Pakistan J. Zool., pp 1-7, 2025. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20221225041249

Association of Single Nucleotide Polymorphisms, rs109075046 and rs136500299 in *ITGB6* Receptor Gene with the Susceptibility of Cattle Breed for Foot and Mouth Disease

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Article Information
Received 25 December 2022
Revised 06 May 2024
Accepted 12 May 2024
Available online 08 July 2025
(early access)

Authors' Contribution
FI: Supervised this study. MA, NM and MN: Collected blood and basic epidemiological data from the subjects. GZ, SA, SJ, NN, AK and AF: Extracted DNA from the blood samples and performed PCRs. FI and MA: Analyzed the data. FI: Prepared the manuscript. All the authors revised and approved the final version of the manuscript.

Key words
Foot and mouth disease, Cattle susceptibility, *ITGB6* receptor gene, rs109075046, rs136500299

ABSTRACT

Foot and mouth disease (FMD) is a prevalent pathological ailment affecting both domestic and wild animals with cloven hooves. It is caused by the Aphtho virus, commonly referred to as the foot and mouth disease virus (FMDV). The disease is characterized by fever, profuse salivation and vesicles on mouth and foot. *ITGB6* receptor gene is one of the major receptor components involved in host tropism of FMD virus in cattle. The aim of this study was to investigate whether there is a correlation existing between rs109075046 and rs136500299 in bovine *ITGB6* receptor gene with the susceptibility of Pakistani cattle breeds to FMD as there is no prior information available in literature. The study population encompassed a total of 225 FMD-negative (control) cattle and 47 FMD infected, at the time of sample collection or in past, (case) cattle, representative of three distinct breeds (Sahiwal, crossbred, and Holstein Friesian), all sourced from Southern Punjab in Pakistan. Tetra-primer ARMS PCR was used for genotyping single nucleotide polymorphism (SNP), rs109075046 (G 29 A) and rs136500299 (T 2145 C). Complete blood count analysis was also carried out for all subjects to report the effect of viral infection on blood composition. Chi-square analysis indicated a significant association with the TC genotype at rs136500299, whereas no substantial associations were observed among the studied genotypes at rs109075046 concerning cattle susceptibility to FMD. Holstein Friesian breed had higher incidence of FMD during present study followed by crossbred cattle. Notably, parameters such as white blood cells, lymphocytes, red blood cells, mean corpuscular hemoglobin, and mean cell hemoglobin demonstrated significant alterations in FMD cases when juxtaposed with the control group. This investigation contributes essential foundational data to establish a linkage between the genotypes rs109075046 and rs136500299 and the susceptibility of cattle to FMD in Pakistan. Such information holds promise for informed selective breeding strategies aimed at minimizing the occurrence of this disease.

INTRODUCTION

Foot and mouth disease (FMD) stands out as an exceptionally contagious and economically ruinous viral ailment affecting animals. It is caused by FMD virus (FMDV), a positive-sense, single stranded RNA virus that is member of the family Picornaviridae (genus *Aphtovirus*) (Ali *et al*., 2022). This virus infects several domesticated (e.g., cattle, Asian buffalo, sheep, goats, and swine) and wild (e.g., African buffalo and wild boar) cloven-hoofed

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0030-9923/2025/0001-0001 $ 9.00/0

animals. While the morbidity rates are notably elevated, the mortality rates among adult hosts generally remain low (Alexandersen and Mowat, 2005).

Following its entry in the host body, the virus replicates locally in primary replication sites such as the nasopharynx and lung during the pre-viremic phase (Arzt *et al*., 2011). The infection then spreads via the bloodstream (viremic phase) to secondary replication sites where the virus grows to high titers and causes typical blisters, erosions and ulcers at specific regions of the oral cavity, feet, and occasionally other sites (Faruk *et al*., 2021). Persistent infection can occur for long periods (30 days –5 years) with virus persisting at certain primary infection sites (e.g. nasopharynx) of infected animals (Zhang and Kitching 2001). Integrins are one of the important biologically active proteins responsible for FMDV host interactions. Functional integrins consist of two non-covalently bound transmembrane glycoprotein subunits viz. alpha and beta, a single transmembrane domain and a small, C-terminal cytoplasmic domain (Stewart and Nemerow, 2007). It is well known that many of the integrins can recognize RGD (Arginine–glycine-aspartic acid) motifs displayed on the extracellular matrix (ECM) proteins of FMD viral capsid, which after binding to the integrin may change the conformational structure (Singh *et al*., 2014). IntegrinaVb6 (ITGB6) is widely believed to be a high-affinity receptor for the FMD tropism among infected animals (Duque and Baxat, 2003).

