

EPIDEMIOLOGICAL STATISTICS OF FMD VIRUS SEROTYPES A, O AND ASIA-1 IN PUNJAB, PAKISTAN DURING 2014-2019

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ABSTRACT: Foot and Mouth Disease is an endemic disease and three serotypes (A, O, Asia-1) are prevalent in Pakistan. The present study shows the frequency of Foot and Mouth Disease Virus (FMDV) serotypes (A, O, Asia-1) in Punjab Province of Pakistan by using antigenic Enzyme-Linked Immunosorbent Assay (ELISA). This study was conducted from 2014-2019 by using a total of 184 epithelial samples from field. Out of 184 samples, 106 samples showed positive results. Out of three serotypes, serotype 'A' was the most prevalent (39.62%) followed by serotype 'O' (33.96%) while the least prevalent was serotype Asia-1 (26.41%). It was marked that no other serotype of FMD reported during this study. This study also described the year wise prevalence of three prevailing FMD serotypes (A, O, Asia-1). During year 2014, the serotype 'A' was more prevalent (95.83%) followed by serotype 'Asia-1' (4.16%) while no case of serotype 'O' was found. During year 2015, the prevalence trend was almost the same as in 2014. In this year, serotype 'A' was 55.55% while serotype 'Asia-1' 37.03% followed by the least prevalent serotype 'O' as 7.40%. During year 2016, 19 positive cases were reported from which the maximum prevalence was serotype 'O' and 'Asia-1' (42.10%) followed by serotype 'A' (15.78%) as least prevalent. During year 2017, the positive cases for FMD increased remarkably i.e. 29 in number. This year serotype 'O' was highest in number with prevalence 75.86%, and serotype 'Asia-1' 20.68% whereas only one case reported for serotype 'A' with only 3.44% prevalence. During year 2018 and 2019, only 7 cases described with no evident case of serotype 'A'. During these two years 4 cases of serotype 'O' were observed followed by 3 cases of serotype 'Asia-1'.

Key words: Prevalence, FMD Virus, Serotype A, O & Asia-1, ELISA, Geographical Distribution, National Control Strategy.

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INTRODUCTION

Livestock is the major sector having share in Pakistan agriculture up to 60.5% and overall share in the GDP is 11.2 percent during year 2018-2019. Gross value addition of livestock in year (2017-2018) was Rs. 1,384 billion that has increased in year (2018-2019) to Rs. 1,440 billion; hence the importance of Livestock can be recognized. The estimated population of cattle and buffalo in Pakistan is 47.8 and 40.0 million respectively in 2018-2019 which provide a lot of number of products in the form of milk, meats, hides and skins (Anonymous, 2018-2019).

Foot and mouth disease (FMD) is a highly contagious viral infectious disease that affects the cloven hoofed animals. (Rehman *et al.*, 2014). It affects both domestic animals including cattle, buffalo, sheep, goat and wild ungulates as well (Habib *et al.*, 2014). The FMDV belongs to genus Aphthovirus, family Picornaviridae and single stranded positive sense RNA virus (Lloyd-Jones *et al.*, 2017). The FMD virus exists in

seven different forms as, O, A, C, Asia 1, South African Territory (SAT-1), (SAT-2) & (SAT-3) depending upon structural proteins which may vary antigenically (Ko *et al.*, 2019). This virus is 25-30nm in diameter and roughly spherical in shape. The genome of this virus is 8.3kb in length and it is enclosed in capsid protein. The capsid is formed by 60 copies of capsomers, and capsomers contain four structural proteins VP1 to VP4. The proteins VP1, VP2, VP3 are present externally on the surface of virus while VP4 is present internally (Jamal and Belsham, 2013). In FMD infected animal may act as a reservoir and continues to shed the virus in secretions for significant period of time infecting the other animals (Li *et al.*, 2007).

In Pakistan serotype O was first time detected in 1952 and up to now it is the major cause of outbreaks all over the country. FMDV type A was first time detected in 1955 in Pakistan. The serotype Asia-1 was first time detected in Okara, Pakistan in 1954 and up to now its cases are reporting (Jamal *et al.*, 2010). But serotype 'C' was also detected in Pakistan first time in 1954 from a

military farm during a disease outbreak. After it, this virus was again detected in 1963 and the last case of this virus was reported in 1995. Since then, up till now no case of FMDV was detected in Pakistan (Jamal *et al.*, 2010).

