Haryana Vet. **47** (December, 2008), pp 28-31

Research Article

RETROSPECTIVE DIAGNOSIS OF FOOT AND MOUTH DISEASE OUTBREAKS BY LIQUID PHASE BLOCKING ENZYME LINKED IMMUNOSORBENT ASSAY

N. K. KAKKER and R. SHARMA[1]
Regional Research Centre on Foot and Mouth Disease
Department of Veterinary Microbiology, College of Veterinary Sciences
CCS Haryana Agricultural University, Hisar -125 004

ABSTRACT

A total of 82 serum samples from 30 foot and mouth disease (FMD) outbreaks occurring in Haryana between January 2002 and December 2003 were analyzed retrospectively for antibody titres using liquid phase blocking enzyme linked immunosorbent assay (LPB-ELISA) against FMD virus serotypes O, A and Asia1. These outbreaks were retrospectively diagnosed and causative agent categorized as belonging to FMD virus serotype O (13), A (10) and Asia1 (1), while in six outbreaks no conclusive FMD virus serotype could be established. Further, of these 30 outbreaks, clinical samples of tongue epithelium were also available for FMD virus serotyping from 12 outbreaks and involved FMD virus serotypes O (10) and A (2) as analyzed by sandwich ELISA. The findings of the retrospective studies on 34 serum samples from these 12 outbreaks using LPB-ELISA corroborated well with the FMD virus serotyping results using sandwich ELISA: Thus LPB-ELISA can be used as a test for establishing the retrospective diagnosis of FMD virus serotype involved in a particular outbreak where suitable clinical sample(s) are not available.

Key words: Retrospective diagnosis, foot and mouth disease, FMD outbreaks, LPB-ELISA, sandwich ELISA

Foot and mouth disease (FMD) has a great potential for causing severe economic losses in susceptible cloven-hoofed animals. Typical clinical cases of FMD are characterised by vesicles formation on feet, buccal mucosa and teats in females. In laboratory, the demonstration of FMD viral antigen in affected tissues or saliva of diseased animals is sufficient for its diagnosis. Serological diagnosis using monoclonal or polyclonal antisera to different FMD virus serotypes is also a valuable tool. Enzyme-linked immunosorbent assay (ELISA) has replaced most of the conventional methods of FMD diagnosis and has the advantage of being faster, not dependent on cell culture, reliable and reproducible. Nucleic acid recognition tests, such as the polymerase chain reaction (PCR), *in situ* hybridisation and real time PCR are being used increasingly as rapid and sensitive diagnostic methods.

For routine diagnosis and serotyping of FMD virus from clinical epithelial tissues, double antibody sandwich ELISA and LPB – ELISA are tests of choice being used for sero-monitoring of antibody response to FMD virus serotypes (Hamblin *et al.*, 1986) specially, when it becomes difficult to collect suitable clinical material due to either late receipt of information about FMD outbreak or mild/ sub-clinical form of the disease in animals.

This communication describes the retrospective diagnosis of FMD outbreaks reported during January 2002 and December 2003 using both techniques.

MATERIALS AND METHODS

Serum samples: Eighty two serum samples were collected from FMD virus affected/ convalescing cattle, buffalo and sheep from 30 FMD outbreaks reported between January 2002 and December 2003. These samples were collected from 12 districts of Haryana during routine surveillance trips/ FMD outbreaks and the animals had a history of absence of FMD virus vaccination. Out of these 30 outbreaks, clinical samples of tongue/gum epithelium were also collected for serotyping of FMD virus from

[1]Corresponding author

12 outbreaks.

Liquid Phase Blocking ELISA: The serum samples were processed for analysis of antibodies against FMD virus serotypes O, A and Asia1 using single dilution (1:128) LPB-ELISA (Hamblin *et al.*, 1986). The serum samples demonstrating >2.1 $\log_{10}$ antibody titres against a particular FMD virus serotype was taken as positive for the presence of antibodies against that particular FMD virus serotype in retrospective diagnosis.

Sandwich ELISA: The epithelial samples collected from the clinical cases of outbreak were processed for FMD virus serotyping by sandwich ELISA (Bhattacharya *et al.*, 1996) with slight modifications as described by Sharma and Kakker (2005).

RESULTS AND DISCUSSION

The year wise distribution of retrospective FMD outbreaks during 2002 and 2003 and number of serum samples processed by LPB-ELISA are presented in Table 1. Of the 30 outbreaks, sera sample showed >2.1 $\log_{10}$ antibody titres in 24 outbreaks against FMD virus serotypes (O: 13, A: 10, Asia1: 1). In the remaining six outbreaks, no conclusive FMD virus serotype could be established.

