



Demonstration of foot-and-mouth disease virus infection specific non-structural protein-antibodies in a vaccinated herd comprising cattle, buffaloes and goats in north India

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ABSTRACT

A serological study employing 3AB3 non-structural protein ELISA and liquid phase blocking ELISA to assess foot-and-mouth disease virus (FMDV) non-structural and structural protein antibodies (NSP- and SP-Ab) was undertaken through sampling from all resident animals of a vaccinated herd comprising 943, 377 and 211 cattle, buffaloes and goats, respectively, in north India. A considerable, though disparate proportions of animals (61.2% cattle, 29.2% buffaloes and 29.9% goats) were positive for NSP-Ab suggesting an exposure to FMDV. From the age-stratified analysis of NSP-Ab prevalence, the probable time point of virus introduction in the farm could be predicted in retrospect to be around 8 months before sampling. The proportion of animals showing $\geq 1.8 \log_{10}$ titre against all 3 serotypes in the vaccine varied from 3.2 to 32.9% in different species indicating poor vaccinal herd immunity, which presumably might have been the reason for the outbreak in the farm.

Key words: Foot-and-mouth disease virus, Liquid phase blocking ELISA, North India, NSP ELISA, Serosurveillance, Vaccinated herd

Foot-and-mouth disease (FMD), a highly contagious and economically devastating disease of livestock, is endemic in India, where 3 different serotypes of FMD virus (FMDV), viz. O, A and Asia 1 are prevalent. As per 19th Livestock Census - 2012 All India Report, the country has a population of 190.9, 108.7, 65.0 and 135.1 million heads of cattle, buffalo, sheep, and goats, respectively. The huge population of livestock in the country remains under constant threat of FMD due to unrestricted movement of animals, poor zoo-sanitary measures enforced during outbreaks and low vaccine coverage. The current FMD control campaign in India does not include the small ruminants and pigs under routine vaccination drive. Usually in the field, lack of real-time reporting and sampling from outbreak areas limit the scope of the disease investigation process. In this scenario,

a systematic herd-based serological study may generate valuable information on FMD prevalence in retrospect and also help improve the sampling and surveillance approaches to be adopted in future. The present work deals with serosurveillance of FMD in a vaccinated herd of north India through detecting and correlating FMDV non-structural and structural protein-antibodies (NSP- and SP-Abs) with the available information on epidemiology and vaccination.

MATERIALS AND METHODS

Study area and sample collection: The sampling was performed in a herd located at NDRI, Karnal, in north India. All resident animals were sampled and 1,531 serum samples (from 943 cattle, 377 buffaloes and 211 goats) were collected. The relevant details of management practices, age and breed of animals, their identification numbers and epidemiological information pertaining to FMD were collected from the farm records.

Non-structural protein (NSP) ELISA: An indirect ELISA was performed using the validated in-house r3AB3 NSP ELISA kit to assess antibodies against 3AB NSP of FMDV in serum samples collected from cattle and buffaloes (Mohapatra *et al.* 2011), whereas serum samples from goats were tested as per Rout *et al.* (2014). The kit has a diagnostic sensitivity and specificity of 95% and 98% on goat and 96% and 96.4% on bovine samples, respectively (Ranabijuli *et al.* 2010, Mohapatra *et al.* 2011).

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Liquid phase blocking (LPB) ELISA: Two-fold dilutions (from 1:16 to 1:128) of serum samples were tested for determining the serotype-specific FMDV structural protein antibody (SP-Ab) titre to assess the overall status of vaccinal immunity against all 3 FMDV serotypes in the vaccine using the in-house LPB ELISA kit as per the procedure described earlier (Ranabijuli *et al.* 2010). The samples showing \log_{10} titre of ≥ 1.8 were considered as having a high titre of antibody. This titre cutoff is being used for the Government sponsored post-vaccination sero-monitoring in the country as a measure of a satisfactory response (Annual Report PDFMD 2014–2015).