This disease causes huge number of economic losses to livestock that lead towards the high morbidity in adult animals, decrease in animal production and failing to come in prime condition due to infection, and high mortality in young ones leading towards the restriction in animal trade and products due to its trans-boundary feature (Mahboob *et al.*, 2016; Ullah *et al.*, 2017; Rafique *et al.*, 2020). Due to poor veterinary infrastructure, improper diagnosis of FMD, high cost of vaccine and unawareness about the clinical signs of disease ascertains towards regular occurrence of this menace (Hussain *et al.*, 2017). The foot and mouth disease has always devastating effects on livestock in a country leading to serious socioeconomic impact and subsequently International trade barrier for livestock products. There are many techniques for the rapid and accurate diagnosis of FMD virus in animals while ELISA is most extensively used detecting tool for the confirmation of FMD serotypes (Wong *et al.*, 2020).

According to OIE FMD manual the antigen can be detected by using different techniques like antigen ELISA, Lateral Flow Devices (LFD), Complement Fixation Test (CFT), Reverse Transcription Polymerase Chain Reaction (RT-PCR), Cell Culture Techniques (cytopathic effect will be observed) and Virus Neutralization Test (VNT). In laboratories ELISA has replaced CFT as it is more sensitive and specific and not affected by pro- or anti-complement factors (OIE FMD Manual).

The objective of this study was to check the prevalence of FMD serotypes (A, O, Asia-1) virus in cattle and buffalo by using antigenic ELISA. The results of this study would also aid in understanding the pattern of circulation of FMD serotype (A, O, Asia-1) in Punjab, Pakistan during 2014-2019.

MATERIALS AND METHODS

Samples Collection: During the year 2014-2019, 184 samples were collected from the tongue epithelium and foot tissue from the diseased animals. The samples were collected aseptically in special transport medium containing the equal amount of glycerol and 0.04M phosphate buffer (PH 7.2-7.6) while antibiotic and antimycotic were added according to OIE Terrestrial Manual 2009. Collected samples were placed in sample preservation box having ice packs and then transported to ELISA Lab. FMDRC, Lahore.

Processing of Field Samples: The field samples were processed by adding epithelium samples in the pestle and

mortar. Onward 10% suspension was prepared by adding Phosphate Buffer Saline (PBS). These samples were then centrifuged at 4500 rpm for 10 minutes at 4°C and stored in deep freezer for further processing.

FMD Virus Serotype Detection by Antigenic ELISA: All the processed samples were detected for different serotypes of FMD virus by using antigen detection ELISA kits (Reference: IZLER, Brescia, Italy) at ELISA Lab. FMDRC, Lahore Pakistan.

RESULTS

All through this study period year 2014 to 2019, a total number of 106 samples were detected as positive for the presence of three serotypes A, O and Asia-1 from the processed 184 field samples. Graph-1 illustrates the percentage prevalence trend of FMD serotypes A, O and Asia-1 for the duration of year 2014 to 2019.

During the year 2014; 23 samples were found positive for serotype 'A' showing percentage prevalence of 95.83% (Table-1). Throughout the year FMD outbreaks were due to serotype 'A' mostly whereas only 1 sample was detected positive for the serotype 'Asia-1' with percentage of only 4.16% while no case of serotype 'O' was detected positive. For the period of year 2015; almost the same prevalence trend was recorded as during the year 2014 being serotype 'A' as most prevalent one, but this year 2 samples were also detected positive for serotype 'O'. The percentage prevalence is documented as 55.5%, 37.03%, and 7.40% respectively for the serotypes A, Asia-1 and O for this year.

The evident shift in the percentage prevalence trend was recorded for the year 2016 and 2017 showing serotype 'O' the most prevalent. In year 2016, the percentage prevalence is observed as 15.78% for serotype 'A' while 42.10% for both serotypes 'O' and 'Asia-1'. Even as in year 2017, 22 samples were detected positive for the serotype 'O' as most prevalent serotype having percentage prevalence of 75.86%. Comparatively, the percentage prevalence for the serotype 'A' and 'Asia-1' was 3.44% and 20.68% respectively. It has been observed that during the years 2016 and 2017, maximum samples of serotype 'O' were detected positive whereas positive samples for serotype 'A' decreased in these two years in comparison to the previous two years 2014 and 2015.

