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Short Communication

Foot and Mouth Disease and Its Differential Diagnosis with Special Perspective to Pakistan

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Abstract | Foot-and-Mouth Disease (FMD) is a highly contagious infection that affects cloven-hoofed animals, the viral infection is prevalent in Pakistan, and serotypes O, A, and Asia 1 are mainly responsible for repeated outbreaks. About 77% of the world's cattle population is affected by the disease, mostly in Asia, Africa and the Middle East. Because of its clinical similarities to other vesicular and viral diseases such as vesicular stomatitis, bluetongue, malignant catarrhal fever, peste des petits ruminants, and orf, an effective laboratory diagnosis is critical for proper treatment and prevention. This document emphasizes the limited utilization of modern diagnostic methods, such as real-time PCR, next-generation sequencing (NGS), and other molecular techniques, in routine diagnosis. Addressing the gaps in diagnostic technologies and monitoring infrastructure is critical to reduce the impact of FMD in Pakistan.

Keywords | Foot and mouth disease, Animal diseases, Differential diagnosis, Pakistan, Molecular diagnostics, Animal disease control

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INTRODUCTION

Livestock products make up about half of global agricultural production. Recent animal health emergencies have highlighted the vulnerabilities of the livestock industry to epidemic outbreaks caused by infectious diseases (Cartín-Rojas, 2012; Houe, 1995). Animal diseases, particularly transboundary animal diseases (TADs), continue to pose a significant threat to animal health, impacting both international trade and agriculture. Based on consultations with the FAO and WOAH, Foot and Mouth disease (FMD) is one of the most important of the TADs (Clemmons *et al*., 2021). FMD is a highly contagious viral disease affecting cattle, buffalo, sheep, goats, pigs, and more than 70 wildlife species (Abubakar *et al*., 2018). High morbidity, rapid transmission, and productivity losses are the main contributing factors to the financial losses in livestock sector. Pakistan is the fourth-largest milk producer in the world, and FMD virus has had a significant impact on the livestock (Abubakar *et al*., 2018). Pakistan is the fourth largest producer of milk in the world, and has severely affected by this disease. Abrupt decline in milk production and rise in the mortality among cattle is due to the endemic nature of FMD. There are 100 million sheep, goat species and 45 million cow, buffalo species reported in Pakistan. FMD, an endemic disease, is estimated to cause annual economic losses of approximately $692 million USD in Pakistan (Abubakar *et al*., 2022). FMD virus belongs to Aphthovirus genus and a member of Picornaviridae family, characterized by icosahedral symmetry (25-30) and having no envelop. virus is composed of a positive sense RNA of about 8400 nucleotides (Aslam and Alkheraije, 2023). There are seven antigenically unique serotypes of FMDV named as O, A, C, Asia-1, Southern African Territories (SAT)1, SAT 2, and SAT 3. The Asia 1 serotype has been reported in

Pakistan. Serotype O is the common among all and has led to huge financial losses while O, A and Asia-1 have resulted in outbreaks making the control difficult (Li *et al*., 2023). FMD symptoms can vary depending on the host species, virus serotype, and strain. Normally adult animals recover within 2-3 weeks of infection, however secondary bacterial infection may further prolong the recovery (Longjam *et al*., 2011). Fever, anorexia, and vesicle appearance on mucous membrane of mouth especially on tongue, dental pad, gum and lips (Rémond *et al*., 2002). However, FMD symptoms overlap with multiple diseases, namely swine vesicular disease (SVD), vesicular stomatitis (SV), vesicular exanthema, malignant catarrhal fever (MCF), rinderpest, peste des petits ruminants (PPR), popular stomatitis, orf, blue tongue (BT) and epizootic haemorrhagic disease (EHD). Therefore, it is necessary to confirm each reported case of FMD by laboratory testing. Furthermore, On the basis of clinical symptoms, viral stereotype cannot be determined which is crucial for the implementation of control and immunization strategies. On the other hand, collected samples pose significant impact on the success and rapid diagnosis.

