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EVALUATION OF COOMBS TEST IN BRUCELLA DIAGNOSIS IN CATTLE AND SHEEP COMPARING WITH OTHER SEROLOGICAL TESTS.

(With 2 Tables)

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تقييم اختبار الكومب فى تشخيص مرض البروسيلة فى الابقار والأغنام مقارنة باختبارات سيرولوجيه اخرى

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فى هذه الدراسه تم فحص عدد ٢٤٠٦ عينة دم للأبقار وعدد ٧٩٤ عينة دم للأغنام سيرولوجيا باختبار التلازن البطئ بالأنابيب وقد ظهر التلازن فى عدد ٣٣ عينه للأبقار وعدد ٢٠ عينه للأغنام . وقد تم فحص العينات التى أظهرت التلازن باختبارات الروزبنجال والاختبار الطبقى الحامضى المتوازن واختبار الكومب . كان اختبار الكومب هو أعلاها حساسيه ثم يليه الطبقى الحامضى المتوازن وأدناها حساسيه كان اختبار الروزبنجال . ولهذا ينصح الباحثون بأن يشمل قائمة مجموعة الاختبارات على اختبار الكومب أساسيا بمراعاة أنه ذو حساسيه عاليه . ومما جعل اختبار الكومب أكثر حساسيه أنه أعطى نتائج ايجابيه فى المعايير المنخفضه (١٠ / ١) لاختبار التلازن .

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SUMMARY

A total of 2406 cattle and 794 sheep blood samples were examined serologically for tube agglutination test (TAT). The reactor samples (33 cattle and 20 sheep)were subjected for Rose Bengal plate test (RBPT), buffered acidified plate test (BAPAT) and coombs tests. The last test was the most sensitive one, then BAPAT, while RBPT was the least sensitive one. The superiority of coombs sensitivity is more evident in case of the 1st titre TAT reactor (1/10). So, it is advisable that coombs test must be essentially involved in brucella profile serological tests as a high sensitive one.

Keywords: Coomb's test, brucella, diagnasis, sheep, cattle.

INTRODUCTION

Brucella diagnosis depends mainly on serological testing for the presence of specific immunogloulins. To make a decision, complete serological tests profile must be employed *(NELSON, 1989)*. TAT is used as the corner-stone of brucellosis eradication schemes throughout the world *(DAVIS, 1971)*. It is a reliable test using B. abortus antigen, but has certain limitations specially in recent infections and in some chronic cases *(NICOLAS, et al., 1968)*. Agglutinins detected by this test are usually either I9M or I9G. In some sera, blocking factor (I9A) may interfere the aggltination developed by I9M and I9G. These blocking antibodies can be detected by the coombs antigtobulin test *(MERCHANT and BAKER, 1983 and BROOK et al., 1991)*.

Coombs test as a brucella diagnostic serological test had been dealt with by many earlier authors, in cattle *(HILL, 1964 and ABDEL-GHAFFAR et al., 1970)* in horse *(McCAUGHEY and KERR, 1967)* and in goat *(CORBEL and LANDER, 1982)*. Most of them had ascertained that its importance resempled in the detection of incomplete or on non agglutinating antibodies. Few available literatures compared between the coombs test and other quantitative tests.

The present work is aiming to study the valiability of the coombs test as a support to confirm TAT comparing with RBPT and BAPAT.

MATERIAL AND METHODS

A- Blood samples:

Through the routine work in Animals Health Instituute, Assiut Lab.-during the period from 1-5-1993 up to 31-10-1993 a total of 2406 cattle and 794 sheep blood samples were collected. TAT was adopted for all sera obtained. All reactors (33 cattle and 20 sheep) were subjected to RBPT, BAPAT and coombs tests.

