Persistence of foot and mouth disease virus type Asia 1 in secretions of vaccinated and unvaccinated calves following experimental infection

S. KAPIL, K.L. AHUJA and S. PRASAD*

Summary: Studies on the excretion in nasal secretion, saliva and oesophageal-pharyngeal fluid of foot and mouth disease virus type Asia 1 in 8 vaccinated and 6 unvaccinated calves were undertaken after exposure of the animals by the intradermolingual route. Vaccinated calves failed to develop viraemia, but virus could be detected in their saliva and nasal secretion during five days.

KEYWORDS: Aphthovirus - Asia - Calf - Cattle diseases - Experimental infection - Secretion - Vaccination.

INTRODUCTION

The major cause of aphthous fever in the north-west region of India during the years 1976 and 1984 was ascribed to foot and mouth disease (FMD) virus type Asia 1 (2). Studies have been carried out on the persistence in saliva and milk of FMD virus after experimental infection of vaccinated cattle with foot and mouth disease virus type C vaccine (15) and in oesophageal-pharyngeal fluid with type O1 vaccine (5). However, such studies with FMD virus type Asia 1 are lacking. The cyclic pattern of Asia 1 as the major type of FMD virus recurring after long intervals necessitated studies on the persistence of the virus in nature and animals.

MATERIALS AND METHODS

Calves

Fourteen cross-bred calves in the age group of 6-8 months were acquired locally. The calves were dewormed with fenbendazole (Panacur®**), sprayed with Malathion®** and their blood smears screened for haemoprotozoa. Oesophageal-pharyngeal fluids were examined for the absence of carrier virus (14). Healthy calves were kept under normal husbandry conditions.

Mice

Swiss albino unweaned mice, 4-5 days of age, were obtained from the Disease-free Small Animal House, Haryana Agricultural University, Hisar.

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** Panacur® (Hoechst Pharmaceuticals Ltd., Hoechst House, Nariman Point, Bombay).

Malathion® (HAFED, Chandigarh).
Virus

Standard FMD virus type Asia 1 was obtained from the Central FMD Laboratory, Indian Veterinary Research Institute, Mukteswar-Kumaon. It was revived in BHK-21 monolayer and given four serial passages. The type of virus was further confirmed by inoculation in suckling mice followed by the complement fixation test (3). The virus was stored at -20°C.

Vaccine

Monovalent Asia 1 vaccine (tissue culture, aluminium hydroxide adsorbed formalin inactivated) was received by courtesy of Bhartiya Agro-Industries Foundation, Pune. Eight calves were vaccinated subcutaneously at the side of the neck with 2.5 ml, while six were kept as controls.

Collection of samples

Before collection of samples, the calves were kept off feed overnight and were only given water. Samples were transported on ice to the laboratory.

Nasal secretion

Nasal secretion was collected using the technique of Matsumoto et al. (7), with slight modifications. Briefly, a tampon weighing approximately 0.5 g was inserted at roughly the area where the pink mucosa starts in the nostril of the calf and was allowed to remain there for 10 minutes. The tampon was then removed with the aid of forceps and weighed. Dulbecco’s phosphate buffer saline (PBS) pH 7.2 was mixed at 1 ml/g, which gave a dilution of 1:2. Later, it was kept at 4°C for 1 hour for proper dissolution of the secretion and then squeezed with the help of a 20 ml syringe. In this way approximately 1 ml of nasal secretion was obtained.

Saliva

Saliva was collected by pulling the tongue of the calf to one side and simultaneously stimulating the oral mucosa with the fingers. Two ml of saliva were obtained from an animal at a time.

Oesophageal-pharyngeal fluid (OPF)

For collection of OPF, a sterilized cup probang was used following the technique of Sutmoller and Gaggero (12). After collection, the probang was immediately washed with 4 per cent sodium carbonate solution. About 2 ml of OPF was easily collected from each calf.

Plasma

Heparinized (10 IU/ml) blood samples were centrifuged at 3,000 rpm for 5 minutes to obtain plasma.

Processing of samples for virus isolation

Nasal secretion

Nasal secretion, collected by the tampon method described earlier, was centrifuged at 5,000 rpm for 15 minutes and the supernatant was inoculated into suckling mice.
Saliva

One part of saliva was mixed with an equal volume of Dulbecco’s PBS, shaken and centrifuged at 5,000 rpm for 5 min. (9). The supernatant was inoculated at 0.05 ml/suckling mouse, intramuscularly.

Oesophageal-pharyngeal fluid

The samples were transported on ice to the laboratory and an equal volume of 0.08M PBS (pH 7.2) was mixed with them (1). Penicillin at 1000 IU/ml and streptomycin at 500 µg/ml were added. The buffered OPF was shaken with an equal volume of fluorocarbon* in a shaker for 30 minutes and then centrifuged at 4,000 rpm for 10 minutes.

All the samples were then inoculated into suckling mice at the dose of 0.05 ml intramuscularly. The mice were observed for 10 days. Affected mice developed listlessness, cyanosis, paralysis of hind limbs and died within 48-72 hours.

The mouse carcasses were eviscerated and fascia and other organs removed. A 10 per cent suspension of skeletal muscle including heart in veronal buffer (pH 7.2) was prepared and was used as a source of antigen for the complement fixation test using the technique of Forman (3).

Challenge

The Asia 1 virus was titrated in BHK-21 cell monolayer and calves were infected with 10,000 TCID$_{50}$/ml of virulent virus by the intradermolingual route using the technique of Henderson (4). The animals were challenged at 21 days post-vaccination.

