

AN OVERVIEW ABOUT PREVALENCE OF FOOT AND MOUTH DISEASE VIRUS IN DISTRICTS KASUR AND LAHORE, PUNJAB, PAKISTAN FROM 2014-2019

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ABSTRACT: During 2014-2019, 109 suspected samples for FMD were received at ELISA Lab Foot and Mouth Disease Center (F&MDRC) Lahore, Pakistan. These samples were collected from Lahore and Kasur districts of Punjab, Pakistan. The prevalence trend of three serotypes (A, O, Asia-1) was checked through antigenic Enzyme Linked Immunosorbent Assay (ELISA). From the processed field samples; 71 samples were detected positive for serotype A, O and Asia-1. Out of the positive samples the most prevalent was serotype 'A' (57.74%) followed by serotype Asia-1 (29.57%) and the least prevalent was serotype 'O' (12.67%). The serotype 'A' was more prevalent in Lahore (82.92%) as compared to Kasur (17.07%). For the period of 2014-2015, no case of serotype 'O' was reported from district Kasur, whereas 9 cases were reported from district Lahore accordingly. Out of 21 cases of Asia-1 received at ELISA Lab FMDRC, Lahore; 18 cases were from Lahore followed by 3 cases from Kasur. From 2014-2019, the prevalence of serotype A, Asia-1 and O in Kasur was 70%, 30% and 0% respectively. The trend of prevalence in Lahore was same as in Kasur with most prevalent serotype A (55.73%) followed by serotype Asia-1 (29.50%) and the least prevalent was serotype O (14.75%). The only difference was that no case of serotype 'O' reported in Kasur while in Lahore 9 cases were reported during the period of six years. In conclusion there are more cases of FMD reported in Lahore as compared to Kasur and prevalence from 2014-2016 is more as compared to years 2017-2019.

Key Words: FMD Serotypes, Surveillance, ELISA, Comparative Perspective, Risk Assessment.

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INTRODUCTION

Livestock is the major subsector of agriculture in Pakistan that contributes up to 60.6 percent to overall agriculture and share in GDP is 11.7 percent. We can judge the importance of livestock from this fact that it provides 35-40 percent income to 8 million rural families. In Pakistan cattle, buffalo, sheep and goats are providing different products and by products that have a huge economic importance (Mahboob *et al.*, 2016; Rafique *et al.*, 2020). The population of cattle, buffalo, sheep and goats is 46.1, 38.8, 30.9 and 76.1 million numbers respectively. These four species are providing up to 60,770,000 tons milk, a product that is huge contributor to Pakistan's economy (Anonymous, 2018-2019).

Foot and Mouth disease is highly contagious, acute viral disease of cloven footed animals (Loth *et al.*, 2011). The clinical signs of this disease include high fever, lesions on tongue, feet, teats and on muzzles with severe lameness (Hayer *et al.*, 2018). This disease is caused by FMDV serotypes (A, O, Asia-1, C, South African Territories (Sat-1), (SAT-2), (SAT-3) and these serotypes immunologically do not confer protection to one another (El-Bayoumy *et al.*, 2014). The FMDV belongs to genus Aphthovirus, family Picornaviridae,

single stranded and positive-sense RNA virus with a genome of 8.4KB (Raletobana 2013). The genome encodes for the Open Reading Frame (ORF), the 5' untranslated region (5'UTR) and 3' untranslated region (3'UTR). The virus consists of four structural (VP1, VP2, VP3 and VP4) and 10 non-structural proteins (Gao *et al.*, 2016).

FMD is endemic and the most widely spread disease in Pakistan (Yasin and Huq 1960). Among the serotypes A, O and Asia-1, the most prevalent is serotype O (Saeed *et al.* 2011) and (Abubakar *et al.* 2015). According to recent study, the most prevalent serotype was Asia-1 (Farooq *et al.*, 2018). A study showed that FMD is more prevalent in developed areas as compared to under developed areas (Nawaz *et al.*, 2014).

