Foot-and-Mouth Disease in Oman (2018-2023): Seroprevalence and Associated Risk Factors

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Abstract

Introduction: Foot-and-Mouth Disease (FMD) is endemic to the Sultanate of Oman and is characterized by recurring outbreaks that generally occur during the winter and spring seasons.

Objective: This study aimed to investigate the seroprevalence of FMD in domestic livestock and identify the potential risk factors associated with the disease.

Method: A total of 315 serum samples were collected from cattle (n=172), sheep (n=88) and goats (n=55) across ten governorates in Oman and tested for Non-Structural Proteins (NSP) using the 3ABC Enzyme-Linked Immuno Sorbent Assay (ELISA). Univariate and binary logistic regression analyses were conducted to assess the relationship between Foot-and-Mouth Disease Virus (FMDV) seropositivity and hypothesized risk factors, including age, sex, animal species and vaccination status.

Results: The overall seroprevalence of FMDV NSP was 43.5%, with the highest rate recorded in south governorate (59.7%), followed by the northern (44.6%) and interior governorates (34.9%). Age (p-value=0.001) and animal species p-value=0.001) were significantly associated with FMDV NSP seropositivity. Animals above 3 years of age exhibited the highest positivity (61.2%), followed by those aged 1 years-3 years (31.6%) and those aged <1 year (25%). Old livestock (above 3 years) were more likely to be seropositive for FMD than younger counterparts (1 year) (OR=4.061, p-value=0.047, CI: 1.016-16.227). Cattle demonstrated a higher likelihood of seropositivity than sheep or goats ($\chi2$=19.09, p-value=0.001). They were 2.763 times more likely to be infected than sheep (OR=2.763, p-value=0.006, CI: 1.345-5.676).

Conclusion: These findings underscore the importance of implementing effective quarantine control measures to minimize the risk of transboundary transmission of FMD associated with the importation of livestock from other endemic countries. Future studies should focus on the post-vaccination responses to field isolates.

Keywords: Seroprevalence • FMDV • Livestock • Risk factors • Oman

Introduction

The livestock industry is of significant socioeconomic importance, particularly for small-scale animal farmers. Despite their significance, livestock production faces various challenges, one of which is the threat of transboundary animal diseases. Foot-and-Mouth Disease (FMD) is a highly contagious and acute viral disease that affects over 70 species of cloven-hoofed animals, including both domestic and wild animals, belonging to the order Artiodactyla [1]. The disease is characterized by a high morbidity rate of 100% and mortality rates ranging from 2%-4% in adults to over 90% in young animals due to a condition known as multifocal myocarditis necrosis [2,3]. From an economic standpoint, FMD is considered one of the most devastating animal diseases globally, resulting in direct losses owing to decreased animal productivity, mortality and abortions. Indirect economic losses stem from impeding the international trade of livestock and their products,

as well as the costs associated with control and eradication efforts. The FMD status of a country is an important factor in determining its access to the global livestock market [4,5].

Foot-and-Mouth Disease Virus (FMDV) is a small, non-enveloped RNA virus belonging to the *Picornaviridae* family and genus *Apthovirus*. The virus has a positive-sense single-stranded RNA genome of 8400 nucleotides, enclosed within an icosahedral capsid composed of four Viral Capsid Proteins (VP) [6]. FMDV is known to have seven distinct serotypes (O, A, Asia1, C, South Africa Territories (SAT)1, SAT2 and SAT3), which are further subdivided into topotypes, lineages and sublineages based on geographical regional occurrences and phylogenetic analysis of the VP1 coding region [7,8]. Antibodies against both Structural Proteins (SP) and Non-Structural Proteins (NSP) are produced in FMD-infected animals. Circulating antibodies can be detected 3 days-5 days after the first appearance of clinical signs [9]. In contrast, vaccinated animals are expected to produce antibodies only against viral structural proteins [10,11].

FMD is endemic in many countries, particularly Asia and Africa and is categorized into seven endemic pools that share relatively similar virus genotypes. Serotypes O and A are the most common in endemic countries, while serotype C has not been reported since 2004 in Kenya and Brazil [12]. The Asia1 serotype is mainly confined to Asia with low incidence and Serotypes SAT 1-3 are primarily detected in Africa, with occasional incursions reported in the Middle East [13].

