J.Egypt.Vet.Med.Ass. 54,No.5: 379 - 384 (1994).

# THE APPLICATION OF ENZYME LINKED IMMUNOSORBENT ASSAY AND OTHER TESTS FOR THE EVALUATION OF THE IMMUNE RESPONSE OF BAFFALO CALVES TO S19 VACCINE.

SANAA F. SALEM;*A. MOHSEN and **M. REFAI
Serum and vaccine Institute, Abbassia, Cairo
*National Research Centre, Dokki, Cairo
**Microbiology Dept., Fac. Vet. Med., Cairo, Egypt.

SUMMARY: Evaluation of immune response in 120 vaccinated buffalo calves with S19 vaccine was achieved by application different serological tests including enzyme linked immunoassay (ELISA), standard agglutination test, mercaptoethanol test, rivanol test and compliment fixation test. The results were compared in terms of sensitivity, specificity and concordance. Considering these terms, ELISA showed superiority over the other conventional tests. In light of these results, ELISA may be used as a final test on vaccinated calves with S19 vaccine before introduction in breeding programs.

## INTRODUCTION

After many years of control and eradication efforts, brucellosis remains a serious problem. Current conventional tests are used for serodiagnosis of brucellosis. All of these tests are agglutination tests. The need for a primary antigen-antibody binding test has been well established (**Anderson et al 1978 and Nielson 1984**). Some reports indicate that detection of B.abortus antibodies can be accomplished by enzyme linked immunoassay (**Reynold, 1983 and Suther et al., 1986**). Other suggest that the procedure is superior to conventional methods for early detection of B.abortus serum antibodies (**Byrd et al., 1979 and Hassanen, 1989**).

The objective of the present investigation was to evaluate the serodiagnostic correlation of ELISA and conventional tests in calves vaccinated with S19 vaccine as a trial to evaluate their immune response before introducing these animals in breeding programs.

Received: 3 .5 .1994

## MATERIAL AND METHODS

A total of 120 animals aged from 4-8 months were selected from dairy herds. They were vaccinated with B. abortus S19 reduced dose (Cooper manufacture, kense, USA). Serum samples were collected from calves 21 days before vaccination. Then other samples were collected at 3 and 6 months after vaccination. Serum samples were kept at -20°C till testing. These samples were subjected to the followkng serological tests, standard agglutination test (SAT), 2 mercaptoethanol test (MET), Rivanol test (Riv. T), Complement fixaton test (CFT). These tests were performed according to **Alton et al., (1988)**. ELISA was done as recommended by **Ruppaner et al., (1980)**. The results were compared in terms of sensitivity, specificity and concordance **(Byrd et al., 1979)**.

## RESULTS

Before vaccination, all animals were negative by all serological tests used. Comparison of sensitivity of ELISA and conventional serological tests in the present study is presented in table (1).

**Table (1): Comparison of sensitivity of ELISA with other conventional tests in vaccinated calves with S19 vaccine.**

| Test / Item | No. of positive | No. of negative | sensitivity % |
|---|---|---|---|
| ELISA | 180 | 60 | 75 |
| SAT | 160 | 80 | 66.7 |
| MET | 40 | 200 | 16.6 |
| RIV. T | 20 | 220 | 8.3 |
| CFT | 40 | 200 | 16.7 |

NO. of samples : 240

The reesults revealed that sensitivity of ELISA for detecting B. abortus antibodies was greater (75%) than other tests. Whereas, the sensitivity of SAT, MET, Riv. T and CFT was 66.7, 16. 6, 8.3 and 16.7% respectivley.

The relative specificity had a wider range from 36.4% (Riv. T) to 100% (SAT) as shown in fig. (1) . In comparison of ELISA with each of the other tests, concordance was between 33.3% (Riv. T) and 100% (SAT) as shown in fig. (2). It was notable that the majority of animals reaching to negative status on all tests at 6 months postvaccination. Moreover, the postvaccinal titres at 4 month of age were lower and reduced to a negative status sooner than those vaccinated in 6 or 8 month of age.

