



Potential transmission of foot-and-mouth disease from pigs to cattle in a mixed animal farming

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ABSTRACT

In the present report, investigation of foot-and-mouth disease (FMD) outbreak in a commercial pig farm located in the outskirts of Bengaluru in February 2018 was carried out. Disease with high morbidity and severity was noticed in the pig herd consisting of 500 animals. Clinically, the animals showed marked dullness, off feeding and limping along with severe vesicular lesions and ulcers on snout and skin around the coronary bands of pigs. The outbreak was caused by FMDV type O as tested by sandwich ELISA of the samples collected from a dead piglet. Demonstration of high levels of antibodies to structural proteins specific to serotype O (as compared to two other serotypes) in the presence of high titres of non-structural antibodies in the randomly collected samples 2 weeks after the episode was suggestive of widespread infection on the farm in the absence of zoo-sanitary measures. Disease transmission in the vaccinated cattle was also evidenced as animals housed in close proximity developed the disease. Vaccination of pigs in addition to large animals is important to avoid transmission of the disease to other animals as pigs may serve as source of active infection as observed in the present outbreak.

Keywords: Antigen detection ELISA, Foot and mouth disease, LPB ELISA, NSP antibody ELISA, Outbreak, Pig

Foot and mouth disease (FMD) is one of the most contagious diseases of farm animals with serious economic threat to the livestock industry. The disease is endemic in many parts of the world including India where it causes substantial economic loss. FMD affects mainly the cloven hoofed animals including cattle, buffalo, sheep, goats, pigs and wild ruminants. The disease is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae*. There are seven distinct serotypes of FMD virus (FMDV), namely O, A, C, SAT 1, SAT 2, SAT 3, Asia-1 and infection with any one serotype does not confer immunity against another (OIE 2018).

FMD is endemic in India with the prevalence of serotypes O, A and Asia-1 amongst which, serotype O accounts for about 80% of the total outbreaks recorded (Subramaniam *et al.* 2012). Karnataka, the eighth largest state in India (19.1 Mha area, 5.8% of the country), is situated in the centre of the western peninsular India. The state has a large FMD-susceptible livestock population which includes 9.50 million cattle, 3.47 million buffaloes, 9.58 million sheep, 4.79 million goats, 0.30 million pigs and an unknown population of wild herbivores as per the 2012 livestock census. Vaccination based FMD control

programme (FMD-CP), which involves biannual vaccination of all cattle, buffaloes and pigs is being implemented in the state since 2011. The disease is endemic and despite vaccination, several sporadic outbreaks in livestock are reported in the state and remain under a constant threat of FMD due to unrestricted movement of huge number of susceptible (unvaccinated) as well as infected animals. Poor management of the disease due to lack of zoo-sanitary measures during the face of outbreaks complicates the situation. Timely investigations of outbreaks are carried out to implement the measures towards efficient control of the disease and abort undue dissemination of the virus in the environment.

Pigs infected with FMDV are considered as robust emitters of airborne FMDV and cattle are highly prone to infection through inhalation. Consequently, pigs are referred to as the amplifier hosts of FMD (Brown 2001). Though the cattle are usually the most frequently involved in epidemics, and play an important role in the maintenance of the FMDV, the role of pigs in several recent FMD epidemics mainly in countries from Eastern Asia, cannot be ignored. Pigs were involved in 7 of 38 immediate notifications made to OIE between January 2010 and May 2011, including three of the first five reported outbreaks in the 2001 epidemic of FMD in UK (Donaldson and Alexandersen 2003, Leon 2012). In the present paper, we describe an outbreak of FMD in pigs in the month of

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February 2018 at a private organized farm and subsequent control measures undertaken. Post-mortem examination of the piglets that died in the outbreak revealed gross lesions typical of FMD, while laboratory investigation for presence of viral antigen in the tissue samples confirmed the disease. Presence of serum antibodies directed towards both structural and non-structural antibodies were demonstrable in the randomly collected samples from the farm at a later stage. The details on disease manifestations, possible causes of outbreak and the risks of disease spread to other farm animals are presented.

