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Experimental infection in guinea pig with *foot and mouth disease virus*

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Abstract

In order to obtain information on murine model for *foot and mouth disease virus*(FMDV) type Asia 1, we studied whether guinea pig was a suitable model for studying FMDV. Apparently healthy 3 months old albino guinea pigs and unweaned 3 days old Swiss albino mice were used for this study. Total of 8 guinea pigs were divided into the infected(n=5) and control(n=3) groups. The incubation period of FMDV in the guinea pigs were roughly 2 days and the viremia persisted for 3 days in the guinea pigs. Mice inoculated with the plasma from control guinea pigs did not show any sign of viremia. The plasma were titrated by virus neutralization test using suckling mice as an indicator host. The mean virus neutralizing antibody titers of infected guinea pig at 3 DPI, 4 DPI and 5 DPI were $\log_{10}$ 2.16, $\log_{10}$ 3.39 and $\log_{10}$ 3.44, respectively whereas there was no neutralizing antibody titer in control group. The difference between the mortality pattern and mean virus neutralizing antibody titer of infected and that of control group at day 3, 4, 5 were statistically significant($p<0.01$).

Key words : Foot and mouth disease, Neutralization test, Bangladesh

Introduction

Foot and mouth disease(FMD) is a highly infectious viral disease of ungulates[1,2] and is still prevalent in many parts of the world, including Europe, Africa, Asia and South America. Due to loss of productivity and the infectious nature of the disease, outbreak of

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FMD has very serious economic consequences, including constraints on international meat and livestock trade.

FMD virus, the sole member of the genus aphthovirus of the family Picornaviridae, consists of seven distinct serotypes. Serotypes O, A and C occur in Europe, South America, Africa and Asia. SAT1, SAT2 and SAT3 are restricted to Africa. Asia-1 is widely distributed throughout Asia[3]. Type Asia-1 is mostly prevalent in Bangladesh, India, Nepal, Thailand, Saudi Arabia, Greece, Turkey, Oman, and Iran[4,12].

Research involving cattle is not only costly but also hamper by an incomplete knowledge of the bovine immune system, such as its immunocompetent subpopulations and its regulatory mechanisms[13]. The murine model has been widely used for experimental study *foot and mouth disease virus*(FMDV). Murine immune systems have been relatively well characterized and are also inexpensive in cost. In addition to the cost savings when using murine rather than cattle, the time required for obtaining the results is significantly shorter. At the beginning of the last century guinea pig was suggested as a suitable animal model for foot and mouth disease[14] and many studies were subsequently performed in this species. Guinea pigs were chosen as experimental models because of the similarity of clinical symptoms in these animals to those of swine and cattle[15]. In the study by Richard et al[15] demonstrated a cell-mediated immune response to FMDV in guinea pig and suggested that this cell-mediated immune response may also constitute part of the immune response to FMDV in swine and cattle. For certain aspects of the disease, e.g. for analysis of the pathogenesis of FMDV and preparation of antisera, there is no substitute for the guinea pig[16]. Dus Santos et al[17] demonstrated a close similarity between murine and cattle in term of both their FMDV antibodies and protection afforded against the challenge following vaccination.

The virus neutralization test using suckling mice has been used for both infected and vaccinated cattle. This test has been utilized to predict the response that are developed following vaccination in the naturally susceptible host, and to predict their degree of protection against virulent challenge[18,21].

In this study, we investigated whether guinea pig was an experimentally suitable model for studying type Asia-1 FMDV. The experiment was conducted for the infectivity of Asia-1 in guinea pig by determining the level of viremia produced by the FMDV and for determination of the neutralizing antibody titer of the virus by the virus neutralization test using unweaned mice.

Materials and Methods

All the experiment was performed in the Department of Microbiology & Hygiene, Faculty of Veterinary Sciences, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The strain of *foot and mouth disease virus* in this study was type Asia-1. This virus sample was obtained from the 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 complement fixation test using known serum collected from Indian Veterinary Research Institute, Izatnagar, India. Collected specimen was triturated with pestle and mortars. A 10% suspension of the

specimen was prepared in PBS(pH 7.0). Then an equal volume of chloroform was added to the suspension and centrifuged at 3500 rpm for 15 minutes. The supernatant was collected and used as inoculum.

