Indian Journal of Animal Sciences **87** (6): 680–684, June 2017/Article

Serological indication of exposure to foot-and-mouth disease virus in comprehensively surveyed traditionally managed small ruminant population of Gujarat plains and hills agro-climatic region of India

MANORANJAN ROUT[1], BIKASH RANJAN PRUSTY[2] and AMIT KANANI[3]

ICAR-Project Directorate on Foot and Mouth Disease, IVRI Campus, Mukteswar, Uttarakhand 263 138 India

Received: 1 October 2016; Accepted: 28 November 2016

ABSTRACT

The present sero-epidemiological study was conducted in Gujarat plains and hills agro-climatic region of India during 2013–2014 to detect infection-specific antibodies against foot-and-mouth disease virus (FMDV) exposure in apparently healthy, unvaccinated, traditionally managed sheep and goat population across 26 districts of Gujarat state. Serum samples from 1,295 small ruminants (381 sheep and 914 goats) were collected and subjected to 3AB3 nonstructural protein (NSP) ELISA for detection of FMDV NSP antibodies (Abs); where 111 of 381 (29.13%) sheep and 201 of 914 (21.99%) goats were positive suggesting their previous exposure to FMDV. Although the animals were not vaccinated, representative numbers of serum samples from both species were also tested in liquid phase blocking ELISA to cross-check the protective antibody titre against all three serotypes in the trivalent vaccine that revealed null or zero 'herd immunity'. The results illustrate the circulation of FMDV in sheep and goats in the particular agro-climatic region of the country and vaccination in these animals needs to be followed to build up desired level of herd immunity against FMD.

Key words: Foot-and-mouth disease virus, Goat, Gujarat, NSP ELISA, Sheep

Gujarat comes under Gujarat plains and hills agro-climatic region. This region includes the hills and plains of Kathiawar, and the fertile valleys of Mahi and Sabarmati rivers. It is an arid and semi-arid region. As per 19th Livestock Census - 2012 All India Report, 1.70 million sheep and 4.95 million goats are found in Gujarat, where these animals are mostly reared in rural areas of the state (Livestock census 2012). More than 70% of sheep and goats are kept by small/marginal farmers and landless labourers. The diverse species and huge number of animals affected by foot-and-mouth disease (FMD) outbreaks produce high ongoing impact in endemic nations (Onono *et al.* 2013). The circulation of FMD virus (FMDV) in livestock poses significant constraints on the trade of animals and derived products, crippling the economic back-bone of the affected countries (Carpenter *et al.* 2011, Muroga *et al.* 2012). Owing to the extreme contagious nature of the disease, rapid action needs to be implemented to check its spread, which necessitates prompt surveillance and tracing of infected farms, and movement restrictions of infected animals (Paton *et al.* 2014).

Present address: [1]Scientist (drmrout@gmail.com), [2]Research Associate (dr.bprusty@gmail.com), [3]Deputy Director (amit_kanani@hotmail.com), Department of Animal Husbandry, FMD Typing Scheme, Animal Disease Investigation Office, Ambawadi, Ahmedabad.

FMD exhibits variable severity, with cattle and pigs showing severe clinical illness whilst the infection tends to be mild or subdued in sheep and goats (Paton *et al.* 2014). The FMD control programme in India at present includes vaccination and serosurveillance only in bovines overlooking sheep and goats. In order to assess the true impact of an ongoing control programme, prevalence studies are useful to gather baseline information (Heffernan *et al.* 2009). For implementation of effective FMD control strategies, a thorough comprehension of the epidemiology of the disease is mandatory. This cannot be realized if the role of small ruminants in the disease epidemiology is overlooked, despite representing a huge fraction of the susceptible domestic livestock in India. With this background, this cross-sectional sero-epidemiological survey in Gujarat plains and hills agro-climatic region of India was aimed at demonstrating the FMDV nonstructural protein antibodies (NSP-Abs) as serological evidence of exposure in sheep and goats across 26 districts of Gujarat state during 2013–2014 with an objective to assess the extent of FMDV circulation in these species, so that their epidemiological role might receive due attention.

MATERIALS AND METHODS

Sampling and study area: Samples were collected from 1,295 small ruminants (age ranging from 1–5 years) including 381 sheep and 914 goats across Gujarat from 26

districts, viz. Anand, Ahmedabad, Rajkot, Banaskantha, Kheda, Bhavnagar, Gandhinagar, Junagadh, Valsad, Tapi, Surat, Jamnagar, Mehsana, Patan, Dang, Navsari, Amreli, Sabarkhantha, Dahod, Panchmahal, Bharuch, Porbandar, Kutchh, Surendranagar, Narmada and Vadodara during 2013–2014. The animals were not vaccinated against FMD. Blood (5 ml) was collected from each animal through jugular venepuncture using sterile vacutainer. The vacutainer tubes filled three-quarters with blood were kept slanting in racks at ambient temperature, followed by serum harvesting with centrifugation at 2,500 rpm for 10 min. Sera were stored at –80° C in the laboratory awaiting analysis.