FMD is endemic in Pakistan, marked by annual seasonal outbreaks across the entire nation. The implementation of enhanced biosecurity measures, along with the avoidance of introducing animals lacking a history of FMD vaccination, combined with regular FMD vaccination protocols, has been proposed as a significant strategy for diminishing the prevalence of this disease among livestock farms in Pakistan (Ali *et al*., 2022).

Seven FMDV pools have been identified from various parts of the World. Pakistan has the endemic pool 3 of FMDV, which is characterized by the presence of three serotypes: O, A, and Asia 1, co-circulating among cloven-hoofed animal populations (Naqvi *et al*., 2022). The local cattle and buffalo populations are generally at risk of getting FMD infection, as high morbidity is reported in these animals resulting in loss in meat and milk production and global trade restrictions on the export of dairy products from endemic areas (Shah *et al*., 2014; Jamal and Belsham, 2018).

It is well known that economic production in livestock breeding depends on raising healthy animals with high breeding value. An ideal approach is to generate animals that are genetically resistant to diseases (Karayel and Karsli, 2022; Rafiq *et al*., 2023; Sheraz *et al*., 2023). It has also been reported that polymorphisms in *ITGB6* gene can affect the susceptibility of animals for FMD (Singh *et al*., 2014, 2015). Until now, no investigation into the potential connection between *ITGB6* gene polymorphisms and cattle's susceptibility or resistance to FMD has surfaced in the context of Pakistan. The primary objective of the current research endeavor was to unveil the genetic variants present at the polymorphic sites, namely rs109075046 and rs136500299, within the *ITGB6* gene across three distinct cattle breeds native to Pakistan. This exploration is conducted through the utilization of the T-ARMS-PCR technique. Furthermore, an endeavor is undertaken to discern any potential correlation between the genotypes observed at the aforementioned polymorphic sites and the cattle's inherent vulnerability to FMDV infection.

MATERIALS AND METHODS

Blood samples and data collection

A grand total of 225 blood samples were gathered from seemingly robust cattle representing three distinct breeds, namely Sahiwal, crossbred, and Holstein Friesian. Additionally, 47 blood samples were acquired from cattle afflicted with or previously affected by FMD. These specimens were meticulously collected from farms chosen at random, situated within the pivotal livestock production zones of Southern Punjab, spanning the period from September 2021 to January 2022. Following the informed consent from the livestock owner, 3-5ml of blood sample was collected from the Jugular vein of each animal by using disposable syringes and preserved in blood collection tubes that contained 0.5 M EDTA solution that act as an anticoagulant. In order to collect epidemiological data associated with the FMD susceptibility in cattle, a questionnaire was also filled for each enrolled animal on the sampling site.

Complete blood count and DNA extraction

Complete blood count of all enrolled cattle was determined by using Automated Sysmex cell counter (Sysmex KX21, Japan). DNA extraction from blood samples was performed by an inorganic method as described by Parveen *et al*. (2021). The quality of the extracted DNA was assessed by measuring the optical density at 260/280 nm (O.R.I. Reinbeker, Hamburg) and by using submerged gel electrophoresis.

Amplification and genotyping at rs109075046 in the bovine ITGB6 receptor gene

A tetra primer amplification refractory mutation

system PCR (T-ARMS–PCR) was conducted to genotype rs109075046, SNP in the 5′untranslated region of the bovine *ITGB6* receptor gene associated with FMD susceptibility in cattle as previously described by Singh *et al*. (2014). The primers used in this T-ARMS PCR were outer forward 5′CTTTCCCTAGCCTGCCTTCT3′, outer reverse 5′GTTCAATCCCCATCCGTTT3′, inner forward 5′ATCATGTTGGAGTTGCTCATG3′ and inner reverse 5′GGTAAAGAAGAAAAGCTGTGATT3′ (Singh *et al*., 2014). A master mixture of 25 µl was prepared to genotype rs109075046 consist of 5 µl of template DNA,10X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM deoxyribonucleotide triphosphate (dNTPs), 2 U Taq DNA polymerase (Parstous, Iran) and 0.2 mM of each primer (12 pm). Amplification of DNA was carried out by using the thermo-profile of Singh *et al*. (2014) that included an initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30s, annealing at 57°C for 30s, elongation at 72°C for 30s and final extension was carried out at 72°C for 10 min.

