

Distribution of Serotypes of Foot and Mouth Disease Virus Among Different Outbreaks Occurring in Punjab Province, Pakistan (2001-2010)

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ABSTRACT

Foot and mouth disease virus (FMDV) comes under genus Aphthovirus and family Picornaviridae and it causes a severe vesicular disease in cloven footed animals especially cattle. It has seven different serotypes but only four of them (O, A, Asia I, C) are prevalent in Pakistan. The present study was aimed to evaluate the prevalence of these serotypes in Punjab province, Pakistan. A total of 659 samples were collected from different outbreaks of FMD occurring in Punjab province from 2001 to 2010. The samples were analysed by complement fixation test by using commercially available hyper immune sera of 4 serotypes. Results showed that 234 samples were positive for FMDV out of which 178 (27.01%) samples were positive for O, 4 (0.60%) for A, 48 (7.28%) for Asia I and 4 (0.60%) for C from 659 number of samples

Key Words: Foot and mouth disease virus, foot and mouth disease, hyper immune serum, complement fixation test

ÖZET

PAKİSTAN, PENCAP İLİNDE ORTAYA ÇIKAN FARKLI SALGINLARDA ŞAP HASTALIĞI VİRÜSÜNÜN SEROTİPLERİNİN DAĞILIMI (2001-2010)

Şap hastalığı virüsü Aphthovirus cinsi ve Picornaviridae ailesine ait olup, başta sığır olmak üzere çift tırnaklı hayvanlarda önemli veziküler hastalığın oluşumuna neden olmaktadır. Yedi farklı serotipi olmasına rağmen bunlardan sadece dördü (O, A, Asya I, C) Pakistan'da yaygındır. Bu çalışmada, Pakistan'da Pencap ilinde bu serotiplerin prevalansının belirlenmesi amaçlanmıştır. 2001 ile 2010 yılları arasında Pencap ilinde meydana gelen farklı şap hastalığı salgınlarından toplam 659 örnek toplanmıştır. Örnekler, ticari olarak temin edilen 4 serotipe ait hiper immun serumlar kullanılarak, komplement fiksasyon testi ile incelendi. Sonuçlar 659 örneğin 234'ünün şap hastalığı virüsü yönünden pozitif olduğunu, 178'inin (%27,01) O serotipinde, 4'ünün (%0,60) A serotipinde, 48'inin (%7,28) Asya I serotipinde ve 4'ünün (%0,60) C serotipinde olduğunu göstermiştir.

Anahtar Kelimeler: Şap hastalığı virüsü, şap hastalığı, hiper immun serum, komplement fiksasyon testi

Introduction

Foot and mouth disease virus (FMDV) belongs to genus Aphthovirus and family Picornaviridae (King et al., 2000; Newman et al., 1973). The virus causes a disease characterised apts and lesions in oral mucosa and foets in especially domestic and wild ruminants. The disease can be fatal in young animals and often tend to form systemic infection (Thomson, 1994; Thomson et al., 2003).

FMDV has seven different serotypes which are prevalent in different areas of the world. Initially, two serotypes of FMDV were recognised as “O” in France and “A” in Germany (Valee and Carre, 1922). Subsequently, type “C” was recognised in Germany (Waldmann and Trautwein, 2003). After a span of 30 years, 3 new serotypes named SAT1, SAT2 and SAT3 were isolated from outbreaks in South Africa (Brooksby, 1958). The last serotype was isolated from outbreaks in Pakistan which was named as “Asia-I” (Brooksby and Roger, 1957). All the serotypes have 30-50% differences (nucleotide sequence) in their VP1 gene (Knowles and Samuel, 2003). There is no existence of cross protection by vaccination among all these 7 serotypes (Kitching et al., 1989; Kitching, 1998). Vaccinated animals which are not completely protected may be a source of infection and spread of disease among healthy animals (Sutmoller et al., 2003).

Among all the serotypes, four of them have been detected in Pakistan i.e. O, A, Asia-I and C (Anjum et al., 2006; Fida et al., 1965; Jamal et al., 2010; Klein et al., 2008; Zulfiqar, 2003). The present study was performed to evaluate the prevalence of four serotypes of FMDV in samples collected from outbreaks of FMD in Punjab province of Pakistan from 2001 to 2010.