Vesicular stomatitis (VS)

VS virus, a Vesiculovirus, belongs to Rhabdoviridae family and has 2 serotypes: New Jersey and Indiana. It is an acute disease that affects the horses but cattle and pigs can also be affected. The virus is spread mainly by biting flies and midges, several other factors including seasonal outbreaks, direct contacts with the lesion of affected animals and contaminated things are responsible for its spread. This virus causes the production of vesicles on the lips, foot, snout, and udder (Kumar *et al*., 2017). According to the Center for Food Security and Public Health (n.d.), VS also got noticed in Pakistan in 2009, however the disease was isolated to particular parts of the country. Viral isolation can be done via tissue culture, chicken eggs and embryonated mice. ELISA, complement fixation, and virus neutralization assays are used to detect viral antigen in vesicular fluid or at the base of the vesicle epithelium.

Blue tongue virus disease

In Pakistan, Bluetongue is one of the dangerous diseases that affect wide variety of animals, and a hurdle in the livestock growth. This Tick-born viral disease belongs to family Reoviridae. It can be spread by cullicoid midges and mechanically by the use of infected surgical tools and needles. In North- west region of Khyber Pakhtunkhwa, overall 48.4% prevalence rate was noted (Kumar *et al*., 2017). It is difficult to distinguish FMD from BT on early stages of the disease but lesions resulting from FMD are vesicular as well as erosive whilst BT lesions are oedematous, hemorrhagic and erosive (Williamson *et al*., 2008). Sheep are easily targeted, but cattle goats are less frequently impacted (Ahmed *et al*., 2019). The result of BTV can differ according to host species. some species exhibit mild to moderate signs whilst other may experience severe outcomes that result in death. For example, some sheep breads are highly susceptible and viremia may occur 4-6 days after infection (Rivera et al., 2021).

Serological tests are used for the diagnosis of BTV by detecting antibodies against it. Two regularly used procedures are ELISA and serum neutralization test (SNT). Commercially available indirect ELISAs are used to detect the antibodies against BTV. This method has proven to be reliable with a specificity of 96.5% and a sensitivity of 98.9% making it valuable for the surveillance. SNT is also highly sensitive and specific to identify the BTV serotypes but it is time consuming and labor intensive (Rojas et al., 2019).

Detecting the genetic material of the BTV allows for a swift and reliable diagnosis from patient samples. RT-PCR is commonly used to detect BTVRT-PCR amplifies specific RNA sections of the virus that code for structural or nonstructural proteins, enabling viral identification and serotype determination possible. Advanced molecular tests can be performed by using the LAMP method, showing rapid and highly specific results to increase diagnostic speed and accuracy (Mehmood *et al*., 2024).

Malignant catarrhal fever

MCF is deadly disease and lymphotropic and vasotropic infection, caused by agents belongs to MCFV complex group and Gammaherpesvirinae subfamily. MCF is being widely identified as a source of major economic losses in many ruminant species, such as cattle, bison, and deer, as well as a hazard to endangered species confined in mixed-species confinement. MCF mostly affects the lymphoid organs, elementary and respiratory tract. After a few days or weeks of the onset of clinical signs death may occur (Headley *et al*., 2020). Viral transmission occurs due to animal movements from place to place for trade as well religious festivals. MCF is a transboundary disease in Pakistan and other countries because goats and sheep can freely travel national boundaries. Since that, there have been no reports of MCF cases in Pakistan. PCR test was used to investigate the prevalence of OvHV-2 in cattle, buffalo, sheep and goats in the Islamabad and Rawalpindi regions of Pakistan, which was about 40%. There is no regular evaluation of this disease in Pakistan, therefore the impact may be overestimated (Riaz *et al*., 2021).

Peste des petits (PPR)

PPR (Peste des petits ruminants) is a serious condition that commonly affects sheep and goats. Small ruminant is essential for the livelihood in a country such as Pakistan, virus has a major financial consequence. Major symptoms are fever, anorexia, ocular and nasal discharges, pneumonia, mouth ulcer, profuse diarrhea, and death. In Pakistan, PPR was diagnosed via postmortem evaluation of signs and lesions. Traditional methods for the detection of PPRV are ELISAs and virus isolation but these are labor-intensive and less effective during the latent phase of infection. However, RT-PCR is gold standard method the detection of PPR diagnosis (Yousaf *et al.*, 2023).