3AB3 NSP ELISA: The serum samples were subjected to 3AB3 NSP ELISA using the validated in-house r3AB3 NSP ELISA kit (ICAR-Project Directorate on Foot and Mouth Disease (PDFMD), Mukteswar) to assess antibodies against 3AB NSP of FMDV (Mohapatra *et al.* 2011). The procedure as mentioned earlier by Rout *et al.* (2014) was followed for the purpose.

Liquid phase blocking (LPB) ELISA: To correlate the unvaccination status of the animals, representative numbers of serum samples from both species were tested in LPB ELISA. Two-fold dilution of serum samples were tested for determining the serotype-specific FMDV SP-Ab titre to assess the overall status of vaccinal immunity (Cut-off for protective antibody being $\log_{10}$ titre of ≥1.8 against all three component serotypes such as O, A and Asia 1 in the vaccine) using the in-house LPB ELISA kit (PDFMD, Mukteswar) as per the procedure described earlier (Ranabijuli *et al.* 2010).

RESULTS AND DISCUSSION

Out of 381 sheep and 914 goats, 111 sheep (29.13%) and 201 (21.99%) goats were positive for NSP-Ab suggesting their exposure to FMDV. In sheep, the NSP-Ab percentage positivity varied from 6.67% in Ahmedabad to 66.67% in Bhavnagar district, whereas in goats it varied widely from 0% in Ahmedabad and Bharuch to 55% in Sabarkhantha district. Total number of sheep and goat sera tested in 3AB3 NSP ELISA and the percentage positivity are presented in Table 1.

Representative numbers of serum samples from both species across all districts tested in LPB ELISA revealed no animals with protective antibody titre (≥1.8 $\log_{10}$ titre against all 3 serotypes in the vaccine) indicating null or zero 'herd immunity'. Traditionally managed goats and sheep are usually not vaccinated against FMD in the country. Therefore, demonstration of FMDV-Abs in these species

Table 1. Total number of sheep and goat sera from Gujarat plains and hills agro-climatic region tested in 3AB3 NSP ELISA with percentage positivity

District	Goat		Sheep	
	Number of samples tested	Number of samples found positive (% positivity)	Number of samples tested	Number of samples found positive (% positivity)
Anand	38	3 (7.89)	12	1 (8.33)
Ahmedabad	35	0 (0)	15	1 (6.67)
Rajkot	43	10 (23.26)	7	2 (28.57)
Banaskantha	35	4 (11.43)	15	3 (20.00)
Kheda	40	5 (12.50)	10	3 (30.00)
Bhavnagar	38	17 (44.74)	12	8 (66.67)
Gandhinagar	37	15 (40.54)	13	4 (30.77)
Junagadh	41	13 (31.71)	9	5 (55.56)
Valsad	36	12 (33.33)	14	4 (28.57)
Tapi	36	10 (27.78)	14	3 (21.43)
Surat	40	15 (37.50)	10	1 (10.00)
Jamnagar	39	7 (17.95)	10	4 (40.00)
Mehsana	39	19 (48.71)	10	5 (50.00)
Patan	29	7 (24.13)	20	8 (40.00)
Dang	40	1 (2.50)	10	1 (10.00)
Navsari	42	3 (7.14)	8	1 (12.50)
Amreli	24	2 (8.33)	25	14 (56.00)
Sabarkhantha	20	11 (55.0)	30	14 (46.67)
Dahod	36	17 (47.22)	14	3 (21.43)
Panchmahal	40	5 (12.5)	10	3 (30.00)
Bharuch	22	0 (0.0)	28	7 (25.00)
Narmada	50	2 (4.0)	0	-
Porbandar	10	3 (30.0)	40	6 (15.00)
Vadodara	50	16 (32.0)	0	-
Kutchh	25	1 (4.0)	25	8 (32.00)
Surendranagar	29	3 (10.34)	20	2 (10.00)
Total samples	914	201 (21.99)	381	111 (29.13)

