

EPIDEMIOLOGICAL STUDIES AND ECONOMIC IMPACT OF FOOT AND MOUTH DISEASE OUTBREAKS IN HARYANA: II (2017-2020)

SWATI DAHIYA*, N.K. KAKKER and ANSHUL LATHER

Department of Veterinary Microbiology

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, Haryana, India

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ABSTRACT

Foot-and-mouth disease (FMD) is a World Organization for Animal Health (OIE)-listed highly contagious viral disease of domestic (cattle, buffaloes, sheep, goats and pigs) and other cloven hooved wild ruminants. FMD virus (FMDV), the causative agent of FMD, belongs to genus *Aphthovirus*, family *Picornaviridae* and order *Picornavirales*. FMDV serotypes O, A and Asia-1 are currently circulating in India. During 2017-2020, 14 FMD outbreaks were recorded from seven districts (Bhiwani, Fatehabad, Hisar, Kaithal, Karnal, Kurukshetra and Sirsa) of Haryana. Clinical samples collected from the suspected animals were subjected to FMDV serotyping using sandwich enzyme linked immunosorbent assay (ELISA) and/or multiplex reverse transcription-polymerase chain reaction (RT-PCR). Only FMDV serotype O was detected from all the 14 outbreaks. The species involved were cattle and/or buffaloes and/or pigs. Of the total economic losses of INR 12.64 million, the maximum loss (INR 5.97 million) was observed due to mortality followed by losses due to the cost of treatment of the affected animals (INR 5.17 million). In addition, 267 serum samples from healthy, infected and recovered animals during and after the outbreak were analyzed by single dilution liquid phase blocking ELISA. The animals showing protective antibody titres ($\geq 1.8 \log_{10}$) against the structural proteins of FMDV serotypes O, A and Asia-1 were 74.2, 80.9 and 88.9%, respectively at the time of outbreak while it was 92.6, 90.9 and 98.3%, respectively one to four months after the outbreak as FMDV vaccination was carried out as soon as outbreaks were suspected. Of the 110 serum samples tested for indirect DIVA ELISA, 27 (24.5%) were found positive against 3AB3 non-structural protein of FMDV, thus indicating virus circulation in that area.

Keywords: Economic impact, Epidemiology, Foot-and-mouth disease outbreaks in Haryana, FMD virus serotype O

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Foot-and-mouth disease (FMD), as described by World Organization for Animal Health (OIE) is the most serious transboundary viral disease affecting cattle, buffaloes, sheep, goats, pigs, several wild life species, etc. FMD is characterized by fever and vesicles with subsequent erosions in the mouth, nares, muzzle, feet, snout and teats along with excessive salivation, lameness, depression and decreased milk production. During 2013-14, the total loss due to FMD in cattle and buffaloes was estimated to be INR 208.97 billion in India and INR 350.4 million in Haryana.

FMD is caused by FMDV which belongs to genus *Aphthovirus*, family *Picornaviridae*, order *Picornavirales*, class *Pisoniviricetes*, phylum *Pisuviricota*, kingdom *Orthornavirae*, realm *Riboviria*. FMDV is having seven serotypes (O, A, C, Asia-1, SAT-1, 2 & 3) prevalent worldwide, of which serotypes O, A and Asia-1 are currently circulating in India.

In 2003-04, the Government of India started FMD-Control Programme (FMD-CP) followed by Extended FMD-CP in 2010-11 carrying out mass vaccination in cattle and buffaloes covering whole of Haryana. In 2019, a National Animal Disease Control Programme (NADCP) was launched for mass vaccination of cattle, buffaloes, sheep, goats and pigs to control FMD by 2025. The

Regional Research Centre on FMD, LUVAS, Hisar has continuously actively participated in implementation of FMD-CP/NADCP in Haryana. The present communication describes the detailed epidemiological studies of FMDV outbreaks in Haryana from January 2017 to December 2020.

MATERIALS AND METHODS

The clinical tissues including tongue and feet epithelium, saliva etc. (from FMD suspected live animals) and heart, kidneys, lungs, etc. (from post mortem cases) were collected in 50% sterile buffered glycerol (0.04 M PBS, pH 7.2-7.6) for FMDV detection and serotyping. In addition, a total of 267 blood samples were collected from cattle, buffalo and pigs during and one-two months after FMD outbreaks. The serum was separated from the blood using standard protocols. The clinical samples were transported to the Department of Veterinary Microbiology, LUVAS, Hisar under cold conditions at 4°C taking all the biosafety measures using triple packing and stored at -20 °C till further use.

