



Clinical Pattern and Therapeutic Approaches in Dairy Cattle Affected with Foot and Mouth Disease Virus (FMDV) Serotype O/2021-22 during an Outbreak in Kashmir, India

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ABSTRACT

Background: Due to the highly contagious nature of Foot and Mouth Disease (FMD) in ruminants, outbreaks require decisive action. Early detection and prompt treatment approaches are crucial to limit the severity of the disease and prevent its widespread transmission.

Methods: The study aimed to assess the clinical pattern, therapeutic approaches and recovery time in crossbred cattle affected by foot-and-mouth disease during the 2021-2022 outbreak in Kashmir Valley. The suspected cases were clinically evaluated and samples were collected. Polymerase chain reaction (PCR) was performed to confirm the FMD-positive cases. A therapeutic trial was conducted on 18 affected cattle, comparing treatment regimens: The first regimen with enrofloxacin, meloxicam and an immunomodulator; the second with gentamicin, meloxicam and an immunomodulator and the third group of cattle with an antibiotic (enrofloxacin) and supportive treatment without an immunomodulator. All cattle received boro-glycerine paste (2%) and potassium permanganate (1:1000) wash for lesions until recovery.

Result: Affected cattle exhibited typical signs of FMD, including fever, altered vital parameters and distinct lesions in the mouth and hooves. PCR confirmed the presence of FMDV in all samples, identifying FMD serotype O. Consistent clinical signs included salivation (100%) and mouth lesions (98.88%) in affected cattle. Therapeutic interventions led to significant ($p \leq 0.05$) improvements in vital parameters, with decreased respiration rate, heart rate and rectal temperature in treated cattle. Group I and II cattle achieved complete clinical recovery in an average of 16.17 and 12.5 days respectively, while Group III had average recovery times of 21 days. The study demonstrated that treatment regimens including antibiotics and immunomodulators showed significantly better clinical recovery and healing results in an average number of days than those without immunomodulators.

Key words: Cattle, Clinical signs, FMD, Serotype O, Therapeutics.

INTRODUCTION

The livestock sector is pivotal in agriculture substantially contributing to the country's economy. However, recently, Asian countries have witnessed FMD outbreaks in livestock, raising concerns and causing substantial economic repercussions (Das *et al.*, 2023). Foot-and-mouth disease (FMD) is a highly contagious infectious disease affecting domestic animals globally (Audarya *et al.*, 2021). Foot-and-mouth disease (FMD) is widespread in India, including Kashmir, where it occurs in outbreak epidemics. Due to the contagious nature of the disease, there are worries about the virus strains spreading to regions or countries where it's not common (Pattnaik *et al.*, 2012). This nature of the disease poses a significant risk of causing substantial economic losses, particularly in highly productive animals, thereby causing major obstacles to the global trade of livestock products (Howlander *et al.*, 2004). The economic impact includes losses in milk and meat production, deaths among young animals, loss in wool quality, reproductive disorders and infertility in the recovered animals (Knight-Jones *et al.*, 2013). The foot and mouth disease virus belongs to the Aphthovirus genus of the Picornaviridae family (Jamal and Belsham, 2013). The Foot and Mouth disease virus is a small, non-enveloped,

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positive-sense, single-stranded RNA virus (Audarya *et al.*, 2021). There are seven serotypes *viz.*, A, O, C, Asia 1 and SAT -1, 2 and 3 of FMDV (Al-Salihi, 2019). Foot and Mouth Disease virus can spread from one animal to another by

inhalation of virus aerosols or by ingestion (Brito-Barbara *et al.*, 2016). The incubation period of the FMD virus varies between 2-7 days (Knight-Jones *et al.*, 2016). The Foot and Mouth disease is suspected based on clinical signs and pathognomic macroscopic lesions in the mouth, udder and feet. Due to multiple strains and sub-strains of FMDV, it is sometimes difficult to control the disease with a routine vaccination approach (Singh *et al.*, 2019). Although there is no standard treatment for FMD, recommendations include anti-inflammatory drugs, mild disinfectants and antibiotics to prevent secondary bacterial infections (Misk *et al.*, 2015). The treatment aims to restore normal appetite in affected animals quickly, reduce the severity of the infection, minimize discomfort and prevent complications and further production losses (Sharma, 2010). Given the importance of foot-and-mouth disease (FMD) in this region, the investigation focused on understanding the clinical picture and implementing immediate therapeutic interventions in animals affected by FMD during an outbreak in Kashmir.