This study depicted that in years 2018 and 2019 there was no sample of serotype 'A' detected positive and percentage prevalence of serotype 'A' also got reduced remarkably in these two years as compared to previous years indicating as a major swing in the FMD serotypes percentage prevalence as depicted in Graph-2.

Percentage Prevalence of Serotype 'A' During Years 2014-2019: The below Pie chart illustrates the percentage prevalence of serotype 'A' during five years. The maximum prevalence was in year 2014 (54.76%)

followed by year 2015 (35.71%). The percentage prevalence decreased in the next three years 2016-2019 gradually, even there was no sample was detected for serotype ‘A’ during the years 2018-2019 (Graph-3).

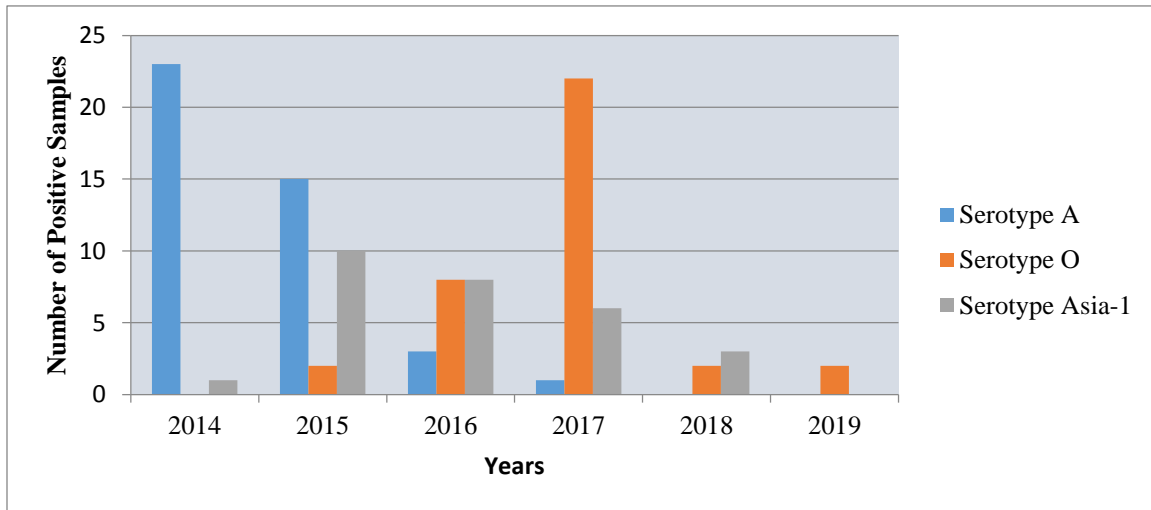
The maximum percentage prevalence of serotype ‘O’ was observed in year 2016 & 2017 as 22.22%, 61.11% respectively while only few samples of

serotype ‘O’ were detected in years 2014-2015 (Graph-4).

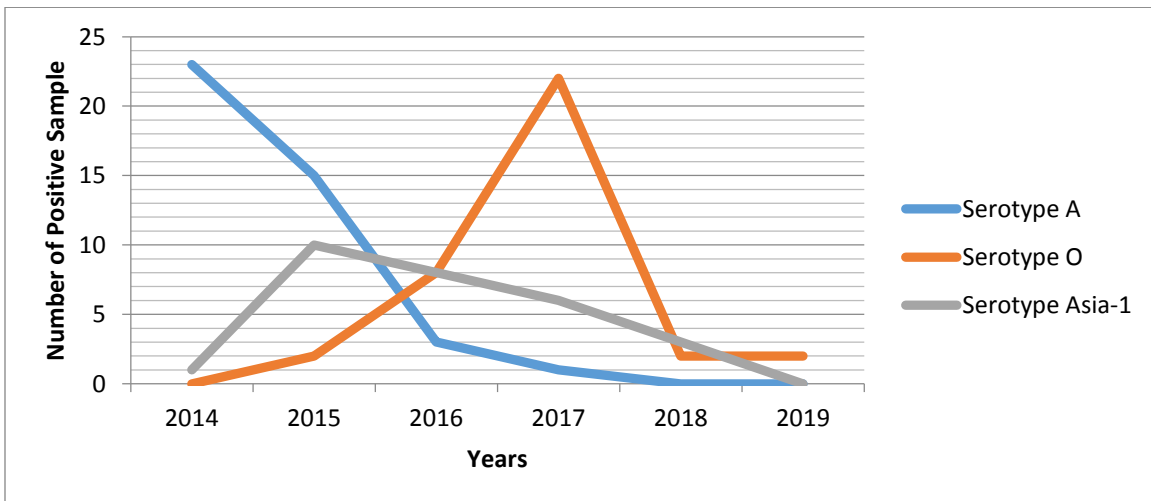
The overall percentage prevalence of serotype ‘Asia-1’ was lower than the other two serotypes ‘A’ and ‘O’. The maximum percentage prevalence was in year 2015 (35.71%) for serotype ‘Asia-1’ (Graph-5).

