Prevalence of Foot and Mouth disease virus and molecular identification of serotype O in bovines of the district Bahawalpur, Punjab, Pakistan using NSP-ELISA

Muhammad Mohsin Abbas[1*], Sajjad Ur Rahman[1], Muhammad Abubakar[2], Muhammad Imran Arshad[1], Khurram Ashfaq[3]

1. Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
2. National Veterinary Laboratory, National Agriculture Research Council, Islamabad, Pakistan
3. Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan

*Corresponding author

Abstract- The current cross-sectional study was conducted in the Bahawalpur district of Punjab, Pakistan, with the objective of finding out the prevalence of the Foot and Mouth Disease (FMD) virus in bovine. As a routine successful running of FAO, FMD eradication program in the country and specifically in the district of Bahawalpur, the bovine population exposed to regular vaccination was compared with the non-vaccinated population. A total of 838 bovine sera samples were analyzed through Nonstructural protein enzyme-linked immunosorbent assay (NSP ELISA), based on 3ABC protein. The overall prevalence in the Bahawalpur district was 13.84% in bovine. The highest prevalence, at the tehsil level, was found in tehsil Ahmedpur East at 26.17% followed by tehsil Khairpur Tamewali at 23.23%, tehsil Bahawalpur at 13.21%, tehsil Hasilpur at 9.65% and tehsil Yazman 2.72% in bovine. More than 4 years of bovine were significantly seropositive (20.51%) followed by >2-4 years (16.05%). Bovine calves of >7-12 months (14.86%) were significantly seropositive followed by3-6 months (10.39%) of age. Prevalence in vaccinated bovines was 17.66%, significantly higher than compared to 9.70% in non-vaccinated bovines. Molecular characterization revealed two FMD isolates (tehsil Ahmedpur East and tehsil Khairpur Tamewali) belong to serotype O, which showed relatedness (62-71%) with the previously reported FMD virus from the Sargodha district.

Index Terms- Prevalence, Bovines, FMD, FMDV, ELISA,

I. INTRODUCTION

The census of livestock in Pakistan recorded 213.1 million population, including cattle (51.5 million), and buffaloes (42.4 million). Livestock share of the district of Bahawalpur is 1.22 million, including cattle (0.84 million), and buffaloes (0.38 million). The major hindrance to the development of this sector is the onset of contagious and non-contagious diseases of economic importance. Foot and mouth disease (FMD) is a disease of main concern in livestock in Pakistan, caused by FMD virus (FMDV) in cloven-hooved animals of the genus Aphtho [1-3], characterized by high fever (up to 104°F), blisters inside the mouth and on the feet are formed initially and then get ruptured in later stages, cause anorexia and lameness [4]. The incubation period of the disease is 1 to 12 days. FMDV has seven serotypes [5]. Out of these, only three serotypes including Asia 1, O, and A, were prevalent in Pakistan [6].

Cattle remained a major reservoir of the FMDV. FMDV serotypes prevalent in cattle have also been reported by antelope, deer, and wild pigs. However, growing evidence in this regard showed that wild animals could not maintain the FMDV for more than a month except for African buffalo. Virus shedding is the principal cause of FMD transmission, dissemination, and its continuous persistence in highly endemic areas. All the body secretions may contain FMDV [7]. Moreover, specific efforts were adapted to understand the role of subclinical infection in the transmission of FMDV in Pakistan. These studies indicate the subclinical persistence of the FMDV in the epithelial cells of the oro-pharynx and milk duct. Similarly, the persistence of the FMDV at the subclinical level in apparently healthy cows had also been reported during 2012-[8].

