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Original Article

Raising of Hyper-immune Serum Against FMD Virus Type "O" Prevailing in Punjab, Pakistan

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ABSTRACT

***Introduction**: Foot and mouth disease (FMD) is supposed to be an imperative disease of domestic and wild ruminants which is a vast reason of high mortality in young animals and production losses in adults. The supreme prevailing stains of FMD in Asia are "O", "A "and "Asia-I", which are supposed to be a big threat to economy and commonly not properly diagnosed. For appropriate diagnoses, hyper-immune serum is required. **Methods**: A study was conducted to produce hyper-immune serum in rabbits which were divided into three groups including Group-I, Group-II and a control group. **Results**: First two groups were weekly inoculated with FMD virus Serotype "O" for six weeks and confirmation of the infection was done with the help of compliment fixation test (CFT), while antibody titer was measured by using ager gel precipitation test (AGPT). Group-II consisting of female rabbits showed earlier and higher titer (Log_2^7) than group-I (Male rabbits) having lower titer (Log_2^5). **Conclusion**: The study recommended the use of females rabbits to raise hyper-immune serum to attain higher titer.*

***Key words:** FMD Virus, Serotype "O", Rabbits, Hyper-immune serum, Pakistan.*

INTRODUCTION

Foot and mouth disease virus (FMDV), a member of genus Aphthovirus of Family Picornavidae produce a contagious and transmissible disease of cloven foot animals termed Foot and mouth disease (FMD) which rapidly spread among domestic flock.[1,2,6,7,8,11] FMD is not only important in domestic animals (cattle, buffalo, Pigs, sheep and goats) but also cause a severe disease in wild species including African buffalos, wild pigs, deer and antelops etc.[6,9] Pigs are found to be very susceptible to the disease and usually act as amplifier host during disease outbreak. [11] In cattle, disease was 1st reported in 1514 by Italian monk "Hieronymus Fracastorius" in Venice but now present everywhere. FMDV has 7 serotypes counting A, O, C, Asia-1, SAT-1, SAT-2 and SAT-3 named after their place of discovery.[2,6] Among all listed, the frequently circulating Serotypes in Asia are O, A and Asia-I.[3]

FMDV is positive sense ssRNA virus of spherical shape with 20-30nm diameter and over 8kb (approx. 8200 nucleotide) in length with spontaneous and high mutation rate of 10^3-10^5 substitutions/nucleotide copied. [6,9,10] Replication of genome without proof reading produces mutation and heterogeneity, which affects the pathogenesis of disease.[10] Because of heterogeneity vaccination against one serotype or sub-serotype not give protection against other.[2] Capsid having 4 structural proteins including VP1, V2P, VP3 externally and VP4 internally of which VP1 is most important for genetic characterization.[6,9]

Clinically cases may be presented with vesicles containing straw color fluid before bursting on oral mucosa, inter-digital spaces and coronary band. But similar signs may also come in vesicular stomatitis and rinderpest so quite difficult to diagnose on clinical basis.[3,6] High elevation in temperature and excessive salivation always give clue towards diagnosis of FMD. As clinical diagnosis is not much reliable so there is needed to go for more authentic serological tests.[3] Most commonly complement fixation tests (CFT), and Serum neutralization tests (SNT) are used

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test now-a-days.[5] Livestock play an imperative role in GDP of Pakistan on which FMD affects acutely by severe mortality in young once and production losses in adults.[2,3,5] whereas on the other hand also interfere with trade on international level.[8,9] Virus Serotype "O" is reported to be endemic in Asia by Davies so the study was planned to produce hyper-immune serum against this Serotype because imported one is expensive and out of reach at farmer level. This hyper-immune serum was aimed to use for proper diagnosis and control of disease ultimately helping the economy.

MATERIALS AND METHODS

1. **Source of Antigen:**

Ag was produced from Foot and Mouth Disease Research Center, Lahore, Pakistan having Tissue Culture Lethal dose ($TCLD_{50}$) $10^{6.7}$. Purification of antigen was done by centrifuging the FMD seed virus at 8500 rpm for 17 min. Then 35ml of supernatant was collected and poured into ultra-centrifugation tubes. After sealing, the tubes were centrifuged at 43000 rpm for 2 hours at 4°C and the resulting pallet of virus was reconstituted in 5cc sterilized phosphate buffer saline (PBS). Sensitivity and sterility tests were conducted to check any unwanted contamination.

