Haryana Vet. **51** (December, 2012), pp 19-23

Research Article

EPIDEMIOLOGICAL STUDIES ON FOOT AND MOUTH DISEASE OUTBREAKS IN HARYANA DURING THE YEARS 2009 AND 2010

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ABSTRACT

A total of four foot-and-mouth disease (FMD) outbreaks were recorded in cattle from Bhiwani, Kurukshetra, Hisar and Rohtak districts of Haryana during the years 2009 (1) and 2010 (3). Clinical samples were collected from the affected animals and subjected to FMD virus serotyping using sandwich ELISA. Of these, FMD virus from two outbreaks belonged to serotype O (Kurukshetra and Rohtak) and one each to serotype A (Hisar) and Asia 1 (Bhiwani). Each of the four outbreaks was investigated under intensive epidemiological studies. Further, 84 serum samples from healthy in-contact, clinically infected and recovered animals in the wake of outbreak and one month after the outbreak were analyzed by liquid phase blocking ELISA and FMD 3AB3 non-structural protein ELISA for sero-epidemiology.

Key words: Foot and mouth disease, outbreaks, epidemiology, virus type distribution, Haryana

Foot-and-mouth disease (FMD) is one of the most economically important and devastating viral disease affecting cloven-hoofed livestock viz. cattle, buffaloes, sheep, goats and pigs as well as several wild life species world wide. In India, the disease is widely distributed, endemic and occurs in all parts of the country throughout the year. The disease causes heavy economic losses to the tune of more than 2 billion US dollars per year (Venkataramanan *et al*., 2006). In order to participate in international trade of animals, animal products and germ plasm, the participating countries have to be free from FMD virus (FMDV) infection. Several nations have attained FMD-free status through stamping out policy and/or systematic vaccination programme and enjoy economic benefits from international trade in animals and livestock products. In light of this, FMD control programme (FMD-CP) was launched in India during 10th Five Year Plan in selective regions to create FMD free zones through mass immunization of cattle and buffalo.

In Haryana, eight of the 21 districts namely Bhiwani, Fatehabad, Hisar, Jhajjar, Jind, Rohtak, Sirsa and Sonepat were covered under FMD-CP. Other 13 districts of Haryana were also covered for FMD vaccination with the help of resources from Central pool: Assistance to States for Control of Animal Diseases (ASCAD). A total of ten phases of mass FMD vaccinations had been carried out since the start of the programme (January 2004) till December 2010 at a regular interval of 6 - 8 months in FMD-CP districts and seven annual vaccinations in 13 districts under ASCAD.

The Regional Research Centre on FMD, Hisar has actively participated in implementation of FMD-CP by providing logistic support in the form of surveillance and sero-monitoring work in all the 21 districts of this state. We have previously reported the FMD outbreaks and distribution of FMDV serotypes in Haryana (Sharma *et al.*, 2002; Kakker and Sharma, 2003; Sharma and Kakker, 2005; Kakker and Sharma, 2007; Sharma and Kakker, 2009). The present communication describes FMD outbreaks in Haryana and virus serotypes involved during the years January 2009 to December 2010.

MATERIALS AND METHODS

Surveillance and constant vigil for FMD outbreaks (if any) was undertaken throughout Haryana by visiting regularly all the 21 districts and also by communicating with the field staff of the Department

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of Animal Husbandry and Dairying, Haryana as described previously (Sharma and Kakker, 2009). The clinical samples were collected from affected animals from four FMD outbreaks. The epidemiological data for each of the four outbreaks was recorded. Serotyping of the FMDV was carried out from the clinical samples by sandwich ELISA (Bhattacharya *et al.*, 1996) and virus isolated as per the standard protocol. The clinical samples, which could not be serotyped in sandwich ELISA, were processed in BHK-21 cell culture system for amplification and isolation of the FMDV and again subjected to serotyping as described above.

In addition, a total of 84 serum samples (varying number from four FMD outbreaks) were collected during the phase of FMD outbreak (74) [healthy in-contact: 56, clinically infected: 10 and recovered: 8] and one month after recovery and vaccination (10). These were tested in liquid phase blocking ELISA (LPB-ELISA, Hamblin *et al.*, 1986) as described previously (Kakker and Sharma, 2008, Sharma and Kakker, 2009) for the detection of vaccinal antibodies against structural proteins of FMDV serotypes O, A and Asia-1 and in 3AB3 non-structural protein ELISA (NSP-ELISA) for the detection of antibodies against NSPs of FMDV as an indicator of recent infection. The serotyping ELISA, LPB-ELISA and 3AB3 NSP-ELISA reagents were supplied by the Central FMD Virus Typing Laboratory of the Project Directorate on FMD, IVRI Campus, Mukteswar, Kumaon, Uttrakhand.