MATERIALS AND METHODS

Study area and population

The study was conducted across outbreak sites between September 2021 and August 2022, in FMD-infected cattle from four districts of Jammu and Kashmir: Baramulla, Bandipora, Ganderbal and Srinagar.

Clinical study

The suspected cases selected for the study were subjected to comprehensive clinical examination as mentioned below and interpretations were framed based on descriptive clinical findings. Visible mucous membranes, vesicles present or absent and salivation (type/consistency) were recorded. Site/examinations of lesions in mouth-tongue, gums, lips, muzzle and foot/corony bands were recorded (Radostits *et al.*, 2010).

Recording of vital signs

The vital parameters (rectal temperature (°F), respiration / min, cardiac evaluation by auscultation/heart rate/min) were recorded in each affected animal before and after the treatment regimen.

Sampling

Samples were collected from FMD-infected animals (cattle). Samples included vesicular tissues from the lesions on the mouth, tongue, hard palate and feet/hooves (Fig 1).

The FMD was confirmed in all samples using PCR, which targeted the untranslated region (5'-UTR) of the FMDV as described by (Madi *et al.*, 2015) generating 328 bp product regardless of the serotype (Madi *et al.*, 2015). The sequence of primers used were 1F: (5'-GCCTGGTCT TTCCAGGTCT-3') and 1R (5'-CCAGTCCCCTTCTCAGATC-3') (Madi *et al.*, 2015). Another PCR was performed to identify the serotype in each FMDV-PCR positive sample using serotype-specific primers for serotypes O, A and Asia-1

(Reid *et al.*, 2000 and Knowles *et al.*, 2005). Furthermore, the plasmids were extracted and sequenced commercially by Bionivid Technology Pvt. Ltd, Bengaluru.

Therapeutic trial study

In a clinical trial, eighteen (18) cattle affected with foot-and-mouth disease virus (FMDV) were selected and divided into three groups, each comprising six animals. Efforts were made to ensure uniformity in age (average more than 2 years) and the severity of the lesions across the groups. Animals of the group I received intramuscular antibiotic (enrofloxacin @ 2.5mg/Kg b.wt.) twice a day for 5 days, meloxicam (0.5 mg/kg b.wt) once a day, along with an immunomodulator/stress modulator (oral Restobal @ 50mL for large animals) for 5 days. The group II cattle received an antibiotic intramuscular injection (gentamicin @ 2.5 mg/Kg b.wt.) for 5 days, meloxicam (0.5 mg/kg b.wt) once a day and a stress modulator (bolus tissue Aid @ 2 boli per day) for 5 days. On the other hand, group III FMD-affected cattle were given only antibiotics (enrofloxacin @ 2.5 mg/Kg b.wt) and supportive treatment (5 days) without any stress modulator. All affected cattle received emollient and antiseptics (2% Boro-glycerine paste and 1:1000 Potassium Permanganate-wash) for oral/mouth lesions



Fig 1: Collection of samples (tongue epithelium and dental pad).



Fig 2: Excessive salivation in FMD- affected animal.

until complete healing was achieved, as well as for the foot lesions. The therapeutic trial aimed to evaluate and compare three approaches by assessing clinical improvement, including vital recovery, healing of oral lesions and overall outcomes regarding the average number of days associated with each treatment.



Fig 3: Eroded and ulcer lesions present on dental pad and tongue in cattle.



Fig 4: Necrosis of inter-digital region in cattle.

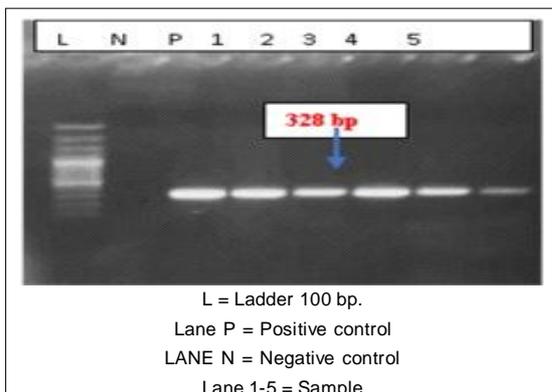


Fig 5: Detection of FMDV by ploymerase chain reaction.