2. **Preparation of Rabbits**

Fifteen healthy rabbits (7 males and 8 females) were procured from Estate section (VRI) and kept in isolated conditions. These rabbits were divided into 3 groups i.e. group-I (Male), group-II (Female) and group-III (Control) having 6 (males), 6 (females) and 3 (1 male and 2 females) respectively. To check Coccidiosis, coccidiostate was given in drinking water for three days. Second course of coccidiostate was given to the rabbits after few days. The rabbits were also fed grains as extra proteinious diet before and after the virus inoculation.

3. **Inoculation of Virus**

Gentamysin was added @ 0.5 cc /5 cc of the total volume of the inoculums and for inoculation following protocol was observed. Each injection was performed with one-week interval in groups I and II while III was kept as control.

Table 1. Schedule for inoculation of virus in rabbits

Time of inoculation (wk.)	Inoculating quantity (cc)		
	Group-I (Male)	Group-II (Female)	Group-III (Control)
0 day	0.5	0.5	Nil
1st	0.5	0.5	-
2nd	0.75	0.75	-
3rd	1	1	-
4th	1	1	-
5th	1.5	1.5	-

4. **Observation of Rabbits**

All rabbits were observed for seven days after each injection. House and bedding was also checked throughout the study.

5. **Antibody titer**

Each rabbit was bleed from ear vain with disposable syringe in aseptic condition and 2cc blood was taken. Serum was collected to know the antibody titer. TCLD of "O" type FMD virus was also performed. All rabbits were bled on day 21st, 28th, 35th, 42nd, 49thand 56thafter the inoculation of virus.

6. **Serological tests:**

For further conformation different serological tests including Ager gel precipitating test (AGPT), Serum neutralization test (SNT) and Compliment Fixation test (CFT) were performed. Out of which AGPT was placed as "gold standard" for conformation. Results for these tests are tabulated under:

RESULTS

Production of hyper-immune serum against FMDV is a procedure which requires a number of inoculations. Similarly, species selection for inoculation is also very significant that entirely depends upon compatibility and amount of hyper-immune serum. For small quantity and in case of laboratory experimentation, rabbits are frequently used because of their small size, long life span and ability to produce antibodies against a number of diseases. Hussain *et al.* (2004) also used rabbits to prepare hyper-immune sera. Antibody titer was measured weekly by using ager gel precipitation test (AGPT). Group-II (Female Rabbits) showed earlier and higher titer than group-I (Male Rabbits) (Table-2) as previously reported by Wenyi *et al.* (2003). Confirmation of infection was done with the help of CFT. It was observed that female rabbits were more sensitive to infection and showed positive result for FMD from third week of inoculation while male rabbits showed positive result from fourth week (Table-3).

Table 2. Antibodies titer Against Type "O" Serotype after inoculation of virus

Sampling day (wk.)	Ab. Titer in Serum		
	Group-I (Male)	Group-II (Female)	Group-III (Control)
1st	Nil	Nil	Nil
2nd	Nil	Nil	-
3rd	Nil	Log_2^3	-
4th	Log_2^3	Log_2^4	-
5th	Log_2^4	Log_2^5	-
6th	Log_2^5	Log_2^6	-
7th	Log_2^5	Log_2^7	-
8th	Log_2^6	Log_2^7	-

Table 3. Weekly results of Compliment fixation test (CFT) and Ager gel precipitation test (AGPT)

Time (wk.)	Group-I (Male)		Group-II (Female)		Group-III (Control)	
	CFT	AGPT	CFT	AGPT	CFT	AGPT
1st week	**-ve**	**-ve**	**-ve**	**-ve**	Negative	Negative
2nd week	-ve	-ve	-ve	-ve	-	-
3th week	-ve	-ve	+ve	+ve	Negative	Negative
4th week	-ve	-ve	+ve	+ve	-	-
5th week	-ve	-ve	+ve	+ve	-	-
6th week	+ve	+ve	+ve	+ve	-	-
7th week	+ve	+ve	+ve	+ve	-	-
8th week	+ve	+ve	+ve	+ve	-	-

For preservation of serum three rabbits from Group-I and II were selected randomly and slaughtered, blood was collected aseptically. Serum was separated and poured in the sterilized aliquots @ 0.5cc/aliquot and were kept at -20º C for further use as a diagnostic agent. It was observed that female rabbits were found +ve for anti-sera throughout the study.