Table-1: Detail of Positive Samples for Serotype A, O and Asia-1 at FMDRC, Lahore during 2014-2019.

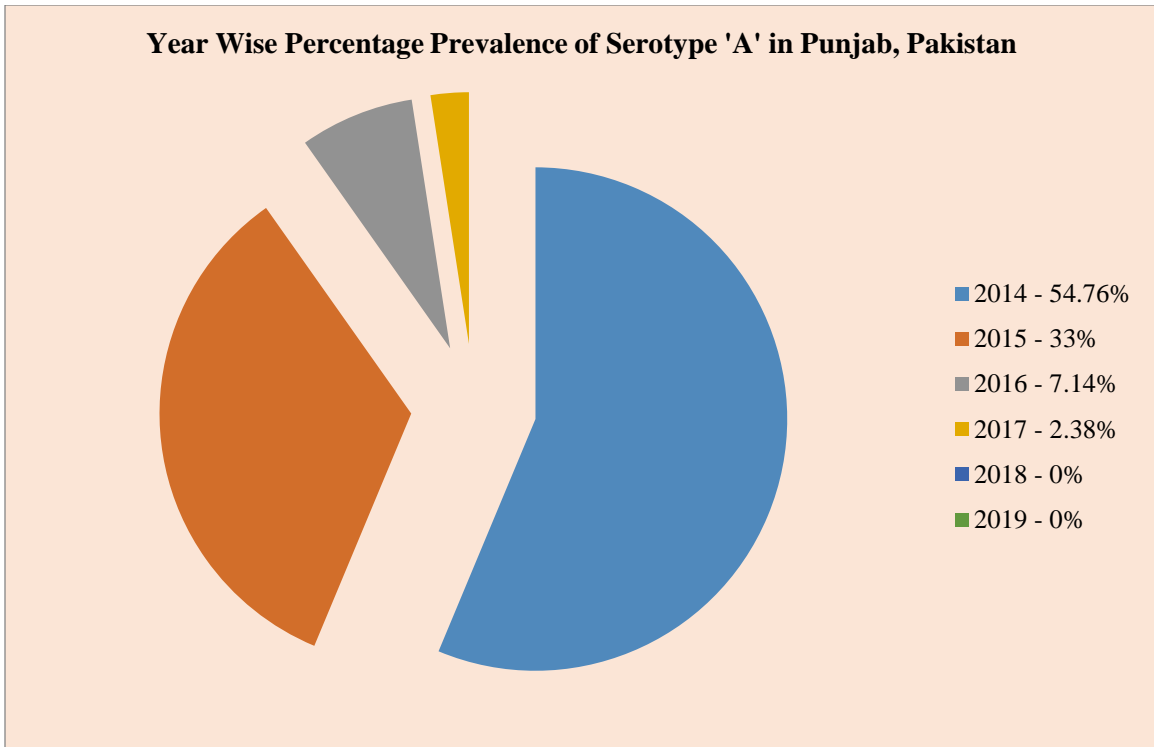
| Positive Samples for Serotype A, O and Asia-1 at FMDRC, Lahore During 2014-2019 | | | |
|---------------------------------------------------------------------------------|------------|------------|-----------------|
| Year | Serotype A | Serotype O | Serotype Asia-1 |
| 2014 | 23 | 0 | 1 |
| 2015 | 15 | 2 | 10 |
| 2016 | 3 | 8 | 8 |
| 2017 | 1 | 22 | 6 |
| 2018 | 0 | 2 | 3 |
| 2019 | 0 | 2 | 0 |
| Total | 42 | 36 | 28 |