Despite the implementation of vaccination programs, effective disease control is challenging due to factors such as the spontaneous evolution of multiple genotypes and the development of an asymptomatic carrier state in some species [14]. Both individual- and herd-like risk factors contribute to the increased prevalence of FMDV. Among individual-level risk factors, age and sex are frequently identified as contributing factors, with a higher seroprevalence observed in adult animals and females. Cattle are more susceptible to FMDV infection than small ruminants and higher seroprevalence rates have been reported in imported animals compared to local breeds [15-18]. At the herd level, farming management systems that involve mixing of different animal species are often associated with increased FMDV seroprevalence [15].

In the Sultanate of Oman, FMDV is endemic, with three primary circulating serotypes (O, A and Asia1) identified [19,20]. The first isolation of FMDV type O dates to the mid-twentieth century from clinically infected cattle in the Dhofar Governorate. In 1978, FMDV serotypes O, A and Asia1 were isolated from clinically and sub clinically infected animals in various regions [19]. However, samples from vaccinated cattle sent to the WRLFMD indicated infection with serotype SAT2 of topotype VII and Alx-12 lineage [21]. The overall seroprevalence of FMD in cattle in the Dhofar region was 52.1%, with 77.7% of the seropositivity being attributed to serotype O and 22.3% to serotype A [22]. A cross-sectional study conducted between 2015 and 2017 showed an overall herd-level seroprevalence of 41.4% for NSP. The seroprevalence rates among cattle, sheep and goats were 55.2%, 38.2% and 37.7%, respectively. Older livestock were more likely to be seropositive (odds ratio (OR) 3.47) and cattle were more likely to be seropositive than sheep or goats (OR 1.88). Female animals (OR 1.27) had increased odds of experiencing an FMD outbreak compared to male

animals. Moreover, vaccinated livestock (OR 5.98) were more likely to be seropositive than non-vaccinated animals [15]. This study aimed to determine the seroprevalence of antibodies against FMDV NSP and assess the potential risk factors associated with seroprevalence in Oman from 2018 to 2023.

Materials and Methods

Study design and sampling on influenza

This study was conducted from 2018 to 2023 to investigate natural infections caused by antibodies against FMDV NSPs. Additionally, the research aimed to evaluate the potential risk factors associated with FMDV NSP infection. A total of 315 serum samples were collected from FMD outbreaks with 172 from cattle, 88 from sheep and 55 from goats. The samples were collected from 109 livestock farms in ten governorates: Musandam (n=20), Muscat (n=5), Ad Dakhiliyah (n=38), Al Batinah North (n=77), Al Batinah South (n=22), Ash Sharqiyah North (n=26), Ash Sharqiyah South (n=21), Ad Dhahirah (n=27), Al Buraimi (n=17) and Dhofar (n=62). This study encompassed the collection of samples across multiple years, specifically in 2018 (n=36), 2019 (n=8), 2020 (n=98), 2021 (n=57), 2022 (n=79) and 2023 (n=37). Serum samples were collected in coagulant collection serum tubes, comprising 5 ml-10 ml of blood drawn from the jugular vein. The samples were transported in a cold chain to the Central Laboratory for Animal Health (Ministry of Agriculture, Fisheries and Water Resources, Oman) and stored at 4°C. The samples were then centrifuged and the sera were separated into cryogenic vials and stored at -80°C until further analysis. Biodata, including age, sex and vaccination status, were collected for each sampled animals to assess their association with FMD seropositivity.

Detection of non-structural protein antibodies against foot-and-mouth disease virus

Sera were screened for antibodies against FMDV non-structural 3ABC proteins using the PrioCHECK® (Thermo Fisher Scientific) FMDV NS blocking ELISA test. The assay was performed according to the manufacturer's instructions. The sensitivity and specificity of the ELISA were determined to be 86%-100% and 87%-99.5%, respectively. The Optical Densities (ODs) of the samples, negative controls, positive controls and internal positive controls were measured at 450 nm using a Multiskan™ FC Microplate Photometer Reader. The mean OD_{450} of the negative controls and the Percentage of Inhibition (PI) of the tested sera were calculated using the following formula: $PI=100- (OD_{450}$ test sample/ OD_{450} max) × 100. The test validity was confirmed by obtaining a mean OD_{450} of the negative control (ODmax) >1.000, mean percentage inhibition of the weak positive control >50% and mean percentage inhibition of the positive control <70%. Samples were considered negative if the PI was <45%, positive if ≥ 50% and doubtful if the PI ranged between 45% and 50%.