There are many methods of diagnosis of FMDV which include virus isolation on cell culture, detection through ELISA, molecular detection through reverse transcriptase- polymerase chain reaction (RT-PCR) and real time PCR (Hussein *et al.*, 2019). The ELISA is more commonly used as compared to Complement Fixation Test (CFT), because it is not affected by pro and anti-complement factors. Serologically FMDV can also be detected by Virus Neutralization Test (VNT) by using cell culture techniques (OIE Terrestrial manual 2009).

MATERIALS AND METHODS

Location of Study: The study was carried out at ELISA Lab. Foot and Mouth Disease Research Center (F&MDRC) Lahore, Pakistan from 2014-2019.

Sample Collection: Epithelial samples of infected animals were collected from districts Kasur and Lahore of Punjab, Pakistan. The samples were collected in the transport media containing the equal amount of glycerol and 0.04 M phosphate buffer with a pH 7.2-7.4 while antibiotic added as described in OIE Terrestrial Manual 2009.

Processing of Samples: The epithelial samples were removed from the transport media and cleaned with the help of dry filter paper. From the cleaned sample up to 2gm of the sample was weighed and triturated in pestle and mortar. Up to 10% suspension was prepared by adding Phosphate Buffer Saline (PBS). In next step the suspected virus and PBS were centrifuged at 3500rpm for 10 minutes at 4°C. The virus was stored at -20°C for further processing.

Identification of Serotype by Antigenic ELISA: All the serotypes were detected by using antigen ELISA kits (IZLER, Brescia, Italy) as described by Wong *et al.* 2020.

RESULTS

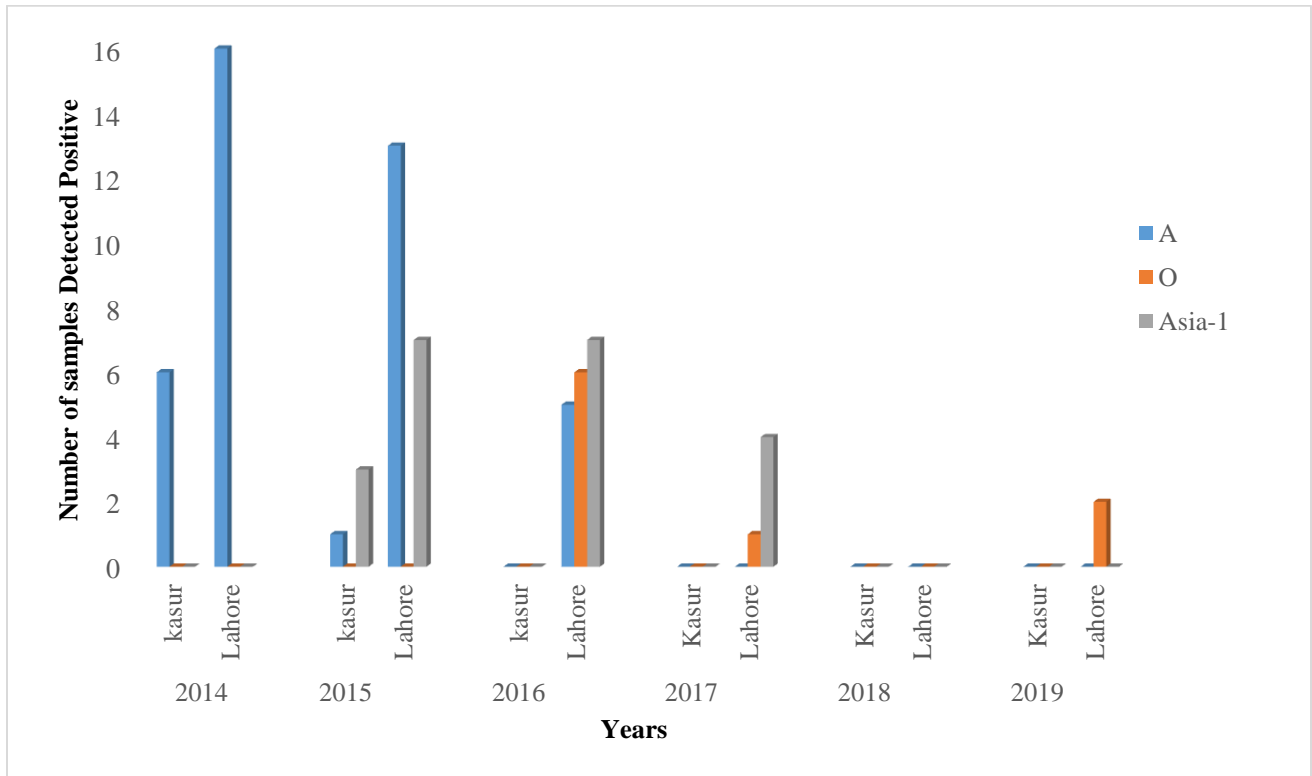
The below bar diagram shows the year wise prevalence trend of three serotypes (A, O, Asia-1) in districts Kasur and Lahore Punjab, Pakistan. During 2014, only serotype ‘O’ was reported in both districts. More number of cases of serotype ‘O’ were reported in Lahore as compared to Kasur.

