LETTER TO THE EDITOR

COMPARISON OF DIFFERENT METHODS OF ROUTINE TYPING OF INDIAN ISOLATES OF FOOT-AND-MOUTH DISEASE VIRUS

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Received March 15, 2006; accepted October 23, 2006

Key words: ELISA; Foot-and-mouth disease virus; RT-PCR, SNAP hybridization

Several methods of routine typing of Foot-and-mouth disease virus (FMDV) have been so far developed: various types of ELISA (*1–2*), an RT-PCR using serotype-specific oligonucleotide primers (*3*), and a RT-PCR targeting the highly variable region of genome combined with a single nucleotide aqueous phase (SNAP) hybridization ELISA (SNAP hybridization ELISA) (*4*).

In the present study, we compared ELISA (*1*), RT-PCR, and RT-PCR with SNAP hybridization ELISA. In the latter method, an asymmetric RT-PCR and multiple biotin-labeled oligonucleotide hybridization probes detected by ELISA were employed. Since the originally described probes for FMDV types A, C and Asia1 (*4*) failed to hybridize with the Indian isolates, they had to be redesigned (data not shown). The three methods were applied to 93 original samples of tongue or feet epithelial cells from positive FMD cases and samples of cell culture supernatants originating from virus isolation tests in which original samples were inoculated into primary cultures of calf thyroid (CTY) or calf kidney (CK) cells (*6*). The virus isolation test on CTY or CK cells evaluated according to CPE was used as reference as both the cells are susceptible to FMDV.

The results showed that 85 of 93 original samples were positive by the virus isolation test. ELISA was positive for 18% of original samples, but for 64% of cultured samples. The RT-PCR positivity increased from 53% for original samples to 74% for cultured samples. Even though the positivity of RT-PCR with SNAP hybridization ELISA for original samples was very high, 80%, it increased to 86% for cultures samples.

Summing up, virus isolation in primary cultures of CTY or CK cells increased the percentage of of positive samples detected by ELISA, RT-PCR and RT-PCR with SNAP hybridization ELISA from 18.28% to 64.52%, from 53.76% to 74.19%, and from 79.57% to 86.02%, respectively. Hence we recommend the virus isolation combined with RT-PCR with SNAP hybridization ELISA for typing FMDV isolates in Indian conditions.

References

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Abbreviations: CK = calf kidney; CTY = calf thyroid; FMDV = Foot-and-mouth disease virus; SNAP = single nucleotide aqueous phase

FMDV	Original samples			Cell cultured samples		
	ELISA	RT-PCR	RT-PCR with SNAP hybridization ELISA	ELISA	RT-PCR	RT-PCR with SNAP hybridization ELISA
	No. of positives					
O	11	42	61	45	56	65
A	2	3	3	3	3	3
C	2	0	2	2	0	2
Asia 1	2	5	8	10	10	10
Total (%)	17 (18.28)	50 (53.76)	74 (79.57)	60 (64.52)	69 (74.19)	80 (86.02)
	No. of negatives					
	76	43	19	33	24	13

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