Data analysis

A formal analysis of the data was conducted, including the percentage and frequency of FMDV cases and their associated risk factors, This analysis was performed using SPSS (IBM SPSS Statistics 20.0), with a confidence level set at 95% and a level of statistical significance set at p<0.05. Univariate analysis was conducted using Pearson's chi-square test to determine the relationship between FMDV seropositivity and potential risk factors, including age, sex, species

and vaccination status. Additionally, binary logistic regression was conducted to calculate the odds ratio for age and species based on the significance level obtained from the univariate analysis.

Results

The study found that out of the 315 samples tested, 137 (43.5 %) were positive for NSP antibodies, which accounts for 43.5%. At the herd level, 60 (55.04%) of the sampled livestock farms were found seropositive for FMDV NSP. The highest seroprevalence was observed in South governorate (59.7%), followed by Northern (44.6%) and Interior governorates (34.9%) (Table 1). The analysis revealed varying seroprevalence rates across the sampled years, with the highest prevalence observed in 2023 (62.16%), followed by 2021 (54.39%), 2020 (43.88%), 2022 (40.51%) and 2018 (27.78%). Notably, no FMDV seropositivity was detected in the samples collected in 2019. To examine the relationship between seropositivity and potential risk factors such as age, sex, animal species and vaccination status, univariable and binary logistic regression analyses were utilized.

Analysis of risk factors for the prevalence of FMDV in the clinically infected population revealed that the sex of animals ($\chi2$=0.14, p-value=0.712) and vaccination status ($\chi2$=2.92, p-value=0.088) were not significantly associated with seropositivity (p-value>0.05). In contrast, age ($\chi2$=28.20, p-value=0.001) and animal species ($\chi2$=19.09, p-value=0.001) were significantly associated with FMDV-NSP seropositivity. The results demonstrated that FMDV infection increased with age, with the highest positivity observed in animals above 3 years of age (61.2%), followed by those between 1 years-3 years (31.6%) and those below and equal to 1 year (25%). Cattle were more likely to be seropositive than sheep or goats ($\chi2$=19.09, p=0.001). The seroprevalence of FMD was highest in cattle (54.1%), followed by goats (35.2%) and sheep (23.6%) (Table 2). The binary logistic regression analysis further revealed that old livestock, defined as those more than 3 years of age, were more likely to be seropositive for FMD compared to younger livestock of one year (OR=4.061, p-value=0.047, CI: 1.016-16.227). Additionally, the analysis showed that cattle were 2.763 times more likely to develop the disease than sheep (OR=2.763, p-value=0.006, CI: 1.345-5.676) (Table 3).

Table 1: Seropositivity (%) of FMDV across various locations in Oman.

Location	No. of tested samples	Seropositivity (%)
North governorates	124	39.4
Musandam	20	6.3
Al Batinah North	77	24.4
Al Batinah South	22	7
Muscat	5	1.6
Interior governorates	129	41
Ad Dakhiliyah	38	12.1
Ad Dhahirah	27	8.6
Al Buraimi	17	5.4
Ash Sharqiyah North	26	8.3
Ash Sharqiyah South	21	6.7
South governorate	62	19.7
Dhofar	62	19.7
Total	315	100

Table 2: Univariate analysis of risk factors associated with FMD seroprevalence.

Variables	Category	Pos./ Tested	Prevalence% (95% CI)	Chi-square	p-value
Age group	<1 years	3/15	25	$\chi2$=28.20	0.001
	1-3 years	55/174	31.6		
	>3 years	79/129	61.2		
Sex	Female	92/208	44.2	$\chi2$=0.14	0.712
	Male	45/107	42.1		
Animal species	Cattle	93/172	54.1	$\chi2$=19.09	0.001
	Goats	31/88	35.2		
	Sheep	13/55	23.6		

No	39/106	36.8		χ2=2.92	0.088	
Yes	98/209	46.9				

Table 3: Binary logistic regression model of risk factors and associated FMD prevalence with odds ratio.