Bovine viral diarrhea

BVD virus is a member of Flaviviridae family, comprises of enveloped, positive sense single stranded RNA (Khodakaram-Tafti and Farjanikish, 2017). BVD is responsible for serious reproductive outcomes, like embryonic loss, stillbirth and congenital abnormalities. Additionally, Fever, depression, lack of appetite, oral ulceration, laboured breathing and diarrhea are the main consequences in older cattle. Internal organ bleeding and death were also seen (Houe, 1995). In Pakistan, no vaccine plan is in place, probably due to the lack of a true picture of BVDV. The main hurdles to adopting the BVDV immunization program are the lack of information among cattle farmers and the cost effectiveness of vaccinations.

A small-scale serological investigation indicated 18.77% BVDV prevalence in southern Punjab, Pakistan (Mushtaq, 2023). Different Studies based on Pakistan mentioned the use of commercially available antibody and antigen captured ELISA kit to detect the antibodies against BVDV and antigen Furthermore PCR was used to validate the results (Ur-Rehman, 2020; Mushtaq, 2023).

Table 1: Comparative studies on differential diagnosis of FMD.

Disease	Title	Findings	Reference
Malignant catarrhal fever	"Molecular detection and characterization of ovine herpesvirus-2 using heminested PCR in Pakistan"	Study underscore the importance of surveillance and control strategies to reduce the viral effect on the ovine population.	(Riaz *et al.*, 2021)
Bovine Viral Diarrhea	"Sero-Epidemiology and Evaluation of First Self-Prepared Bovine Viral Diarrhea Virus Vaccine in Cattle of Punjab, Pakistan"	The study demonstrated the effectiveness of a self-prepared vaccine against BVD Virus in Punjab cattle, emphasizing its potential for improving herd immunity and disease management in the region.	(Mushtaq *et al.*, 2023)
	"Detection of bovine viral diarrhea virus prevalent in dairy herds of Punjab, Pakistan"	The research identified a significant prevalence of BVD virus in dairy herd of punjab region in Pakistan, pointing out the need control strategies to protect the productivity and health of livestock.	(Gohar *et al.*, 2013)
Bovine Viral Diarrhea	Determination of Sero-Prevalence and Associated Risk Factors of Bovine Viral Diarrhea Virus (Bvdv) in Bovine Population from Southern Punjab, Pakistan.	The study reported the seroprevalence of BVD Virus in the bovine population, along with identifying key risk factors that necessitate targeted interventions for disease management.	(Ur-Reman *et al.*, 2020)
	"Suitability of ear notch biopsy and serum pairs for detecting nature of bovine viral diarrhoea virus infection in dairy herds, Pakistan"	The study emphasizes the importance of accurate diagnostic techniques in managing BVDV in dairy herds, advocating for the use of biopsy and serum as effective tools in disease surveillance and control strategies in Pakistan's dairy sector	(Ahmad *et al.*, 2012)
Orf	"Seroprevalence of Contagious Ecthyma and Its Associated Risk Factors in Sheep and Goats of Punjab, Pakistan"	The study revealed a significant seroprevalence of Contagious Ecthyma in sheep and goats in Punjab region of Pakistan, along with various associated risk factors that warrant targeted control measures.	(Hussain *et al.*, 2023)
	"First isolation and genetic characterization of bovine herpesvirus 1 from cattle in Pakistan"	This study represents the first report of BoHV-1 isolation in Pakistan, providing critical insights into the epidemiology of this pathogen and its potential impact on livestock health.	(Rehman *et al.*, 2023)

Rinderpest

Rinderpest (cattle plague) is caused by virus which belongs to Paramyxo-viridae family, affects both animals as well as humans. In cattle and buffaloes, usual signs of rinderpest are nasal and ocular discharge. Death rate may increase due to ulcers in mouth and GIT, dysentery, protein loss, dehydration and immunity suppression (Roeder *et al*., 2013). Rinderpest is thought to have started in Asia and spread to other regions via cattle migration. World Organisation for Animal Health (WOAH) declared the Rinderpest as a second disease to be eradicated from the world after human small pox (Jain, 2023).