Amplification and genotyping at rs136500299 in the bovine ITGB6 receptor gene

A tetra primer amplification refractory mutation system PCR (T-ARMS–PCR) was conducted to genotype rs136500299, SNP in the 5′untranslated region of the bovine *ITGB6* receptor gene associated with FMD susceptibility in cattle as previously described by Singh *et al*. (2015). The primers used in this T-ARMS PCR were: outer forward 5'TGCATAATAAAACTCAATAC3', outer reverse 5'ATTCATCAGCCACCTTTTTG3', inner forward 5′ CAGATTTCTCAAAGGATAGCTT 3′ and inner reverse 5′ CTTGCAGAGAACAGGAAACAG 3′ (Singh *et al*., 2015). A master mixture of 25 µl was prepared to genotype rs136500299 consist of 2.5ul of 10X reaction buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 1U of Taq polymerase (New England Biolabs Inc. USA), 12 pm of each primer and 5µl genomic DNA. Amplification of DNA was carried out by using the thermo-profile of Singh *et al*. (2015) that included initial denaturation at 94°C for 5 min, followed by 25 cycles of denaturation at 94°C for 30s, annealing at 58°C for 30s and elongation at 72°C for 30s. Final extension was carried out at 72°C for 5 min.

Statistical analysis

Data was analysed using Minitab version 18 (Mini Tab, USA). Significance level was set at $P < 0.05$. Data was expressed as mean ± standard error of mean (SEM). Chi square test was used to compare the genotype and allele frequency between the enrolled FMD positive and negative cattle breeds. Two sample t-test was applied to compare complete blood count between enrolled FMD positive and negative cattle breeds.

RESULTS

Genotyping at rs109075046 in ITGB6 receptor gene of analyzed cattle

Tetra Arms PCR based amplification of cattle DNA for SNP rs109075046 in 5′untranslated region of *ITGB6* receptor gene resulted in a 659 base pair fragment for outer primers. While primers specific for GG (wild) genotype and AA (mutant) genotype amplified products of 257 bp and 445 bp, respectively. Cattle having heterozygous genotype (GA) amplified both 257 and 445 bp bands.

When the genotype at rs109075046 in *ITGB6* receptor gene was compared among cattle negative for FMD, it was observed that GA (0.4) was the most common genotype followed by GG (0.38) and AA (0.2), respectively (Table I). Similar trend was also observed in cattle suffering from FMD enrolled during present study [GA (0.55) > GG (0.31) > AA (0.12)] (Table I). Chi square test analysis revealed that the genotype frequency varied non significantly ($P = 0.2$) when compared between FMD negative (control) and FMD positive cattle (case) (Table I).

Table I. Over all comparison of the genotype and allele frequency at SNP rs109075046 and rs136500299 in *ITGB6* receptor gene of cattle negative (control) or positive (case) for FMD enrolled from Southern Punjab. Chi square test was calculated to compare the genotype and allele frequency among the enrolled cattle breeds.

Analyzed SNP	Status	Genotype frequency			Total	P value	Allele frequency		P value
		AA	GG	GA			A	G	
rs109075046	Control	47(0.2)	87(0.38)	91(0.4)	225	0.2	185(0.41)	265(0.58)	0.9
	Case	6(0.12)	15(0.31)	26(0.55)	47		38(0.4)	56(0.59)	
	Status	**TT**	**CC**	**TC**	**Total**	**P value**	**T**	**C**	**P value**
rs136500299	Control	10(0.04)	151(0.67)	64(0.28)	225	0.03*	84(0.18)	366(0.81)	0.02*
	Case	2(0.04)	21(0.44)	24(0.51)	47		28(0.3)	66(0.7)	

P > 0.05 = Non significant; P < 0.05 = Least significant (*)

When the allelic frequency at rs109075046 in *ITGB6* receptor gene was compared among cattle negative for FMD it was observed that G allele (0.58) was most common followed by A allele (0.1) (Table I). Similar trend was observed in cattle suffering from FMD enrolled during present study [G (0.59) > A (0.4)] (Table I). Chi square test revealed that allelic frequency varied non significantly (P = 0.9) when compared between FMD negative (control) and FMD positive (case) (Table I).

When genotypic and allelic frequency at rs109075046 in *ITGB6* gene was compared between FMD negative (control) and positive (case) cattle belonging to different breeds enrolled in present study. Chi-square test revealed that genotypic frequency (P = 0.3) and allelic frequency (P = 0.1) varied non significantly between them (Table I).

