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DIFFERENTIATION BETWEEN INFECTED, REPEATEDLY VACCINATED AND FREE ANIMAL WITH FMD VIRUS USING BIO-ENGINEERED VIA

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SUMMARY

Sera from infected, vaccinated and negative cattle were tested using 3D ELISA to allow each category of animals to be clearly differentiated based on the presence of antibody to 3D antigen. The assay can recognize infected animals in countries where FMD outbreaks have occurred, specially if ring vaccination has been performed, also at the same time vaccinated animals can become infected without apparent clinical symptoms. 3D ELISA test is simple and rapid to discriminate between antibodies elicited by FMD infection or by vaccination.

INTRODUCTION

Differentiation of infection from vaccination based on the detection of antibody to non-structural proteins of FMD virus, assays that detect antibody to the non-structural proteins of FMD virus to achieve an actual solution (Berger et al., 1990; Neitzert et al., 1991; Bergman et al., 1993; Lubroth and Brown, 1995). Antibodies to the capsid proteins are induced by both vaccination and infection. It is not therefor possible to differentiate animals that have been infected from those that have been vaccinated by the detection of antibody to structural proteins, non-structural proteins have the possibility to detect previous exposure to FMD virus type. Several authors used 3D protein expressed as recombinant antigen (Villinger et al., 1989; Mackay et al., 1994; O'Donnel et al., 1996). However these assays still suffer from several limitation, such as low specificity and extensive antigen purification procedures.

MATERIAL AND METHODS

1 - Preparation of recombinant proteins:

The sequence corresponding to the 3CD region of FMD virus type "O" was amplified by PCR and cloned into pET21a-d vector (fig.1) and transformed to expression host induced by IPTG to express the protein of the insert sequence. The molecular weight of the final products were confirmed by polyacrylamide gel elctrophoresis (PAGE) and by liquid phase blocking sandwich ELISA using two reference polyclonal antibodies (O'Donnel et al., 1996).

2-ELISA:

Indirect ELISA was developed for the detection of antibody to FMD virus 3D in cattle sera. Coating of ELISA plate with antigen using carbonate bicarbonate buffer, pH 9.6 by incubation at 4°C overnight, then washing with phosphate buffered saline containing 0.05% tween 20, non specific protein binding sites were blocked by incubation for one hour at 37°C, blocking buffer (1% lactalbumin in PBS with "tween 20" 0.05%) 50 µl per well, 50 µl of each test. Serum was added to two wells and plates were incubated for a further hour at 37 O°C. Guinea pig reference hyperimmune was added to each well in the same time negative control with negative serum was made, incubated for one hour, conjugate was added anti -guinea pig conjugated with horse radish peroxidase enzyme for a further hour at 37°C. Finally, the reaction was developed using 50 µl per well orthophenyl diamine (OPD) and 0.005% H_2O_2. The reaction was stopped after 15 minutes by adition of 50 µl per well 1.25 M H_2SO_4 and the absorbence of well was read at 492 nm.

3-Agar Gel Immunodiffusion test:

The test was made using bio-engineered VIA antigen on central wells and sera samples and positive control sera were placed peripherally in agar 1%, plates were incubated at room temperature and read after 48 hours (McVicar and Sutmoller, 1970).

4-Serum samples:

Hundred serum samples from FMD free area, free of antibodies to type "O" of FMD virus measured by serum neutralization test and ELISA to establish specificity of the test.

One hundred and twenty sera samples from experimentally infected cattle from 7^{th} day post infection until 3 months after infection, also 12 sera samples from experimentally infected sheep.

One hundred and fifty serum samples collected from cattle after several vaccinations with inactivated alhydrogel adjuvant vaccine type "O_1".

RESULTS

All 100 negative cattle sera gave negative result by 3D ELISA. More than 80% of negative cattle sera showed titer below log10 = 0.3 and the remaining