RESULTS

Results showing the persistence of virus in different secretions are given in Table I. Virus could be recovered from all the animals on each day.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appearance</th>
<th>Disappearance</th>
<th>Persistence</th>
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<tr>
<td>NS</td>
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<td>OPF</td>
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NS = Nasal secretion; SAL = Saliva; OPF = Oesophageal-pharyngeal fluid; PL = Plasma; Vac. = Vaccinated; Cont. = Control.
a-1 DPC = 16 hours, b- = Virus absent.

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Nasal secretion

Vaccinated and non-vaccinated calves did not differ in the persistence of virus in nasal secretion. The virus could be detected for 5 days in both groups.

Saliva

Asia 1 virus could be detected in saliva of control calves even before the appearance of lesions. The virus persistence was longer in controls (7 days) compared to vaccinated calves (5 days).

Oesophageal-pharyngeal fluid (OPF)

Virus was detected earlier in the OPF of control calves (1 day) compared to vaccinated calves (2 days). Persistence in the pharyngeal area was also longer in the control group.

Viraemia

Viraemia developed only in control calves and lasted for 3 days. Vaccinated calves failed to develop viraemia.

Lesions

In vaccinated challenged calves only primary lesions at the site of inoculation (i.e. the tongue) could be seen. However, one calf did not develop any lesion. Unvaccinated infected calves developed lesions on both tongue and feet. However, one calf developed lesions on the tongue only, while another calf did not develop any lesion.

DISCUSSION AND CONCLUSION

In the present study, FMD virus could be detected in saliva and oesophageal-pharyngeal fluid of control calves following challenge before the appearance of lesions. In unvaccinated challenged calves virus could be detected in buccal and nasal secretions from days 1 and 2, respectively. The virus could be detected up to 6 days post-challenge in nasal secretion. Similar observations have also been reported (10). Prasad and Kumar (9) detected FMD virus type Asia 1 in nasal secretion of susceptible calves for up to 184 hours.

Following challenge, virus could be detected for 5 days in saliva of vaccinated calves while, in controls, it could be detected for a week. Maximum persistence of foot and mouth disease virus in saliva was 9 days (10), while Prasad and Kumar (9) detected Asia 1 in saliva of susceptible calves for 7 days post-infection. Weyhe (15) reported that animals vaccinated with type C monovalent vaccine excreted the virus in saliva, mostly between 4 to 6 days post-challenge, but without showing any clinical signs.

Oesophageal-pharyngeal fluid was treated with fluorocarbon. Treatment with fluorocarbon eliminated fungous and bacterial contaminants and apparently reactivated aphthovirus from neutralising antibody and other inhibitors (11). Increase in the virus titre by log 1.0-2.0 has been reported following treatment with fluorocarbon (14). Virus could be detected in OPF up to 13 days post-challenge in...
vaccinated animals and for 21 days in control animals (maximum period of observation). Kaaden et al. (5) detected FMD virus type O1 in OPF samples using bovine thyroid cell culture for 9 months after challenge. This might be due to higher sensitivity of thyroid cell culture compared to the BHK-21 or the suckling mouse system. The suckling mouse and BHK-21 system almost reacted in parallel as far as a recovery of FMD virus was concerned (14).

Leeuw et al. (6) reported that all the 10 vaccinated animals infected 2-9 months after the last vaccination by intranasal route resisted the challenge, but virus multiplied in the pharyngeal area to a lesser extent than in susceptible control animals. Immunoelectromicroscopy (8) and immunofluorescence (9, 13) have also been employed for detection of foot and mouth disease virus in oesophageal-pharyngeal fluid. These have high sensitivity and are useful in detecting low concentration of virus. None of the vaccinated calves developed disease — i.e. neither viraemia nor secondary lesions, indicating that all were protected. However, virus could be detected in their secretions.

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PERSISTANCE DU VIRUS APHTEUX DE TYPE ASIA 1 DANS LES SÉCRÉTIONS DE VEAUX VACCINÉS ET NON VACCINÉS, APRÈS INFECTION EXPÉRIMENTALE. —

S. Kapil, K.L. Ahuja et S. Prasad.

Résumé : L'étude porte sur l'excrétion du virus aphteux de type Asia 1 chez des veaux de 6 à 8 mois. Les prélèvements ont été réalisés aux niveaux de la sécrétion nasale, de la salive et du liquide oesophago-pharyngien chez 8 veaux vaccinés et 6 non vaccinés. Les animaux ont été soumis à une infection expérimentale par voie intradermolinguale. Les veaux vaccinés n'ont pas présenté de virémie mais le virus a été détecté dans leur salive et leur sécrétion nasale pendant cinq jours après l'infection expérimentale.


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PERSISTENCIA DEL VIRUS AFTOSO DE TIPO ASIA 1 EN LAS SECRECIONES DE TERNEROS VACUNADOS Y SIN VACUNAR, PREVIA INFECCIÓN EXPERIMENTAL. — S. Kapil, K.L. Ahuja y S. Prasad.

Resumen: Se refiere el estudio a la excreción del virus aftoso de tipo Asia 1 en terneros de 6 a 8 meses de edad. Se realizaron las muestras a niveles de la secreción nasal, saliva y líquido esofagogástrico en 8 terneros vacunados y 6 sin vacunar. Se sometieron los animales a una infección experimental por vía intradermolingual. Los terneros vacunados no presentaron viremia, pero se les detectó el virus en la saliva y secreción nasal durante 5 días después de la infección experimental.


REFERENCES