becomes an indicator of definite exposure to the field virus (Hyera *et al.* 2006). The seropositivity in small ruminants might have been mostly derived from the persistent or the subclinical infection of FMD. Muhammed Saleh *et al.* (2013) having reported a high prevalence of seropositivity to virus-infection-associated antigen (VIAA) in previously diseased and healthy sheep in Basra, concluded it to be due to subclinical infection or to carrier state. Recovered sheep and goats can become carriers for 4–6 months (Alexanderson *et al.* 2002), whereas persistence of NSP-Ab up to 3 years post-infection in absence of virus recovery in small ruminants have been reported earlier (Paton *et al.* 2009). Because of mild clinical signs of FMD, the disease gets frequently ignored or misdiagnosed in small ruminants (Ganter *et al.* 2001) posing them as a concealed threat for virus transmission to cattle (Radostitis *et al.* 2007). Sheep without obvious symptoms may excrete considerable amounts of FMDV (de Bravo Rueda *et al.* 2014) thereby playing a significant role in FMD epidemiology even as source of infection to other susceptible animals. It has been speculated that transmission of FMDV from sheep to cattle might have occurred during the 1994 type O epidemic in Greece (Donaldson 2000), the 1999 type O epidemic in Morocco (Blanco *et al.* 2002) and the 2001 type O epidemic in UK (Gibbens *et al.* 2001).

Solid diagnosis is pertinent to any disease control programme (Namatovu *et al.* 2013) and serological testing is a valuable tool for FMD surveys that can promptly detect antibodies in infected as well as mildly symptomatic or even asymptomatic animals, where collection of lesions is not feasible (Brocchi *et al.* 2006). NSP ELISA detecting NSP-Ab is useful in providing evidence of previous or current FMDV infection irrespective of vaccination status and the serotype involved and this is the only way of serological identification of infected as well as carrier animals (Bronsvoort *et al.* 2004, Barnett *et al.* 2015). Following infection, NSP-Abs can be detected in serum for months or years (Chen *et al.* 2007). NSP seropositivity in the sampled small ruminants can be ascribed to the fact that the animals might have been infected recently, or some time before, or they might have fully recovered from FMDV infection and have become carrier (Paton *et al.* 2014). Such serosurveys have been reliably applied to determine the prevalence of FMD in small ruminants in FMD-endemic country Uganda (Balinda *et al.* 2009) and India (Rout *et al.* 2014).

The significant difference in prevalence estimates between the districts could be explained by the probable difference in husbandry practices and cross-border movements. The husbandry systems practiced in the sampled herds in surveyed districts were mainly extensive with free access to animal movement, which favours the spread of FMD. Another husbandry practice reflects both large and small ruminants being reared in close proximity. Communal grazing is practiced in most of the areas, where both small and large ruminants are allowed to use the same pasture land and water points. In such circumstances, the possibility of transmission of virus from infected cattle and buffaloes in contact to small ruminants cannot be ignored. Fernandez *et al.* (1976) reported that high percentage of subclinical infection of FMD occurred in sheep population kept in close contact with cattle. Intha *et al.* (2009) in Vientiane reported that FMD outbreaks were common where cattle were comingled with goats. Hence, there might be a predictive link between FMD seropositive small ruminants and the intermixing of domestic animals by the farmers. In Gujarat, shepherds in the unorganized sectors usually keep mixed flock of sheep and goats and they lead a nomadic life with frequent movement/migration of their flocks for grazing. In addition, these areas vary in terms of pasture and water availability, which might have caused the demand-driven movement of animals and herdsmen. Other studies also report that the movement of herds in search of pasture and water from one area to another is a significant risk factor for FMD (Fevre *et al.* 2006, Megersa *et al.* 2009, Habiela *et al.* 2010). Uncontrolled livestock movements play a key role in the spread of FMD that is always a threat to susceptible herds (Knight-Jones and Rushton 2013). Moreover, the state of Gujarat experiences periodical FMD outbreaks and the prevalence estimate for NSP-Ab in bovines of the state has been reported to be 17.56% (Annual Report PDFMD 2014–2015). Such episodic incidences of FMD in the districts might have caused the spillover of infection from domestic bovines to small ruminants.

As the control on livestock movement in a large sub-continent like India with numerous states having porous borders with an extensive livestock production system is practically tedious, alternative strategies involving regular surveillance and prophylactic vaccination appear to be imperative for FMD control. Moreover, vaccination is known to reduce the transmission of FMDV from infected to susceptible animals and also check silent amplification of virus (Orsel *et al.* 2005). Further such studies are necessary to characterize the circulating FMDV serotype(s) in the studied areas and determine the duration of carrier status in sheep and goats. Any control programme requires updating the status of knowledge on the current disease epidemiology, so that pertinent measures can be formulated and implemented. However, the above findings shed a ray of light on an indication that FMD control programme in an endemic country like India should strategically include small ruminants to achieve a targeted success. As sheep and goats in Gujarat plains and hills agro-climatic region are mostly reared in rural set up by marginal farmers for earning livelihood, it needs programmed attention on the livestock health. In these village areas, rural and transhumant production systems are practiced, where community grasslands and forests are the major source of feeding. Livestock rearing should be strongly integrated with various farming systems in this agro-climatic region for income supplementation of the farmers. Again, more surveillance studies targeting other infectious diseases need to be proposed and formulated so as to augment the animal health and profitability of the poor farming communities

in the region.