DISCUSSION

Virus lodge and multiply in epithelium of respiratory tract causing viremia after entering where it forms vesicles after passing the Incubation period which may vary from 2-3 days to 14 days. Antibodies develop after few days terminating the viremic phase by helaing lesions.[3]

Control of FMD is only possible with continuous monitoring of Serotype circulating in population. Usually inactivated vaccines are used supplementary to disease control but their efficacy is low because of antigenic heterogeneity of the virus.[1,10] As vaccination control the clinical aspects of a disease but not control the persistence infection so it is necessary to find out such test which may able to identify the infected animals or previously exposed with live virus but not vaccinated. For this World Health Organization (WHO/OIE) has developed a diagnostic test to eradicate the disease which depends on screening NSP ELISA, followed by an enzyme linked immune-electotransfer blot test (EITB) to clear up false positive reactions**.**

For an event of outbreak FMD free countries established a reserve of emergency vaccines e.g European Union Vaccine Bank (EUVB) and International Vaccine Bank (IVB) etc.[1]

REFERENCES

1. Barnett PV, Carabin H. A review of emergency foot-and-mouth disease (FMD) vaccines. Vaccine. 2002; 20:1505–1514.
2. Carrillo C, Lu Z, Borca MV, Vagnozzi A, Kutish GF, Rock DL. Genetic and Phenotypic Variation of Foot-and-Mouth Disease Virus during Serial Passages in a Natural Host. JOURNAL OF VIROLOGY. 2007; 81(20):11341–11351.
3. Davies G. Foot and mouth disease. Research in veterinary science. 2002; 73:195-199.
4. Hussain I, Rasool MH, Mahmood MS. Production of hyperimmune serum against Infectious Bursal Disease Virus in rabbits. Pakistan Vet. J. 2004; 24(4):179-183.
5. Iqbal MS, Rahman SU, RASOOL MH, MANSOOR MK. Serodiagnosis of Foot and Mouth Disease Virus in Buffalo Through Counter Immunoelectrophoresis and ELISA in Pakistan. Int. J. Agri. Biol. 2002; 4(4):510–512.
6. Jamal SM, BELSHAM GJ. Foot-and-mouth disease: past, present and future. *Vet. Res.* 2013; 44:116-130.
7. Keeling MJ, Woolhouse MEJ, Shaw DJ, Matthews L, Chase-Topping M, Haydon DT, Cornell SJ, Kappey J, Wilesmith J, Grenfell BT. Dynamics of the 2001 UK Foot and Mouth Epidemic: Stochastic Dispersal in a Heterogeneous Landscape. SCIENCE. 2001; 294: 813.
8. Lin T, Li J, Shao J, Cong G, Du J, Gao S, Chang H. Development of Monoclonal Antibody against Foot-and-mouth Disease Virus A Type [J]. VIROLOGICA SINICA. 2011; 26(4): 273-278.
9. Li-na M, Zhang J, Hao-tai C, Jian-hua Z, Yao-zhong D, Yong-sheng L. An overview on ELISA techniques for FMD. Virology Journal. 2011; 8:419.
10. Martín V, Perales C, Abia D, Ortíz AR, Domingo E, Briones C. Microarray-based identification of antigenic variants of foot-and-mouth disease virus: a bioinformatics quality assessment. BMC Genomics. 2006; 7:117.
11. Ward MP, Laffan SW, Highfield LD. The potential role of wild and feral animals as reservoirs of foot-and-mouth disease. Preventive Veterinary Medicine. 2007; 80:9–23.
12. Wenyi GU, Holland M, Janssens P, Kerr P. Antibody response in the female rabbit reproductive tract to influenza haemagglutinin encoded by a recombinant myxoma virus. Virol. 2003; 313:286–295.

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