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QUESTIONABLE REACTIONS TO ROSE ENGAL TEST

(With 2 Tables)

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تفاعلات غير محدده لاختيار اللروزبنجال

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عند فحص قطيع من الأبقار مكون من ٤٥٠بقره ومصاب بالبروسيلا سيرولوجيا باختبار الروزبنجال والتلازن الانبوبى وجد أن هناك تضارب فى نتائج الفحص فى ٤٦ حاله منهم. توالى فحص هذه العينات ٣ مرات بمعدل مره كل شهرين بهذين الاختبارين وتم تحديد الحاله السيرولوجيه أما ايجابيه أو سلبيه لعدد ٣١ حاله منهم وظلت ١٥ حاله تعطى نتائج متضاربه تم فحص ال١٥ حاله الباقيه باختبار الروزبنجال والتلازن الانبوبى والروزبنجال المعدل ووجد أن هناك أتفاق بين نتائج الروزبنجال المعدل والتلازن الانبوبى فى ١٤ حاله من هذه الحالات.

SUMMARY

Disagreement between the results of Serum Agglutination Test (SAT) and Rose Bengal Test (RBT) was detected in 46 animals among 450 tested cattle in infected brucella herd. Repeated testing of these animals for three times at 2 months interval clarified the serological status of 31 out 46 cases. Modified RBT was applied with SAT and standard RBT on the rest 15 cases after heat inactivation of sera. Modified RBT results agreed with that of SAT in 14 out of the 15 cases.

Keywords: Questionable reactions to rose bengal test.

INRTRODUCTION

Conventional seroagglutination tests as Serum Agglutination Test (SAT) and Rose Bengal Test (RBT) are still the corner stone in detection of brucella antibody response to infection and/or vaccination by certain vaccines. Bovine IgM and IgG are the active immunoglobulins in these two tests, *(TIZZARD 1982).*

SAT performed at a neutral pH demonstrates a high analytical sensitivity in detection of IgM and IgGl i.e. lessing of immunoglobulin isotypes are requried to give positive aggl-utination reaction under standard test conditions,*(WRIGHT and NIELSEN 1987).*

However, SAT diagnostic specificity is poor especially when the test is interpreted at low titres,*(DOHO et al., 1986).*

RBT was developed to improve the specificity of agglutination tests and is used as screening or supplementary test in detection of specific bovine IgM and IgG to brucella, *(ALTON et al., 1988).* Its acidic pH (3.65), help in activation of the non agglutinating IgGl to be active agglutinins in addition to its inhibitory effect on the non specific agglutinins, *(SUTHERLAND, 1980).*

Contradictory results between SAT and RBT were encountered in which SAT was positive to some samples that gave negative reactions with RBT. Such results can conistitute a problem during interpretation of serum tests applied on suspected herd and hinder eradication programms.

One aspect in dealing of such cases is the retesting of these problem samples with its high cost, labour and time needed. Another approach is to modify RBT in order to increase its sensitivity in clarifying the serological status of these animals.

In this study, samples from infected and vaccinated herd which gave different rections to RBT and SAT were subjected to a modified RBT using double of the serum volume needed in the routine test.

MATERIAL and METHODS

Serum sample used in this study was encountered during surveying an infected brucella herd of 450 cattle by SAT and RBT. It was noticed that there was no agreement between results of the 2 tests in 46 cases (10.02%). Repeated testing for 3 times at 2 months interval were applied on these samples to clarify their srological reactions. Sera that still gave different results were sbjected to heat in-activation at 56 °C for 50 minutes then tested by SAT and RBT as described by *MORGAN et al., (1978).* Modified RBT using double serum volume (0.06 ml.) of the routine test was applied on these samples.

RESULTS

Out of the 46 examined sera, agreement between results of the 2 tests was acheived in 31 cases after repeated examina-tions, Table (1).

The results of the rest 15 samples that were subjected to SAT, RBT and modified RBT after heat inactvation of sera were illustrated in Table (2). Table 2 showed that modified RBT agreed with SAT in 14 out of 15 tested samples.

DISCUSSION

Disagreement between results of SAT and RBT was not uncommon and reported by *MORGAN et al., (1969), NICOLETTI (1969) and NICOLETTI and BURCH (1969).* Although, SAT has acquired the status of traditional test for bovine brucellosis and was helpful in brucella eradication schemes throughout the

world, yet in recent years some doubts has been laid on its efficacy, *(ALTON et al., 1975).*

DAVIES (1971) and *CORBEL (1972)* reported that RBT had the ability to eliminate non specific reaction to brucellosis which might give doubtful result in SAT.

ALLAN et al., (1976) stated that non specific reactions to brucellosis agglutination tests can be classified to immunologically non specific and diagnostically non specific reactions. Immunologically non specific reaction, due to non specific agglutinins which can agglutinate a variety of unrelated bacterial antigens, *(HESS 1953),* conistituted about 60% of non specific agglutination reactions. These reactions are inhibited by the acidic pH of the RBT.

Diagnostically non specific reaction, although immunologically specific, may be attributed to residual vaccination titres after strain 19 vaccination and very recent or choronic brucella infection in herds suffering from brucellosis in the final stages of eradication programm, *(MORGAN 1969).* It is strongly believed that it is the case in which SAT gave reaction while RBT is negative.

Sera examined by modified RBT in this study were heat inactivated in order to avoid the immunologically non specific reaction that may arised from doubling the volume of serum which bring the serum antigen mixture at higher pH value than the standard test.

It is commonly known that in case of contradictory results, other supplemental tests than SAT and RBT should be applied. Most of supplementary tests is directed towards detection of the IgG isotype while missing IgM. Dismissing the diagnostic importance of IgM may be an inherent danger as it is the first immunoglobulin class produced in response to early infection.

Control of brucellosis in egypt depends upon early detection of infected animals by SAT and RBT with the use of strain 19 vaccination. Leaving animals designed as negative by RBT after first testing among other free animals, till proved to be free after repeated testing may lead to spread of the disease and complicate the eradication programms.

Previously detected questionable reactions to RBT are arised in infected herds where strain 19 vaccination is applied. Humoral antibody response to brucella in these animals appeared to be quite different and necessiate intensive studies to modify tests applied for diagnosis of brucellosis to detect these cases.

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Table 1: Results of repeated testing of 46 samples that gave contradictory reaction to SAT and RBT

No. of samples agreed to SAT & RBT			No. of samples
Both tests positive	Both tests negative	Total agreed	disagreed
24	7	31	15°

° All these samples were RBT negative and SAT positive or suspecious.

Table 2: Results of SAT, RBT and modified RBT on sera after heat inactivation.

Serial	Animal status	SAT		RBT	Modified RBT
		40 IU	80 IU		
1	HV	+	+	-	+
2	HV	+	+	-	+
3	HV	+	-	-	+
4	HV	+	+	-	+
5	HV	+	+	-	+
6	HV	+	+	-	+
7	HV	+	-	-	+
8	HNV	+	-	-	+
9	HNV	+	-	-	+
10	CV	+	-	-	+
11	CV	+	-	-	-
12	CV	±	-	-	+
13	CV	+	±	-	+
14	CV	+	-	-	+
15	CNV	+	-	-	+

HV = Heifer vaccinated at calfhood
CV = Cattle vaccinated at calfhood
\+ = Positive reaction
HNV = Heifer not vaccinated
CNV = Cattle not vaccinated
\- = Negative reaction