MATERIALS AND METHODS

Disease outbreak and clinical manifestations: The outbreak of FMD occurred at a private commercial piggery (K G Srikantapura, Bengaluru North, Bengaluru Urban district, latitude 13°03'N longitude 77°47'E). The farmer was not aware of the disease but noticed that the growing pigs in all the sheds had become dull and lame. On close inspection, it was observed that the pigs showed variable degree of lameness and most of the time were recumbent in a huddle at particular place. Squealing due to pain was observed when they were made to move. The pigs were unable to move to the feeders resulting in lower than normal feed intake further leading to loss in the weight gain. Initially, death in piglets was observed and within a week the farmer lost more than 30 piglets due to infection. Two piglets were presented for post mortem and the gross lesions typically were suggestive of FMD. Heart and foot lesions were collected during post mortem and the samples were processed and screened by using serotype detection antigen differentiating ELISA (sandwich-ELISA)

Outbreak investigation: The farm was situated in an isolated area away from the village where about 500 Large White Yorkshire, Duroc and Landrace breeds of different age groups were housed. During the visit it was observed that nearly 300 pigs were affected with the disease. The pigs were fed with kitchen-waste procured from the local restaurants. The food was not boiled before being fed to the animals. The infected animals were not isolated and no disinfection was carried out. Severe vesicular lesions typically on the snout and skin around the coronary bands were observed in the affected pigs. Almost all the affected animals were lame and recumbent due to the severity of lesions. Epithelial surface showed necrosis with prominent raw ulcer on the snout of affected pigs. Severe erosions and ulcerations around the coronary band with sloughing of the claw were also evident in few affected pigs. The farmer also was rearing 12 number of cattle and regularly vaccinated against FMD, but three heifers which had received fewer vaccinations, showed clinical signs first, before the disease spread to other animals with mild clinical signs. Animals recovered within 3–4 days.

Sample collection: Samples from heart and foot lesions were collected during post-mortem in buffered glycerine saline. Blood samples were collected (n=24) from the ear vein and sera were screened for FMDV antibodies.

Serotype differentiating antigen detection ELISA: Supernatants of the homogenized clinical tissue materials were used in a serotype differentiating antigen detection ELISA as per Bhattacharya *et al.* (1996) for confirmation of serotype of the virus involved in the outbreaks.

Liquid phase blocking ELISA (LPBE): The test was performed as per the procedure described in OIE manual of standards for diagnostic tests and vaccines. A two fold dilution of test sera was carried out from 1:4 to 1:1024 for titration of antibodies.

NSP antibody detection ELISA: The test was performed using a Prionics FMD NS kit as per the manufacturer's instructions. After colour development and stopping the reaction, the plate was read in ELISA reader (450 nm wavelength) and the absorbance values obtained were used to determine the percent-inhibition (PI) values. Serum samples having $\geq 50\%$ PI values were considered as positive to NSP antibodies.

RESULTS AND DISCUSSION

The present outbreak of FMD in pigs in an organized farm was due to FMDV serotype O as confirmed by both sandwich-ELISA and multiplex-PCR at the ICAR-DFMD laboratory, Mukteswar. Outbreak of FMD in a private pig farm as well as organized cattle and pig farm due to FMDV serotype O has been reported earlier (Rout *et al.* 2016, Rout *et al.* 2017). In VP1 region-based phylogenetic analysis, the serotype O isolates causing the outbreak were found to conglomerate within Ind2001 lineage. Since 2013, the FMD outbreaks in the state of Karnataka are due to serotype O alone, affecting mainly the cattle. The pigs in the farm were not vaccinated against FMD. The farm was situated away from the village and no outbreaks in the surrounding village were reported. The probable source may be the hotel waste feed procured from restaurants located in nearby town. Pigs are much more susceptible to infection by the oral route than ruminants (Grubman and Baxt 2004). Outbreak in a South African pig farm in 2000 due to swill feeding, UK outbreak of FMD during the 2001 involving a swill-fed pig unit, feeding of contraband abattoir offal to pigs reared in close proximity to cattle causing the outbreak in Uruguay were the recent episodes of disease transmission in pigs attributed to animal feed contamination (Sutmoller *et al.* 2003, Paton *et al.* 2009). The clinical signs in the present outbreak were observed in the growing pigs with initial mortality in piglets. Once infection is detected in a pig farm, the spread of contact infection within the farm/herd begins sooner, through animal-to-animal transmission (Leon 2012). In the present episode, because of lack of awareness, the animals were not isolated and zoo-sanitary methods were not followed resulting in the disease spread to the whole farm.