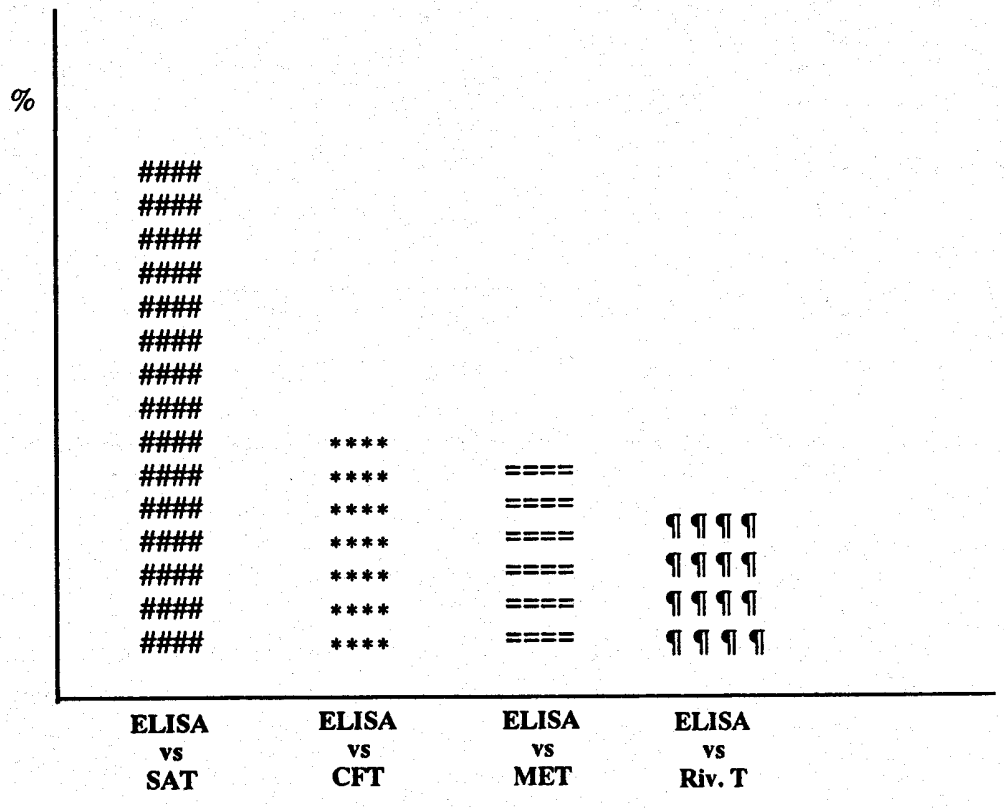


**Fig. 1: Comparison of relative specificity of ELISA to other conventional tests for brucella antibodies of vaccinatied calves.**

