Research Article

Circulation of Predominantly Prevalent Serotypes of Foot and Mouth Disease Virus in Bovine Animals in Bahawalpur, Pakistan

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Abstract | Vaccination of the foot and mouth disease (FMD) virus needs the examination of the prevalent virus serotypes in the area. The current cross-sectional study was conducted in the Bahawalpur district of Punjab, Pakistan, with the purpose to determine the prevalence of FMD virus serotypes Asia 1, O and A in bovines. A total of 838 bovine sera samples were tested through Solid phase competitive enzyme-linked immunosorbent assay (SPC-ELISA), based on structural proteins at the National Veterinary Laboratory (NVL), Islamabad from 10 January 2021 to 09 January 2022. The overall bovine population was carrying maximum seroprevalence of serotype O (28.52%) followed by serotype Asia 1 (23.63%) and serotype A (13.6%) in district Bahawalpur. Majority of the bovine population (50.26%) was detected seropositive for type O of foot and mouth disease virus (FMDv) followed by serotype Asia 1 with (37.17%) in tehsil Ahmedpur East and type A with 21.83% prevalence in tehsil Bahawalpur. Moreover, it was emphasized that seropositive cases of type O (42.88%), Asia 1(36.69%) and type A (21.33%) were significantly higher in vaccinated bovine group as compared to non-vaccinated bovine with (12.93%), (9.45%) and (5.22%) respectively across the district. More specifically the cow population was carrying maximum seroprevalence of type O with (60.55%) compared to buffalo with (36.58%). It was concluded that serotype O was more prevalent in district Bahawalpur followed by serotype Asia 1 and serotype A and cow population was found more susceptible than buffalo.

Received | November 02, 2022; **Accepted** | January 12, 2023; **Published** | April 17, 2023
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Citation | Abbasm N.M., S. Rahman, M. Abubakar, M.I. Arshad and K. Ashfaq. 2023. Circulation of predominantly prevalent serotypes of foot and mouth disease virus in bovine animals in Bahawalpur, Pakistan. *Sarhad Journal of Agriculture,* 39(2): 308-315.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39.2.308.315
Keywords | Seroprevalence, FMD, FMDV, Serotypes, Vaccinated, Non-vaccinated, Anti-Structural Protein, structural protein, Monoclonal antibodies

Introduction

Foot and mouth disease (FMD) is a transboundary disease and economically it is an important disease of livestock in Pakistan (Qureshi *et al.*, 2022). Pakistan was considered at stage 2 in 2015 (Hussain *et al.*, 2019) and achieved stage 3 in 2021 of the Progressive Control Pathway in targeted areas of

the country objecting to decrease viral load through implementation of control strategy endorsed by the government (Lyons *et al*., 2021). Further development needs efforts to decrease viral transmission that may finally result in being awarded certified disease-free status by the World Organization for Animal Health. FMD is commonly seen in cattle, sheep, goats, pigs and domestic water buffalo, and in wild cloven-footed animals (Wong *et al*., 2020; Yar *et al*., 2021).

FMD has long-term imprints including loss of productivity and restriction in trade (Abubakar *et al*., 2022). FMD is an illness of cloven-hoofed animals and is caused by the FMD virus of the genus *Aphthovirus* (Sanaullah *et al*., 2019), 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 (Abubakar *et al*., 2012). Bahawalpur district comprises of five tehsils with 1.22 million heads of bovine and is located at southern border region of Pakistan. Most of the medium and small-scale farmers maintain their valuable animal resource as mainstay of their livelihood in the district of Bahawalpur. FMD virus has seven serotypes, out of which, only three serotypes including Asia 1, O, and A are prevalent in Pakistan (Medina *et al*., 2020).

Animals infected or vaccinated with FMDv produce antibodies against viral structural proteins (SP). Anti-Structural Protein (Anti-SP) antibodies may not differentiate between infected and immunized animals and are mostly serotype specific. Therefore, serotype-specific ELISA tests were standardized and recommended for the detection of serotype Asia 1, O, and A prevailing in Pakistan (Abubakar *et al*., 2015).