ACKNOWLEDGEMENTS

This research is an output from Indian Council of Agricultural Research (ICAR) funding. The authors wish to acknowledge the assistance of field and laboratory staff of FMD Typing Scheme, Ahmedabad, Gujarat in sample collection. Finally, we wish to wholeheartedly thank the farmers and livestock owners for their co-operation.

REFERENCES

Alexanderson S, Zhang Z and Donaldson A I. 2002. Aspects of the persistence of foot and mouth disease virus in animals - The carrier problem. *Microbes and Infection* **4**: 1099–1110.

Annual Report, PDFMD. 2014–2015. Project Directorate on Foot and Mouth Disease, Mukteswar, Nainital, India.

Balinda S N, Tjørnehøj K, Muwanika V B, Sangula A K, Mwiine F N, Ayebazibwe C, Masembe C, Siegismund H R and Alexandersen S. 2009. Prevalence estimates of antibodies towards foot-and-mouth disease virus in small ruminants in Uganda. *Transboundary and Emerging Diseases* **56**: 362–71.

Barnett P V, Geale D W, Clarke G, Davis J and Kasari T R. 2015. A review of OIE country status recovery using vaccinate-to-live versus vaccinate-to-die foot-and-mouth disease response policies I: benefits of higher potency vaccines and associated DIVA test systems in post-outbreak surveillance. *Transboundary and Emerging Diseases* **62**(4): 367–87.

Blanco E, Romero L J, El Harrach M and Sanchez-Vizcaino J M. 2002. Serological evidence of FMD subclinical infection in sheep population during the 1999 epidemic in Morocco. *Veterinary Microbiology* **85**: 13–21.

Brocchi E, Bergmann I E, Dekker A, Paton D J, Sammin D J, Greiner M, Grazioli S, De Simone F, Yadin H, Haas B, Bulut N, Malirat V, Neizert E, Goris N, Parida S, Sÿrensen K and De Clercq K. 2006. Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine* **24**: 6966–79.

Bronosvoort B M C, Sorensen K J, Anderson J, Corteyn A, Tanya V N, Kitching R P and Morgan K L. 2004. A comparison of two 2ABC ELISAs in a cattle population with endemic, multiple serotype foot-and-mouth disease. *Journal of Clinical Microbiology* **42**(5): 2108–14.

Carpenter T E, O'Brien J M, Hagerman A D and McCarl B A. 2011. Epidemic and economic impacts of delayed detection of foot-and-mouth disease: a case study of a simulated outbreak in California. *Journal of Veterinary Diagnostic Investigation* **23**: 26–33.

Chen S P, Lee M C, Sun Y F, Cheng I C, Yang P C, Lin Y L, Jong M H, Robertson I D, Edwards J R and Ellis T M. 2007. Immune responses of pigs to commercialized emulsion FMD vaccines and live virus challenge. *Vaccine* **25**(22): 4464–69.

de Bravo Rueda C, Dekker A, Eble P L and de Jong M C M. 2014. Identification of factors associated with increased excretion of foot-and-mouth disease virus. *Preventive Veterinary Medicine* **113**: 23–33.

Donaldson A. 2000. The role of sheep in the epidemiology of foot-and-mouth disease and proposasls for control and eradication in animal populations with a high density of sheep. *Session of the Research Group of the Standing Technical Committee of EuFMD.* 5–8 September 2000. Borovets: FAO of the United Nations.

Fernandez T F, Quition P A, Bulman G M, Ferria M E V and Alonso Fernandez A. 1976. Serological survey of FMD in sheep in the central valley of cockabamba, Bolivia. *Boletin del Centro Panamericano de Fiebre Aftosa* **21/22**: 35–43.

Fevre E M, Bronsvoort B M C, Hamilton K A and Cleaveland S. 2006. Animal movements and the spread of infectious diseases. *Trends in Microbiology* **14**: 125–31.

Ganter M, Graunke W D, Steng G and Worbes H. 2001. Foot and mouth disease in sheep and goats. *Deutsche Tierärztliche Wochenschrift* **108**(12): 499–503.