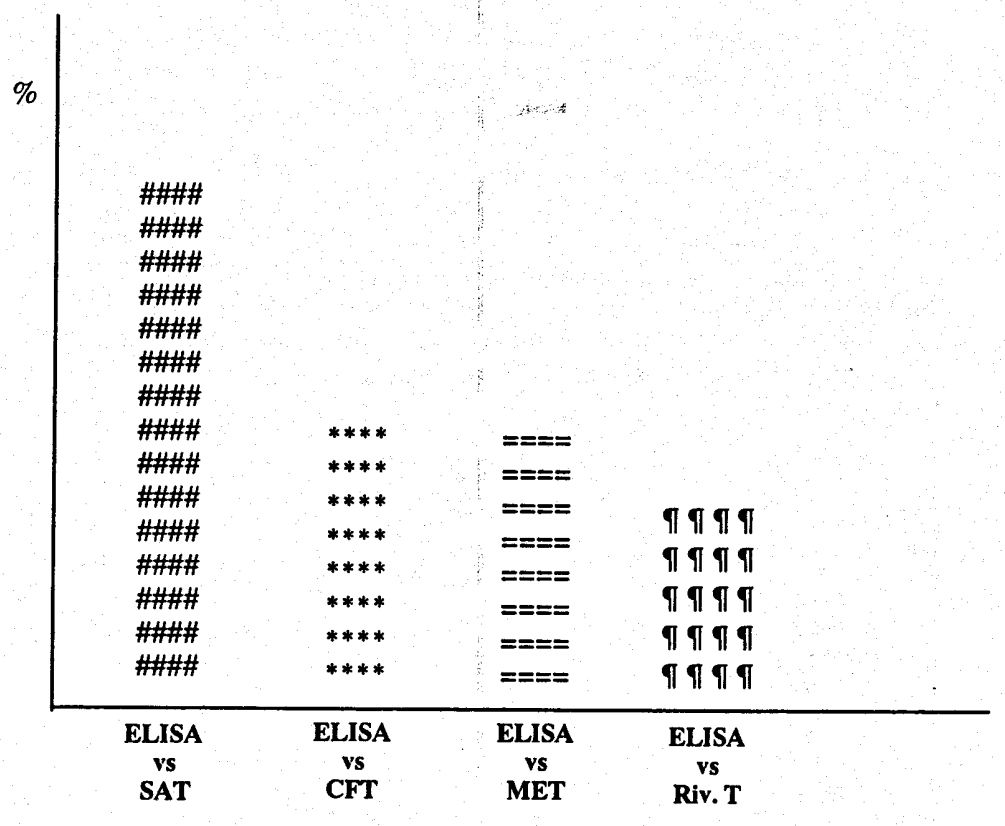


**Fig. 2: Comparison concordance results of ELISA with results from other serological tests.**

## DISCUSSION

The performance of ELISA was detected by comparing its sensitivity, spcificity and concordance with those of four conventional serological tests aiming to evaluate the immune response of vaccinated calves to S19 vaccine.

The apparent differences in sensitivity among serological methods for detecting brucella antibodies reflects inherent variables distinctive to various procedure. This may be due to the difference of classes of immunoglobulins detected by conventional serologic methods (**Sutherland**

1985). However, the sensitivity of these methods varies between tests. ELISA for bovine antibrucella IgG was delvelopged because the various agglutionation tests used for the serolgic diagnosis of bruceloosis in cattle have limited ability to detect brucella specific IgG1 and IgG2 (**Hassanen 1987**). This is substantiated by the results of the present study where the sensitivity of ELISA for detecting brucella antibodies was greater than other agglutination tests. Also, ELISA correlated at a higher degree with SAT and CFT. However, primary antigen antibody binding tests such ELISA are not thought to be affected by the prozone phenomenon that occurs with SAT and CFT (**Suther et al., 1986**). This advantage may further enhance its value as a diagnostic test. Our findings support the conclusions of **Shrivastava et al. (1991)** and **Kulkarni et al., (1991)** regarding usage of ELISA for detection of antibodies to B. abortus in bovine sera. It could be recommended to apply ELISA as a final test in calves vaccinated with S19 before introducing them in breeding programs. thus, ELISA can be a useful adition to the battery of serological tests.

## REFERENCES

**Alton, G. G.; Jones, L. M.; Angus, R. D. and Veger, J. M. (1988):** Technique for brucellosis laboratory. INRA, Paris, ISBN, France.

**Anderson, R. K.; Berma, D. T.; Berry, W.; Hopkin, J. A. and Wise, R. (1979):** Report of the National Brucellosis Technical Commision USDA, Aug. 28.

**Byrd, J. W.; Heck, R. J. and Hidalgo, F. C. (1979):** Evaluation of the enzyme linked immunosorbent assay for detecting brucella abortus antibodies. Am. J. Vet. Res. 40 896-898.

**Hassanen, A. S. (1987):** Some immunological studies in brucelosis. Ph. D. Thesis (Microbiology), Fac. Med., Assiut university.

**Kulkarni, S. B.; Khot, J. B.; Sherikar, A. A. and Joshi, M. M. (1991):** Detection of bruce lla antibodies in bovine by ELISA and its comparison with SAT and RBPT Indian Vet. Med. J. 15, 256 - 259.

**Nielson, K. (1984):** Comparative assement of antibody isotypes to brucella abortus by primary binding assay. Preventive Vet. Med. 2. 197-204.

**Reynold. S. L. (1983):** The use of ELISA and CFT in managing field outbreaks of brucella. Proceedings of the 87th Annual meeting of the USAHA 129-135.

**Ruppanner, R.; Meyer, M. E.; Willebery, P. and Behymer, D. E. (1980):** Comparison of ELISA with other tests for brucellosis, using sera from experimentally infected heifers. Am. J. Vet. res. 41, 1329-1332.

**Shrivestava, P. K.; Tongaonkar, S. S.; Mukherjee, F. and Rana, S. K. (1991):** A comparison of dot ELISA with other conventional tests for the serodiagnosis of bovine brucellosis. Indian J. of Animal Sciences 61, 123-125.

**Suther, D. E.; Cooper, R. S. and Vanderwagen, L. C. (1986):** description and evalua-

tion of an enzyme linked immunosorbent assay test used for brucella screening of dairy cattle. Proceedings of 19th annual meeting of USAHA, 167-176.

**Sutherland, S. S. (1985):** Comparison of ELISA and CFT for the detection of specific antibody in cattle vaccinated with B.abortus.J. of Clin. Microbiol. 22, 44-47.