FMDV detection and serotyping: The clinical tissues were processed for FMDV detection and serotyping by sandwich enzyme linked immunosorbent assay (ELISA) (Bhattacharya *et al.*, 1996) using reagents provided by Indian Council of Agricultural Research-Directorate of FMD (ICAR-DFMD), Mukteswar, India. The optical density (OD) was measured at 492 nm in ELISA Reader

*Corresponding: swatidahiya@luvas.edu.in

(Tecan, Austria). The samples were processed by multiplex reverse transcription-polymerase chain reaction (mRT-PCR) assay (Giridharan *et al.*, 2005). The PCR products were analyzed using ethidium bromide stained 1.5% agarose gel electrophoresis. Amplified products of 249 bp, 376 bp and 537 bp indicated the presence of FMDV serotype O, A and Asia-1, respectively.

Detection of antibodies against structural and non-structural proteins of FMDV: The serum samples were subjected to single dilution liquid phase blocking ELISA (sdLPBE) for detection of antibodies against structural proteins (SP) of FMDV serotype O, A and Asia-1 (Sharma *et al.*, 2015). The animals exhibiting antibody titres $\geq 1.8 \log_{10}$ were considered as protective against that particular serotype. Antibodies against 3AB3 non-structural protein (NSP) of FMDV were detected in serum samples using indirect ELISA to differentiate between infected and vaccinated animals (DIVA) (Bora *et al.*, 2014). The reagents for sdLPBE and NSP ELISA were provided by ICAR-DFMD. The sample exhibiting ≥ 40 percent positivity was interpreted as positive for antibodies against NSP 3AB of FMDV.

Economic impact: Direct economic losses were calculated taking into consideration the four major parameters i.e. loss due to mortality, draught power, milk production and cost of treatment of the affected animals. The average milk production of the affected milch animal came down from 12 to 3 litres (average) per day per buffalo and 8 to 2 litres (average) per day per cattle. The cost of buffalo and cow milk in village was estimated to be INR 35 and 30 per litre of milk, respectively. There were no productivity losses in calves but treatment expenses were there. The treatment cost per adult, heifers and calves were estimated to be INR 2000, 1500 and 1000, respectively. The treatment cost per adult pig and piglet was estimated to be INR 500 and 200, respectively. The economic losses of mortality are calculated by assessing the approximate cost per adult, heifer and calves in case of buffaloes as INR 28000, 20000 and 15000, respectively while for cattle as INR 20000, 15000 and 10000, respectively. The cost of adult pig and piglet was INR 1000 and 600, respectively.

RESULTS AND DISCUSSION

During the period January 2017 to December 2020, a total of 14 FMD outbreaks (five in 2017, six in 2018, three in 2019 and nil in 2020) were recorded from seven districts in the state of Haryana (Table 1). The FMD outbreaks and distribution of FMDV serotype in Haryana have been reported previously also (Kakker and Sharma, 2012).

Maximum outbreaks were observed in Hisar (06) followed by two each from Kurukshetra and Sirsa and one each from Kaithal, Karnal, Fatehabad and Bhiwani (Table 1). The species-wise epidemiological data of FMD outbreaks are shown in Table 2. The species involved in each of the two outbreaks were only cattle (No. 8 & 14), only buffaloes (No. 4 & 6), only pigs (No. 12 & 13) and both cattle and buffaloes in seven outbreaks (No. 1-3, 5, 9-11). The maximum mortality was recorded in the outbreak (No. 7) in which besides cattle and buffaloes, pigs were also involved which is the amplifier for FMDV. It has been reported that the cattle can secrete 1,20,000 aerosolized TCID50 virus particles/day compared with 400 million TCID50 dose/day for pig (Foot and Mouth Disease <https://s3.wp.wsu.edu/uploads/sites/2050/2016/03/FMD-Overview.pdf>).

In tropical areas, most of the outbreaks occur in winters when the ambient temperature is low which is suitable for FMDV survival (Bhattacharya *et al.*, 2005). During 2017-2020, out of 14 FMD outbreaks in Haryana, 11 were recorded during the winter season i.e. January to March (Fig. 1). During the previous 14 years (2003-2016) also all the FMD outbreaks reported from Haryana were in the months of December to April.