RESULTS AND DISCUSSION

During January 2009 to December 2010, four FMD outbreaks were recorded including one each in March 2009 (village Lalhana, Bhiwani), January 2010 (Shri Krishan Gaushala, Kurukshetra), March 2010 (CCS HAU Animal Farm, Hisar) and April 2010 (village Madina, Rohtak) in the state of Haryana. The intensive epidemiological studies undertaken to investigate these four FMD outbreaks are as under:

1. FMD Outbreak at Village Lalhana, Bhiwani: The Bhiwani district is covered under FMD-CP of the Haryana state and VIIIth phase of FMD vaccination was completed in this village during Nov. 2008. The outbreak occurred in a private dairy farm (0.5 sq. km. area) located at the outskirts of the village where the dairy owner did not get his animals vaccinated in spite of the visit of the FMD vaccination team. Of the 72 animals (5 buffaloes and 67 cross-bred cattle), eight cattle (11.11%) were affected with the disease. The duration of the FMD outbreak was five days and FMDV serotype Asia-1 was isolated from the affected animals. The authorities were advised to immediately re-vaccinate the animals against FMD in surrounding area. Further investigations revealed that FMD outbreak was not recorded in the area since more than five years before.

Sero-epidemiology: A total of 10 serum samples were collected from healthy in-contact (4), clinically infected (3) and FMD recovered (3) cattle and subjected to LPB-ELISA. All the four healthy animals and three clinically infected cattle demonstrated <1.8 log10 antibodies against FMD virus serotype O, A and Asia 1 except one animal which didn't exhibit antibodies against FMD virus serotype A, but had antibodies against serotype O and Asia-1 (Table 1). The antibody titres of in-contact animals and clinically infected cattle confirmed that these animals were not vaccinated. All the three FMD recovered animals demonstrated higher level of antibodies against FMD virus serotype Asia-1. In NSP-ELISA, these animals were highly positive for anti-NSP antibodies indicating recent infection with FMDV.

This outbreak involved a dairy farm which may be considered as an isolated unit on the outskirts of the village. As the animals were not vaccinated, they had very low levels of antibodies against all the prevalent FMD virus serotypes in LPB-ELISA. The village Lalhana is situated on the migratory route of animals from Rajasthan to Delhi and animals may have contracted the virus from the infected animals in transit. On the other hand FMD vaccination was done in the animals surrounding this dairy since no FMD cases were detected in the neighboring livestock population in the village.

2. FMD Outbreak at Shri Gopal Krishan Gaushala, Kurukshetra: The Kurukshetra city has Shri Gopal Krishan Gaushala in a total area of about 50 hectares,

housing an elite herd of 170 Holstein-Frisian (HF) cattle (80 adults, 60 heifers and 30 calves) and 6 buffaloes. The animals are taken care by the Gaushala management. The Kurukshtera district is covered under ASCAD funding and VIth round of FMD vaccination was completed in July 2009. The Gaushala management got vaccinated their animals again (VIIth round) in first week of January 2010.

Of the 170 cattle, a total of 10 animals (6 milch cattle, 2 heifers and 2 calves) went down with the disease, clinical samples collected and FMDV serotype 'O' was isolated from the affected animals. The affected animals were immediately isolated and healthy in-contact animals were relocated to new housing unit quite away from the infected site. The infected site was disinfected. All the healthy stocks were revaccinated using oil adjuvanted FMD vaccine. The duration of the FMD outbreak was 10 days and none of the animals suffered after 10th day. No indigenous cattle and buffaloes present in the Gaushala were involved in the FMD outbreak.

Sero-epidemiology: A total of 15 serum samples were collected from healthy in-contact (5), clinically infected (5) and recovered (5) animals and subjected to LPB-ELISA. The sera from healthy as well as recovered animals demonstrated persistent vaccinal antibodies >1.8 log10 against FMDV serotypes O, A and Asia 1 (10 out of 10). It appears that all these animals have been vaccinated very recently (Table 1). However, antibody response to type A and Asia 1 was poor in serum samples collected from clinically infected animals. The authorities were advised to undertake ring vaccination immediately in surrounding areas of Kurukshetra.