During the clinical episode of the disease in the present outbreak, severe vesicular lesions typically on the snout and skin and around the coronary bands were observed in the affected pigs. Almost all affected animals were lame and recumbent due to the severity of lesions. The initial

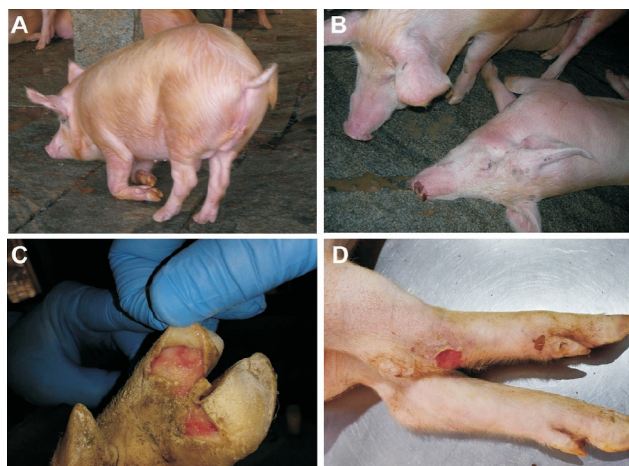


Fig. 1. Clinical signs/lesions of FMD in the pig. Inability or reluctance to stand on the feet (A) due to foot lesions ranging from mild vesicular lesions to severe erosions on the snout (B), foot (D) and especially in and around the coronary band with sloughing of the claw of FMD affected pig (C).

clinical signs were loss of appetite and lameness with mild fever, inability to rise up, reluctance to move and body tremors due to pain. Epithelial surface showed necrosis with prominent raw ulcer observed. Severe erosions and ulcerations around the coronary band with sloughing of the claw were also evident in many affected pigs (Fig. 1).

Coincident gross findings have earlier been reported (Leon 2012). Similar to the present outbreak, the incidence of the disease in non-immunized populations can be as high as 100% and mortality in piglets was also recorded in the affected herd as previously reported by others (Rovid *et*

al. 2010). The farmer was immediately advised to separate the infected animals followed by disinfection of the premises on a daily basis, and other sanitary measures. Within 10 days the disease was under control without any further cases of FMD and associated mortality. The clinical materials collected during post mortem were found positive for serotype O antigen in antigen detection ELISA and mRT-PCR. In LPBE, 23 out of 24 pigs demonstrated log₁₀ Ab titre of ≥ 1.8 , against FMDV serotype O, while the titres against other serotypes were very low. Although any evidence of SP-Ab is taken as an indicator of virus infection in disease-free countries that do not practice vaccination, only a clear spike in SP-Ab response against any serotype can demonstrate infection associated antibodies reliably, in the context of an endemic region practicing vaccination, even with low coverage. Hence, a serotype specific ≥ 4 -fold rise of LPBE titre (≥ 0.6 log₁₀ titre difference) in comparison to other two component serotypes in the vaccine was the criterion to assess infection antibody response. Serotype O specific rise in the antibody titre was observed in pigs compared to serotype A and Asia-1 as a consequence of exposure to infection rather than vaccination with a trivalent vaccine. In NSP ELISA, infection associated antibodies could be detected in all serum samples confirming the virus activity in the herd. Simultaneous detection of both NSP-Ab positivity and serotype specific rise in SP-Ab titre substantiated the infection status of the animals (Fig. 2).

FMD affected pigs may liberate vast quantities of airborne virus in their exhaled breath and the aerosol spread of the disease reportedly occurs over more than 10 km from

