Vet.Med.J.,Giza. Vol.44, No.1.(1996): 95-99.

RAPID DETECTION AND TITRATION OF RINDERPEST ANTIBODIES IN BOVINE SERA USING THE CHAMBER IMMUNOFLUORESCENT TECHNIQUE

SAMIA, A.A. AYAD; KHODEIR, M.H. and MOUAZ, M.A.

Rinderpest Res. Vacc. Dep., Vet. Ser. Vacc. Res. Inst., Abassia, Cairo.

SUMMARY

Chamber immunofluorescent technique (CIT) was compared to the serum neutralization (SNT) and enzyme-linked immunosorbent assay (ELISA) for the detection of rinderpest virus antibodies in bovine sera. ELISA was found to be the most sensitive test while CIT compared to SNT was more specific than ELISA. Also results of CIT were obtained in less than 2 hours. Moreover titers detected by CIT were comparable to those obtained by SNT. In conclusion, CIT was found to be a reliable, rapid, specific and sensitive technique for the detection of rinderpest antibodies in bovine sera.

INTRODUCTION

An increasing awareness of rinderpest in Egypt prompted a comparative evaluation of the techniques for the detection of rinderpest antibody in sera. Several techniques have been developed for screening the antibodies which have a dirct relevance to in-vivo immunity to rinderpest (Scott et al. 1986).

The serum neutralization test is considered the most reliable test for assessment of the immune response to tissue culture rinderpest vaccine as well as the sero-epidemiological studies among farm animals. Other serological techniques have been also employed in combination for obtaining presumptive results.

The indirect fluorescent antibody technique has been used by Liess and Plowright (1963), Liess (1966), Hassan (1987) and Afaf (1994) for detection of rinderpest antibodies in bovine sera.

Kobune et al. (1976) found that the IFA was sensitive as the SNT and detected rinderpest antibodies before neutralizing antibodies appeared. It was concluded that the IFA is a rapid and reliable method for detection of rinderpest antibodies (Prabhunda and Sambamurti, 1976).

Enzyme linked immunosorbent assay (ELISA) could be used for detecton of rinderpest antibodies in animal sera with a noticeable degree of sensitivity. It was found to be potentially rapid and economic for screening large number of sera for antibodies to rinderpest (Rossiter et al., 1981); (Anderson et al., 1982) and (Sharma et al., 1983). The speed of testing was considered important in selecting the method tested., other considerations to be taken into account were the sensitivity and specificity of the tests, cost and case of adaptation to automation. Three serological tests were selected for comparison: the serum neutralization test (SNT), the enzyme linked immunosorbent assay (ELISA) and the chamber immunofluorescent technique (CIT).

MATERIAL AND METHODS

1- Serum Samples:

The initial comparative exercise using SNT, IFA and ELISA was performed on 200 bovine serum samples collected from known vaccinated animals from different Egyptian Governorates.

2- Rinderpest virus (RPV):

Rinderpest Kabete (O) strain of RPV at its 99th passage on bovine kidney cell cultures (Singh et al., 1967) was used for SNT and preparation of the viral antigen. The vaccine was given by serum and Vaccine Research Institute. Dept. of Rinderpest. Abassia. Cairo, Egypt.

3- Rinderpest antigen:

It was prepared by harvestation of the supernatant fluic of infected Vero cells at full cytopathic effect. It was used as a viral antigen for ELISA.

4- Cell culture and media:

African green monkey kidney (Vero) cell culture was kindly supplied by NAMRU-3 Abbassia, Cairo, Egypt. The minimum essential medium with Hanks salts (Eagle, 1959) was used for cell culture passages. The medium was supplemented with new born calf serum (virus and mycoplasma free).

5- Serum neutralization test (SNT):

The test was performed as described by Rossiter and Jessett (1982).

6- Enzyme linked immunosorbent assay (ELISA):

The ELISA employed was based on the indirect method for the detection of antibody as described by Voller et al. (1976) and Anderson et al. (1982).