In the very next year (2015) the trend was different from 2014. In this year both serotypes ‘A’ and ‘Asia-1’ were reported. In Kasur more cases of serotype ‘Asia-1’ were reported as compared to serotype ‘A’ while in Lahore serotype ‘A’ was more prevalent as compared to serotype ‘Asia-1’.

During year 2016, there was no case reported from Kasur at F&MDRC but all three serotypes (A, O, Asia-1) were reported from Lahore. The most prevalent serotype in Lahore was ‘Asia-1’ (38.88%) followed by serotype ‘O’ (33.33%) and the least reported was serotype ‘A’ (27.77%).

During year 2017, again there was no case reported at F&MDRC from Kasur and same as in 2016. From Lahore only two serotypes (O, Asia-1) were detected and the most prevalent was serotype Asia-1.

There was no case reported from Kasur and Lahore during year 2018. In 2019, only serotype ‘O’ was detected in Lahore. No other serotype was detected in Lahore and Kasur during this year.



Graph-1: Prevalence of FMDV Strains in District Kasur and Lahore during 2014-2019

Table-1: Prevalence of Serotype A, O and Asia-1 in Two Districts of Punjab, Pakistan During 2014-2019.

Districts	Serotype A	Serotype O	Serotype Asia-1
Kasur	7	0	3
Lahore	34	9	18

DISCUSSION

During the study from 2014-2019, only three serotypes (A, O, Asia-1) were found. From these serotypes, the maximum prevalent strain was serotype A followed by serotype Asia-1 and the least prevalent was serotype 'O'. During this study the maximum cases were in Lahore as compared to Kasur. From year 2014-2016 the most prevalent serotype was 'A' followed by 'Asia-1' and the least prevalent was serotype 'O'. But during 2017-2019 the most prevalent was serotype 'Asia-1' followed by serotype 'O' and no case of serotype 'A' was reported.

During year 2005-2006, 25 samples were collected from Kasur and 25 samples from Lahore. In both districts serotype O was 100% prevalent and no case of serotype A and Asia-1 was found during these years. During this study the maximum prevalence of serotype O was in Lahore and Kasur as compared to other districts (Awan, 2009).

A study was also reported by Food and Agriculture Organization (FAO) from January 2014 to March 2016. In 2014 the most prevalent serotype was 'O' followed by 'A' and the least prevalent was serotype 'Asia-1' in Punjab, Pakistan. This trend was same in 2015 with serotype 'O' as most prevalent (50.68%), followed by 'A' (26.71%) and the least prevalent was 'Asia-1' (22.60%). In 2016, the serotype 'A' was least prevalent and the serotype 'O' was most prevalent (FAO 2016). But during this study serotype 'O' was least prevalent. It may be due to comprehensive, integrated and well-focused vaccine campaigns and improved vaccine quality.