The district wise distribution of FMD outbreaks diagnosed retrospectively, number of samples processed and FMD virus serotypes involved in 12 districts of Haryana during 2002 and 2003 are presented in Table 2. Maximum numbers of outbreaks were retrospectively diagnosed as caused due to FMD virus in Hisar (9 outbreaks). Of the 82 sera sample collected from these 30 outbreaks, 30 samples were found to demonstrate antibodies against FMD virus serotype O, 19 against A and three against Asia1 by LPB-ELISA. In the remaining 30 sera sample no conclusive FMDV serotype(s) could be established.

The species wise distribution of retrospectively diagnosed FMD outbreaks is presented in Table 3. Of the 30 FMD outbreaks,

Table 1

Year wise distribution of retrospective FMD outbreaks and serotypes involved between January 2002 and December 2003

Year	No. of samples	No. of outbreaks	Virus types involved			VNE*
			O	A	Asia 1	
2002	20	5	4	-	1	-
2003	62	25	9	10	-	6
Total	82	30	13	10	1	6

*VNE = No conclusive FMD virus serotype established

Table 2

District wise incidence of retrospective FMD outbreaks in Haryana and serotypes involved between January 2002 and December 2003

District	No. of outbreaks	No. of samples	FMDV serotype(s) involved			VNE*
			O	A	Asia 1	
Ambala	1	2	0	0	0	2
Bhiwani	1	5	3	0	0	2
Fatehabad	2	4	0	0	0	4
Gurgaon	1	2	1	0	0	1
Hisar	9	21	3	12	0	6
Jhajjar	2	9	2	1	0	6
Jind	2	4	2	0	0	2
Karnal	2	6	5	1	0	0
Kurukshetra	2	6	0	4	0	2
Rohtak	5	12	8	1	0	3
Sonipat	1	3	0	0	3	0
Yamuna Nagar	2	8	6	0	0	2
Total	30	82	30	19	3	30

*VNE = No conclusive FMD virus serotype established

Table 3

Species wise distribution of serum samples from retrospective FMD outbreaks and serotype involved between January 2002 and December 2003

Species	No. of outbreaks	No. of samples	Virus type involved			VNE*
			O	A	Asia 1	
Cattle	5	11	8	-	-	3
Buffalo	13	29	7	9	3	10
Sheep	2	8	4	-	-	4
Cattle and Buffalo	10	34	11	10	-	13
Total	30	82	30	19	3	30

*VNE = No conclusive FMD virus serotype established.

five involved cattle only, 13 involved buffalo only and two outbreaks involved sheep only. The remaining 10 outbreaks involved both cattle and buffalo simultaneously.

The clinical samples of tongue epithelium from 12 outbreaks demonstrated the involvement of FMD virus serotype O and A in 10 and two outbreaks, respectively by sandwich ELISA (Table 4). The findings of the retrospective studies on 34 serum samples from these 12 outbreaks using LPB-ELISA corroborated well with the FMDV serotyping results using sandwich ELISA in 10 outbreaks. However, in one FMD outbreak in Jhajjar district, the clinical material demonstrated the presence of FMD virus serotype O, whereas the serum sample tested by LPB-ELISA demonstrated antibodies both against FMD virus serotypes O and A. Hence, no conclusive FMD virus serotype could be established from this particular outbreak on the basis of LPB-ELISA. Likewise, in another FMD outbreak in Bobua, Hisar, the clinical material demonstrated the presence of FMD virus serotype O, whereas none of the serum samples tested by LPB-ELISA demonstrated antibodies against any of the FMD virus serotypes and thus, the virus type assigned was FMD virus serotype O on the basis of sandwich ELISA. Of the 12 outbreaks, the FMD virus types identified retrospectively were found to be belonging to FMD virus serotype O (9), A (2), and from one outbreak no virus serotype could be established (Table 4).

Hamblin *et al.* (1986, 1987) developed LPB-ELISA for measuring antibodies against FMD virus in sera from sheep and cattle for evaluation of the immunological response of animals following infection as well vaccination and compared results with virus neutralization test (VNT). The ELISA was considered more reliable and useful for evaluation of the immunological response of animals following infection as well vaccination (Hamblin *et al.*, 1987). Araujo *et al.* (1996) also developed LPB-ELISA for the quantification of antibodies against three FMDV strains and compared with VNT in 158 water buffaloes from various premises of Sao Paulo State-Brazil. These results also indicated that the LPB-ELISA might replace the conventional VNT for detection and quantification of antibodies to FMDV.