RESULTS AND DISCUSSION

Out of 943 cattle, 377 buffalo and 211 goat serum samples tested by 3AB3 NSP ELISA, 577 (61.2%), 110 (29.2%) and 63 (29.9%) animals were found positive, respectively, suggesting a high apparent prevalence of NSP-Ab in the farm. When an age-stratified seropositivity was considered, the proportion of positive animals increased considerably in all species at '8–12 months' age group compared to <8 months age groups (Table 1). A relatively higher proportion of kids (14.2%) in the <4 months age category revealed seropositivity by 3AB3 NSP ELISA as compared to cattle (6.4%) and buffalo (2.4%) calves. The dams of the seropositive young animals were also found positive for 3AB3 NSP-Ab. Very low seropositivity was observed in the 4–6 months age group for cattle (5%). However, no samples from buffalo and goat tested positive in NSP ELISA in this age group. Interestingly, the entire population was seronegative for NSP-Ab in the 6–8 months age category. In LPB ELISA, 32.9% (310/943) cattle, 3.2% (12/377) buffaloes and 5.2% (11/211) goats showed \log_{10} titre of ≥ 1.8 against all 3 serotypes in the trivalent vaccine.

Serosurveillance for antibodies to FMDV NSPs clearly substantiates the status of occult virus infections (Paton *et al.* 2014) and also helps in retrospective diagnosis of FMD outbreaks that is considered to be an important adjunct to molecular techniques in locating hot-spots of FMDV circulation and potential sources of infection. In India, 3AB3 NSP ELISA is being used for detecting FMDV infection in

cattle and buffalo population under intensive bi-annual vaccination. The apparent seroprevalence of NSP-Ab in the country stands at about 27% over the last 5 years (Annual Report PDFMD 2014–2015). In this study, presence of a high level of 3AB NSP-Ab in sampled herd raised suspicion over recent virus circulation. An age-stratified analysis of the samples revealed significantly lower percentage positivity for NSP-Ab in <8 month-old animals in comparison to other 4 age strata in >8 months age group. An abrupt increase in NSP-Ab percentage seropositivity was evident in the 8–12 month age category in all the 3 constituent species in the farm after taking a dip in the 6–8 months category and remained consistently high for age groups beyond 1 year of age. When the <8 months age category was further resolved, the seropositive buffalo calves and kids were found exclusively in the <4 months age group, while all of the seropositive cattle calves clustered in the <6 months age (Table 1). The distribution of NSP-Ab level in the age-wise sub-populations is suggestive of a recent wave of infection in the farm and the probable time point of virus introduction could be estimated in retrospect to be around 8 months before the time of sample collection, i.e. during February 2011. Such retrospective serological evidence in favour of an outbreak correlated with the epidemiological information obtained from the farm supervisor and animal care-takers who admitted to an FMD-like outbreak, heavy mortality in young unvaccinated calves, abortion in a few pregnant animals and drastic drop in the average milk production of the farm during that time. The technique of detecting recent virus activity by such serological analysis of young animals beyond the age at which maternal antibodies are lost has also been described for other infectious diseases like bovine viral diarrhoea virus (BVDV) infection in cattle populations (Sayers *et al.* 2015).

It is evident that distributing the entire population into different age groups and assessing the proportion of NSP reactors effectively helps in deciding the approximate time point of infection. One constraint in determining the exact point of infection, if it has occurred within 6 months before collection, could be the interference and misinterpretation

Table 1. Age-stratified distribution of seropositivity for FMDV NSP-Abs

Species		<4 months	4–6 months	6–8 months	8–12 months	1–2 years	2–3 years	>3 years	Overall
Cattle	Total number sampled	77	60	37	36	120	113	500	943
	% NSP-Ab +ve	6.4%	5%	0%	23.8%	61.7%	83.2%	78.2%	61.2%
Buffalo	Total number sampled	41	9	4	11	52	46	214	377
	% NSP-Ab +ve	2.4%	0%	0%	16.7%	46.2%	56.5%	26.6%	29.2%
Goat	Total number sampled	49	0	2	11	65	58	26	211
	% NSP-Ab +ve	14.2%	0%	0%	27.3%	50.8%	24.1%	23.1%	29.9%