Genotyping at rs136500299 in ITGB6 *receptor gene of analyzed cattle*

Tetra Arms PCR based amplification of cattle DNA for SNP rs136500299 in *ITGB6* receptor gene resulted in a 433 base pair fragment for outer primers. While primers specific for TT (wild) genotype and CC (mutant) genotype amplified products of 347 bp and 128 bp, respectively. Cattle having heterozygous genotype (TC) amplified both 128 bp and 347 bp bands.

When the genotype at rs136500299 in *ITGB6* receptor gene was compared among cattle negative for FMD, it was observed that CC (67%) was the most common genotype followed by TC (28%) and TT (4%), respectively. When rs136500299 was analyzed in cattle suffering from FMD enrolled during present study, it was observed that majority of cattle had heterozygous (TC) genotype (51%) followed by mutant (CC, 44%) and homozygous wild (TT, 4%) (Table I). Chi square test analysis revealed that the genotype frequency at rs136500299 varied significantly (P = 0.03) among case and FMD control with FMD negative animals had significantly higher mutant while FMD positive cattle had higher heterozygous genotype (Table I).

When the allelic frequency at rs136500299 in *ITGB6* receptor gene was compared among cattle negative for FMD it was observed that C allele (81%) was most common followed by T allele (18%) (Table I). A similar trend was observed in FMD positive cattle enrolled during present study (C allele 70%, T allele 30%) (Table I). Chi square test revealed that allelic frequency varied significantly with mutant allele (C) being more common than wild allele (T) (P = 0.02) when compared between control and case (Table I).

When genotypic and allelic frequency at rs136500299 in *ITGB6* gene was compared between FMD control and case cattle belonging to different breeds enrolled in present study. Chi-square test revealed that genotypic frequency (P = 0.9) and allelic frequency (P = 0.8) varied non significantly between them (Table II).

Table II. Breed wise comparison of genotype and allele frequency at SNP rs109075046 and rs136500299 in *ITGB6* receptor gene between FMD negative (control) and FMD positive (case) cattle enrolled from Southern Punjab during present study. Chi square test was calculated to compare the genotype and allele frequency among the enrolled cattle breeds.

SNP	Breeds	Genotype frequency									Allele frequency						
		Control				Case					Control			Case			
		AA	GG	GA	Total	AA	GG	GA	Total	P value	A	G	Total	A	G	Total	P value
rs109075046	Holstein Friesian	12 (0.16)	31 (0.41)	32 (0.42)	75	6 (0.21)	9 (0.32)	13 (0.46)	28	0.3	56 (0.37)	94 (0.62)	150	25 (0.4)	31 (0.5)	56	0.1
	Crossbred	18 (0.24)	27 (0.36)	30 (0.4)	75	0	6 (0.31)	13 (0.68)	19		66 (0.44)	84 (0.56)	150	13 (0.34)	25 (0.65)	38	
		Control				Case					Control			Case			
		TT	CC	TC	Total	TT	CC	TC	Total	P value	T	C	Total	T	C	Total	P value
rs136500299	Holstein Friesian	3 (0.04)	46 (0.61)	26 (0.35)	75	1 (0.04)	9 (0.39)	13 (0.56)	23	0.9	32 (0.21)	118 (0.48)	150	15 (0.32)	31 (0.67)	46	0.8
	Crossbred	3 (0.04)	51 (0.68)	21 (0.28)	75	1 (0.04)	12 (0.5)	11 (0.46)	24		27 (0.18)	123 (0.48)	150	13 (0.27)	35 (0.72)	48	

P > 0.05 = Non significant

Hematological parameters of FMD negative and positive cattle

In the current investigation, it was observed that exclusively Holstein Friesian and crossbreed cattle exhibited FMD infection. To elucidate the impact of FMD on blood counts, we conducted a comparative analysis of the CBC parameters between two distinct groups: FMD-positive (cases) and FMD-negative (controls) cattle breeds: Holstein Friesian case vs control and crossbred, case vs control. Comparison was not made for Sahiwal breed cattle as none of enrolled FMD infected cattle belonged to Sahiwal breed. Two sample t-test results revealed that control Holstein Friesian cattle had higher white blood cell count, lymphocytes and red blood cell count while they had lower mean corpuscular volume and mean corpuscular hemoglobin than control animals (Table II). FMD infected Crossbred cattle had higher white blood cell count, lymphocytes and red blood cell count while they had lower mean corpuscular volume and mean corpuscular hemoglobin concentration than control animals. All other studied parameters varied non significantly for both breed when compared between FMD positive and negative cattle ($P > 0.05$) (Table III).