Orf

Orf virus belongs to family Poxviridae family and a prototype species of Parapoxvirus. It is distributed worldwide and characterized by severe skin condition in sheep and goat and also referred to as contagious ecthyma and scabbymouth.

In sheep, goats and other wild ruminants, ORFV causes proliferative and ulcerative skin lesions on gums, lips, muzzles, nostrils, teats and commissures. Humans may acquire lesions as a result of intimate contact with diseased animal. Molecular testing for particular ORFV strains have also been utilized successfully for diagnosis, however they are ineffective for herd screening (Chan *et al*., 2009). Molecular techniques such as PCR is known for its high specificity, sensitivity and reliability but they have limitations including need of advanced labs, trained personnel, high cost per test and long result time (Yang and Rothman, 2004). On the other hand, qualitative ELISA is economical and sensitive for the diagnosis of ORF viruses at herd level (Chan *et al*., 2009).

There is lack of enough research regarding this disease in Pakistan, so it is impossible to estimate the economic consequences. A confirming diagnostic method for diseases is the PCR test. This test is the best choice for differential diagnosis of this disease from other diseases having similar signs and symptoms (Hussain *et al*., 2023).

DISCUSSION

Foot and mouth disease, caused by a viral infection, affects ruminants, pigs, and at least 30 wild animal species. To address economic problems related to international commerce, it is crucial to prioritize illness management and early detection (Ullah *et al*., 2023). Although the disease has been effectively managed in many areas of the world via frequent vaccination of sensitive animals and killing of diseased animals, no country has been regarded safe due of the extremely infectious nature and rapid spread of the infection. For controlling the disease, early stage detection of outbreaks is necessary. This can be possible when diagnostic tools available are specific and sensitive and at the same time rapid (Rweyemamu*et al*., 1978). When it comes to herd screening, conventional methods of detecting FMDV infection are either not very sensitive or extremely specific. Since there is an urgent need for better diagnostic tests to identify viral infections, attention must be focused on creating quick, easy, noninvasive diagnostics that don't require costly lab equipment (Longjam *et al*., 2011).

In conclusion, addressing the gaps in diagnostic technologies and monitoring infrastructure is critical to reduce the impact of FMD in Pakistan. One of the main obstacles in dealing with FMD is the limited utilization of modern diagnostic methods, such as real-time PCR, next-generation sequencing (NGS), and other molecular techniques, in routine diagnosis Integrating modern diagnostic technologies with a comprehensive monitoring network can significantly improve the country's ability to control FMD, protect animal health, and promote agricultural growth. There is a lack of research based investigations in Pakistan, and in several cases outbreaks remain unreported.

Advancements not only direct the control of FMD but also provide the foundation for long lasting improvements in animal health and in production as well. Large production of meat, milk and other animal-derived products directly improve the livelihood of farmers. Furthermore, improved disease control reduces reliance on emergency responses, limiting the costs associated with disease outbreaks. These initiatives improve the position of Pakistan as an agricultural country.

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NOVELTY STATEMENT

To date, there is no study that specifically focuses on the diagnostic techniques lacking in Pakistan, particularly in relation to the above-mentioned animal diseases.