Similar study was performed to observe the prevalence of FMD throughout the Pakistan. During 2005-2007 the maximum prevalent serotype was serotype O (66.66%) followed by serotype Asia-1 (21.66%) and the least prevalent was A (3.33%). The remaining 8.33% was mixed infection. From 2008-2009, the trend totally changed with maximum prevalence of serotype A (53.12%) followed by O (43.75%) and mixed infection was 3.12%. There was no case of serotype Asia-1 reported during these two years (Abubakar *et al.*, 2012).

Another study was also conducted to record the prevalence in three districts of Punjab. The maximum prevalence was in Faisalabad (29.33%) followed by Khanewal (17.66%) and the least prevalent was in Chakwal (11%). This showed that maximum prevalence was in developed districts as compared to

underdeveloped (Nawaz *et al.*, 2014). The same trend was in study conducted at FMDRC, Lahore in which maximum prevalence was in Lahore as compared to Kasur.

Related study was carried out to check the prevalence of three serotypes (A, O, Asia-1) in six districts of Punjab, Pakistan. The results of this study showed that maximum prevalent was serotype O (67.11%) followed by serotype A (50.15%) and serotype Asia-1 as least prevalence (39.31%) (Akram and Khan, 2011).

Likely a comparable study was carried out to access the prevalence of FMD in different districts of Punjab, Pakistan. From the 77 positive cases serotype O (62.33%) was most prevalent followed by Asia-1 (33.77%) and serotype A (3.90%) (Nawaz *et al.*, 2018). The reason for decrease in number of FMD cases from 2017-2019 would be due to improvement in the quality of FMD vaccine produced locally at F&MDRC Lahore. Furthermore, newly adapted virus strains prevalent in the field were also incorporated in vaccine production process successfully. Moreover, FMD vaccine is more commonly used in district Lahore for livestock population as compared to district Kasur because of mass vaccination campaigns and farmer's awareness.

Conclusion: The prevalence of FMD in districts Lahore and Kasur during 2017-2019 was decreased as compared to the year 2014-2016. The decrease in percentage prevalence of FMD in studied districts may be due to the FMD Progressive Control Program started with the collaboration of FAO and well integrated mass vaccination campaigns.

Author Contribution Statement: Dr. Rehan Rafique, Dr. Muhammad Shoaib Noor, Dr. Sajjad Hussain, Dr. Rashad Munir, Dr. Abeera Mubarak, and Dr. Shaukat Ali executed an overview study regarding the prevalence of FMD in two different districts of Punjab including Lahore and Kasur as a team. Dr. Sajjad Hussain and Dr. Rashad Munir made available every possible support as the head of F&MDRC and supervised the whole project while Dr. Shaukat Ali and Dr. Abeera Mubarak provided support in technical aspects of this study. Moreover, Dr. Muhammad Shoaib Noor and Dr. Rehan Rafique analyzed prevalence based field trials and all technical aspects of Antigen ELISA. Dr. Rehan Rafique wrote the manuscript while Dr. Shaukat Ali reviewed the study and Dr. Rizwan Rafique helped in the write up process along with graphical representation of this study.

Conflict of Interest: The authors declared that they had no conflict of interest.

