Transmission of foot-and-mouth disease virus from experimentally infected Indian buffalo (Bubalus bubalis) to in-contact naïve and vaccinated Indian buffalo and cattle

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Abstract

This study investigated the transmission of foot-and-mouth disease virus (FMDV) from experimentally infected Indian buffalo to in-contact naïve and vaccinated cattle and buffalo. In each of six rooms, two donor buffalo that had been inoculated with FMDV were housed for five days with four recipient animals, comprising one vaccinated buffalo, one vaccinated calf, one unvaccinated buffalo and one unvaccinated calf. Vaccination was carried out with current Indian vaccine strain (O/IND/R2/75) and challenged on 28 days post-vaccination with an antigenically similar strain (O/HAS/34/05). All 12 donor buffalo and the six unvaccinated cattle and six unvaccinated calves developed clinical signs of foot-and-mouth disease (FMD). In contrast, all six vaccinated cattle (100%) and four out of six vaccinated buffalo (66.6%) were protected from disease but all became infected with FMDV. This confirms that buffalo have the potential to spread FMD by direct contact and that vaccination can block this spread. The numbers of animals in the study were too small to determine if the differences in clinical protection afforded by vaccination of cattle and buffalo are significant and warrant a different dose regime.

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1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of livestock and a major threat to trade and commodity markets worldwide [1]. FMD is endemic in India with serotypes O, A and Asia 1 virus in circulation and outbreaks are recorded throughout the year [2]. India has the world’s largest cattle and buffalo population and the 105 million buffalo constitute 57.3% of the world population according to the 2007 census. Indian (Asian) buffalo (Bubalus bubalis) are reared for milk, meat and draft purposes and thereby play an important role in the Indian economy. Buffalo contributed more than half (53.4%) of the total milk production in India during 2010–2011. In India, a mixed farming of cattle and buffalo is commonly practiced. The role of Indian buffalo in FMD epidemiology, disease transmission and immune response to vaccination has been poorly studied. Transmission of FMD virus from infected cattle to naïve buffalo and further transmission of virus from buffalo to naïve goats were reported previously [3]. Transmission of FMDV from affected cattle and pigs to naïve buffalo as a result of close contact has also been cited in the literature [4].

In a sub-clinical episode of FMD, introduction of Indian buffalo into a cattle herd was postulated as the probable cause of an outbreak [5]. African buffalo (Syncerus caffer) are known to be susceptible to FMDV, to carry virus for long periods without showing clinical signs, and to be efficient maintenance hosts of the Southern African Territories (SAT) type viruses [6]. African buffalo can carry the virus for a period of 5 years, and isolated herds up to 24 years, although the persistence in individual buffalo is probably not lifelong [7]. Transmission of SAT-type virus from persistently infected African buffalo to cattle under experimental and natural conditions has been demonstrated [8] and possibly occurs via sexual contact [9]. Findings for African buffalo may not hold good for Asian buffalo since the two species are distinct, and their roles in FMD epidemiology probably differ. In our earlier study [10], a buffalo infected...
via the dental pad transmitted infection to naïve cattle and buffalo after 24 h direct contact. Considering the large population of buffalo in India, the practice of mixed farming of buffalo and cattle and the inclusion of buffalo in the current national vaccination control program along with cattle, we investigated the possibility of transmission of FMDV from experimentally tongue inoculated Indian buffalo to in-contact naïve and vaccinated buffalo and cattle. The efficacy of FMD vaccine in buffalo was also studied by simulating a direct contact challenge experiment as knowledge of vaccine efficacy is limited in buffalo and assumptions have been made from cattle studies.

2. Materials and methods

2.1. Cell lines and viruses

Baby Hamster kidney 21 (BHK-21), primary calf thyroid (CTY) and the Instituto Biologico Renale Swine-2 (IBRS2) cells were provided by the tissue culture laboratory, Research and Development Centre, Indian Immunologicals Limited (IIL), Hyderabad. O/IND/R2/75 vaccine strain was received from the virus seed laboratory, IIL, Hyderabad. O/HAS/34/05 virus was used for experimental infection of buffalo. O/HAS/34/05 virus is homologous to O/IND/R2/75 (rY value > 1.00) [11]; and was isolated from epithelial tissue of a suspected FMD case in a non-vaccinated buffalo from Sirsa District, Haryana State.