7- Chamber immunofluorescent technique (CIT):

The test was performed as described by Soliman et al. (1989), using multi-chamber slides.

RESULTS

The comparative results using the three tests on 200 bovine sera are shown in (Table 1). The ELISA test recorded more positive samples than the other two tests, while the least was recorded by the CIT. Table 2 and 3 demonstrated the evaluation of CIT and ELISA versus SNT for the detection of rinderpest antibody, with emphasis to the sensitivity, specificity and agreement between them.

The agreement between CIT and ELISA to SNT was 95% and 97% respectively. Although ELISA was found to be more sensitive than the CIT, the latter reported to be more specific, when both tests were compared to the SNT.

Titers obtained by SNT (mean value, 44.8), were higher than those obtained by CIT (mean value, 37.2), but the overall sensitivity in terms of positive and negative is comparable. (Table 4).

Table 1 : Results of SNT, CIT and ELISA for the detection of antibody in bovine sera

No. of Samples	SNT		CIT		ELISA	
	+ *	- **	+	-	+	-
200	108	92	98	102	112	88

* + = Detected of rinderpest antibodies
** - = Not detected of rinderpest antibodies

Table 2 : Results of CIT and ELISA compared to SNT for the detection of rinderpest antibody

SNT	CIT		ELISA	
	+	-	+	-
Pos. (108)	98	10	107	1
Neg. (92)	0	92	5	87

Table 3 : Specificity , sensitivity , positive and negative prediction values and agreement between CIT, ELISA and SNT

Test	Specificity (%)	Sensitivity (%)	Pos. Pred. Value (%)	Neg.Pred. Value (%)	Agreement (%)
CIT	102/92 (111)	98/108 (91)	98/98 (100)	92/102 (90)	190/200 (95)
ELISA	88/92 (96)	112/108 (104)	107/112 (96)	87/88 (99)	194/200 (97)

Table(4) : Titers obtained by SNT and CIT on twenty positive SNT sera

Sample no.	SNT titers	CIT titers
1	64	32
2	64	32
3	64	64
4	64	64
5	32	16
6	16	<16
7	64	32
8	64	32
9	64	64
10	64	64
11	64	32
12	16	<16
13	16	<16
14	32	16
15	64	32
16	32	16
17	64	64
18	64	32
19	64	64
20	64	64
Mean Value	44.8	37.2

DISCUSSION

A major problem in any comparative exercise is the optimization of the respective techniques in order that a fair comparison may be made. Rapid diagnosis of rinderpest disease is an essential requirement of any control or eradication program.

The used routine serological test was the SNT, which produces a result in a period not less than 7 days. The average duration from the commencement to the result of either CIT or ELISA was significantly less than that of SNT (2 to 4 hours, respectively). One of the problems inherent in the tissue culture based system such as the SNT is that sera which are cytotoxic because of hemolysis or contamination fail to give a result. (Saliki et al., 1993). Moreover the use of aseptic techniques in the SNT is, however, the only area in any of the other methods where the training and skill of the operator plays a significant role.

The efficiency of any control and in particular any eradication scheme depends largely upon the sensitivity of the technique emplayed for the diagnosis of the disease. ELISA has been recommended before (Libeau et al., 1994) as a suitable test for routine diagnosis of field specimens. The results obtained here indicated that the ELISA detected more positive than the SNT and CIT. But the use of a highly sensitive system such as ELISA introduces question of the specificity i.e. false positive, (Anderson et a al., 1982). Comparing the results obtained by ELISA and CIT to those obtained by SNT (The gold standard test), indicated that CIT is more specific than ELISA i.e. four sera were ELISA positive and not confirmed by SNT or even CIT. Approximate results were previously obtained by

Hassan (1987), Kobune et al., (1976) and Prabhunda and Sambamurti (1976). On the other hand, all CIT positive were confirmed by SNT. The overall agreement between CIT and ELISA with the SNT is high (95% and 97%, respectively), indicating that both tests are reliable for replacing the SNT.