Alonso *et al.* (1992) and Blacksell *et al.* (1994) reported indirect sandwich ELISA for the detection and serotyping of FMDV antigen. Callens *et al.* (1998) described a test for detection of FMDV by RT-PCR and virus isolation in in-contact sheep without clinical signs of FMD. Mackay *et al.* (2001) reported a solid-

Table 4

Comparative analysis of FMD virus serotyping by sandwich ELISA and LPB ELISA

Village/ District	FMD virus type involved		Remarks
	Sandwich ELISA	LPB ELISA (no. tested)	
Yamuna Nagar	O	O (5)	Type O
Fatehabad	O	O (2)	Type O
Rohtak	O	O (4)	Type O
Jind	O	O (2)	Type O
Bhojraj, Hisar	O	O (3)	Type O
Assandh, Karnal	O	O (4)	Type O
Jhajjar	O	O, A (3)	VNE*
Tarawari, Karnal	O	O (2)	Type O
Badala, Hisar	A	A (1)	Type A
Bass, Hisar	A	A (4)	Type A
Bobua, Hisar	O	- (2)	Type O
Dhana, Hisar	O	O (2)	Type O

*VNE = No conclusive FMD virus serotype established

phase competitive ELISA for measuring antibodies in an extensive range of sera from cattle. Likewise, Chenard *et al.* (2003) developed a solid-phase blocking ELISA for mass serology for the detection of antibodies against FMDV serotype O in sera collected from non-infected cattle, pigs and sheep.

In mass vaccinated population, the antibody titres detected in LPB-ELISA might interfere with the retrospective diagnosis in convalescent/ recovering animals. Therefore, before considering this test for FMD virus serotyping, information on vaccination history be ensured as in some cases the antibody titres may be due to vaccination. Moreover, diagnosis based on serological response may also be problematic in endemic areas due to the possibility of previous infection. Detection of type specific antibody along with history of absence of vaccination can be used for retrospective diagnosis in serum samples of recovered/convalescent animals. This is particularly useful in mild cases or where epithelial tissue could not be collected. Based on the findings of the retrospective studies on FMD outbreaks in the present study by measuring antibody titres in serum samples and FMDV serotyping results along with the history of absence of vaccination, it is suggested that LPB-ELISA can be employed for establishing the FMD virus type involved in a particular outbreak where suitable clinical sample(s) are not available.

Acknowledgements

The authors wish to thank the Indian Council of Agricultural Research (ICAR), New Delhi, Project Directorate on FMD, IVRI Campus, Mukteswar-Kumaon (Uttrakhand) and the CCS Haryana Agricultural University, Hisar for providing funds and facilities. The authors are also thankful to the Director General and Field Officers of the Department of Animal Husbandry and Dairying, Govt. of Haryana for help in collecting FMD specimens. The help rendered by the laboratory staff particularly, Sh. Narender Paul, VLDA and Chandan Singh, V. L. A. is also acknowledged.

REFERENCES

Alonso, A., Martins, M.A., Gomes, M.P., Allende, R. and Sondahl, M.S. (1992). Foot and mouth disease virus typing by complement fixation and enzyme-linked immunosorbent assay using monovalent and polyvalent antisera. *J. Vet. Diagn. Invest.* **4**: 249-53.

Araujo, J.P., Montassier, H.J. and Pinto, A.A. (1996). Liquid-phase blocking sandwich enzyme-linked immunosorbent assay for detection of antibodies against foot and mouth disease virus in water buffalo sera. *Am. J. Vet. Res.* **57**: 840-43.

Bhattacharya, S., Pattnaik, B. and Venkataramanan, R. (1996). Development and application of sandwich enzyme-linked immunosorbent assay (ELISA) for type identification of foot and mouth disease (FMD) virus in direct field materials. *Indian J. Anim. Sci.* **66**: 1-9.

Blacksell, S.D., Lunt, R.A., Chamnanpood, C., Linchongsubongkoch, W., Nakarungkul, N., Gleeson, L.J. and Megkamol, C. (1994). Establishment of a typing enzyme-linked immunosorbent assay for foot and mouth disease antigen, using reagents against viruses endemic in Thailand. *Rev. Sci. Tech.* **13**: 701-709.

Callens, M., De Clercq, K., Gruia, M. and Danes, M. (1998). Detection of foot-and-mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot-and-mouth disease. *Vet Q. 20 Suppl.* **2**: S37-40.

Chenard, G., Miedema, K., Moonen, P., Schrijver, R.S. and Dekker, A. (2003). A solid-phase blocking ELISA for detection of type O foot-and-mouth disease virus antibodies suitable for mass serology. *J. Virol. Methods* **107**: 89-98.

Hamblin, C., Barnett, I.T. and Crowther, J.R. (1986). A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouth disease virus. II. Application. *J. Immunol. Methods* **93**: 123-29.

Hamblin, C., Kitching, R.P., Donaldson, A.I., Crowther, J.R. and Barnett, I.T. (1987). Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouth disease virus. III. Evaluation of antibodies after infection and vaccination. *Epidemiol. Infect.* **99**: 733-44.

Mackay, D.K., Bulut, A.N., Rendle, T., Davidson, F. and Ferris, N.P. (2001). A solid-phase competition ELISA for measuring antibody to foot and mouth disease virus. *J. Virol. Methods* **97**: 33-48.

Sharma, R. and Kakker, N.K. (2005). Scenario of foot-and-mouth disease outbreaks in Haryana state during the years 2003 and 2004. *Haryana Vet.* **44**: 47-51.