2.2. Challenge virus preparation

Challenge virus O/HAS/34/05 was prepared by passing in the tongues of buffalo calves as described for cattle by NagendraKumar et al. [12]. Briefly, one buffalo calf was inoculated intradermally with BHK 21 monolayer adapted O/HAS/34/05 virus (10⁵ TCID₅₀). The tongue epithelium was collected 48 h post inoculation. For a second passage, epithelial tissue was collected from vesicles and after trituration in 0.04 M phosphate buffer followed by centrifugation at 3000 rpm; the clear supernatant was used to inoculate (intradermally) the 2nd buffalo. The same procedure was followed for third buffalo passage. Then the tongue epithelium was collected from third passage buffalo and 20% W/V virus suspension was prepared. To make the glycerol stock 50% of sterile glycerol was added to the virus suspension and stored at −20 °C. The virus was then titrated in buffalo calves to establish the buffalo infective dose 50 values (BID₅₀).

2.3. Experimental animals

Murrah male buffalo calves (n = 24; 6–12 months of age) and crossbred male cattle calves (n = 12; 6–12 months of age) were obtained from the holding farm of IIL, Hyderabad. These animals were reared in the farm from one month of age and were screened by 3 rounds of testing for FMDV-non-structural protein (NSP) antibodies using PrioCHECK® FMDV NS kit (PrioLynks Lelystad B.V., The Netherlands) and structural antibodies [13]. All the animals were negative against both NSP and structural antibodies in all the three rounds of testing. In addition, the animals were tested for the absence of virus in the oesophago-pharyngeal fluids (Probang samples) by inoculation of primary bovine thyroid cells [14] followed by antigen ELISA [15] and RT-PCR [16].

2.4. Vaccines, vaccination and experimental design

Monovalent FMD vaccine incorporating O/IND/R2/75 (7 μg/dose) FMDV inactivated antigen was formulated with Montanide ISA 206 (Seppic, France) as a water-in-oil-in-water (W/O/W) emulsion. One group of buffalo calves (GrI; n = 6) and a second group of cattle calves (GrII; n = 6) were administered with 2.0 ml of formulated vaccine by intra-muscular route whereas a third and a fourth group of buffalo (GrIII; n = 6) and cattle (GrIV; n = 6) calves remained unvaccinated. Donor buffalo (n = 12) were inoculated with 10⁶ BID₅₀ of buffalo passaged O/HAS/34/05 FMDV by the intradermologing route at 24 h before contact challenge. At 28 days post-vaccination, one animal from each group (GrI, II, III and IV) was housed for 5 days in an individual room along with two donor buffalo that were inoculated with FMDV 24 h prior to introduction. After 5 days of contact challenge, the vaccinated and non-vaccinated animals were separated from the donors. These animals were rehoused with their original groups (Fig. 1). Clinical signs and rectal temperatures were monitored for 15 days post challenge. Experiments were conducted in a bio-secure animal isolation unit at IIL.

2.5. Sample collection and processing

Clotted blood for serology to detect antibodies to both structural and non-structural proteins was collected from in-contact vaccinated and non-vaccinated cattle and buffalo on 0, 7, 14, 21 and 28 days post-vaccination and on 9, 14, 19, 25, 32 and 39 days post exposure. The sera were separated, inactivated at 56 °C for 30 min and stored at −20 °C until further use.

2.6. Virus neutralizing antibody test (VNT)

Titres of neutralising antibodies against FMDV O/IND/R2/75 virus were measured by micro-neutralization assay as described in the OIE Manual of Diagnostic Tests and vaccines [13].

2.7. Non-structural protein antibodies

Antibodies to FMDV NSP 3ABC were tested using PrioCHECK® FMDV NS kit (PrioLynks Lelystad B.V., The Netherlands) [17].

2.8. Statistical analysis

A linear mixed model was used to compare neutralising antibody titres, with log₁₀ titre as the response variable and time post challenge (as a factor), species and vaccination status as fixed effects and animal as a random effect. Model selection proceeded by stepwise deletion of non-significant terms (as judged by the Akaike information criterion (AIC)) starting from a model including time post challenge, species and vaccination status together with pairwise interactions between each variable.

Similarly, a linear mixed model was used to compare NSP antibodies responses, with percentage inhibition as the response variable and time post challenge (as a factor), species and vaccination status as fixed effects and animal as a random effect. Model selection proceeded by stepwise deletion of non-significant terms (as judged by the AIC) starting from a model including time post challenge, species and vaccination status together with an interaction between species and vaccination status.

Correlation between pre-challenge serum neutralising antibody titres (i.e. those on day 0 post challenge) and post-challenge NSP antibody responses (on day 32 and 39 days post challenge) were assessed for vaccinated buffalo and cattle using Spearman’s rank correlation coefficient. Correlations between serum neutralising antibody titres and NSP antibody responses at each time point, post challenge, were also examined using Spearman’s rank correlation coefficient for unvaccinated and vaccinated cattle and buffalo.