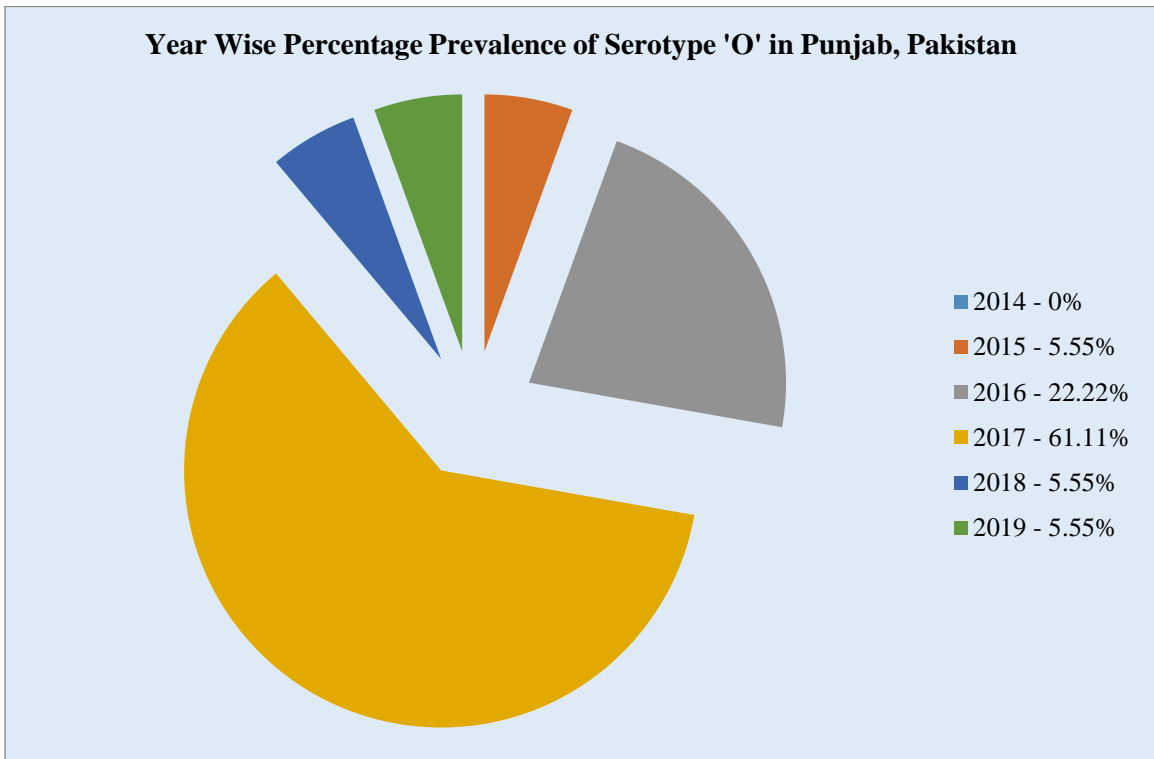
Graph-1: Prevalence of FMD Virus Serotypes in Punjab, Pakistan from 2014-2019



