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Experimental Viremia in Guineapigs Against Foot and Mouth Disease Virus

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Abstract: Experimental production of viremia in guineapigs against foot and mouth disease virus was studied. Post infection viremia was observed in post inoculated guineapigs only. Viremia appeared within 3DPI and persisted up to 5 DPI in post inoculated guineapigs, but control animals did not show any signs of viremia. Viremial level was also measured in post-inoculated guineapigs. The mean virus titres at 3, 4 and 5 DPI were $\log_{10}$ 2.16, 3.39 and 3.44 respectively. It is concluded that guineapigs might be an excellent mode for studying the viremia against foot and mouth disease virus.

Key words: FMD, viremia, guineapigs, post inoculation, LD_{50}, immunology

Introduction

Overall, the economic loss due to foot and mouth disease (FMD) is very high and although no detailed study has been carried out to calculate this loss in Bangladesh. It has been estimated that about 15.7 million $ is lost every year due to the loss of milk and draft power alone (Hasan, 1985). The types of FMD virus identified in Bangladesh are A, O, C, Asia-1 and sub type A_{22} (Islam *et al*., 1985 and Chowdhury *et al*., 1996). Viremia is a suitable marker for differentiating between immune and susceptible animals. But report of such studies is scanty in literature. Therefore the experiment was conducted to determine the level of viremia produced by type Asia-1 in experimental guinea pigs. To our knowledge, this is the first report about experimental viremia in guineapigs against foot and mouth disease virus in Bangladesh.

Materials and Methods

The experiment was done in Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh. The study period was one year.

Collection and preparation of specimens: The strain of foot and mouth disease virus used in this study was type Asia-1. Original sample was obtained from Animal Health Research Division of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. The strain was collected from Savar dairy farm situated nearby BLRI and confirmed by known serum collected from Indian Veterinary Research Institute, Izzatnagar, India using complement fixation test. Collected specimens were triturated with pestle and mortars. A 10% suspension of the specimen was prepared in PBS. Then an equal volume of chloroform was added to the suspension and centrifuged at 3500 rpm for 15 minutes. The supernatant was collected for inoculum. The inoculum was tested for safety and sterility as per standard procedure (Anonymous, 1992) before inoculation.

Post infection viremia: This was conducted using apparently healthy albino guineapigs of 3 months old and Swiss albino unweaned mice of 3 days old, which were infected with FMD virus type Asia-1. At first eight guineapigs were selected and grouped as "A" with 5 guineapigs and "B" with 3 guineapigs. Each guinea pig of "A" group was infected with Asia-1 FMDV (adapted) at the doze rate of 100 Mice Lethal $Doze_{50}$ (100 MLD_{50}) 0.1 ml inoculum. Group "B" was kept as control throughout the experiment. Blood samples were collected through cardiac puncture at 24,48,72,96,120,144 and 168 hours of post inoculation from each guineapigs. Plasma from the collected blood was separated. Five plasma samples collected from the guineapigs were treated with penicillin 10000 IU. and streptomycin 10 mg ml^{-1} before inoculation in suckling mice. Each plasma sample was assessed in terms of presence or absence of virus in five suckling mice in each occasion. The suckling mouse was inoculated with 0.05 ml of plasma per mouse in thigh muscle. The inoculated mice were observed for the detection of infection up to seven days and the observations were recorded. The mice and guineapigs were maintained in separate cages in a well ventilated room and during this period they were given normal laboratory diet and fresh drinking water.

Level of viremia: Plasma samples of postinfected animals were used to determine the titres of virus. To determine the titer of virus, 10 fold diluted plasma in phosphate buffer saline solution ranging from 10^{-1} to 10^{-7} was inoculated at the rate of 0.05 ml per unweaned mice intramuscularly. For each dilution 3 mice were used. Mortality of mice was observed up to 7 days. The virus titer was calculated as per the method of Reed and Muench (1938)

Results

Post infection viremia: Table 1 represents the postinoculated viremia in FMD type Asia-1 virus infected guineapigs. Viremia studies were conducted up to 7 days post infection (DPI). In post inoculated guinea pigs viremia appeared in 3DPI and persisted up to 5DPI. Viremia was detected only in postinoculated guinea pigs but control animals did not show any viremia. Out of 5 baby mice inoculated with collected plasma from virus inoculated guineapigs of 3 DPI causes death of all 5 mice indicated the onset of viremia and this result were observed up to 5th day of the experiment but from 6th day no death was observed indicate termination of viremic stage. This indicates probably the antibody produced against inoculated FMD virus in the body after 6th day interval which helps guineapigs to withstand the challenge.

Table 1: Post inoculated viremia in FMDV type Asia-1 virus infected guineapigs

	Animal numbers			Days post inoculation				
		1	2	3	4	5	6	7
Post inoculated	5	0/5	0/5	5/5	5/5	5/5	0/5	0/5
Control	3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Denominator represents the number of baby mice inoculated.
Numerator represents the number of baby mice died

Table 2: Level of viremia in post inoculated type Aisa-1 MDV infected guineapigs up to 7 DPI

Titers of virus $\log_{10}$ MLD_{50}/0.05 ml plasma

	Animal numbers	Days post inoculation						
		1	2	3	4	5	6	7
Post inoculated	5	-	-	3.25	4.24	4.24	-	-
Control	3	-	-	-	-	-	-	-
Mean		-	-	2.16	3.39	3.44	-	-

Level of viremia: The level of viremia in post inoculated guinea pigs up to 7 DPI is shown (Table 2). Level of viremia was determined in baby mice from the plasma collected from the infected guinea pig. This was determined in post inoculated animals (viremia appeared only in post inoculated animals). The mean virus titres of 3, 4 and 5 DPI were $\log_{10}$ 2.16, 3.39 and $\log_{10}$ 3.44 respectively indicating as the DPI increasing the titre of virus also increases.

Discussion

Viremia is suitable indicator for differentiating between immune animals from susceptible ones during challenge test against foot and mouth disease. Post infection viremia was determined in the plasma of postinfected animals up to 7 days using baby mice as indicator host system. In these findings viremia appeared only in post infected mice. Viremia appeared in post infected animals as early as 72 hours post infection. Similar findings were also reported by Sharma (1979), Charan and Prasad (1981). They could detect postinfected viremia in pigs up to 3 DPI only. There was a positive correlation between the appearance and nonappearance of viremia. Charan and Prasad, (1981) observed similar correlation in vaccinated animals. Similar observation were also recorded by other workers in buffalo and cattle (Moussa *et al.*, 1977). Following intranasal inoculation in sheep viraemia developed within 24 hours and it disappeared after 68 hours (Sharma, 1979). Present findings also positively correlated with this result. These findings are also in close agreement with Dhenin *et al.* (1979) who found that FMD virus appeared in the blood and in muscles in 32 and 20 hours respectively in artificially infected pigs. Som *et al.* (1980) found that viremia of Aujesky's disease developed in the rabbit after 2-3 days of post-infection but no viremia developed within 24 hours. Average titer of virus of 3, 4 and 5 DPI were observed as $\log_{10}$ 2.16, 3.39 and $\log_{10}$ 3.44, respectively. Present findings are also in close agreement with Perryman *et al.* (1988) and Nair and Sen (1993). The results of the present study suggest the utility of this laboratory animal for studying the viremia against foot and mouth disease virus.

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