Purposes of the FMDV detection test included to certify animals for trade, to confirm suspected cases of FMD, to test vaccine efficacy, and to provide confirmation of absence of infection [9]. Knowledge sharing regarding FMDV detection may be a valuable tool for FMD control in susceptible animals and also pave a way for better productive ability of the susceptible animals [10]. Vaccinated animals produce antibodies only against nonstructural proteins (NSPs). The non-structural proteins are highly conserved and are not serotype specific [11]. Among the NSPs of FMDV, 3ABC polyprotein is the most reliable [12] and antigenic indicator for differentiation of infected from vaccinated animals (DIVA) [13]. It may be detected within 10 days post recent infection in infected animals and also may be detected up to 2 years (Moonen *et al*., 2004). NSP ELISA may be utilized to detect viral circulation at the herd level and also to validate absence of infection [14]. The development of NSP ELISA has improved the surveillance of FMD even in vaccinated herds [15].

Our research hypothesis is that FMDV may perpetuate in the bovine animal population. The purpose of the current study is to explore the dynamics of the FMDV and to detect the circulation of field virus in vaccinated and non-vaccinated bovines of different gender and age groups by determining the prevalence of FMDV in Bahawalpur district of Punjab Province to develop FMD-free zones initially in targeted areas

II. IDENTIFY, RESEARCH AND COLLECT IDEA

Collection of serum samples

A total of 838 sera were collected through convenient sampling from the district of Bahawalpur. Out of which 436 samples were collected from vaccinated bovine and 402 were collected from the non-vaccinated bovine population.

NSP ELISA

The presence of anti-FMDV antibodies in serum samples was tested by using the FMD NSP ELISA kit (3ABC-Trapping ELISA Rev 0-0313), according to manufacturer's instruction IZSLER, Biotechnology Laboratory, Italy. Microtitre plates were supplied pre-coated with the 3ABC antigen captured by the MAb. A colorimetric reaction developed when the conjugate had bound to the sample antibody. The color development was proportional to the number of antibodies present in the test sample. After the addition of a stop solution, the optical density (OD) of the developed color was read by a micro-plate photometer (Thermo, 590741) [16].

Molecular Identification of FMDV

A total of 18 oral swab samples from suspected cases were processed through RT-PCR, a technique used in the laboratory in which RNA extraction using Thermo scientific, GeneJet RNA purification kit, formation of c DNA through reverse transcription, polymerase chain reaction, and agarose gel electrophoresis was performed to identify the amplified product [17].

Sequence Analysis

All positive PCR samples were submitted to Advance Bioscience International Lahore for sequence analysis. The phylogenic tree was constructed and visualized using the Mega 6.0 program.

Analysis of data

Minitab software was utilized for statistical analysis of data. A p-value ≤ 0.05 indicated that prevalence was statistically significant, whereas a p-value > 0.05 showed that prevalence was statistically non-significant and it may be denoted by steric sign (*).

III. WRITE DOWN YOUR STUDIES AND FINDINGS

Tehsil wise seroprevalence of FMD in bovine population

The prevalence of antibodies to FMDV in the bovine population within five tehsils of the Bahawalpur district was studied. The highest seroprevalence was found in the bovine of tehsil Ahmedpur East 26.17% followed by tehsil Khairpur Tamewali 23.23%, tehsil Bahawalpur 13.21%, tehsil Hasilpur 9.65% and in tehsil Yazman 2.72% as indicated in Fig 1.

The comparative seroprevalence of FMDV within tehsils of Bahawalpur district were segregated into cows and buffaloes. It was recorded that the overall buffalo population was carrying maximum seroprevalence with (37.8%) in tehsil Ahmedpur East area followed by 26% in tehsil Khairpur Tamewali. However, The Cow population was carrying a maximum of (17.99%) of FMD seroprevalence from tehsil Hasilpur followed by 17.43% in tehsil Ahmedpur East as detailed in Fig 2.

Age wise seroprevalence of FMD in bovine population

The data indicated that age wise maximum seroprevalence of 20.51% was noted in adult bovine population having more than 4 years of age followed by 16.05% and 14.15% in bovine of 2-4 years and 1-2 years of age, respectively. Within the bovine calve population, maximum seroprevalence was noted in 7-12 months (14.86%) followed by 10.39% and. 6.66% in 3-6 months and less than 3 months of age respectively, as detailed in table 1.