DISCUSSION

FMD stands as one of the most formidable infectious diseases affecting livestock on a global scale. Its far-reaching impact results in substantial economic losses for farming communities and the dairy industry, particularly in low and middle-income countries. Pakistan, in particular, bears the brunt of these losses due to its heavy reliance on livestock, which constitutes a fundamental pillar of the agriculture-based economy (Di Nardo *et al*., 2021; Ranjan *et al*., 2016). The estimated production losses due to endemic FMD are between US$ 1.2 to 2.3 billion with over 50 million animals gets affected every year (Knight-Jones and Rushton, 2013). In some recent reports, incidence of susceptibility to FMD has been linked with the genotype of various genes from different countries across the globe (Singh *et al*., 2014, 2015) but from Pakistan, to the best of our knowledge, there is no information available in literature regarding the susceptible cattle breed or the cattle gene(s) the polymorphisms of whom can lead to development of FMD. In agricultural economy of developing countries, domestic cattle plays important role and its contribution goes beyond the direct production of meat and milk to skin, fiber, fertilizers and fuel production (Iqbal *et al*., 2019). In developing countries, cattle improvements largely depend on the selective breeding of the individual animals with superior phenotype (Meuwissen *et al*., 2016; Olschewsky and Hinrichs, 2021). By understanding the genetic basis of host resistance using advanced molecular tools, especially by identifying polymorphisms in the genes that determine the specificity of the immune response and play a rolein conferring resistance or susceptibility, will offer new alternatives in various disease control programs,

Table III. Hematological parameters of foot and mouth disease negative (control) and positive Holstein Friesian breed and crossbred (case) of cattle enrolled during present study. Data is expressed as mean ± standard error of mean. P-value indicates the results of two sample t-test calculated for each studied parameter.

Parameters (unit)	Holstein Friesian breed			Crossbred cattle		
	FMD –ive control	FMD +ive case	p-value	FMD –ive control	FMD +ive case	p-value
White blood cells (10^3/µl)	11.30±0.76	29.97±5.69	0.01**	10.09±0.66	36.62±3.57	P < 0.001***
Lymphocyte (%)	43.76±1.89	65.56±5.34	0.003**	38.40±2.17	57.58±4.69	0.001***
Red blood cells (10^6/µl)	5.37±0.14	6.95±0.66	0.04*	5.07±0.14	6.89±0.44	0.001***
Monocyte (%)	4.69±0.51	6.25±1.53	0.4	-	-	-
Hemoglobin (g/dl)	9.33±0.14	10.2±1.22	0.5	9.5±0.2	9.89±0.69	0.5
Hematocrit (%)	28.5±0.71	29.82±3.39	0.7	34.84±0.85	29.7±2.36	0.05*
Mean corpuscular volume (fl)	55.6±1.92	43.04±1.38	P < 0.001***	69.79±1.9	43.21±1.43	P < 0.001***
Mean corpuscular hemoglobin (pg)	19.82±0.7	14.51±0.5	P < 0.001***	26.10±0.78	14.54±0.41	P < 0.001***
Mean corpuscular hemoglobin concentration (g/dl)	35.33±0.41	34±0.68	0.1	33.75±0.37	33.67±0.48	0.8
Platelet (10^3/µl)	255.85±10.72	622.44±183.12	0.08	267.15±10.72	206.79±45.43	0.2

P > 0.05 = Non significant; P < 0.05 = Least significant (*); P < 0.01 = Significant (**); P < 0.001 = Highly significant (***)

especially for infectious diseases (Singh *et al*., 2015). Therefore, the objective of this study was to ascertain the genotype and allelic frequencies at rs109075046 and rs136500299 within the *ITGB6* receptor gene among cattle in Punjab, Pakistan, both those affected by FMD and those unaffected.