A total of eight FMDV outbreaks (No. 4, 6-11 and 14) were recorded after five months of last vaccination (Table 2). The decrease in protective antibody titres against FMDV in animals is evident from the sero-surveillance data (Table 3). During outbreak no. 4, 50% positivity (DIVA reactivity) against 3AB3 NSP of FMDV was observed which indicated FMDV circulation in the area. There were two outbreaks (No. 12 & 13) in which only pigs were involved in which no FMDV vaccination was being practiced during that period (Table 2). The protective antibody titres ($\geq 1.8 \log_{10}$) were exhibited in 100% of cattle and buffalo serum samples tested using sdLPBE (Table 3) which could be the reason for low morbidity in other susceptible animals of the area. In two outbreaks (No. 1 and 5) where FMDV vaccination was carried out less than a month ago, 255 and 150 animals, respectively were affected. This indicated the probability of presence of FMDV in that area even prior to vaccination (Table 2). The high DIVA reactivity (35 and 18.2%, respectively) indicated virus circulation in the area (Table 3).

A large number of animals were affected and died in two FMDV outbreaks (No. 2 & 3) in Sirsa during February-March 2017. The distance between the two villages was approximately 13 km. Apart from FMDV serotype O, *Pasteurella multocida* was also recovered from the affected animals indicating combined outbreak with

Table 1
Details of clinical samples collected from FMD suspected animals from different parts of Haryana (2017-2020*)

Outbreak No.	Month and Year	Location/ Village (District)	Species	Tissue material collected	No. of samples collected
1	January 2017	Garhi (Hisar)	Buffalo	Gum epithelium	5
2	February 2017	Bacher (Sirsa)	Cattle & Buffalo	Heart, gum, saliva, tongue & teat epithelium	7
3	March 2017	Keharwala (Sirsa)	Cattle & buffalo	Saliva, foot, gum, tongue mouth & epithelium	11
4	August 2017	Gamra (Hisar)	Cattle & buffalo	Tongue epithelium & heart	5
5	September 2017	Kheri Jalab (Hisar)	Cattle & buffalo	Saliva & tongue epithelium	2
6	February 2018	Bir Hansi (Hisar)	Buffalo	Tongue epithelium & saliva	3
7	February 2018	Samain (Fatehabad)	Cattle, buffalo & pig	Saliva, lung, heart, small intestine, spleen, foot, pharynx, gum, snout & epithelium	21
8	March 2018	Kurukshetra	Cattle	Gum & tongue epithelium	6
9	March 2018	Barsana (Kaithal)	Cattle & buffalo	Tongue, hoof & gum	3
10	March 2018	Uchani (Karnal)	Cattle & buffalo	Gum & tongue epithelium	4
11	September 2018	Bhagana (Hisar)	Buffalo	Feet, oral lesions and heart, spleen & lungs	4
12	January 2019	Tosham (Bhiwani)	Pig	Skin epithelium	1
13	February 2019	Satrod (Hisar)	Pig	Skin & heart	5
14	February 2019	Chunia Farm (Kurukshetra)	Cattle	Saliva & heart	4
				TOTAL	81

*No FMD outbreak was reported during 2020.

Table 2
Species-wise epidemiological details of FMD outbreaks in Haryana (2017-2020)

Outbreak No.	Number of animals at risk	Number of animals affected	Number of animals died	Month & year of last FMD vaccination (Time elapsed)
1	C= 690 B= 2510 G = 50	C= 5 B= 250	B= 27	December 2016 (<One month)
2	C= 895 B=710 S= 191 G= 153 Camel=5	C= 160 B= 110	C= 49 B= 22	December 2016 (Two months)
3	C= 1399 B=1597 S= 52 G= 33 Camel= 56	C=282 B=410	C= 95 B= 49	December 2016 (Three months)
4	C= 378 B=1488 G= 1 P= 3	B=7	Nil	December 2016 (Eight months)
5	C= 453 B=2333 S= 116 G= 51 P= 25	C= 15 B=135	B=16	August 2017 (<One month)
6	C= 137 B= 603	B= 3	Nil	August 2017 (Six months)