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REFERENCES

- Abubakar, M., M. J. Arshed, Q. Ali and M. Hussain (2012). Spatial trend of Foot and Mouth Disease virus (FMDV) serotypes in cattle and buffaloes, Pakistan. *Viol. Sin.* 27(5): 320-323.
- Abubakar, M., M. J. Arshed, J. Gonzales, G. Ferrari, M. Hussain and Q. Ali (2015). An appraisal on the occurrence of foot-and-mouth disease virus serotypes in cattle and buffaloes, Pakistan. *Arch. Virol.* 160(6): 1561-1564.
- Akram, M. and M. A. Khan (2011). Sero-prevalence of foot and mouth disease in large ruminants in central Punjab, Pakistan. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 32(1and2): 6-11.
- Awan, F. N. (2009). Epidemiology of foot and mouth disease in buffaloes of Punjab province. Doctoral dissertation, University of Veterinary and Animal Sciences, Lahore.
- El-Bayoumy, M. K., K. A. Abdelrahman, A. M. Allam, T. K. Farag, H. A. Abou-Zeina and M. A. Kutkat (2014). Molecular characterization of foot and mouth disease virus collected from Al Fayoum and Beni-Suef governorates in Egypt. *Glob. Vet.* 13(5), 828-835.
- Farooq, U., Z. Ahmed, K. Naeem, M. Bertram, B. Brito, C. Stenfeldt, J. P. Steven, M. LaRocco, L. Rodriguez and J. Arzt (2018). Characterization of naturally occurring, new and persistent subclinical foot-and-mouth disease virus infection in vaccinated Asian buffalo in Islamabad Capital Territory, Pakistan. *Transbound. Emerg. Dis.* 65(6): 1836-1850.
- Gao, Y., S. Q. Sun and H. C. Guo (2016). Biological function of Foot-and-mouth disease virus non-structural proteins and non-coding elements. *Virol. J.* 13(1): 1-17.
- Hayer, S. S., K. VanderWaal, R. Ranjan, J. K. Biswal, S. Subramaniam, J. K. Mohapatra, G. K. Sharma, M. Rout, B. B. Dash, B. Das and B. R. Prusty (2018). Foot-and-mouth disease virus transmission dynamics and persistence in a herd of vaccinated dairy cattle in India. *Transbound. Emerg. Dis.* 65(2): 404-415.
- Hussein, H. A., R. Y. Hassan, R. M. Nashar, S. A. Khalil, S. A. Salem, and I. M. El-Sherbiny (2019). Designing and fabrication of new VIP biosensor for the rapid and selective detection of foot-and-mouth disease virus (FMDV). *Biosens. Bioelectron.* 141: 111467.
- Loth, L., M. G. Osmani, M. A. Kalam, R. K. Chakraborty, J. Wadsworth, N. J. Knowles, J. M. Hammond and C. Benigno (2011). Molecular characterization of foot-and-mouth disease virus: implications for disease control in Bangladesh. *Transbound. Emerg. Dis.* 58(3): 240-246.
- Mahboob, K., R. Rafique, T. Farooq, I. Huma, S. Parveen and M. N. Khan (2016). Raising of Hyper-Immune Serum against FMD Virus Type "O" Prevailing in Punjab, Pakistan. *Int. Arch. BioMed. Clin. Res.* 2(3): 104-106.
- Nawaz, Z., M. Arshad, and Z. Iqbal (2014). Epidemiology of foot and mouth disease in buffaloes and cattle of Punjab using nonstructural proteins ELISA. *Pak. J. Agri. Sci.* 51(2).
- Nawaz, Z., S. U. Rehman, A. B. Siddique, M. A. Zahoor and S. Ali (2018). Investigations of foot and mouth disease outbreaks in different districts of Punjab, Pakistan. *Buffalo Bull.* 37(1): 81-87.
- Raletobana, J. G. (2014). Sero-prevalence and risk factors of foot and mouth disease in goats in Ngamiland and North East Districts of Botswana (Doctoral dissertation).
- Rafique, R., A. Mubarak, M. S. Noor, S. Hussain, R. Munir and S. Ali (2020). Adaptation of Foot and Mouth Disease Virus Subtypes Pan Asia-II, Tur-06 and Sindh-08 towards the Vaccine Development and Serological Findings by SPC ELISA and Serum Neutralization Test. *Pak. J. Sci.* 72(3): 169-175.
- Saeed, A., Q. M. Khan, U. Waheed, M. Arshad, M. Asif and M. Farooq (2011). RT-PCR evaluation for identification and sequence analysis of foot-and-mouth disease serotype O from 2006 to 2007 in Punjab, Pakistan. *Com. Immunol. Microbiol. Infect. Dis.* 34(2): 95-101.
- Yasin, S. A. and M. M. Huq (1960). Foot-and-mouth disease in Pakistan. *Bull. Off. Intl. Epizoot.* (54).
- Wong, C. L., C. Y. Yong, H. K. Ong, K. L. Ho and W. S. Tan (2020). Advances in the Diagnosis of Foot-and-Mouth Disease. *Front. Vet. Sci.* (7): 477.