All statistical analyses were implemented in R [18].
3. Results

3.1. Clinical findings

All twelve of the needle challenged donor buffalo showed tongue and foot lesions as expected. All the vaccinated cattle (6/6) and four vaccinated buffalo (4/6) were protected from clinical disease after 5 days direct contact challenge with these clinically infected donor buffalo. This difference in protection (6/6 in cattle vs 4/6 in buffalo) is not statistically significant (Fisher exact test: $P=0.45$). Out of the two unprotected vaccinated buffalo, one showed foot lesions at 10 days post-challenge (dpc) whereas the other one showed foot lesions only in one foot starting from 12 dpc (Table 1). The lesions observed were smaller in size in comparison to those seen in the non-vaccinated infected animals. No tongue lesions were observed in these two unprotected vaccinated animals. Foot lesions in two of the non-vaccinated buffalo were observed at 7 dpc, whereas foot lesions in the other four non-vaccinated buffalo were observed at 11 dpc. Only one non-vaccinated buffalo developed a tongue lesion, which was observed at 7 dpc. Five non-vaccinated cattle showed foot lesions at 10 dpc and one showed a foot lesion at 11 dpc. Four of these six

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$+$—number of foot revealed lesion, TL—tongue lesion, DL—dental pad lesion.
unprotected cattle showed tongue or dental pad lesions at 10 dpc, one showed at 7 dpc and the 6th one did not show any tongue or dental pad lesion. Pyrexia (≥39.0°C to 40.2°C) was recorded at the same time as the appearance of vesicles, but was less evident in the vaccinated unprotected animals in comparison to the unprotected non-vaccinated animals.

3.2. Neutralising antibody response

A neutralizing antibody titre to FMDV O/IND/R2/75 was detected as early as 14 dvp and peak antibody titres were obtained at 28 dvp in vaccinated buffalo and cattle. The mean antibody titre in vaccinated buffalo and cattle were $10^{1.2}$ (95% confidence interval (CI): $10^{0.8} – 10^{1.7}$) and $10^{1.5}$ (95% CI: $10^{1.2} – 10^{1.8}$), respectively, at the time of exposure. Two vaccinated buffalo that showed clinical signs had low serum neutralizing antibody titres ($10^{0.8}$; $10^{1.3}$) whereas a third vaccinated buffalo with low neutralizing antibodies ($10^{1.1}$) at the time of exposure was protected.

Following the challenge exposure, the serum neutralising antibody titres were observed in the range of $10^{1.2}$ to $10^{1.8}$ up to 32–39 days post challenge in vaccinated buffalo and cattle (Fig. 2). In non-vaccinated control buffalo and cattle a rapid seroconversion was evident following exposure to challenge and the antibody titres ($10^{1.0}$ to $10^{4.0}$) were detected up to 32–39 dpc (Fig. 2). Both vaccinated buffalo and cattle had significantly higher neutralising antibody titres than non-vaccinated control buffalo and cattle at all time points post exposure, but there was no significant difference in serum neutralising antibody titres between vaccinated buffalo and cattle at any time point post exposure.

3.3. NSP antibody response

NSP antibodies appeared at 9 dpc in three non-vaccinated buffalo and four non-vaccinated cattle, at 14 dpc in two non-vaccinated buffalo and two non-vaccinated cattle and at 19 dpc in one non-vaccinated buffalo. NSP antibodies were detected at 14 dpc in three vaccinated buffalo and two vaccinated cattle while two vaccinated buffalo and one vaccinated cattle showed NSP antibodies at 32 dpc. One vaccinated buffalo and two vaccinated cattle were not positive for NSP antibodies. Virus replication occurred earlier in non-vaccinated control animals than in the vaccinated animals as was evident from antibody responses against NSP (Fig. 3).

NSP antibody responses were significantly lower in vaccinated cattle compared with unvaccinated cattle and unvaccinated buffalo, but not significantly different when compared with vaccinated buffalo. Responses did not vary significantly amongst any of the other groups (i.e. unvaccinated cattle, unvaccinated buffalo and vaccinated buffalo).

3.4. Correlation between neutralising and NSP antibody responses

There was no significant correlation between pre-challenge serum neutralising antibody titres and post-challenge NSP antibody responses (at either 32 or 39 days post challenge) in vaccinated buffalo or cattle. Furthermore, there was no significant correlation between neutralising antibody titres and NSP antibody responses at any time point post exposure for vaccinated or unvaccinated cattle or buffalo.