	S= 50 G= 40			
7	C= 1452 B=5550 S= 103 G= 40 P= 212	C= 303 B= 477 P= 170	C= 5 B=133 P= 70	August 2017 (Six months)
8	C= 50	C=23	C= 4	August 2017(Seven months)
9	C= 42 B= 18	C=9 B= 2	C= 8	August 2017 (Seven months)
10	C= 24 B= 8 P= 20	C=2 B= 2	Nil	August 2017 (Seven months). Vaccination denied by farmer
11	C= 328 B= 2024 S= 42 G= 16	C= 11B= 84	C= 1B= 10	March 2018(Six months)
12	P= 5	P= 1	Nil	No FMD vaccination in pigs
13	P= 140	P= 45	P= 30	No FMD vaccination in pigs
14	C= 1604 B= 6107 S= 145 G= 123 P= 30	C= 64	C= 1	September 2018(Five months)
TOTAL	C= 7452 B= 22948 S= 699 G= 507 P= 435 Camel=61	C= 874 B= 1480 P= 216	C= 163 B= 257 P= 100	

C= Cattle, B= Buffalo, S= Sheep, G= Goat, P= Pig

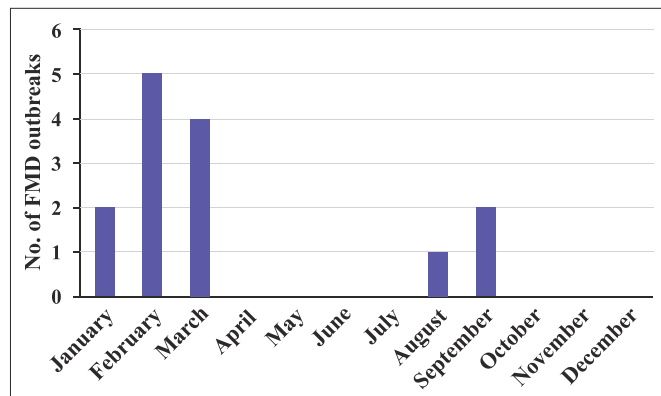


Fig. 1. Month-wise distribution of FMD outbreaks in Haryana (2017-2020)

Hemorrhagic Septicemia (HS) which might be the reason for high mortality in village Bacher. Further, the animals had very low levels of antibodies against *Pasteurella multocida* in serum samples (data not shown).

Of the 110 serum samples tested for indirect DIVA ELISA, 27 (24.5%) were positive for antibodies against 3AB3 NSP of FMDV. For the five outbreaks (No. 2, 8-10 and 14) serum samples were either not available or not tested. The overall case attack rate per thousand animals for FMDV in cattle, buffalo and pig was found to be 117.3,

64.5 and 496.6, respectively during the overall period of four years (2017-2020). The mortality rate per thousand animals for FMDV in cattle, buffalo, and pig was 21.9, 11.2 and 229.9, respectively for these four years.

Overall, a total of 267 serum samples from healthy, infected and recovered animals during and after the outbreak were analyzed by sdLPBE. The animals showing protective antibody titres ($\geq 1.8 \log_{10}$) against the structural proteins of FMDV serotypes O, A and Asia-1 were 74.2, 80.9 and 88.9%, respectively at the time of outbreak while it was 92.6, 90.9 and 98.3%, respectively one to four months after the outbreak as FMDV vaccination was carried out as soon as outbreaks were suspected.

FMDV serotype O was detected by ELISA and/or mRT-PCR in 58 samples out of 81 tested during 2017-2020 from all the 14 outbreaks in Haryana (Table 4). None of the sample demonstrated FMDV serotype A or Asia-1. In India during 2019, FMDV serotype O continues to be the most predominant serotype and was responsible for 98% of the outbreaks (ICAR-DFMD, Annual Report 2019). FMDV serotype C has not been reported from India since 1995 (Bhattacharya *et al.*, 2005) while serotype Asia-1 and A have not been reported from Haryana since 2009 and 2010,

Table 3

Serum antibody titres in susceptible animals against FMDV structural and non-structural proteins during and after the outbreaks in Haryana (2017-2020)