After a month of FMD outbreak, eight serum samples from recovered animals were collected and analyzed for 3AB3 NSP-ELISA as well as LPB-ELISA. All the infected animals demonstrated antibodies against 3AB3 NSP of FMDV indicating thereby recent FMDV infection. Further, all the FMD recovered animals had antibody levels >2.4 log10 against FMDV serotype O and A (except in one animal). The antibody titres against FMDV serotype Asia 1 were in the range of 1.8 – 1.95 log10.

It is believed that the contact of farm personnel with the infected stray animals/ migratory herd might be a pre-disposing factor for this outbreak. Further investigations revealed that no FMD outbreak was recorded in the area for more than five years before.

3. FMD Outbreak at CCS HAU Animal Farm, Hisar: The Hisar district is covered under FMD-CP of the Haryana state and so far nine round of vaccinations had been completed. The CCS HAU Animal Farm maintains a total of 1396 animals comprising 500 cattle (275 cross bred) and 225 indigenous (125 Hariana and 100 Sahiwal), besides 309 buffalo, 437 sheep and 150 goats. The farm is well organized and has adopted biannual FMD vaccination regimen along with seromonitoring using SNT/LPBE since 1995. The last vaccination at the Farm was done in October 2009 and FMD outbreak occurred in March 2010. Of the 500 cattle, five calves (1%) of 5-12 months of age group were affected with the disease. FMDV serotype 'A' was isolated from the affected calves and duration of the FMD outbreak was five days.

Sero-epidemiology: Serum samples were collected randomly from 44 (adult 25, heifers 10 and calves 9) healthy animals and subjected to LPB-ELISA. The serum samples demonstrated overall protective antibody titres (>1.8 log10) against FMDV serotypes O, A and Asia-1 in 56.8, 45.45 and 38.64% animals, respectively (Table 1). The outbreak occurred exactly after 5½ months after the last vaccination when most of the animals were in the window of susceptibility, particularly against FMDV serotypes A and Asia-1. Further, young calves had even less persistence of protective antibodies (Table 1). The serum samples collected from affected animals processed for 3AB3 NSP-ELISA and LPB-ELISA were positive for 3AB3 antibodies and demonstrated rise in antibody titres against FMDV serotype A.

The FMD outbreak at CCS HAU Animal Farm can be attributed to the absence of vaccinal antibody titres against all the three FMDV serotypes. Few sporadic and recovered cases were also reported in the adjacent areas (Azadnagar and Kamdhenu Dairy Farm, Suryanagar, Hisar) before the initiation of FMD outbreak at the CCS HAU Animal Farm in March 2010,

but FMDV could not be detected from these areas due to late reporting. However, serum samples from Kamdhenu Dairy Farm were highly positive for 3AB3 NSP antibodies demonstrating recent FMDV infection. These cases were recorded immediately after influx of migratory animals from neighbouring state through Hisar. An advisory was issued to the Animal Husbandry Department for immediate vaccination of all the susceptible livestock as well as elite herds in the state.

4. FMD Outbreak at Village Madina, Rohtak: The village Madina has approximately 6902 livestock population. The Rohtak district is covered under FMD-CP and so far nine round of FMD vaccinations were done in all the susceptible animals. The last vaccination in the village was done in Nov 2009. In April 2010, two milch cattle were affected with the disease. Clinical samples were found positive for FMDV serotype 'O'. The affected animals were immediately isolated and healthy in-contact animals were relocated to new housing unit quite away from the infected site. The infected site was disinfected. All the healthy stocks were revaccinated using oil adjuvanted FMDV trivalent vaccine. The duration of the FMD outbreak was only 2 days. None of the animals suffered after the day following the visit to the place of FMD outbreak. No indigenous cattle and buffaloes present in the house were involved in the FMD outbreak. On investigation, it was revealed that the two milch cattle were purchased recently and were unvaccinated. The FMD outbreak occurred approximately 6 months post vaccination.

Sero-epidemiology: Five serum samples were collected from healthy in-contact (3) and clinically infected (2) animals in the phase of the FMD outbreak and subjected to LPB-ELISA. The sera from healthy animals demonstrated protective vaccinal antibodies >1.8 log10 in all the three animals against FMDV serotypes O and A (Table 1). However, antibody response to serotype Asia-1 was <1.8 log10 in one of the in-contact animal. The serum samples collected from clinically infected animals demonstrated <1.8 log10 antibodies level against serotypes O (only one animal), A and Asia-1 which confirmed that these two animals were not vaccinated. The authorities were advised to undertake ring vaccination immediately in surrounding areas of the village. After one month of FMD outbreak, serum samples from two infected (and recovered subsequently) animals were collected and analyzed for 3AB3 NSP-ELISA. Both the animals demonstrated antibodies against 3AB3 NSP of FMDV indicating recent FMDV infection.