This study was conducted using apparently healthy 3 months old albino guinea pigs and unweaned 3 days old Swiss albino mice. 5 guinea pigs were infected in the footpad with type Asia-1 FMDV(adapted) at the doze rate of 100 mice lethal dose$_{50}$ (100MLD$_{50}$) of 0.1 ㎖ inoculum. Three uninfected guinea pigs were used as control. The mice and guinea pigs were maintained in separate cages in a well ventilated room and during this period they were given normal laboratory diet and fresh drinking water. Blood samples were collected from all the guinea pigs through cardiac puncture at 24, 48, 72, 96, 120, 144 and 168 hours post inoculation. Plasma from the collected blood was prepared and was treated with penicillin (10000 I.U.) and streptomycin(10 ㎎/㎖) before inoculation into suckling mice. Each plasma sample was assessed for presence of virus using five suckling mice in per occasion. 0.05 ㎖ each plasma was inoculated into thigh muscle of mice. Monitored for seven days whether these animals showed signs of FMDV infection and mortality.

The collected plasma were titrated by virus neutralization test following standard procedure using suckling mice as an indicator host. The plasma samples were diluted(1 : 2 to 1 : 56) in PBS(pH 7.0). One hundred mice lethal dose fifty (MLD$_{50}$/0.1 ㎖) containing FMD type Asia 1 virus mixed with individual plasma sample. The mixtures were taken, mixed properly and incubated at room temperature for one hour, 0.05 ㎖ of each serum virus mixture was inoculated intraperitoneally into each of 5 suckling mice and the observations were recorded for the determination of neutralizing antibody titers. The highest dilution of plasma protecting more than 50% of inoculated mice was taken as the end point titer calculated as per method of Ahad et al[22]. The values obtained were log transformed.

To find out interaction between infected and control group and also time of post inoculation results of the mortality pattern of mice and mean virus neutralizing antibody titres were statistically analyzed by 2 way analysis of variance.

Results

The mortality pattern of mice inoculated with plasma of guinea pigs at 7 days post infection have been shown in Table 1. The plasma from guinea pigs on days 3, 4 and 5 post infection with FMDV caused death of all mice. These indicated that viremia of FMDV were present in guinea pigs. Mice inoculated with the plasma from guinea pigs on day 1, 2, 6, and 7 post infection with FMDV did not show significant sign of viremia or mortality. These indicated that the incubation period of FMDV in the guinea pig were roughly 2 days and that the viremia persisted for 3 days in the guinea pigs. Mice inoculated with the plasma from control guinea pigs did not show any sign of viremia.

The virus neutralization antibody titer of the plasma from infected and control group have been presented in Table 2. The mean virus neutralization antibody titers of infected guinea pig at 3 DPI, 4 DPI and 5 DPI were $\log_{10}$2.16, $\log_{10}$3.39 and $\log_{10}$3.44, respectively whereas there was no neutralizing antibody titer in control group.

The differences between the mortality

pattern and mean virus neutralizing antibody titer of infected and that of control group at day 3, 4, 5 were statistically significant ($p<0.01$). There was no significant difference of mortality pattern and mean virus neutralizing antibody titer between infected and control group at day 1, 2, 6, 7.

Table 1. Mortality pattern of mice inoculated intraperitoneally with plasma of guinea pig from infected and control groups

Days post inoculation	Mortality / 0.05㎖ plasma (No of died mice / No of inoculated mice)	
	Infected guinea pigs(n = 5)	Control guinea pigs(n = 3)
1	0/5	0/5
2	0/5	0/5
3	5/5**	0/5
4	5/5**	0/5
5	5/5**	0/5
6	0/5	0/5
7	0/5	0/5

n = Number of guinea pig;
** = $p<0.01$

Table 2. Virus neutralizing antibody titer in plasma of guinea pig from infected and control groups

Days post inoculation	Mean titer of virus log10 / 0.05 ㎖ plasma	
	Infected guinea pigs(n=5)	Control guinea pigs(n=3)
1	0.0	0.0
2	0.0	0.0
3	2.16**	0.0
4	3.39**	0.0
5	3.44**	0.0
6	0	0.0
7	0.0	0.0

n = Number of guinea pig;
** = $p<0.01$

Discussion

Evaluation of the neutralizing antibody titer against virus has largely depended on measurement of the antibody response elicited in inoculated animals. For FMDV, the test is conducted in cattle and based either on the anti-FMDV humoral immune response at 60 dpv or on a protection test at 90 dpv. Both methods require the use of cattle, making them expensive and time consuming. Consequently the development of faster and less expensive technologies would be advantageous.

