# DEMONSTRATION OF ANTIBODIES AGAINST FOOT AND MOUTH DISEASE VIRUS (FMDV) TYPE O AND ASIA-1 IN NON-DESCRIPTIVE CROSSBRED CALVES

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**Summary.** – Sera from non-descriptive crossbred calves were screened for the presence of neutralizating antibodies against FMDV type O and Asia-1 for a period up to 215 days. The antibody titer of 16 remained constant up to 215 days against type O and up to 190 days against type Asia-1 virus in some animals. In majority of the animals the antibody titers remained constant up to three months. The possible reason for a frequent breakdown of immunity in the vaccinated animals even 3 – 4 months after vaccination could be the fact that the calves were vaccinated repeatedly at their very early age with an ill effect of immunization in the presence of persisting antibodies.

**Key words:** Foot and mouth disease virus; maternally derived antibodies; serum neutralization antibodies

FMD is widely prevalent in India for the past few decades. Four types of FMDV O, A, C and Asia-1 have been recorded. For the control of FMD, the application of a polyvalent vaccine contaming types $O_5$, $A_{22}$, $C_1$ and Asia-1/1 is recommended at intervals of 4 to 6 months. For the past 10 years a majority outbreaks reported from different parts of India were due to virus type O (more than 50%). Next to type O was type Asia-1 (20 – 30%) (Rama Rao and Rao, 1988). For both the types there were also reports of vaccine breakdown.

In the present study the emphasis was given on the estimation of serum neutralization (SN) antibody titers in calves against FMDV type O and Asia-1 procured from unknown sources.

Non-descriptive young calves with no available history were procured at regular intervals from the local market. The animals were screened for the absence of SN antibodies against FMDV type $O_5$, $A_{22}$, $C_1$, and Asia-1 before subsequent use in inocuity and safety testing of FMD vaccine. A total of 24 non-descriptive crossbred calves in the age group of 6 to 12 months were purchased from the local market. The sera were screened for neutralizing antibodies against FMDV type O and Asia-1.

Vaccine strains of FMDV type O and Asia-1 maintained in cattle tongue epithelium were adapted to BHK-21 cells (5 – 6 serial passages). The viruses were titrated in 96-well microtiter plates and stored at -70 °C after adding equal volume of preservatives i.e. sucrose and lactalbumin hydrolysate.

The calves were bled periodically and the serum after separation was inactivated at 56 °C for 30 mins. Aliquots of the serum samples were distributed into polypropylene vials and were stored at -20 °C.

BHK-21 (Glasgow) cells were used for the SN test, that was carried out in 96-well microtiter plates. Foetal calf serum (Flow) was used for growth and maintenance of cells. Serial dilutions of the serum from 1:4 to 1:16 was added to cell monolayers in microplates (50 µl per well). Then 100 $TCID_{50}$ of virus in 50 µl was added into each well. The results were expressed as the reciprocal of the highest dilution of serum which neutralized the virus. Groups of eight animals were tested for the presence of SN antibodies against both FMDV types (O and Asia-1).

The results show (Table 1) that the SN antibodies in calves which had antibody titer 16 against FMDV types O and Asia-1 at day 0 persisted for quite a long time. Against FMDV type O , the titer remained constant in 7 out of 8 calves for a period of 54 – 98 days. Four out of 8 calves had the titer 16 even after an observation period od 94 – 215 days. However, a fall of the titer was noticed in 3 animals (to 4) and one animal (to 8) after a period of 215 days. The same trend was noticed with regard to the persistence of SN antibodies against type O in 8 animals tested for a period of

**Table 1. Neutralization antibodies against FMDV types O and Asia-1 in sera collected at different intervals**

| Animal | 1st bleeding | | 2nd bleeding | | 3rd bleeding | |
|---|---|---|---|---|---|---|
| No. | Day | Titer | Day | Titer | Day | Titer |
| FMDV type O | | | | | | |
| 746 | 0 | 16 | 54 | 16 | 94 | 16 |
| 768 | 0 | 16 | 98 | 16 | 215 | 16 |
| 742 | 0 | 16 | 54 | 16 | 94 | 16 |
| 748 | 0 | 16 | 54 | 16 | 94 | 16 |
| 771 | 0 | 16 | 98 | 16 | 215 | 4 |
| 772 | 0 | 16 | 98 | 8 | 215 | 4 |
| 775 | 0 | 16 | 98 | 16 | 215 | 8 |
| 776 | 0 | 16 | 98 | 16 | 215 | 4 |
| FMDV type Asia-1 | | | | | | |
| 746 | 0 | 16 | 60 | 16 | 88 | 16 |
| 768 | 0 | 16 | 93 | 16 | 190 | 16 |
| 742 | 0 | 16 | 60 | 8 | 88 | 8 |
| 748 | 0 | 16 | 60 | 8 | 88 | 8 |
| 771 | 0 | 16 | 93 | 16 | 190 | 8 |
| 772 | 0 | 16 | 93 | 16 | 190 | 8 |
| 775 | 0 | 16 | 93 | 16 | 190 | 8 |
| 776 | 0 | 16 | 93 | 16 | 190 | 16 |

Titers are expressed as reciprocals of highest dilutions of sera which neutralize the virus.