All the four FMD outbreaks recorded during 2009 and 2010 were in four of the 21 districts of Haryana involving cattle only. The number of outbreaks recorded during the earlier years were 52 in 2001(Sharma *et al.*, 2002), 26 in 2002 (Kakker and Sharma, 2003), 111 in 2003 (Sharma and Kakker, 2005), 15 in 2004 (Sharma and Kakker, 2005), three in 2005 (Kakker and Sharma, 2007), one in 2006 (Kakker and Sharma, 2007), one in 2007 (Sharma and Kakker, 2009) and two in 2008 (Sharma and Kakker, 2009) in various districts of Haryana. Moreover, there were very few animals involved in all the four outbreaks after the start of FMD-CP, whereas before the launch of FMD-CP, a large number of susceptible animals used to be involved during most of the FMD outbreaks (Maan *et al.*, 1998; Sharma *et al.*, 2002; Kakker and Sharma, 2003; Sharma and Kakker, 2005; Sharma *et al.*, 2006). The less number of FMD outbreaks during 2009 and 2010 in the state may perhaps be due to the development of herd immunity through mass vaccination of susceptible animals. Further, no

Table 1
Sero monitoring studies during foot and mouth disease outbreaks at different places in Haryana

Animal's status	No. of animals tested	No. of animals showing titres >1.8 log10 against FMD virus serotype		
		O	A	Asia-1
Village Lalhana, Bhiwani				
Healthy (in contact)	4	1	0	1
Clinically infected	3	0	0	0
Recovered	3	0	0	3
Shree Gopal Krishan Gaushala, Kurukshetra				
Healthy (in contact)	5	5	5	5
Clinically infected	5	4	2	2
Recovered	5	5	5	5
CCS HAU Animal Farm, Hisar				
Adult Cattle	25	20 (80)	16 (64)	14 (56)
Heifers	10	5 (50)	4 (40)	3 (30)
Calves	9	0 (0)	0 (0)	0 (0)
Total	44	25 (56.8)	20 (45.45)	17 (38.64)
Shree Gopal Krishan Gaushala, Kurukshetra				
Healthy (in contact)	3	3	3	2
Clinically infected	2	1	0	0

mortality was observed in any of the four FMD outbreaks during this period. In contrast, 32 and 18 animals died in 2006 and 2005, respectively due to combined infection with *Pasteurella multocida* (Kakker and Sharma, 2007).

The clinical samples collected from these outbreaks were successfully diagnosed using sandwich ELISA and all the three FMD virus serotypes were recorded from these outbreaks: FMDV serotype O (2 in 2010), serotype A (1 in 2010) and Asia-1 (1, 2009). On the other hand the FMD virus serotype Asia-1 was recorded during 2008 and serotype O during 2007 (Sharma and Kakker, 2009).

The FMD outbreaks were recorded from Jan. to April. On the basis of FMD outbreaks recorded since the start of FMD–CP in January 2004, it has been observed that all the FMD outbreaks had occurred during January to March each year i.e. 2004 (January, February and March, Sharma and Kakker, 2005), 2005 (January and March, Kakker and Sharma, 2007), 2006 (February, Kakker and Sharma, 2007), 2007 (February, Sharma and Kakker, 2009) and 2008 (January, Sharma and Kakker, 2009). It is, therefore, pertinent to initiate the FMD vaccination during the months of November and December, so that the animals exhibit heightened immune response during next quarter.

It is worthwhile to mention that after the start of FMD-CP in Haryana, most of the sporadic cases of FMD during current years involved cross-bred cattle, particularly HF and the fact that in-contact buffaloes and indigenous cattle remained unaffected in most of the outbreaks suggesting a predilection of FMDV for the HF cattle. It is, therefore, suggested that cross-bred cattle should be vaccinated at four months interval or vaccine of high potency i.e. 6 PD50 may be used to immunize cross-bred cattle. Further, the incidence of FMD outbreaks has come down as a result of the mass vaccination programme. This can serve as a model towards "FMD Free Haryana" and pave the way for launching a National FMD Eradication Programme in the country.

ACKNOWLEDGEMENTS

The authors wish to thank the Indian Council of Agricultural Research, New Delhi; Project Directorate on FMD, IVRI Campus, Mukteswar-Kumaon (Uttrakhand) and the CCS HAU, 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 providing help in collecting FMD specimens. The help rendered by the laboratory staff particularly, S/Sh. Narender Paul, VLDA, Chandan Singh and Meenu, Lab Attendants is also acknowledged.

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