An important feature of the methodology presented here is that it does not require the use of cattle, which, beside the obvious financial advantage, eliminates the problems generated by the variability in genetic background, age, nutrition and sanitary status of cattle.

The neutralization test results confirmed the observations of Skinner[23] that, by this test, it is possible to detect virus type antibody in the first weeks after infection. After intraperitoneal injection of living FMDV particles involve viral replication which spontaneously subsides in 3 days simultaneously with the appearance of high titer of neutralizing antibody as reported in mice[24]. Cunha and Honigman[19], Borca et al[13] also used neutralization test using mice to determine the neutralizing antibody titer against FMDV.

Viremia is described as a suitable indicator for differentiating immune animals from susceptible ones and as an index of protective immunity during challenge test against FMDV[25,26]. Viremia provide the active role of immune response in the elimination of FMDV in experimental model and its prolongation confirms the importance

of antibody in the elimination of FMDV[13].

The use of unweaned mice in neutralization test for FMDV antibodies has been advocated since Skinner[27] first reported the susceptibility of these animals to the virus of this disease. Cunha et al[18] using unweaned mice, compared a serum-protection test with the neutralization test in the evaluation of the FMDV antibody content of cattle serums.

The viremia was determined in the plasma of post infected guinea pigs using baby mice as an indicator host system. In this study we found that guinea pig showed viremia for at least 3 days. Viremia appeared in post infected guinea pig as early as 72 hour post infection. Sharma[28], Charan and Prasad[26] detected post infected viremia in pigs upto 3 DPI only. Other workers[25,26,29] detected viremia and virus neutralizing antibody titer in sheep, cattle and pig. Moussa et al[30], Auge de Mellow and Sutmoller[29] also recorded viremia and virus neutralizing antibody titer in buffalo and cattle. Viremia of FMDV in sheep was detected[31] 12 hours after experimental inoculation and persisted for about 54 hours but in another study in sheep[32], viremia was detected in 14 hours after injection intradermally with FMDV types O, A and C. Viremia in sheep of FMDV was also studied by Sharma[28]. Following intranasal inoculation in sheep, viremia developed within 24 hours and it disappeared after 68 hours. In our findings viremia detected in 3 DPI and continued for 5 DPI only. Dhenin et al[33] found that FMD virus appeared in the blood and in muscles in 32 hours and 20 hours respectively in artificially infected pigs. Viremia of Aujesky's disease in rabbits was studied by Som et al[34]. They found that viremia developed in the rabbit after 2~3 days infection but no viremia developed within 24 hours. Average titre of virus of 3 DPI, 4 DPI and 5 DPI were observed as $\log_{10}2.16$, $\log_{10}3.39$, and $\log_{10}3.44$, respectively. Mastan and Dabouchard[35] detected viremia in sheep with FMDV. Fernandez et al[36] detected high virus titre against FMDV in experimentally infected adult mice blood at 12~24 hour after infection that persisted up to 48~72 hours. Perryman et al[37] detected immune response to terminate viremia in horse. Van Bekkum[38] and Pay et al[39] reported the correlation between plasma antibody level and protection against challenge induced by FMD virus. Muther et al[40] reported virus neutralizing antibody titer above $\log_{10}1.33$ in cattle against type O FMDV. Nair and Sen[41] observed plasma neutralizing antibody titer more than $\log_{10}1.60$ with other type of FMDV in cattle.

The results presented here indicate that the antibody response obtained in mice by VN agree with the protective test in mice suggesting the feasibility of using assay as an indicator. The implementation of virus neutralization gives faster, easier and more economical results making it the procedure of choice for evaluating the potency of virus with the benefits of yielding rapid and applicable results from an inexpensive methodology.

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