**Table 2. Neutralization antibodies against FMDV types O and Asia-1 in sera collected at different intervals**

| Animal | 1st bleeding | | 2nd bleeding | | 3rd bleeding | |
|---|---|---|---|---|---|---|
| No. | Day | Titer | Day | Titer | Day | Titer |
| FMDV type O | | | | | | |
| 747 | 0 | 16 | 54 | 16 | nd | nd |
| 743 | 0 | 16 | 54 | 16 | nd | nd |
| 745 | 0 | 16 | 54 | 16 | nd | nd |
| 749 | 0 | 16 | 54 | 16 | nd | nd |
| 731 | 0 | 16 | 40 | 16 | nd | nd |
| 735 | 0 | 16 | 40 | 4 | nd | nd |
| 759 | 0 | 16 | 98 | 16 | nd | nd |
| 763 | 0 | 16 | 98 | 16 | nd | nd |
| FMDV type Asia-1 | | | | | | |
| 769 | 0 | 16 | 93 | 16 | 190 | 16 |
| 770 | 0 | 16 | 93 | 16 | 190 | 16 |
| 271 | 0 | 16 | 30 | 16 | nd | nd |
| 275 | 0 | 16 | 30 | 16 | nd | nd |
| 278 | 0 | 16 | 30 | 16 | nd | nd |
| 732 | 0 | 16 | 60 | 16 | nd | nd |
| 735 | 0 | 16 | 60 | 16 | nd | nd |
| 738 | 0 | 16 | 60 | 16 | nd | nd |

Titers are expressed as reciprocals of highest dilutions of sera which neutralize the virus. nd – not done.

98 days only (Table 2). Only 1 out of 8 animals showed a fall of the titer (to 4) after 98 days.

With FMDV type Asia-1 (Table 1), 2 calves showed a fall of the antibody titer (to 8) after 60 days, whereas in 5 calves there was a persistence of the titer (to 16) up to the period of 90 days. The titer remained constant in 2 calves even up to 190 days. However, a fall of the antibody titer (to 8) was noticed in 3 animals after 190 days. In the third group (Table 2) it was observed that in 7 out of 8 calves the titer remained constant up to 60 days.

Presence of antibodies against both FMDV types O and Asia-1 were observed. The animals in the age group of 6 – 12 months used in this study were procured from the local market. It is possible that the animals did not receive any vaccination during their early age. As a result, their immune mechanism might function normally. The calves were young and their low SN antibody titers indicated only maternally derived antibodies. The animals used in this study could have been born out of immune mothers but during the period of their first 4 months they have not been exposed to FMDV antigen type O or Asia-1 by way of vaccination or have not actually suffered from the disease.

For the past 12 years, almost every year outbreaks caused by FMDV type O and Asia-1 (58% and 20% respectively) were noticed throughout the country and particularly in South India. During the course of an outbreak it was noticed that the breakdown of immunity of type O took place even after 2 – 4 months of vaccination. It is interesting to note that in this study the calves without receiving any vaccine after procurement and with no possible contact with live FMDV (as they were housed in a restricted area) could maintain their SN antibodies for 6 – 7 months. Repeated outbreaks in the vaccinated animals in the organized farm may be due to the fact that the calves were vaccinated repeatedly at their very early age without considering the ill effects of immunization in the presence of maternally derived antibodies. Nicholls *et al.*, (1984) reported that in the areas where foot and mouth disease is controlled by regular vaccination, the incidence of disease is the greatest in young stock under 2 years of age, suggesting that calves may not respond as well as adults to vaccination. It was reported by Sen (1985) that the protection conferred against FMD in cattle does not correlate with the SN antibody titer. The role of macrophages in optimum immune status of animals with regard to FMD (McCullough and Kihm, 1990) is also important. Besides that the contribution of T cells to the immune status of the animals requires a critical examination in order to find a possible reason of vaccine failure.

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## References

McCullough, K.C., and Kim, U. (1990): *The Immunology of Foot and Mouth Disease*. Proceedings of Joint OIE and FAVA symposium, Pattaya, Thailand, pp. 136–147.

Nicholls, M.J., Black, M.M., Rweyemamu. M.M., Genovese, J., Ferrari, R., Hammant, C.A., de Silva, E., and Umehara, O. (1984): The effect of maternally derived antibodies on the response of calves to vaccination against foot and mouth disease. *J. Hyg. Camb.* **92**, 105–106

Rweyemamu , M.M., Pay, T.W.F., and Simms, J.J. (1982): The control of foot and mouth disease by vaccination. In C.S.G. Grunsell and F.W.G. Hill (Eds): *The Veterinary Annual,* 22nd ed., Bristol Publ. Scienteogmica.

Rama Rao, D., and Rao, B.U. (1988): The occurence and distribution of foot and mouth disease virus types in India from 1971 to 1985. *Rev. Sci. Tech. Off. int. Epiz.* **7,** 357–364.

Sen, A.K. (1985): Correlation of cattle potency test and neutralization test in foot and mouth disease vaccine. *Indian J. anim. Sci.* **55**, 173–175.