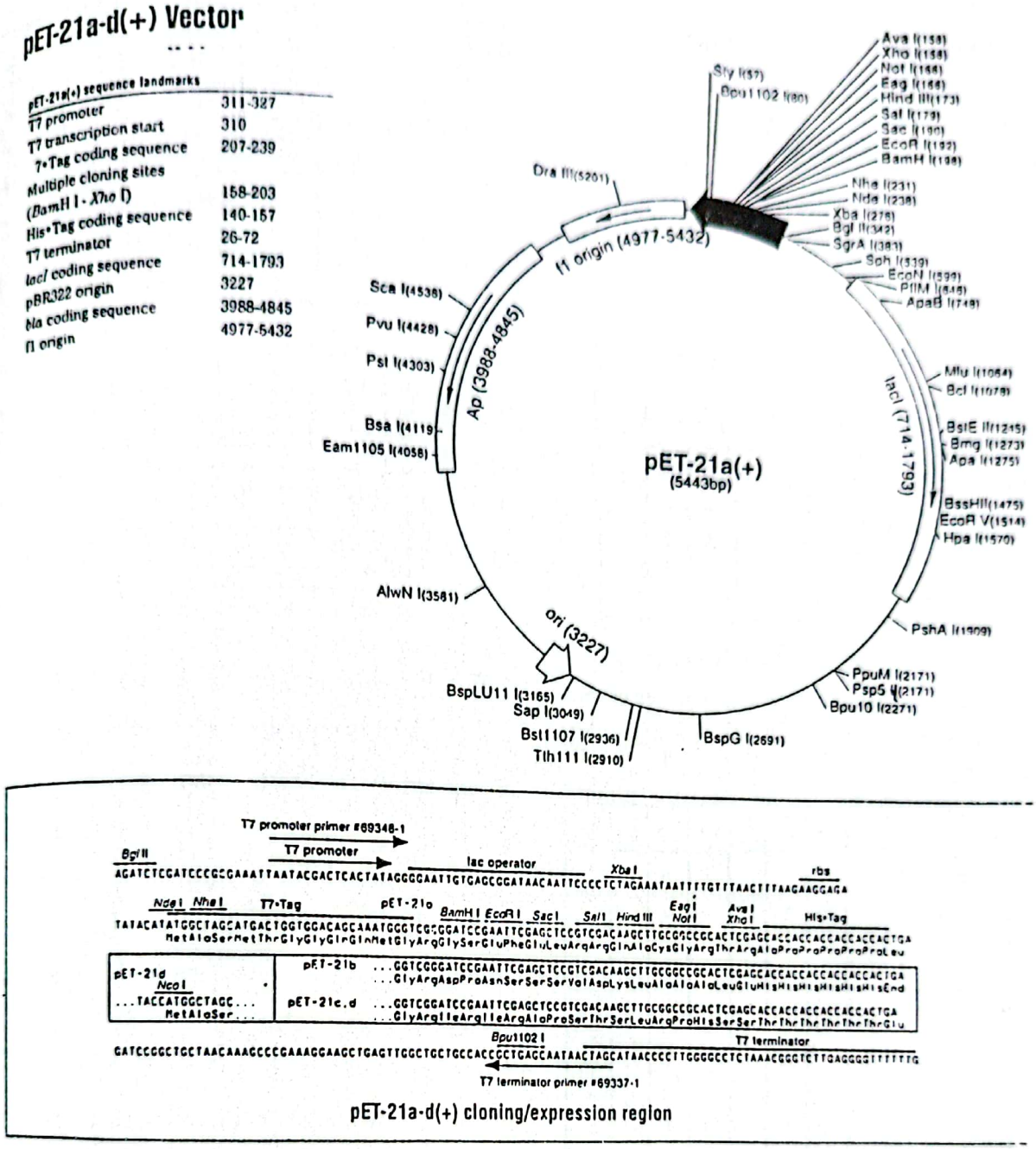

Fig 1. pET 21 a-d vector

Table (1): Comparative specificity and sensitivity of 3D ELISA and AGID

Assay	Cattle sera from FMD free region	Sera from infected cattle
3D ELISA	0/100 (0%)	115/120 (95.8%)
AGID	0/100 (0%)	70/120 (58.3%)

Table (2): Frequency of 3D ELISA titers of 100 negative sera

Log_{10}	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Number of animals	80	10	3	--	5	2	--

Table (3): Frequency distribution of log10 values of sera from negative, vaccinated and infected in 3D ELISA

Log10	0.3	0.4	0.5	0.6	0.7	0.8	1.0	1.1	1.2	1.3	1.4
No. of negative animals	80	10	3	--	80	2	--	--	--	--	.
No. of vaccinated animals	10	20	40	18	--	62	--	--	--	--	--
No. of infected animals	--	--	--	--	--	--	4	16	95	5	--

percent between log10 = 0.3 and log10 = 0.8-0.9 (fig.2).

The sensitivity and specificity of ELISA 3D, and AGID is shown in (table 1).

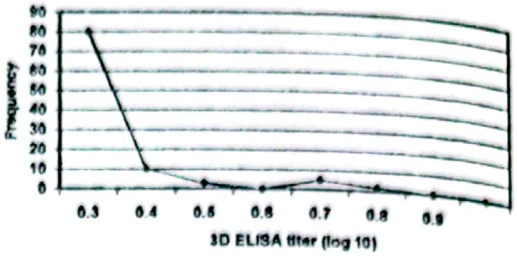

Fig 2: Frequency of 3D ELISA titers of 100 negative sera.