Statistical analysis

The qualitative data were presented in terms of percentage and the quantitative data were analysed by One-way ANOVA. Post-hoc analysis was performed by Duncan's multiple range test (DMRT) using SPSS-20. The statistical differences were determined at the 5% level of significance (Snedecor and Cochran., 1994).

RESULTS AND DISCUSSION

Affected cattle exhibited characteristic signs of foot-and-mouth disease (FMD), with fever, altered vital parameters and distinct lesions in the mouth, muzzle, hoof and udder. In all affected cattle (100%), salivation was consistently observed and mouth lesions were seen in 98.88% of cases (Fig 2). Eroded and ulcerous lesions were found in the mouth, dental pad, tongue, foot/h hoof (Fig 3, 4) (Table 1). Other signs observed were anorexia and a reduction in milk yield in all affected animals. In this study all the samples subjected to a polymerase chain reaction (PCR) confirmed positive for foot-and-mouth disease virus (FMDV) (Fig 5). Subsequent PCR amplification and sequencing efforts validated the identification of serotype O in all FMDV-positive samples.

The mean rectal temperature ($^{\circ}\text{F}$), the mean respiration rate (per minute) and the mean heart rate (per minute) recorded in infected cattle and post-treated cattle (5-day interval) are detailed in Table 2. Notably, a decrease in mean rectal temperature was evident in all treatment groups (100.38 ± 0.04 , 101.24 ± 0.02 and 101.97 ± 0.05). Also, there was a significantly ($p \leq 0.05$) higher respiration rate (40.43 ± 2.18 , 43.00 ± 2.46 and $42.19 \pm 1.82/\text{min}$) observed in affected cattle compared to the post-treated cattle. There was a significant ($p \leq 0.05$) decrease in the recorded heart rate (per minute) in post-treated cattle (61.42 ± 0.14 , 61.51 ± 0.18 and 61.42 ± 0.14) than in affected cattle. Before administering the treatment regimen, all animals across the three groups experienced an increase in body temperature, respiratory rate and heart rate, with no significant differences observed between the groups (Table 2).

Therapeutic trials were conducted on clinically affected cattle and the results for different treatment regimens are presented in Table 3. In group I cattle (treated with enrofloxacin, meloxicam and restobal), the complete clinical recovery based on the healing of oral lesions and complete return of appetite was recorded at 16.17 ± 1.14 average number of days. However, signs of recovery of vital parameters were evident from the 5th day in four animals and on the 7th day in two animals. Likewise, in Group II, animals treated with the regimen (antibiotic-Gentamicin, antipyretic) and a stress modulator (tissue

Table 1: Clinical Lesions presented in FMD-affected cattle.

Lesions	Salivation	Mouth lesions	hoof/udder lesions
Percentage (%) of affected animals	100.00	98.88	3.74

aid), complete clinical recovery was seen at 12.5 ± 0.76 average number of days. In group III, animals treated with the regimen (antibiotic-enrofloxacin, antipyretic) without an immunomodulator, complete recovery was observed at 21.00 ± 1.06 average numbers of days. Therapeutic trials on clinical cases demonstrated significantly ($P \leq 0.05$) better results in terms of the number of days taken for the resolution of clinical signs and precisely healing of oral lesions and return of complete appetite in animals treated with antibiotics and antistressors/immunomodulators compared to those treated with therapeutic regimens only (Fig 6).

A prompt response to an FMD outbreak not only limits the direct effects of the disease but also plays a vital role in protecting unaffected herds by preventing the virus from establishing itself in new populations (Grubman and Baxt, 2004). Additionally, early intervention can help to avoid long-term complications that may arise from delayed treatment, such as chronic infections and reduced productivity in affected animals (Windsor *et al.*, 2020). In addition to the hallmark clinical features of high fever, excessive salivation, oral lesions and lameness, severe chronic cases often