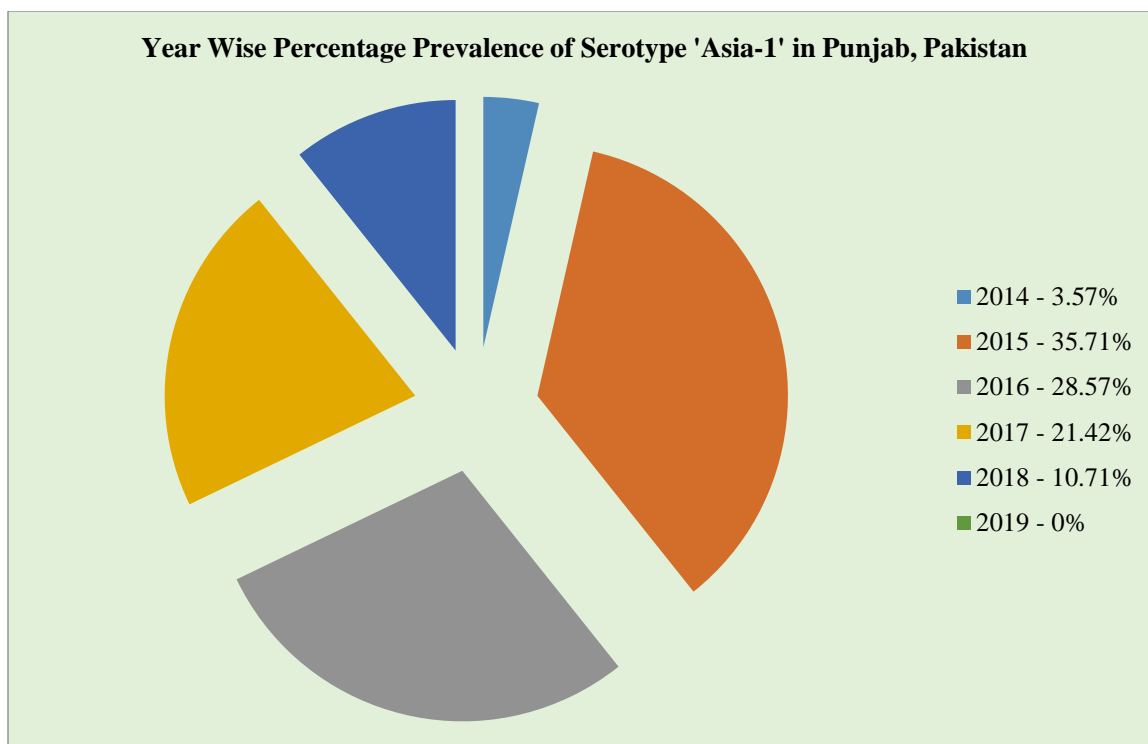
Graph-2: Prevalence of Serotype A, O & Asia-1 in Punjab, Pakistan from 2014-2019.



Graph-3: Percentage Prevalence of Serotype 'A' During Years 2014-2019.



Graph-4: Percentage Prevalence of Serotype 'O' During Years 2014-2019



Graph-5: Percentage Prevalence of Serotype ‘Asia-1’ During Years 2014-2019

DISCUSSION

FMD is the most important trans-boundary disease of livestock in Pakistan and almost all over the world. It is also a serious threat to cattle and buffalo population rather than sheep & goats. The FMD virus serotypes are detected through the antigenic ELISA kit (IZLER, Brescia, Italy) and all field samples of this study were also serotyped through this technique as conducted by Sulayeman *et al.*, 2018; Kamal and Hassan, 2017.

The prevalence of FMD virus serotypes A, O, Asia-1 and C was compared by Jamal *et al.*, 2010 from 1952-2007. Out of the positive cases the most prevalent was serotype O that was (61%) whereas 24% and 15% cases were positive for Asia-1 and type A respectively. The serotype C was only 0.006% positive (Jamal *et al.* 2010). In Pakistan only three serotypes are present and serotype ‘O’ was most prevalent (70%) followed by Asia-1 (25%) and least prevalent serotype ‘A’(4.67%) as narrated by Rehman *et al.*, 2014.

A study was conducted to check the seroprevalence of FMD in bovine that was 19.33% and prevalence was more in cattle (23.09%) than buffalo (16.04%) as described by Nawaz *et al.*, 2014. To Access FMD serotypes prevalence a study was also evaluated in Bangladesh Agriculture University in which out of 85 samples, 31 (36.47%) positive for serotype A, 26 (30.58%) positive for serotype O and 10 (11.76%) for serotype Asia-1 and the remaining 4 (4.7%) positive for

mixed infection of serotype ‘A’ and ‘Asia-1’. The study concluded serotype ‘A’ and ‘O’ as the most prevalent strains in Bangladesh (Hossen *et al.*, 2014).