Seroprevalence of FMDV in vaccinated and non-vaccinated bovine population

Seroprevalence of FMDV in vaccinated bovine was 17.66%, compared with 9.70% from non-vaccinated bovine. FMD seroprevalence was distributed variably within the cow and buffalo population of district Bahawalpur and it was recorded that seropositive cases were significantly higher in vaccinated cows (20.28%) and buffaloes (18.6%) compared to the male population across the district as showed in Fig 3.

Gender wise seroprevalence of FMDV in bovine population

Irrespective of the age and vaccination status, gender wise, the antibody-based prevalence against FMDV (13.98%) in bulls compared with (18.38%) from cows. Whereas Seroprevalence in the ox population was lower (9.24%), compared with (12.82%) from buffaloes, as mentioned in table 2.

Molecular characterization of FMDV in bovine population

Results of RT-PCR revealed that two of 18 samples were positive for FMDV. One of these two positive samples was taken from a cow of tehsil Ahmedpur East, and one was taken from a buffalo of tehsil Khairpur Tamewali. For more verification, phylogenetic analysis was completed.

BLAST analysis

The BLAST results verified the incidence of FMDV in samples. BLAST analysis of FMDV isolated from cows of tehsil Ahmedpur East showed 62% homology with FMD-type O, previously reported in cattle of Janwala, Sargodha, Pakistan. BLAST analysis of FMDV isolated from buffalo of tehsil Khairpur Tamewali showed 71% identity with FMD-type O, previously reported in cattle of Janwala, Sargodha, Pakistan, as indicated in Fig4. Isolates of the present study are available in NCBI GenBank under Accession Numbers, OK359042 and OK359043.

Discussion:

The present study was conducted to address the seroprevalence of FMDV in the bovine population of the Bahawalpur district of Punjab, Pakistan. Overall seroprevalence in bovine was 13.84% which coincides with the results of the sero-epidemiological study conducted in Ethiopia that indicated the overall seroprevalence was 12.04% in ruminants [18]. Whereas the results of two studies indicated that overall seroprevalence of FMD in bovine was 19.33% [19] and 22.11% in Punjab, Pakistan [20]. An

epidemiological study conducted in Afghanistan indicated that seroprevalence of 51.4% was noted in bovine species [21]. This difference may be due to the fact that better immunization of bovine is being maintained in the Bahawalpur district.

Results indicated that the highest seroprevalence was found in the bovine population of tehsil Ahmedpur East 26.17% followed by tehsil Khairpur Tamewali 23.23%, tehsil Bahawalpur 13.21%, tehsil Hasilpur 9.65% and in tehsil Yazman 2.72%. High prevalence of antibodies to FMDV described the endemic nature of disease in tehsil Ahmedpur East and tehsil Khairpur Tamewali of the district Bahawalpur. It was recorded that the buffalo population was carrying higher seroprevalence with (37.8%) than cow population (17.99%). These results coincide with the results of another epidemiological study which showed the higher seroprevalence of 61% was noted in buffaloes compared to 41% of cattle in Laos [22]. The variation in seroprevalence of FMDV in two species may be due to the reason that both cows and buffaloes are at different feeding systems, therefore having different immune responses, different animal population density and have a different probability of receiving virus [23].

Results of our study indicated that age wise maximum seroprevalence of 20.51% was recorded in adult bovine population having more than 4 years of age followed by 16.05% and 14.15% in bovine population of 2-4 years and 1-2 years of age respectively which coincides with the results of another epidemiological investigation conducted in Pakistan that the rate of susceptibility was maximum (23.43%) in animals of > 4 years of age and lowest (13.33%) in animals of < 2 years age [19]. A study conducted in Iraq indicated that higher prevalence was reported in > 4 years of age animals followed 2 to 4 years old animals [24].