exhibit additional complications such as sore feet, as highlighted by Kandel *et al.* (2018) and Muthukrishnan *et al.* (2020). Elevated temperatures, congested interdigital skin and fresh lesions on the tongue, mouth and feet in FMD-affected animals have also been reported in studies by Parida *et al.* (2008) and El-Bayoumy *et al.* (2014). Consistent with these observations, 80% of confirmed clinical outbreaks in India are attributed to serotype O, which aligns with our findings (Subramaniam *et al.*, 2012; Baro *et al.*, 2019; Audarya, 2021). Our results are corroborated by Rhaymah *et al.* (2010), Lotfollahzadeh *et al.* (2012) and Alagmy *et al.* (2022), who also reported a significant increase in rectal temperature, respiration rate and heart rates in FMD-infected animals compared to control/ treated groups. There is no specific antiviral treatment for FMD, making antibiotics and supportive care essential to manage symptoms and prevent secondary bacterial infections (Munazza and Khalid, 2023). The increase in body vitals especially temperature/inflammatory response is likely due to the release of endogenous pyrogens, such as interleukins and tumour necrosis factor, in response to antigens (Mariappan *et al.*, 2012). Kandel *et al.* (2018) observed that cattle affected by FMD when presented with high fever, responded well to meloxicam administered intramuscularly at a dose of 0.5 mg/kg body weight. Tufani (2013) found that FMD-affected animals treated with gentamicin and supportive measures exhibited a higher recovery rate (91.30%) than those treated with enrofloxacin. Sivajothi *et al.* (2018) and Bhamare *et al.* (2022) reported that oral administration of Restobal-herbal product, acts as a potent immunomodulator and anti-stressor, helping to enhance the immune response. Similarly, tissue Aid, a blend of proteolytic enzymes derived from plant, fungal and bacterial sources, helps accelerate wound healing, reduce edema and other inflammatory conditions and enhance the immune response (Shah and Amini, 2017). The topical application of glycerine, boric acid and potassium



Fig 6: Healing of mouth lesions/erosions.

Table 2: Vital parameters in FMD-affected cattle: Pre-treatment and Post-treatment (Mean±SE).

Groups (n= 18)	Rectal temperature (°F)		Respiration rate /min		Heart rate (bpm)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
I	102.30±0.16 ^b	100.38±0.04 ^a	40.43±2.18 ^b	21.36±0.26 ^a	75.04±2.01 ^b	61.21±0.23 ^a
II	103.26±0.16 ^b	101.24±0.02 ^a	43.00±2.46 ^b	22.28±0.18 ^a	79.00±2.02 ^b	61.51±0.18 ^a
III	102.30±0.13 ^b	101.97±0.05 ^b	42.19±1.82 ^b	21.99±0.15 ^a	77.75±1.53 ^b	61.42±0.14 ^a

Values with dissimilar superscripts differ significantly ($P \leq 0.05$).

Table 3: Therapeutic study in clinically FMD-affected cattle.

Treatment group	Number of cattle		Average no. of days taken for recovery Mean±SE
	Treated	Complete recovery	
Group I (n=6)	6	6	16.17±1.14
Group II (n=6)	6	6	12.5±0.76
Group III (n=6)	6	6	21.00±1.06

permanganate solution to lesions has been shown to provide a soothing emollient effect with specific antiseptic properties. Moreover, it has been observed to reduce excessive salivation, contributing to overall symptom relief (Pawar *et al.*, 2010). In clinical cases, antibiotics have proven vital in treating secondary bacterial infections, particularly in animals with severe mouth and hoof lesions (Radostits *et al.*, 2010). The findings of this study indicate that animals receiving a combination of antibiotics and immunomodulators demonstrated a marked improvement in clinical severity, with complete healing of lesions observed within a few weeks. In addition to antibiotics, a comprehensive treatment plan included using mild antiseptics, disinfectants and anti-inflammatory drugs, which helped mitigate the severity of the disease. This approach effectively addresses the immediate bacterial challenges while promoting faster recovery in FMD-affected animals.

CONCLUSION

The study confirmed the presence of FMDV serotype O in all affected cattle, which exhibited consistent clinical signs, including excessive salivation and lesions in the mouth and hooves, characteristic of FMD. The therapeutic trials demonstrated that cattle administered with regimens combining antibiotics and immunomodulators showed faster and more complete recovery compared to those that did not receive immunomodulators/stress modulators. This underscores the critical role of tailored treatment strategies in managing FMD, highlighting the need for early detection, appropriate therapeutic interventions and the inclusion of immunomodulatory/stress modulator agents to effectively reduce the impact of the disease and limit its spread during outbreaks.

Conflict of interest

All authors declare that they have no conflict of interest.

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