA	SAT		
				FAO	CVL	USDA
	RBPT	-	97.5	88	95	96
	BAPA		-	86	97.5	93.6
	FAO			-	84	92
SAT	CVL				-	90
	USDA					-

Table (6):Percentage of agreement between RBPT, BAPA and SAT in animals classified as suspicious.

		RBPT	BAPA	SAT		
				FAO	CVL	USDA
	RBPT	-	92	55	61	55
	BAPA		-	60	67	55
	FAO			-	90	100
SAT	CVL				-	90
	USDA					-

Table (7): Precentage of agreement between RBPT, BAPA and SAT in animals classified as negative

		BRPT	BAPA	SAT		
				FAO	CVL	USDA
SAT	RBPT	-	87	100	52	74
	BAPA		-	87	60	85
	FAO			-	52	71
	CVL				-	90
	USDA					-

2- Sheep sera:

Both the locally prepared and imported antigens gave the same results with sheep sera, but the small number of sheep serum samples made the analysis of such results of little value.

DISCUSSION

The results obtained in the present work revealed that the locally prepared RBPT and BAPA antigens, whether from S 99 or S 1119/3, were as efficient as the imported antigen. This was expected because the locally prepared antigen were prepared according to the methods described by Alton et al. (1975) using the recommended chemicals, equipments and bacterial strains.

The differences observed in the results obained by the different interpretation of SAT were due to the international limits of each category (positive, suspicious and negative). The question is which one of these interpretations is the convenient and suits our circumstances in Egypt. In the CVL interpretation, the lower limit of positive (61.5 I.U.) allows the detection of

all positive animals even those with low I.U. content. In Egypt, where the number of bovines is relatively small and also the bad hygienic measures, which may lead to the sensitization of the animals by a variety of microorganisms, many of which cross-react with brucella antigens, the use of test and slaughter method of eradication depending on the CVL interpretation will result in a great loss of animals. Another disadvantage of such interpretation is the narrow range of the suspicious group (31.5 - 61.5 I.U) while in high prevalence, an increase in false positive cases occurs (Tizard, 1982), so many cases of suspicious animals will be considered positive. From our results, it is clear that the USDA interpretation is the best as it could detect more animals than the FAO interpretation and it also had a good range of suspicious thus allowing animals with false positive reactions to be retested and properly diagnosed.

Statistically, the harmonic coefficient ranged from 0.70 to 0.74. That means that 70-74% of the animals tested showed a degree of reaction to RBPT and BAPA proportional with the I.U. content. The absence of complete harmony may be due to the differences in immunoglobulin types and concentration of the tested sera (Allan et al., 1976).

The very good agreement between RBPT and BAPA identifying positive, suspicious and negative animals is due to the fact that both tests have the same role and detects the same immunoglobulins (Joint FAO/WHO, 1986). However, the absence of complete agreement may be explained by that the pH of RBPT antigen and serum reaches 3.85 (Angus, 1990, personal communication) while that of BAPA and serum is 4.0 (Angus and Burton, 1984). This difference in the final pH may lead to the inhibition of non-specific agglutinins in one test more than in the other.

Also, there is a very good agreement between RBPT, BAPA and USDA interpretation in the positive and negative animals. This comes in accordance with the findings of Contini et al., (1973). Also, Badjevic and Bajrovic (1981) stated the closer correlation between RBPT and SAT. The moderate agreement observed in the suspicious group may be explained by the concept of Allan et al., (1976) who mentioned that these tests detect the same classes of immunoglobulins, but at different concentrations (SAT is less sensitive). Another suggestion is that of Corbel (1972) who mentioned that RBPT detects only the IgG_1 class. Also Huber(1989) stated that RBPT is more sensitive in the IgG_1 class while the SAT possesses the higher sensitivity in the IgG_1 and IgM classes.

There was one serum sample that was negative in SAT but positive in both RBPT and BAPA. This may be explained by the findings of Allan et al., (1976) that RBPT is more sensitive than SAT, so such sample may have immunoglobulin concentration lower than that needed to be detected by SAT, but enough to be detected by RBPT. Another explantion is the statement of Davies (1971) that RBPT could detect infection earlier than SAT.

ON the other hand, one serum sample was negative in RBPT and BAPA, but had a positive I.U. content in SAT. This may be due to the prozone phenomenon of RBPT suggested by Herr (1982).

In conclusion, it will be expensive to depend only on importation of antigens, and our results indicate the capability and success of perparing RBPT and BAPA antigens and it is clear that the use of such antigens in diagnosis of bovine brucellosis is very satisfactory. This opens the door for the local production of such antigens on large scale which result in self-sufficiency of the country and in saving hard currency.

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