Materials and Methods

Collection of samples

Samples were collected from animals suspected for foot and mouth disease from different areas of Punjab, Pakistan for analysis

in Foot and mouth disease research centre (F&MDRC) Lahore, Pakistan. Samples were collected from only those animals having high temperature and blisters between inter-digital spaces of foot and on the tongue. A total of 659 samples were tested. Number of samples tested per year from 2001 to 2010 has been shown in Table 1. Blisters collected from tongue or epithelial tissue from inter-digital spaces were preserved in buffered glycerine (with pH 7.4) till further processing.

Complement Fixation Test

Complement fixation test was used for detection of antigen in the samples according to method explained by Forman (1974) with few modifications. Briefly, the test comprised of two sets of antigen and antibody. First set contained three components i.e. suspected sample (antigen), FMD hyper immune serum serving as antibody (purchased from Veterinary Laboratories Agency New Haw, Addlestone, Surrey, KT15 3NB, UK) and complement (guinea pig serum) while second set contained washed Red Blood Cells (R.B.Cs) from sheep (antigen) and hemolysin or amboceptor (hyper immune serum against sheep R.B.Cs prepared in rabbits) which would serve as an antibody.

To perform the test, 100, 250 and 200µL positive serum antigen, and complement, respectively were added in a Cohn's tube. For detection of each serotype, two tubes were prepared and placed in water bath at 37°C for 30 minutes. After that, 500µL of hemolysin system (250µL of hemolysin premixed with 250µL washed R.B.Cs) was sensitized by incubation at 20-40°C for 30 minutes. The tubes were observed for the presence or absence of hemolysis of R.B.Cs and compared with positive and negative controls. Tubes containing hemolysis were negative while tubes with hemolysis were considered as positive. The principle of the test was that if antigen and antibody of first set were homologous then complement would be fixed with them and no hemolysis of R.B.Cs would occur in the presence of hemolysin system. However, if antigen and antibody were not homologous, complement would not be fixed with them and

would cause the lysis of R.B.Cs when hemolysin system was added.

Results

The number of positive samples for FMDV and yearly status of four serotypes from the samples collected from different outbreaks of Punjab province Pakistan has been shown in

Table 1. From total 659 samples, 234 (35.50%) samples were found positive for FMDV whereas 425 (64.49%) samples were negative for FMDV. From all of the samples, 178 (27.01%) samples were positive for serotype O; 4 (0.60%) for A; 48 (7.28%) for Asia I and 4 (0.60%) samples for serotype C from 659 number of samples.

Table 1. Number of samples tested each year, number of positive samples for FMDV and number of samples positive for four serotypes.

Tablo 1. Yıllara ait test edilen örnek sayısı, Şap hastalığı virüsü pozitif örneklerin sayısı ve dört serotipe ait pozitif örneklerin sayısı.

Year	Samples Tested	Positive for FMDV	Serotype O	Serotype A	Serotype Asia I	Serotype C
2001	49	13	13	-	-	-
2002	49	3	1	-	2	-
2003	31	22	2	4	12	4
2004	70	34	2	-	32	-
2005	19	2	2	-	-	-
2006	98	35	33	-	2	-
2007	88	31	31	-	-	-
2008	99	15	15	-	-	-
2009	97	44	44	-	-	-
2010	59	35	35	-	-	-
Total	659	234 (35.50%)	178 (27.01%)	4 (0.60%)	48 (7.28%)	4 (0.60%)

Discussion

A total of 659 samples collected from outbreaks of FMD in Punjab province Pakistan over a span of ten years were analysed in this study for the evaluation of specific serotypes. 234 samples were found positive for FMDV. 178 (27.01%) samples were positive for serotype O, 4 (0.60%) for A; 48 (7.28%) for Asia I and 4 (0.60%) samples for serotype C from 659 number of samples. Data available regarding the prevalence of FMDV in Pakistan is very rare. Serotype O was in highest percentage. Similar results of high percentage of O (35.42%) have been reported by Khan et al. (1989) who studied the prevalence of FMDV from 1962 to 1988 in Pakistan. The occurrence of O at highest percentage as observed in this study is also in accordance with findings of Fida et al. (1965); Rauf et al. (1981) and Jamal et al. (2010). The results of this study are in

great accordance with Jamal et al. (2010) and Iqbal et al. (2011).

Conclusion

It was concluded from the study that serotype O was more prevalent in Punjab province Pakistan than other three serotypes of FMDV with Asia I as the second highest occurring serotype.

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