of test results due to maternally derived antibodies (MDA) received through colostrum in young ruminants. The duration of MDA in agriculturally important animal species was reported to be usually 3–6 months (Niewiesk 2014). But in this study, a clear cut increase in NSP-Ab prevalence could be noticed on samples derived from >8 months old animals. A few cases of NSP-Ab seropositivity in the <6 months old category (8 out of 137 cattle) and in the <4 months old category (1 out of 50 buffaloes and 7 out of 49 goats) could be attributed to persistent MDA in the young animals. All seropositive young calves demonstrated perfect correlation in NSP-Ab status with their respective dams. Calves which were seronegative in spite of their dams being positive could be due to the effect of time and quantity of colostrum feeding and capacity of absorption by the newborn and titre of antibody in dams during nursing, which ultimately determine duration of persistence of detectable MDA. In any case, evidence of NSP-Ab in young animals, even if derived maternally, is indicative of FMDV circulation in a herd. Further, not a single 6–8 months old animal (out of 37 cattle, 4 buffaloes and 2 goats), expectedly free from MDA, revealed NSP-Ab reiterating the point of infection to be beyond 8 months before sampling.

Cattle, buffaloes and goats revealed wide variation in 3AB NSP-Ab status. However, the rate of infection in buffalo and goats was either lower than in cattle or resulted in less seroconversion, perhaps due to lower levels of virus replication. Such subdued virus replication might have led to a weaker/low titred NSP-Ab response, which had dropped below the detection threshold by the time of collection, nearly 8 months post-outbreak. Such findings corroborated with clinically milder or asymptomatic form of sickness observed in buffaloes and goats, compared to cattle during the outbreak as informed by the caretakers and the farm officials. It was also reported that NSP seroconversion or development of antibodies to NSPs is related to the extent of virus replication, which in turn depends upon levels of host susceptibility, immune status and the nature and severity of exposure (Paton *et al.* 2014). Even the duration of detection of NSP-Abs was previously correlated with the severity of clinical disease and level of virus replication (Kitching 2002). Animals showing no clinical disease reportedly had shown only transient level of NSP-Ab (Huang *et al.* 2002). In goats, the disease manifestations were less evident as observed previously, which confirms their silent involvement in FMD epidemics in a mixed farming situation (Ranabijuli *et al.* 2010). Other presumptions could be that either buffaloes or goats are inherently poor NSP seroconverters or the rate of NSP-Ab decay in these species is faster than that in cattle making a majority of the infected buffaloes and goats seronegative for NSP-Ab by the time of sampling. Alternatively, either the rate of infected-carrier conversion or persistence of carrier state might be higher in cattle compared to buffalo and goat. It is known that in ruminants, FMDV is capable of causing a persistent subclinical infection during which the animals may continue to carry infectious virus for

prolonged periods of time; up to 3.5 years in cattle, 9 months in goat and 1–2 years in water buffalo (Weaver *et al.* 2013). The factors such as relative proportion of carrier animals, duration of persistence of anti-FMDV Abs could not be studied in greater detail due to unavailability of oropharyngeal fluid for virus detection and serial bleeds at earlier points post-infection.

To conclude, this exhaustive serosurveillance using SP- and NSP-Ab detection systems in an organized herd provided useful insights into the extent of FMDV activity in retrospect. Poor herd immunity might have presented the virus with a ‘window of susceptibility’ making the entire population prone to FMDV challenge and because of rearing density and unrestricted movement of care-takers between the enclosures; an index case could have led to the outbreak, in turn producing such high percentage of NSP-Ab prevalence. Interestingly, the species showing the lowest level of SP-Ab manifested less severe clinical symptoms during the outbreak and a relatively lower level of NSP-seroconversion suggesting the possible inherent difference in their susceptibility to clinical FMD. Finally, we believe that this integrated serosurveillance approach could be intelligently extrapolated to monitor FMDV circulation in the country with a mixed farming set up, which is slowly entering into the phase 3 of the progressive control pathway so that risks from localized virus activity and impact of implemented control programmes could be assessed.

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