Outbreak No.	Species	Time of Serum sample collection	Samples tested for SP anti-bodies using sdLPBE	Number (%) of animals showing protective antibody titres ($\geq 1.8 \log_{10}$) against SP of FMDV serotypes			Serum samples tested for anti-3AB NSP anti-bodies using DIVA ELISA		
				O	A	Asia-1	Total samples tested	Samples positive	Percent Positivity
1	Cattle + Buffalo	Healthy (in-contact) animals at the time of outbreak	20	20 (100.0)	20 (100.0)	20 (100.0)	20	7	35.0
	Cattle + Buffalo	One month post outbreak	15	12 (80.0)	13 (86.7)	15 (100.0)	15	5	33.3
2	Cattle + Buffalo	At the time of outbreak	NA	-	-	-	-	-	-
3	Cattle + Buffalo	At the time of outbreak	73	63 (86.3)	68 (93.2)	72 (98.6)	3	1	33.3
4	Cattle + Buffalo	One month post outbreak	12	12 (100.0)	12 (100.0)	12 (100.0)	12	6	50.0
5	Cattle + Buffalo	At the time of outbreak	11	8 (72.7)	11 (100.0)	11 (100.0)	11	2	18.2
6	Cattle + Buffalo	At the time of outbreak	19	11 (57.9)	11 (57.9)	16 (84.2)	19	5	26.3
7	Cattle + Buffalo + pig	At the time of outbreak	49	22 (44.9)	25 (51.0)	28 (57.1)	30	1	3.3
	Cattle + Buffalo	Two months post outbreak	15	9 (60.0)	9 (60.0)	12 (80.0)	NA	-	-
8	Cattle	-	NA	-	-	-	-	-	-
9	Cattle + Buffalo	-	NA	-	-	-	-	-	-
10	Cattle + Buffalo	-	NA	-	-	-	-	-	-
11	Cattle + Buffalo	At the time of outbreak	45	35 (77.8)	39 (86.7)	45 (100.0)	NA	-	-
12	Pig	At the time of outbreak	6	6 (100.0)	6 (100.0)	6 (100.0)	NA	-	-
13	Pig	At the time of outbreak	2	2 (100.0)	2 (100.0)	2 (100.0)	NA	-	-
14	Cattle	-	NA	-	-	-	-	-	-
	TOTAL	At the time of outbreak	225	167 (74.2)	182 (80.9)	200 (88.9)	110	27	24.5
		1-2 months after the outbreak	42	33 (78.6)	34 (81.0)	39 (92.9)			

NA: Not available

Table 4

FMDV serotypes detected from clinical samples from different parts of Haryana by sandwich ELISA and/or mRT-PCR (2017-2020)

Outbreak Number	No. of clinical sample(s) tested	Samples tested positive for FMDV serotype		
		O	A	Asia-1
1	5	2	-	-
2	7	4	-	-
3	11	8	-	-
4	5	4	-	-
5	2	1	-	-
6	3	3	-	-
7	21	10	-	-
8	6	6	-	-
9	3	3	-	-
10	4	3	-	-
11	4	4	-	-
12	1	1	-	-
13	5	5	-	-
14	4	4	-	-
TOTAL	81	58	0	00

Table 5
Economic losses (INR) due to FMDV outbreaks in Haryana (2017-2020)

Year	Number of outbreaks	Loss due to mortality	Loss of draught power	Loss of milk production	Loss due to cost of the treatment	Total losses
2014	4	19000	68550	0	39000	126550
2017	5	4161000	670950	28800	3831000	8691750
2018	6	1781000	393525	360000	1228500	3763025
2019	3	28400	45900	0	115500	189800
2020	0	-	-	-	-	-
Total	14	5970400	1110375	388800	5175000	12644575

respectively. It is a matter of investigation whether FMDV serotypes A and Asia-1 could be considered as a candidate for eradication at least from Haryana as per the guidelines of OIE/FAO on the same pattern as adopted for eradication of FMDV serotype C (Paton *et al.*, 2021).

Of the total economic losses of more than INR 126 lakhs, the maximum economic loss (INR 59.704 lakhs) was observed due to mortality followed by the cost of treatment (INR 51.75 lakhs) of the affected animals, loss due to draught power (INR 11.10375 lakhs) and loss of milk production (INR 3.888 lakhs) (Table 5).

This report suggests that there should be timely vaccination of susceptible livestock population. On the basis of scientific data generated and presented by the Scientists of LUVAS as per the guidelines of a committee constituted by the Department of Animal Husbandry and Dairying (DAHD), Government of India, New Delhi for “Recommendations on usage of FMD+HS combined vaccine”, the Haryana state has been allowed (first in the country) to use the combined vaccine in animals. As a result there has been no incidence of FMD and HS (data not shown) in the State after the start of combined vaccination since April 2019, thus paving the way for other states to follow the same.

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