Variables	β	SE β	p-value	Odds ratio	95% CI	
					lower	upper
Age						
<1 years	0	-	-	1	-	-
1-3 years	0.287	0.709	0.686	1.332	0.332	5.351
>3 years	1.401	0.707	0.047	4.061	1.016	16.227
Animal species						
Sheep	0	-	-	1	-	-
Cattle	1.016	0.367	0.006	2.763	1.345	5.676
Goats	0.694	0.398	0.081	2.002	0.917	4.368

Note: β: logistic coefficients, SE: standard error, CI: confidence interval

Discussion

This study presents analysis of the epidemiology of FMD in clinically infected animals (cattle, sheep and goats) across various governorates in the country, excluding Al Wusta, where FMD outbreaks were unreported. The desert environment could play a critical role in the absence of FMD cases in the region, where small ruminant herds are reared in scattered holding areas without any cattle population. This study focused on the seroprevalence of FMDV NSP in animals infected with FMDV. According to the results of this study, approximately 43.5% of the sampled animals tested positive for FMDV NSP.

The findings of this study align with retrospective data from studies conducted in Oman and other endemic countries, supporting the robustness of the reported prevalence rate [24-28]. Furthermore, similar results were observed in a study by Mohanty, et al. [27] who reported a seroprevalence of 41.1% in livestock populations that experienced FMDV outbreaks.

It is anticipated that the prevalence of FMD in animals with ongoing FMDV infections will be higher than that reported in cross-sectional studies. This discrepancy can be attributed to the timing of sample collection, which occurs before infected animals produce antibodies against NSP. Following FMDV infection, antibodies are generally detectable 3days-5 days after the onset of clinical signs with diminishing sensitivity over time [28]. The FMD PrioCHECK NSP ELISA used in our study was previously found to have a high specificity of 99.5% and 97.2% in vaccinated and non-vaccinated cattle, respectively and a diagnostic sensitivity of 97.2% [29]. However, its sensitivity decreased to 21.6%-28.4% in naturally infected and non-vaccinated animals [30].

In the categorization of animals by age, sex, species and vaccination status, there was no statistically significant difference in the infection rate between males and females. This finding is consistent with studies showing no significant association between animal sex and FMDV seropositivity [31]. However, contrasting results exist, with some studies reporting a higher FMDV incidence in female animals, which may be attributed to prolonged exposure related to production purposes [32].

According to our study, the seropositivity rate was highest in cattle (54.1%), followed by goats (35.2%) and sheep (23.6%). This aligns with previous studies showing similar seroprevalence rates of 55.2%, 38.2% and 37.7% in cattle, goats and sheep, respectively [15]. The higher infectiousness of cattle compared to sheep has been attributed to factors such as profuse salivation and nasal discharge, which contribute to environmental contamination and virus transmission. Furthermore, other researchers documented that both cattle and sheep show comparable susceptibility to FMD. Additionally, the higher transmission rate from cattle to cattle compared to the transmission rate from sheep to cattle indicates that cattle are more infectious than sheep. Consequently, cattle predominantly drive the transmission of FMDV in mixed populations comprising both sheep and cattel [33].

The seroprevalence of FMD was found to be significantly associated with the age of the animals, with adults being four times more likely to contract the infection than young animals. This finding aligns with prior research, which suggests that adults are more susceptible to FMD due to their frequent exposure to various virus serotypes and the persistence of FMDV antibodies over an extended period [34]. Additionally, commercial FMD vaccines are believed to produce antibodies only against SP, which was the rationale for using NSP ELISA as DIVA test. It has been speculated that substandard FMD vaccines may contain residual NSPs. Repeated vaccination with substandard FMD vaccines could potentially result in the development of NSP antibodies, making it difficult to differentiate between naturally infected animals and vaccinated animals [35]. These factors could account for the high proportion of NSP antibody-positive animals observed in the vaccinated livestock in this study.

Conclusion

This study showed high endemicity of FMD in the country, with an overall prevalence rate of 43.5% and evident differences among the various study areas. The multivariable logistic regression analysis identified age and animal species as statistically significant risk factors for FMD, demonstrating that cattle and animals over three years of age were more likely to contract the disease. The high endemicity of FMD in the country emphasizes the necessity of implementing strategic vaccination efforts alongside preventative control measures. Targeted vaccination, considering the prevalent serotypes and regional variations, can play a pivotal role in decreasing the incidence of FMD and minimizing its economic consequences on livestock industry.

Acknowledgement

Conflict of Interest

The authors declare that they have no conflicts of interest.

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