B- Antigens:

1- Standard B. abortus agglutination for TAT was obtained from Veterinary Serum and Vaccine Institute-Abbasia. The method was emplyed to *ALTON* et al., *1975.*
2- Rose Bengal antigen was spplied from Merieux Institue-France, also according to *Alton* et al., *1975.*
3- BAP antigen 0.5% phenol was obtained from SAS- Scientific San Antonios, Texas. USA, and was carried out according to Angus and Barton, 1984.
4- Coombs antiglobulin was derived from Biotest Diagnostics, Frankurt, Germany. The method was that recommended by *BEH and LASCELLES (1973).*

RESULTS

The results are manifested in tables 1 & 2.

DISCUSSION

As the brucella diagnosis is referred mainly to serological tests, it is of great importance to study the perference of some tests than others. In Egypt, TAT is considered as the corner stone of brucellosis eradication scheme. So, it is the routine screening test but due to having certain limitations, some serological tests must be added to confirm the TAT results.

From the present study, it is obviously noticed that the coombs test is the most sensitive test than the other two tests tried (RBPT and BAPAT) as shown in tables 1 & 2. The superiority of coombs test sensitivity was evident in the first titre reactor (1/10) as the positive reaction percentage for coombs, BAPAT and RBPT were 61%, 38% and 23% in cattle and 83.3%, 66.6% and 16.6 in sheep respectively. BAPAT sensitivity comes always behind that of the coombs, while RBPT has the least sensitivity allover cases. As well as this high sensitivity of coombs to detect the agglutinating antibodies, it can detect the incomplete or blocking anitbodies specially in chronic form of brucellosis when 19M decrease and 19G lose

their aggltinating power *(BAKER and BREACH, 1980).*

These findings may be due to the fact that RBPT antigen has acidity of pH 3.6 which is lower than that of TAT. This lower pH inhibits the activity of 19M and inhance the agglutination of 19G *(HUBER, 1989).* While BAPAT has pH4-inbetween RBPT and TAT-which permits the detection of 19M as well as 18G *(STEMSHORN et al., 1985).* This explain why BAPAT is more sensitive than RBPT. Coombs test was designed to detect the incomplete antibodies to red cells but was quickly appreciated as a sensitive technique for the detection of the presence of all immunoglobulins when bound to cells. These immunoglobulins can be detected after the addition of the antisera directed against the immunoglobuulins under study *(DICK, 1984).* The specificity of the coombs test in brucella diagnosis is good as that of complement fixation test but it is 4-8 times more sensitive *(HILL, 1964). WILSON and SMITH, 1984* recommended that the most specific test is the coombs, but even this it is positive only in 70% of infected animals. To obtain more accurate results, combination of coombs test with other serological tests were recommended. *LE PENNEC and GOYON, 1966* stated that the most sensitive diagnostic method is the concurrent use of coombs and complement fixation tests. While *ABDEL-GHAFFAR et al., 1970* adviced to use TAT incombination with either coombs or complement fixation test. The three tests were recommended together by *CORBEL and LANDER, 1982.*

From the present findings and above recommendations for the specificity and sensitivity of the coombs test, it is advisable to envolve it on the top of serological tests profile specially in cases of low TAT reactors.

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Table (1): Serological tests for cattle.

Titre of TAT	No	RBPT		BAPAT		Coombs test	
		No	%	No	%	No	%
1/10	13	3	23%	5	38%	8	61%
1/20	6	1	16.6%	1	16.6%	2	33.3%
1/40	5	1	20%	2	40%	4	80%
1/80	3	3	100%	3	100%	3	100%
1/320	6	6	100%	6	100%	6	100%
Total	33	14	42%	17	51%	23	70%

Table (2): Serological tests for sheep.

Titre of TAT	No	RBPT		BAPAT		Coombs test	
		No	%	No	%	No	%
1/10	6	1	16.6%	4	66.6%	5	83.3%
1/20	8	7	87%	8	100%	8	100%
1/40	3	3	100%	3	100%	3	100%
1/80	1	1	100%	1	100%	1	100%
1/160	1	1	100%	1	100%	1	100%
1/320	1	1	100%	1	100%	1	100%
Total	20	14	70%	18	90%	19	95%