CIT has several advantages (i) results are obtained in less than two hours (ii) more specific than ELISA but less sensitive than the SNT. (iii) hundreds of sera can be tested daily. (iv) virus-cell relationship can be easily demonstrated in intact sheet of cells. (v) the preparation of chamber slides is easier and faster than the preparation of purified antigen for ELISA.

In conclusion, CIT is a reliable, rapid, specific and sensitive test for the qualitative and quantitative detection of rinderpest antibody in bovine sera, for surveillance and diagnosis of rinderpest disease.

REFERENCES

Afaf; A.A. (1994): Studies on the immune status to rinderpest virus in different species of animals in Egypt. M. Vet. Med. Thesis (Infectious diseases). Fac. Vet. Med. Cairo Univ.

Anderson J.; Rowe L.W., Taylor W.P. and Crowther J.R. (1982): An enzyme-linked immunosorbent assay for the detection of IgG, IgA, IgM. antibodies to rinderpest virus in experimentaly infected cattle. Res. Vet. Sci. 32: 242-247.

Eagle, E. (1959): Amino acid metabolism in mammalian cell cultures. Science, 130: 432-437.

Hassan, H.M.M. (1987): Comparative studies on the diagnostic tests used for rinderpest. Ph. D. Vet. Thesis (Microbiology) Fac. Vet. Med. Cairo Univ.

Kobune, F., Ito, M. and Yamanouchi, D. (1976): Detection of antibody to rinderpest virus by indirect fluorescent antibody technique. Jap. J. Med. Soc. Biol. 29 (3), 171-176.

Libeau G.; Diallo A.; Colas F. and Guerre L. (1994): Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. Vet. Rec. 134: 300-304.

Liess, B. (1966): Studies on rinderpest virus by the use of cell culture. Arch. Exp. Vet. Med. 20; 157-202 and 203-257.

Liess, B. and Plowright, W. (1963): The propagation and growth characteristics of rinderpest virus in Hela cells. Arch. Ges. Virus forsch, 14; 27-38.

Prabhunda and Sambamurti, B. (1976): A note on fluorescent antibody technique for rapid diagnosis of rinderpest Ind. J. Anim. Sci. 46 (8) 454-457.

Rossiter P.B. and Jessett D.M. (1982): Microtiter techniques for the assay of rinderpest virus and neutralizing antibody. Res. Vet. Sci. 32: 253-256.

Rossiter, P.B., Jessett, D.M. and Holmes, P. (1981): Micro-ELISA test for detecting antibodies to rinderpest virus antigens Trop. Anim. Hlth. Prod. 13 No. 2, 113-116.

Saliki J.T. Libeau G.; House J.A.; Mebus C.a. and Dubovi E.J. (1993): Monoclonal antibody-based blocking enzyme-linked immunosorbent assay for specific detection and titration of peste des-petits rumminants virus antibody in caprine and ovine sera. J. Clin. Microbiol. 31: 1075-1082.

Scott, G.R.; Taylor, W.P. and Rossiter, P.B. (1986): Manual on the Diagnosis of Rinderpest FAO, Agriculture studies, Italy, 11: 199-205.

Sharma, B.; Joshi, R.C.l Bansal, R.P. and Lahiri, D.K. (1983): Evaluation of enzyme linked Immuno Sorbent Assay in rinderpest. Ind. J. Vet. Sci. 53: 1292-1295.

Singh K.V.; Osman, O.A., Ivon, F.E. and Thanaa, I. Baz. (1967): The use of tissue culture rinderpest vaccine for Egyptian cattle and water buffaloes. Corn. vet., 57 (2): 465-479.

Soliman A.K.; Botros B.A.M. Ksiazek T.G.; Hoogstraal H.; Helmy I. and Morrill J.C. (1989): Seroprevalence of Rickettsia typhus and Rickettsia conrii infection among rodents and dogs in egypt. J. Trop. Med. Hyg. 92: 345-349.

Voller A., Bidwell D.E. and Bartlett A. (1976): Enzyme immunoassays in diagnostic medicine. Bull W.H.O. 53: 55-65.