THE USE OF A LIQUID PHASE BLOCKING ELISA KIT FOR DETECTION OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS IN COLOMBIA

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Abstract

THE USE OF A LIQUID PHASE BLOCKING ELISA KIT FOR DETECTION OF ANTIBODIES AGAINST FOOT-AND MOUTH DISEASE VIRUS IN COLOMBIA.

The objective of this study was to undertake an interlaboratory comparison of a liquid phase blocking ELISA for detection of antibodies to FMD virus. For that purpose sera from 120 vaccinated, 120 infected and 120 FMD negative cattle were tested. All sera were tested in a screening assay at a dilution of 1/32. Positive sera were tested in a titration assay (1/10, 1/50, 1/250, 1/1250). For serotype O_1 Cruzeiro 108 sera from the FMD-free group were classified as negatives giving a specificity of 90%. For the same serotype the group of infected/ vaccinated cattle gave 114/115 positive results showing a sensitivity of 95% respectively 96%. For serotype A_{24} Cruzeiro from the FMD-free group 85 sera were classified as negatives giving a specificity 71%. For the same serotype the group of infected/ vaccinated cattle gave 90/99 positive results showing a sensitivity of 75% respectively 82%. The predictive value of the assay was good as results expected for the different serum categories were mainly confirmed in the test. Nevertheless a high number of plates were rejected due to "outside limits" and further adjustments are necessary to obtain more reliable results.

1. INTRODCUTION

In some regions of Colombia foot-and-mouth disease virus serotypes O_1 Campos and A_{24} Cruzeiro exist endemically. At present the country is involved in the hemispheric foot-and-mouth disease eradication plan. To achieve this objective it is necessary to use techniques with a higher sensitivity and specificity than the traditional diagnostic serological tests [1,2,3,4,5]. The use of a liquid phase blocking ELISA, LPBE is of great benefit in areas, where FMD prevention, control and eradication programs are carried out. The LPBE provides more reliable results because it is very sensitive and specific. Other advantages are the fast delivery of results - usually within the same day - and the fact that the technique is easy to perform and does not require special laboratory conditions e.g. cell culture or CO_2 environment.

2. MATERIAL AND METHODS

The assay is based on specific blocking of a defined amount of FMDV antigen by antibodies in the test sample during the liquid phase [6,7]. After the test serum is allowed to react with specific FMDV antigen, the test serum/antigen mixture is transferred to an ELISA plate coated with FMDV serotype specific trapping antibodies. The presence of antibodies to FMDV in the serum sample will result in the formation of immune complexes and consequently reduce the amount of free antigen trapped by the immobilized rabbit antisera. In turn, less amount of guinea pig anti-FMDV detecting antibodies will react in the next incubation step. After the addition of enzyme labeled (horseradish peroxidase, HRP) anti-guinea pig immunoglobulin and substrate/chromogen solution a reduction of color development will be observed when compared to control containing free antigens only. The bench protocol of the Joint FAO/IAEA Division was followed [8].

A total of 360 sera from cattle were tested from 3 different categories as shown below:

- 120 bovine sera from free areas of FMD (provided by CPFA)
- 120 bovine sera from vaccinated cattle with trivalent vaccine (provided by CPFA)
- 120 bovine sera from FMD infected animals obtained from outbreaks which naturally occurred in different regions of Colombia.

3. RESULTS

For serotype O_1 Cruzeiro 108 sera from the FMD-free group were classified as negatives giving a specificity of 90%. For the same serotype the group of infected/ vaccinated cattle gave 114/115 positive results showing a sensitivity of 95% respectively 96%. For serotype A_{24} Cruzeiro from the FMD-free group 85 sera were classified as negatives giving a specificity 71%. For the same serotype the group of infected/ vaccinated cattle gave 90/99 positive results showing a sensitivity of 75% respectively 82% (Table I).

TABLE I. RESULTS ACCORDING TO GROUP OF SERA AND SEROTYPE

Bovines	Samples	O Virus			A Virus		
		P	N	R	P	N	R
Free	120	5	108	7	21	85	14
Infected	120	114	4	2	90	15	15
Vaccinated	120	115	0	5	99	10	11

P = positive
N = negative
R = retest

A high number of plates was classified "outside limits" because the positive serum controls were out of the upper and lower limits, although the negative serum control and the antigen control were within limits.

4. CONCLUSIONS AND DISCUSSION

Although most of the plates were rejected due to "outside limits" the predictive value of the assay remained good as results expected for the different serum categories were confirmed in the test as shown in Table I.

Further adjustment for the upper and lower control limits is necessary to obtain reliable results. It could be observed that the problem was more noticeable for the disease free sera, where a small percentage of the samples were positives. In the case of sera from infected animals, the highest percentage was positive. A similar result was observed in the group of sera from vaccinated animals.

In the group of sera from infected animals positive results were obtained for both serotypes. The reason for this is that these animals live in FMD endemic areas where additionally vaccination is carried out.

Once having standardized this technique it will be used as a routine test all over the country to monitor the success of the vaccination programme, which is being applied systematically every six months.

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