The bovine *ITGB6* (integrin beta 6) gene is located on chromosome 2 and consists of 16 exons (Singh *et al*., 2014). This gene encodes a protein that is a member of the integrin superfamily. Members of this family are adhesion receptors that function in signaling from the extracellular matrix to the cell (Karayel and Karsli, 2022). The 5′UTR region of bovine ITGB6 is around 145 bp in size and rs109075046 is a G > A change in this region. During the present study, although GA (heterozygous) was the most common genotype at rs109075046 in *ITGB6* receptor gene observed in both FMD positive (case) and FMD negative (control) cattle but analysis of our data revealed that this genotype was not associated with the susceptibility of FMD (Table I). There is only one previously published study on this topic, conducted by Singh *et al*. (2014), which examined 33 FMD-negative and 55 FMD-positive cattle from Andhra Pradesh, India. Their study reported that the GA genotype was the most prevalent among both case and control cattle. Although our findings did not achieve statistical significance as observed in the study of Singh *et al*. (2014), the overall distribution of genotypes was consistent between the cattle populations examined in both studies. Additionally, Singh *et al*. (2014) also highlighted a strong and highly significant relationship between the incidence of FMD and factors such as the season of calving, year of calving, and genotype.

During the present investigation, it was observed that genotype at rs 136500299 in *ITGB6* receptor gene varied significantly when compared between FMD positive (case) and FMD negative (control) cattle. Wild genotype "CC" was abundant in control while majority of cases exhibited "TC" at rs 136500299 (Table I). Our results are in agreement with the only study available in literature where Singh *et al*. (2015) had reported that genotype at rs 136500299 receptor gene varied significantly among FMD virus infected and uninfected cattle and majority of FMD positive cattle had "TC" genotype at studied SNP concluding that heterozygosity (TC) at rs 136500299 in ITGB6 receptor gene had strong association with FMD susceptibility among the enrolled Crossbred cattle.

It has been reported in the literature that FMD virus affects the cell count and serum biochemistry of the infected cattle resulting in development of anemia and pancreatic dysfunction (Gökçe *et al*., 2004; Barkakati *et al*., 2015). Hence, during present study, we have compared the complete blood count parameters between FMD positive and negative cattle (Holstein Friesian and Crossbred). We observed that white blood cells, lymphocyte, red blood cells, mean corpuscular volume and mean cell hemoglobin varied significantly when compared between FMD positive and negative cattle (Table III). In a recent study from Bangladesh Faruk *et al*. (2021) reported that red blood cell count and mean corpuscular volume varied significantly when compared between FMD negative and positive cattle. It was observed that red blood cell count decreased and mean corpuscular hemoglobin increased gradually as cattle passed from primary to advance stages of FMD. While an increase in red blood cells and decrease in mean corpuscular volume was observed in recovered and uninfected cattle. In another study from Assam in India, (Barkakati *et al*., 2015) reported a significant decrease of RBC count, total protein, albumin, blood urea nitrogen, calcium level in serum whereas a significant increase of blood glucose concentration in FMD affected cattle that were in recovery phase. In present study, we observed an increase in the red blood cells and decrease in mean corpuscular volume in FMD affected cattle indicating that enrolled cattle were in the recovery phase from FMD. Reduction in mean corpuscular hemoglobin concentration in our results can be due to some sort of anemia in cattle suffering from FMD. While high level of white blood cells and lymphocytes in FMD positive cattle indicates the condition of inflammation and infection (Alfaro *et al*., 2021).

CONCLUSION

In conclusion, for the first time from Pakistan, we are reporting the genotype at rs109075046 and rs136500299 in *ITGB6* receptor gene in three cattle breeds. The "TC" genotype at rs136500299 was found associated with the susceptibility of enrolled cattle to FMD, while none of the studied genotypes at rs109075046 were associated with susceptibility to FMD in enrolled cattle. We are also reporting that Holstein Friesian breed had higher incidence of FMD during present study followed by crossbred. It was observed that white blood cells, lymphocytes, red blood cells, mean corpuscular and mean cell hemoglobin were the complete blood count parameters that were significantly affected in FMD affected cattle. This study seeks to provide fundamental data that can serve as a foundation for establishing a correlation between rs109075046 and rs136500299 genotypes and cattle susceptibility to FMD in Pakistan. Such data can be instrumental in implementing genotyping-based selective breeding strategies for cattle, ultimately contributing to a reduction in disease incidence and the associated economic losses.

DECLARATIONS

Acknowledgement

Authors are grateful to livestock owners upon providing the blood and epidemiological data from their animals.

Funding

No specific research grant was available for this project.

Ethics approval

Ethical Research Committee of the Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan (Pakistan) approved all the animal handling procedures and laboratory protocols applied in this study via letter number IPAB/Ethics/59/2021.

Consent to participate

Informed consent was obtained from all the livestock owners before including their animals in this study.

Data availability statement

All the data generated during this study is presented in the manuscript.

Statement of conflicts of interest

The authors have declared no conflict of interest.

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