AUTHOR'S CONTRIBUTION

Authors were contributing in selection of topic, reviewing different papers and organizing in scientific way for publication.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abubakar M, Manzoor S, Ahmed A (2018). Interplay of foot and mouth disease virus with cell-mediated and humoral immunity of host. Rev. Med. Virol., 28(2): e1966. https://doi.org/10.1002/rmv.1966

Abubakar M, Syed Z, Manzoor S, Arshed MJ (2022). Deciphering molecular dynamics of foot and mouth disease virus (FMDV): A looming threat to Pakistan's dairy industry. Dairy, 3(1): 123-136. https://doi.org/10.3390/dairy3010010

Ahmed S, Shafee M, Razzaq A, Niazi MR, Wahid A, Khan G, Khan AH (2019). Status of animal health with emphasis of blue tongue virus disease in Kalat Region, Balochistan. Pak-Eur. J. Med. Life Sci., 2(2): 44-46. https://doi.org/10.31580/pjmls.v2i2.1144

Aslam M, Alkheraije KA (2023). The prevalence of foot-and-mouth disease in Asia. Front. Vet. Sci., 10: 1201578. https://doi.org/10.3389/fvets.2023.1201578

Cartín-Rojas A (2012). Transboundary animal diseases and international trade. Int. Trade Econ. Policy Perspect., 143: 166. https://doi.org/10.5772/48151

Center for Food Security and Public Health, (n.d.). *Vesicular stomatitis virus: Disease information for animal health professionals and veterinarians*. Iowa State University, College of Veterinary Medicine. Retrieved January 13, 2025, from https://www.cfsph.iastate.edu/DiseaseInfo/notes/VesicularStomatitisVirus.pdf

Chan KW, Yang CH, Lin JW, Wang HC, Lin FY, Kuo ST, Hsu WL (2009). Phylogenetic analysis of parapoxviruses and the C-terminal heterogeneity of viral ATPase proteins. Gene, 432(1-2): 44-53. https://doi.org/10.1016/j.gene.2008.10.029

Clemmons EA, Alfson KJ, Dutton III JW (2021). Transboundary animal diseases, an overview of 17 diseases with potential for global spread and serious consequences. Animals, 11(7): 2039. https://doi.org/10.3390/ani11072039

Fernández J, Agüero M, Romero L, Sánchez C, Belák S, Arias M, Sánchez-Vizcaíno JM (2008). Rapid and differential diagnosis of foot-and-mouth disease, swine vesicular disease, and vesicular stomatitis by a new multiplex RT-PCR assay. J. Virol. Methods, 147(2): 301-311. https://doi.org/10.1016/j.jviromet.2007.09.010

Headley SA, de Oliveira TES, Cunha CW (2020). A review of the epidemiological, clinical, and pathological aspects of malignant catarrhal fever in Brazil. Braz. J. Microbiol., 51: 1405-1432. https://doi.org/10.1007/s42770-020-00273-6

Houe H (1995). Epidemiology of bovine viral diarrhea virus. Vet. Clin. North Am. Food Anim. Pract., 11(3): 521-547. https://doi.org/10.1016/S0749-0720(15)30465-5

Hussain I, Aslam A, Rabbani M, Anjum A (2023). Seroprevalence of contagious ecthyma and its associated risk factors in sheep and goats of Punjab, Pakistan. Pak. J. Zool., 55(5). https://doi.org/10.17582/journal.pjz/20220331030325

Jain NR (2023). The first animal disease to be eradicated in the history of pandemic-rinderpest. mLAC J. Arts, Commerce Sci., 1(1): 2-8. https://doi.org/10.59415/mjacs.v1i1.77

Jamal SM, Belsham GJ (2013). Foot-and-mouth disease: Past, present and future. Vet. Res., 44: 1-14. https://doi.org/10.1186/1297-9716-44-116

Khodakaram-Tafti A, Farjanikish GH (2017). Persistent bovine viral diarrhea virus (BVDV) infection in cattle herds. Iran. J. Vet. Res., 18(3): 154.