4. Discussion

India has the world’s largest buffalo population and mixed farming of cattle and buffalo is practiced by farmers. The current FMD control programme in India mainly involves mass vaccination of cattle and buffalo. However, the efficacy of FMD vaccination of buffalo is poorly understood and assumptions have been made by extrapolation from cattle studies. Although, some studies have investigated the transmission of FMDV from infected buffalo to naive buffalo and cattle [3–5], no detailed study has been made until now to find out the efficacy of FMD vaccines in buffalo, in particular to investigate the ability of vaccine to block the transmission of FMDV from in-contact infected buffalo to vaccinated buffalo and cattle. Therefore, this study was designed to investigate the efficacy of current Indian FMD vaccine (O/IND/R2/75) in buffalo and its ability to prevent the disease transmission from in-contact infected buffalo that were challenged with a homologous ($r_1$ value > 1.00) virulent strain (O/HAS/34/05). Both the vaccine and challenge viruses belong to the Middle East-South Asia (ME-SA) topotype. Simultaneously, we compared the transmission of disease from in-contact infected buffalo to vaccinated cattle.

Intradermolingual inoculation of FMDV resulted in generalised disease in all the donor buffalo. The donor buffalo showed both tongue and foot lesions. These results differ from the observations of Madder et al. [19], in which the reaction of buffalo to experimental infection was mild. It may be significant that the virus used in that experiment was of bovine origin, without adaptation to buffalo. However, in the present study, buffalo origin virus, further adapted by three passages in buffalo was used which might be the reason for prominent FMD clinical signs in buffalo. This might also have contributed to more prominent signs in the non-vaccinated buffalo compared to the non-vaccinated cattle. However, the dental pad/tongue lesions were less prominent in in-contact, non-vaccinated, infected buffalo compared to in-contact non-vaccinated infected cattle. This finding is in agreement with earlier studies [5,10,19–21].

In India, it has been observed that FMD is often overt in cattle but covert in buffalo, making it difficult to establish the origin and source of infection. The present study showed that buffalo may be infected as readily as cattle and they can also act as a source of infection for healthy cattle and buffalo upon direct contact, as reported in the field by Gomes et al. [5].

All the vaccinated cattle and four out of six vaccinated buffalo were protected. However, two vaccinated buffalo and all the non-vaccinated cattle and buffalo were clinically affected. The study indicated that FMD could be transmitted from infected buffalo to in-contact non-vaccinated buffalo and cattle. The study also indicated that FMDV transmission could be reduced by vaccinating buffalo. Although two vaccinated buffalo were clinically infected, the delayed and low level of non-structural antibody response indicated that there was less viral replication in these animals than the unvaccinated in-contact infected animals. Though the challenge virus is antigenically homologous to vaccine strain, these two vaccinated buffalo with $10^{0.65}$ and $10^{1.1}$ neutralising antibody response were not protected whereas a third vaccinated buffalo with similar range ($10^{1.1}$) of neutralizing antibody response was protected. Similar observations were made in cattle previously where the animals with medium to high neutralising antibody responses were not able to protect against challenge in contrast to animals with low neutralising antibody response that were protected [22,23]. Moreover, protection against FMDV infection has been observed in the absence of a detectable specific humoral response [24]. Furthermore, it has been recently reported that not only humoral antibody, but also the cell-mediated immune response have a role in FMD vaccine-induced protection [25]. However, in this study measurement of cell-mediated immune response has not been characterized. In the present study, serum neutralizing antibody responses were detected at 14 dvp and peak serum neutralizing antibody titre were reached at 28 dvp in both vaccinated buffalo and cattle. The antibody response elicited by vaccinated and non-vaccinated buffalo was comparable with antibody responses induced in vaccinated and non-vaccinated cattle, respectively. This
finding is in agreement with our earlier vaccine work (unpublished) and also in non-vaccinated cattle and buffalo [5].

There was no essential difference in the detection of FMD NSP antibodies after infection between non-vaccinated cattle and buffalo. All the vaccinated and non-vaccinated buffalo and cattle showed NSP antibodies after challenge indicating virus multiplication in these animals. This clearly indicated that sterile immunity could not be induced even though the dose of the vaccine was adequate to offer clinical protection in cattle. Although the titres of neutralising antibodies were similar for vaccinated cattle and
buffalo, two out of six vaccinated buffalo were clinically infected. Higher payloads of antigen than the usual cattle dose may possibly confer protection in buffalo and therefore a dose response study in buffalo may be warranted.

In conclusion, the study indicated that FMDV could be transmitted from infected buffalo to susceptible in-contact naïve buffalo and cattle by direct contact. FMDV vaccination of buffalo could reduce the transmission of disease by reducing virus replication, but for completely blocking the transmission of FMDV, higher doses of antigen payload might be required in the vaccine formulation. The study highlights the potential role of Indian buffalo in FMDV transmission, and this is something that may have an impact on future control strategy.

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References