Our results indicated within bovine calves' population, the seroprevalence of maximum was noted in 7-12 months (14.86%) followed by 10.39% and 6.66% in 3-6 months and less than 3 months of age respectively. Higher prevalence in older animals may be due to repeated prolonged exposure of older bovine with field virus and unrestricted animal movement. Passive maternal immunity may provide protection to the calves against field virus [25].

Our data indicated that seroprevalence in cows was (18.38%), compared with from bulls (13.98%), whereas the buffalo population seroprevalence was (12.82%), compared with (9.24%) from ox, which match with the study arranged in three districts of Pakistan (Faisalabad, Chakwal and Khanewal) that seroprevalences of FMD were higher in females (24.20%) as compared to males of cattle (21.42%). Similar pattern was observed in buffalo population and noted that seroprevalence was 17.22% in buffaloes and 13.42% in ox [19]. A study conducted in Ethiopia indicated the similar trend of higher seroprevalence of FMD among cows (15.7%) than in bulls (8.27%) (Esayas*et al*., 2009). A Study arranged in Nigeria also indicated that prevalence of FMDV was higher in female cattle (72.8%) than males (50%) [26]. A study conducted in Iraq concluded that higher seroprevalence (82.5%) from cows was noted as compared to (17.5%) from bulls [24].

Lower prevalence in males may be associated due to the reason that male cows and buffaloes are provided with improved nourishment than female in investigated district. Females are reared for longer period to get production, which may lead to higher incidence of FMDV in female animal population [27].

Seroprevalence in vaccinated bovine was 17.66%, compared with 9.70% from non-vaccinated bovine which coincided with the results of a study conducted in Iraq indicated that prevalence was higher (41%) in vaccinated cattle compared to (39%) in non-vaccinated cattle [24]. Such findings may indicate that the natural challenge of FMDV in remained high in studied areas.

Molecular characterization of FMDV indicated that FMDV type O, OK359042**,** isolated from the cow of tehsil Ahmedpur East, showed 62% identity with previously reported FMD-type O from cattle of Janwala, Sargodha, Pakistan. Analysis of FMDV, OK359043, isolated from buffalo of tehsil Khairpur Tamewali showed 71% identity with FMD-type O which was previously isolated from cattle of Jhannala, Sargodha, Pakistan. The 5′UTR region of FMDV is a conserved region among all serotypes. The sequences indicated maximum homology to FMD-type O isolates, which is in accordance with the results of a molecular study that demonstrated the molecular characterization of 31 FMD-Type O viruses [28]. Another molecular epidemiological study conducted in southwest Niger indicated that testing of clinical samples revealed the maximum homology to the FMDV serotype O [29].

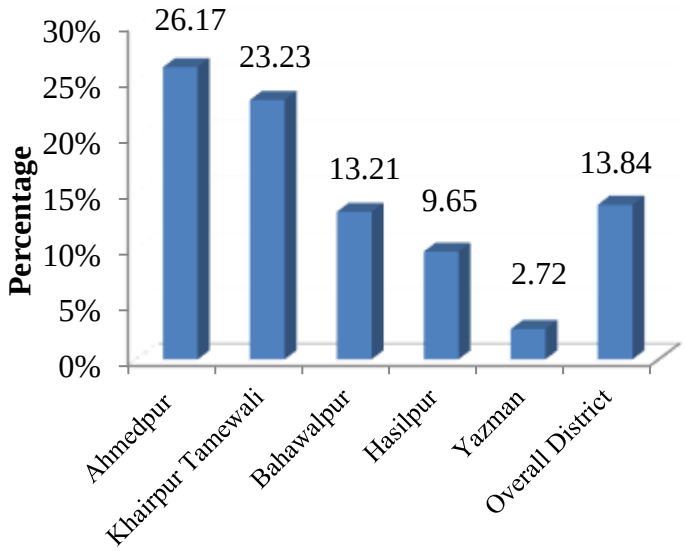

Fig 1: Tehsil wise distribution of seroprevalence of FMDV in Bovine population in district of Bahawalpur