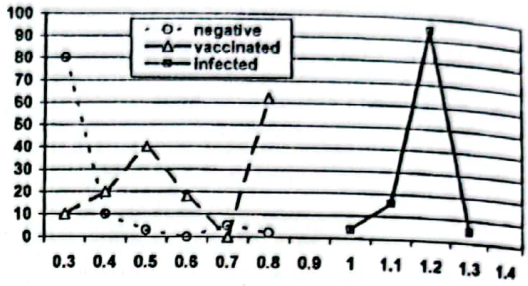

Fig 3: Frequency distribution of log10 values of sera from negative, vaccinated and infected in 3D ELISA.

Sera samples from experimentally infected cattle showed antibodies to 3D protein from 7th day post infection until the last sample 3 months post infection.

Antibody response to bio-engineered VIA antigen after vaccination, sera from cattle vaccinated with inactivated alhydrogel adjuvant vaccine showed high titers of antibodies by SNT and ELISA.

3D antibodies were detected in about 60% of repeated vaccinated cattle and the level of 3D antibodies in repeated vaccinated cattle less than the threshold level of 3D antibodies detected in infected animals and positive control (fig.3).

2DISCUSSION

The antigenicity of the 3D protein is conserved amongst all FMD virus serotypes. Anti-VIA antibody response induced by virus infection may be detected at least for 6 months and persists for 1-2 years after infection. However, inactivated vaccine preparations produced from virus infected cultures contain only low levels of VIA antigen and antibody responses of VIA antigen after vaccination are therefore generally low and transient (Alonso et al., 1988; Pinto and Garland, 1979).

The assay uses bio-engineered antigen that does not represent a biohazard therefore reducing the amount of manipulation of live FMD virus that is required for serology.

The following criteria were defined for differentiating between sera from negative, infected and vaccinated cattle based on their antibody status with regard to structural and non-structural antigens.

Sera from negative or free animals were negative for antibody to both structural and non-structural proteins. The proportion of sera from negative animals showed a low non-specific reaction against 3CD protein (as showed in fig. 2).

Sera from infected animals were positive for antibody to both the structural proteins and non-structural proteins (3CD).

Sera from vaccinated animals were positive for antibody to structural proteins and the majority was also positive for antibody to 3CD but not reach the threshold level of infected animals (as shown in fig.3).

The assay described have represents a rapid, simple and reproducible method of differentiating animals that have been infected from those that have been merely vaccinated.

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REFERENCES

Alonso Fernandez,A.; Gomes,I. And Bahnemann,H.G. (1988): Bol.Centro Panam Fiebre Aftosa. 54: 43-50.

Berger,H.G.; Straub,O.C.; Ahl,R.; Tesar,M. and Marquardt,O. (1990): Identification of foot anf mouth disease virus replication in vaccinated cattle by antibodies to non-structural virus proteins .Vaccine. 8, 213-216.

Bergmann,I.E.; Auge de Mello,P.; Neizert,E.; Becke,E. and Gomes,I. (1993): Diagnosis of persistant aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme linked immunotransfer blot analysis with bio-engineered non-structural viral antigens

. Am.J.Vet.Res. 54, No.6, 825-831.

Lubroth,J. and Brown,F. (1995): Identification of native foot and mouth disease virus non-structural protein 2C as a serological indicator to differentiate infected from vaccinated livestock . Res. in Vet. Science. 59, 70-80.

Neitzert,E.; Beck,E.; Auge de Mello,P.; Gomes,I. And Bergmann,I.E. (1991): Expression of the Aphthovirus RNA polymerase gene in Escherichia coli and its use together with other bio-engineered non-structural antigens in detection of late persistant infection . Virology. 184, 799-809.

Mackay,D.; Forsyth,M. and Davis,P. (1994): as a diagnostic antigen in ELISA . Rep. Sess. Res. Group Euro-Comm. Contr. FMD. Vienna 1994.

McVicar, J.W. and Sutmoller,P. (1970): Am.J.Epidemio. 92,4:273-278.

Pinto,A.A. and Garland,A.J. (1979): J.Hyg. 82,41-50.

O'Donnel,V.K.; Boyle,D.B.; Sprout, K.; Fondevil,N.A.; Forman, A.; Schudel, A. and Smitsart, E.N. (1996): Detection of antibodies against foot and mouth disease virus using a liquid phase block sandwich ELISA (LBPE) with a bio - engineered 3D protein.

J. Vet. Diagn.Invest .8, 143 - 150.

Villinger,F.; Muller,H.K.; Bruckner, L.; Ackerman,M. and Kihm,U. (1989): Antibodies to foot and mouth disease virus infection associated (VIA) antigen: use of bioengineered VIA protein as antigen in an ELISA . Vet.Microbiol. 20, 235-246.