Similar study was reported in India to check the status of three serotypes (A, O, Asia-1) of FMDV from 2007-2011. The most prevalent serotype was type ‘A’ (80%) followed by Asia-1 (12%) and the least prevalent was type ‘O’ (8%) (Subramaniam *et al.*, 2013). A study was also carried out to check the prevalence of different serotypes of FMD in Punjab, Pakistan from 2001-2010. A total of 659 samples were collected and 234 samples were positive. From these 234 samples 178 (27.01%) were positive for serotype ‘O’, 48 (7.28%) cases for Asia-1, for serotype ‘A’ only 4 (0.60%) positive. The remaining four samples were found positive for serotype ‘C’(Ahmad *et al.*, 2013). Here serotype ‘O’ was the most prevalent, but type ‘A’ as the least prevalent that was different from the results of FMDRC, Lahore.

Another comparable study was performed to determine the prevalence of FMD serotypes in ruminants from different areas of Punjab, Pakistan by using indirect ELISA. From 109 epithelial tissue samples 77 cases were positive. Out of the 77 cases 62.33% were positive for type O followed by 33.77% positive for Asia-1 and only 3.90% for serotype ‘A’ (Nawaz *et al.*, 2018).

FAO observed sixty-six cases in February 2018 in two provinces (Azad Kashmir and Punjab) of Pakistan. Out of 66 cases the most prevalent was type A (40.90%) followed by serotype ‘O’ (27.27%), the least one was Asia-1 (1.51%). The mixed infection case was 1.51% and

the un typed was 22.72% (Report on Progressive Control of FMD in Pakistan). This trend was almost the same as the results of prevalence of different serotypes observed in this study.

A related study was also conducted in Karak and Haripur districts of Khyber Pakhtunkhwa Province of Pakistan during 2008-2011 to check the burden of FMD in buffalo, cattle, sheep and goats. These results showed that the mass vaccination strategy decreased the burden of FMD in these areas (Mushtaq *et al.*, 2014). A decreased in number of cases of FMD in 2017-2019 may be due to mass vaccination and advancement of vaccine production strategies. From these three, serotypes 'A' is most prevalent up to 2016 followed by serotype 'Asia-1'. During 2014-2016 there were only few cases of serotype 'O'. This trend is totally changed from 2017 to 2019, in these years' serotype 'O' was most prevalent and few cases of serotype 'A' were detected.

Out of the 106 positive cases of all three serotypes, serotype 'A' had maximum percentage 39.62% (42 cases) followed by serotype 'O' 33.96% (36 cases) and the serotype 'Asia-1' least percentage 26.41% (28 cases). Serotype 'A' continued to decrease from year 2014 to 2019 while serotype 'O' continued to increase from 2014-2017. These results are in accordance with the surveillance reports of FAO 2018. The above mentioned research work showed that serotype 'A' and 'O' are most prevalent as compared to Asia-1 and the results of this study are also in line for the prevalence of FMD serotypes in Punjab, Pakistan during 2014-2019.

These results expressed the percentage prevalence of three serotypes of FMD virus in Punjab, Pakistan. From year 2014-2017, the overall prevalence was more as compared to the year 2018-2019. The decrease in the prevalence may be due to different FMD control projects and awareness campaigns. During 2014, Pakistan signed different projects in collaboration with Food and Agriculture Organization (FAO) of United Nations and United States Department of Agriculture (USDA) to control FMD. Likewise in 2018, similar projects were also signed between Ministry of National Food Security & Research Pakistan and Food and Agriculture Organization (FAO) of United Nations to accelerate the FMD control strategies.

The other reason for decrease in incidence of serotypes is due to improvement in quality of FMD vaccine. With the advancement in the research & development activities prevailing field isolates of FMD virus were incorporated for the production of vaccine. Because incorporated strains used in vaccine production is closely related to the newly prevalent field strains ultimately giving better protective results against foot & mouth disease.

Conclusion: The present study confirmed serotype A, O and Asia-1 are currently circulating in Pakistan as

detected by Antigen ELISA kits. These isolated viruses can be used to improve vaccine quality ultimately leading to better health coverage of livestock population and expounding exportable surpluses as well.

Author Contribution Statement: Dr. Rehan Rafique, Dr. Muhammad Shoaib Noor, Dr. Rashad Munir, Dr. Sajjad Hussain, Dr. Abeera Mubarak, and Dr. Shaukat Ali designed the comprehensive epidemiological study as a team. Dr. Sajjad Hussain and Dr. Rashad Munir provided institutional support as the head of F&MDRC and supervised the whole study 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 practically performed this research based field trial 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 graphical representation along with the write up process.

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

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