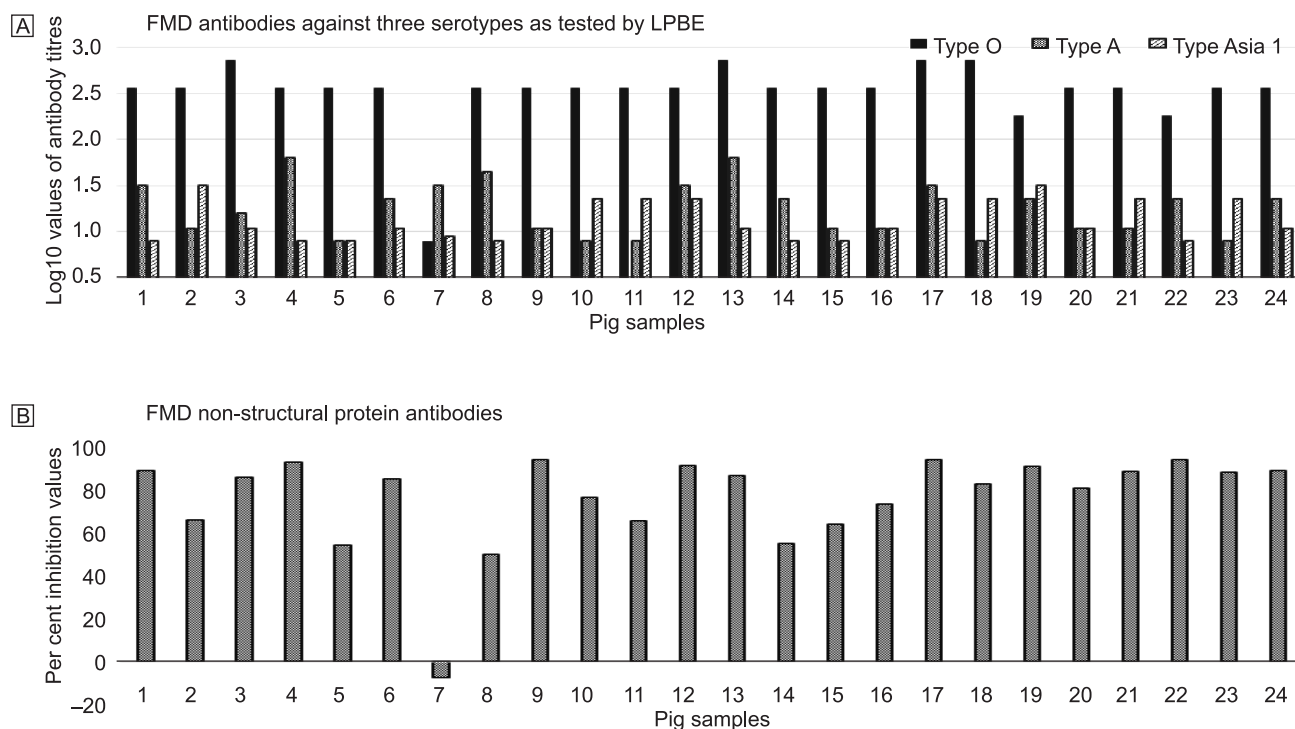


Fig. 1. FMD antibodies in pigs after the FMD outbreak on the farm. Structural and non-structural protein antibodies were measured by liquid phase blocking ELISA (A) and blocking ELISA (B) using commercial kit (Prionics FMD NS kit), respectively.

infected pigs to cattle downwind (Gloster *et al.* 1982, Donaldson 1987). In the present episode, the virus had spread to vaccinated cattle housed away from the pig herd and clinical lesions were observed in three vaccinated heifers. Movement of men or materials prone to contamination might have also played a role in spread of infection to the cattle. Most often, the spread of FMDV is associated with the movement of infected pigs which excrete large amount of virus in aerosol and ruminants get infected via inhalation of the infectious droplets (Alexandersen *et al.* 2003). Besides air borne spread from pigs to cattle, the disease transmission through animal attendants would have also played a role as no bio-sanitary measures were followed during the phase of outbreak.

In the present investigation, cattle housed in close proximity developed the disease though vaccinated 15 weeks back. Frank clinical signs including oral lesions in all the 12 animals with presence or absence of foot lesions were observed. However the animals recovered in 3–5 days. FMD infection was confirmed by detection of antibodies in the sera collected 18–22 days post recovery in cattle. Antibodies to both structural proteins (LPBE test) and non-structural protein (PrioCHECK FMD NS test) were detectable in the sera samples confirming the infection in cattle. This observation confirms that FMD transmission can occur in cattle despite FMDV vaccination status as animals had high exposure to virus following the wide spread infection in pigs in the adjacent herd. Though the vaccine-induced immunity was not assessed in this case, it could be expected that the serum titres would have been on the decline at 4 months post vaccination.

Though the vaccination based FMD-CP which involves biannual vaccination of all cattle, buffaloes and pigs is being implemented in the state since 2011, the farmer had not vaccinated the pigs. Hence creating awareness among farmers is necessary for controlling the disease. Training to improve the ability of disease recognition and reporting along with village-level biosecurity measures is of paramount importance in FMD ‘hotspots’ if sustainable initiatives at regional level meant for FMD control are to be actualized (Nampanya *et al.* 2013). The farming community should be well informed and trained about the clinical signs of FMD and strong awareness of the importance of prompt reporting of the disease to the official authorities must be created. Though pigs are covered under the FMD mass vaccination campaign, there is no focused approach on the vaccination of pigs in the commercial herds as they are maintained only for their productive life. However, this is important in view of the possible threat of transmission of the disease from pigs to the susceptible dairy herds.

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