Gibbens J C, Sharpe C E, Wilesmith J W, Mansley L M, Michalopoulou E, Ryan J B and Hudson M. 2001. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. *Veterinary Record* **149**: 729–43.

Habiela M, Alamin M A G, Raouf Y A, Yahia H and Ali Y H. 2010. Epizootiological study of foot and mouth disease in the Sudan: the situation after two decades. *Veterinarski Arhiv* **80**: 11–26.

Heffernan C, Misturelli F, Nielsen L, Gunn G J and Yu J. 2009. Analysis of Pan-European attitudes to the eradication and control of bovine viral diarrhoea. *Veterinary Record* **164**: 163–67.

Hyera J M K, Letshwenyo M, Monyame K B, Thobokwe G, Pilane A R, Mapitse N and Baipoledi E K. 2006. A serological survey for antibodies to foot-and-mouth disease virus in indigenous Tswana goats and sheep in Kasane, Maun and Shakawe districts in northwestern Botswana. *Onderstepoort Journal of Veterinary Research* **73**: 143–47.

Intha P, Phitsanu T and Suvicha K. 2009. Descriptive study on risk factors associated with foot and mouth disease outbreak in cattle in Vientiane, the capital city of Lao People's Democratic Republic. *Chiang Mai Veterinary Journal* **7**(2): 97–106.

Knight-Jones T J D and Rushton J. 2013. The economic impacts of foot and mouth disease – What are they, how big are they and where do they occur? *Preventive Veterinary Medicine* **112**: 161–73.

Livestock Census (19th). 2012. All India Report, Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi. Pp. 1–120.

Megersa B, Beyene B, Abunna F, Regassa A, Amenu K and Rufael T. 2009. Risk factors for foot and mouth disease seroprevalence in indigenous cattle in southern Ethiopia: the effect of production system. *Tropical Animal Health and Production* **41**: 891–98.

Mohapatra J K, Pandey L K, Sanyal A and Pattnaik B. 2011. Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. *Journal of Virological Methods* **177**: 184–92.

Muhammed Saleh W M, Hasso S A and Abdulla F A. 2013. Serological diagnosis of FMD in sheep in Basra by ELISA test. *Iraqi Journal of Veterinary Sciences* **27**(2): 79–84.

Muroga N, Hayama Y, Yamamoto T, Kurogi A, Tsuda T and Tsutsui T. 2012. The 2010 foot-and-mouth disease epidemic in Japan. *Journal of Veterinary Medical Science* **74**: 399–404.

Namatovu A, Wekesa S N, Tjørnehøj K, Dhikusooka M T, Muwanika V B, Siegsmund H R and Ayebazibwe C. 2013. Laboratory capacity for diagnosis of foot-and-mouth disease in Eastern Africa: implications for the progressive control pathway. *BMC Veterinary Research* **9**: 19–29.

Onono J O, Wieland B and Rushton J. 2013. Constraints to cattle

production in a semi arid pastoral system in Kenya. *Tropical Animal Health and Production* **45**: 1415–22.

Orsel K, Dekker A, Bouma A, Stegeman J A and de Jong M C. 2005. Vaccination against foot and mouth disease reduces virus transmission in groups of calves. *Vaccine* **23** (41): 4887–94.

Paton D J, Ferris N P, Hutchings G H, Li Y, Swabey K, Keel P, Hamblin P, King D P, Reid S M, Ebert K, Parida S, Savva S, Georgiou K and Kakoyiannis C. 2009. Investigations into the cause of foot-and-mouth disease virus seropositive small ruminants in Cyprus during 2007. *Transboundary and Emerging Diseases* **56**: 321–28.

Paton D J, Füssel A E, Vosloo W, Dekker A and De Clercq K. 2014. The use of serosurveys following emergency vaccination, to recover the status of "foot-and-mouth disease free where vaccination is not practiced". *Vaccine* **32**: 7050–56.

Radostitis O M, Gay C C, Hinchcliff K W and Constable P D. 2007. *Text Book of the Disease of Cattle, Horses, Sheep, Pigs and Goats.* 10th edn, Pp. 1223–30. Saunders Elsevier Company Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

Ranabijuli S, Mohapatra J K, Pandey L K, Rout M, Sanyal A, Dash B B, Sarangi L N, Panda H K and Pattnaik B. 2010. Serological evidence of foot and mouth disease infection in randomly surveyed goat population of Orissa, India. *Transboundary and Emerging Diseases* **57**: 448–54.

Rout M, Senapati M R, Mohapatra J K, Dash B B, Sanyal A and Pattnaik B. 2014. Serosurveillance of foot-and-mouth disease in sheep and goat population of India. *Preventive Veterinary Medicine* **113**: 273–77.