Kumar R, Balena V, Patel SK (2017). Vesicular diseases in livestock with special reference to foot and mouth disease. J. Pure Appl. Microbiol., 11(1): 417-422. https://doi.org/10.22207/JPAM.11.1.54

Li F, Li Y, Ma J, Wu R, Zou X, Liu Y, Zhu Y (2023). Molecular evolution, diversity, and adaptation of foot-and-mouth disease virus serotype O in Asia. Front. Microbiol., 14: 1147652. https://doi.org/10.3389/fmicb.2023.1147652

Longjam N, Deb R, Sarmah AK, Tayo T, Awachat VB, Saxena VK (2011). A brief review on diagnosis of foot-and-mouth disease of livestock: Conventional to molecular tools. Vet. Med. Int., 2011(1): 905768. https://doi.org/10.4061/2011/905768

Mehmood F, Dastgir S, Ayaz MM, Naz M, Mujahid U, Ghaffar MJ, Naseem L (2024). Understanding and managing emerging and re-emerging viral diseases of animals. Complement. Altern. Med. Non–Convent., pp. 59. https://doi.org/10.47278/book.CAM/2024.149

Mushtaq MH (2023). Sero-epidemiology and evaluation of first self-prepared bovine viral diarrhea virus vaccine in cattle of Punjab, Pakistan.

Rehman HU, Rabbani M, Ghafoor A, Riaz A, Awan FN, Raza S (2020). First isolation and genetic characterization of bovine herpesvirus 1 from cattle in Pakistan. Pak. Vet. J., 41: 163-165. https://doi.org/10.29261/pakvetj/2020.092

Rémond M, Kaiser C, Lebreton F (2002). Diagnosis and screening of foot-and-mouth disease. Comparat. Immunol. Microbiol. Infect. Dis., 25(5-6): 309-320. https://doi.org/10.1016/S0147-9571(02)00028-0

Riaz A, Dry I, Dalziel R, Rehman SU, Shah MA, Akhtar HMN, Baig R (2021). Molecular detection and characterization of ovine herpesvirus-2 using heminested PCR in Pakistan. J. Vet. Sci., 22(4). https://doi.org/10.4142/jvs.2021.22.e51

Rivera, N. A., Varga, C., Ruder, M. G., Dorak, S. J., Roca, A. L., Novakofski, J. E., Mateus-Pinilla, N. E. (2021). Bluetongue and epizootic hemorrhagic disease in the United States of America at the wildlife–livestock interface. Pathogens, 10(8), 915.

Roeder P, Mariner J, Kock R (2013). Rinderpest: The veterinary perspective on eradication. Philos. Trans. R. Soc. B Biol. Sci., 368(1623): 20120139. https://doi.org/10.1098/rstb.2012.0139

Rojas, J. M., Rodríguez-Martín, D., Martín, V., & Sevilla, N. (2019). Diagnosing bluetongue virus in domestic ruminants: current perspectives. Veterinary Medicine: Research and Reports, 17-27.

Rweyemamu MM, Booth JC, Head M, Pay TW (1978). Microneutralization tests for serological typing and subtyping of foot-and-mouth disease virus strains. Epidemiol. Infect., 81(1): 107-123. https://doi.org/10.1017/S002217240005381X

Teifke JP, Breithaupt A, Haas B (2012). Foot-and-mouth disease and its differential diagnoses. Tierarztliche Praxis. Ausgabe G, Grosstiere/Nutztiere, 40(4): 225-237. https://doi.org/10.1055/s-0038-1623119

Ullah M, Li Y, Munib K, Rahman HU, Zhang Z (2023). Sero-epidemiology and associated risk factors of foot-and-mouth disease (FMD) in the Northern Border regions of Pakistan. Vet. Sci., 10(5): 356. https://doi.org/10.3390/vetsci10050356

Ur-Rehman H (2020). Determination of sero-prevalence and associated risk factors of bovine viral diarrhea virus (Bvdv) in bovine population from Southern Punjab, Pakistan.

Williamson S, Woodger N, Darpel K (2008). Differential diagnosis of bluetongue in cattle and sheep. In Practice, 30(5): 242-251. https://doi.org/10.1136/inpract.30.5.242

Yang, S., Rothman, R.E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. The Lancet infectious diseases, 4(6), 337-348.

Yousaf A, Anwar DM, Ahmed M, Qaiser I, Rabbani RA, Berjang AS, Habib F (2023). Diagnosis and control of peste des petits (PPR) in small ruminants: A comprehensive review. Anim. Vet. Sci., 11(5): 111-127.