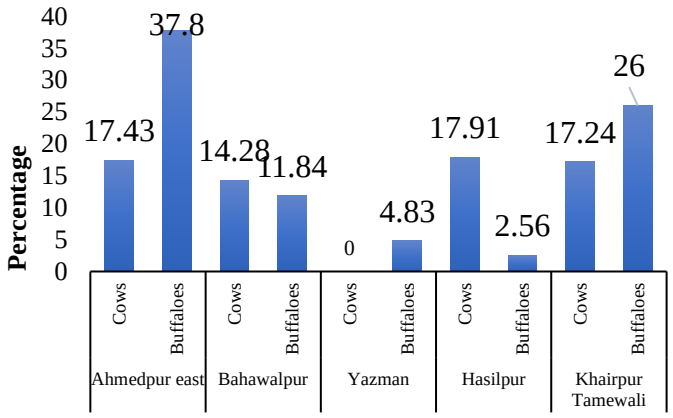

Fig 2: Tehsil wise distribution of comparative seroprevalence of FMD virus between cows and buffalo population in district Bahawalpur

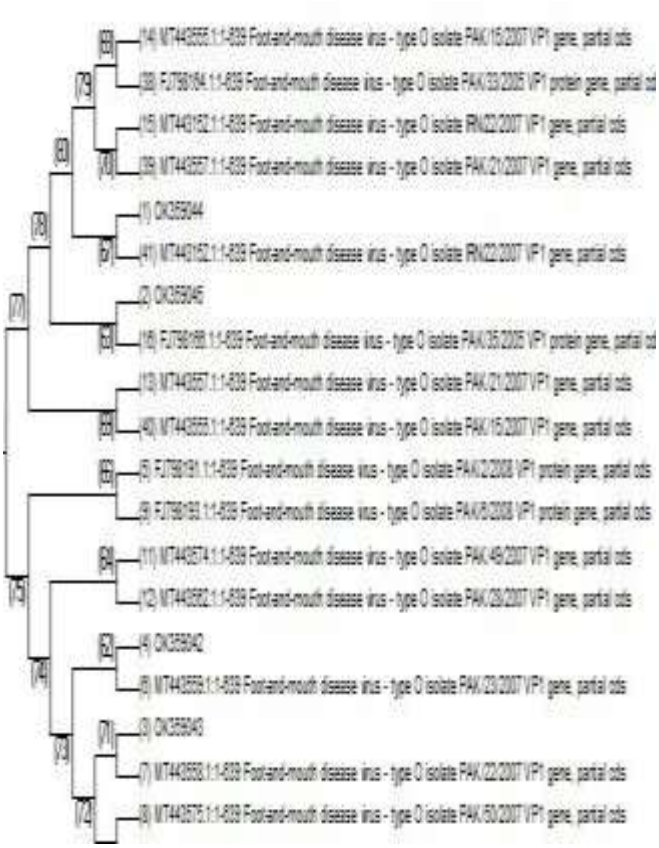

Fig 4: Phylogenetic analysis of FMDV conducted through partial sequence of VP1 gene indicated location of Accession No OK359042 and OK359043 isolates

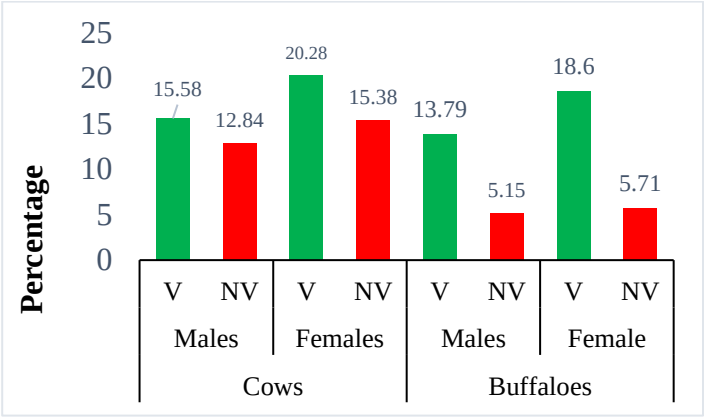

Fig 3: Results of gender wise and vaccination status wise seroprevalence of FMDV among cow and buffalo population in district of Bahawalpur.

Table 1: Age wise distribution of FMDV in Bovine population in district Bahawalpur

Groups of Species	Calves and adults	Age-wise	Total number of samples analyzed	Number of the positive sample	Prevalence (%)
Bovines	Bovine calves	< 3 Months	105	07	6.66
		3-6 Months	154	16	10.39
		7-12 Months	74	11	14.86
	Bovine adults	>1Y-2Y	226	32	14.15
		>2Y-4Y	162	26	16.05
		> 4 Years	117	24	20.51

Values comparable to each other statistically ($p>0.05$) are denoted as "*" sign

Table 2 Gender wise seroprevalence of FMDV distributed between cow and buffalo population in district Bahawalpur

Species	Gender	Total no of the samples tested	Number of the positive sample	% Prevalence
Cows	Male (Bulls)	186	26	13.98
	Female	234	43	18.38
Buffaloe	Male (Ox)	184	17	9.24
	Female	234	30	12.82

Values comparable to each other statistically ($p>0.05$) are denoted as "*" sign

IV. Conflict of Interest:

There is no conflict of interest among the authors, with the institution of authors, with previous publications, etc.

V. Ethics Approval:

The blood collection process was conducted in the context of routine veterinary medicine, with the consent of the animal owners to blood sampling and in line with institutional bioethics committee

VI. CONCLUSION

The study concluded the persistence of high antibodies titer in response to vaccination than non-vaccinated bovine population, specifically buffaloes of more than 4 years of age. Moreover, the incidence and severity of FMD were mitigated through regular vaccination program in the district of Bahawalpur. Regular vaccination program may be continued to maintain better protection of bovine population in the district of Bahawalpur.

.

Acknowledgment

We are very thankful for the financial support provided by the project for the enhancement of Foot and Mouth Disease control in Pakistan, FAO FMD Project (OSRO/PAK/801/JPN). Authors acknowledge the technical support of NVL/FAO/Laboratories, Islamabad and office of the ORIC, UAF. Authors also acknowledge Dr. Muhammad Afzal, FAO, Islamabad for his continuous guidance throughout the study.

References

[1] G. O. Young, "Synthetic structure of industrial plastics (Book style with paper title and editor)," in *Plastics*, 2nd ed. vol. 3, J. Peters, Ed. New York: McGraw-Hill, 1964, pp. 15–64.

[2] W.-K. Chen, *Linear Networks and Systems* (Book style). Belmont, CA: Wadsworth, 1993, pp. 123–135.

[3] H. Poor, *An Introduction to Signal Detection and Estimation*. New York: Springer-Verlag, 1985, ch. 4.

[4] B. Smith, "An approach to graphs of linear forms (Unpublished work style)," unpublished.

[5] E. H. Miller, "A note on reflector arrays (Periodical style—Accepted for publication)," *IEEE Trans. Antennas Propagat.*, to be published.

[6] J. Wang, "Fundamentals of erbium-doped fiber amplifiers arrays (Periodical style—Submitted for publication)," *IEEE J. Quantum Electron.*, submitted for publication.

Authors

First Author – **Muhammad Mohsin Abbas (Ph.D.),** Institute of Microbiology, University of Agriculture Faisalabad.
Second Author – **Sajjad Ur Rahman**, (Ph.D.), Institute of Microbiology, University of Agriculture Faisalabad
Third Author – **Muhammad Abubakar**, National Veterinary Laboratory, National Agriculture Research Council, Islamabad, Pakistan, address.

Correspondence Author – **Muhammad Mohsin Abbas (Ph.D.),** Institute of Microbiology, University of Agriculture Faisalabad,