More emphasis was made in recent years to promote this region as FMD free zone through transboundary disease eradication program in the country. For proper herd immunity to be attained by the livestock we need to immunize a specific percentage of livestock of that area for successful. For example, 95% of a population needs to be immunized to attain protection as a whole. (Galanis *et al*., 2021). The estimate for COVID-19 is 70% to 80% whereas flu needs 33% to 44% to reach herd immunity level (MacIntyre *et al*., 2022; Moghnieh *et al*., 2022). To attain herd immunity level more than 80 percent bovine animal population must be immunized (Sarker *et al*., 2020). Sporadic occurrence of FMD even in the vaccinated animals may demand determining the carrier status of animal with respect the specific serotype of FMDv. The purpose of the present research efforts was to explore the structural protein serology-based distribution of FMDv and to detect the circulation of field virus serotypes (Type Asia 1, type O and type A) in vaccinated and nonvaccinated bovine in Bahawalpur district, which may lead to promote better understanding and to further materialize the efforts for the prevention and control of transboundary transmission and to promote FMD free zones across Asia.

Materials and Methods

Collection of serum samples

In the district of Bahawalpur bovine population was vaccinated with FAO recommended vaccine (FGBI, Federal Center for Animal Health, Russia) which contained A-Iran-05, O Panasia-2, Asia-1 and Sind-08 FMD serotypes. Based on expected 50% prevalence of FMD, the statistically appropriate number of total 838 sera were collected from the district of Bahawalpur. Out of which 436 from vaccinated and 402 samples were collected from non-vaccinated bovine population (Woodrum *et al*., 1996).

Solid-phase competitive ELISA (SPC ELISA)

The presence of serotype specific anti-SP antibodies in serum samples was tested by using the FMD SPC ELISA kits (IZSLER, Biotech Lab, Italy). Selected neutralizing anti-FMDV monoclonal antibodies were used in solid phase competitive ELISA, specific for FMDV serotype Asia 1, O, and A to measure antibodies against these serotypes (Kang *et al*., 2018). Optical density of the test wells compared to the control wells was read by a microplate photometer (Thermo, 590741) and interpreted as negative or positive based on the percentage inhibition (PI). PI values of less than 70% were measured negative, and values equal to or greater than 70% were measured positive for the occurrence of antibodies (Cao *et al*., 2022).

Data analysis

Minitab software was utilized for statistical analysis of data. Chi Square test was used to determine any association of risk factors (Age, gender, vaccination status and area) with incidence of FMD. Chi-square values ≥ 5 indicated a high correlation between two sets of data. Chi-square values < 5 indicated a low correlation between two sets of data. To determine

statistical significance, p-value was also calculated. A p-value ≤ 0.05 indicated that prevalence was statistically significant whereas a p-value > 0.05 showed that prevalence was statistically non-significant (Chan, 2003).

Results and Discussion

Tehsil wise seroprevalence of serotype Asia 1, O and A of FMDV in bovine of district Bahawalpur

The comparative seroprevalence of FMD serotypes within tehsils of Bahawalpur district showed type O with 50.26% in tehsil Ahmedpur East area followed by 33.33% in tehsil Khairpur Tamewali. Maximum prevalence of serotype Asia1 was noted 37.17% in tehsil Ahmedpur East followed by 31.60% in tehsil Bahawalpur. Maximum seroprevalence of type A was recorded 21.83% in tehsil Bahawalpur followed by 20.94% in tehsil Ahmedpur East as mentioned in Figure 1.

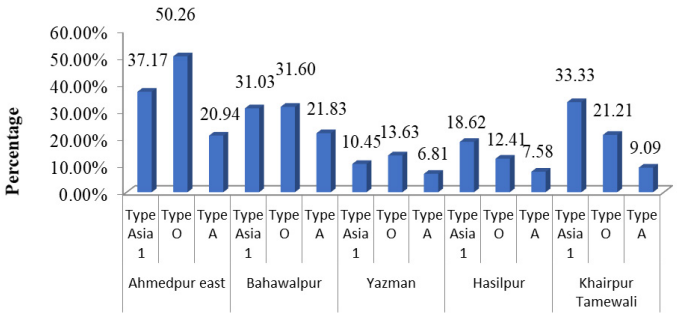

Figure 1: *Tehsil wise seroprevalence of serotype Asia 1, O and A of FMDV in bovine of District Bahawalpur.*

Overall seroprevalence of FMD serotype Asia 1, O and A distribution in bovine of district Bahawalpur

Overall bovine population was carrying maximum seroprevalence of type O (28.52%) followed by Asia 1 (23.63%) and type A (13.6%) in district Bahawalpur respectively as shown in Figure 2.

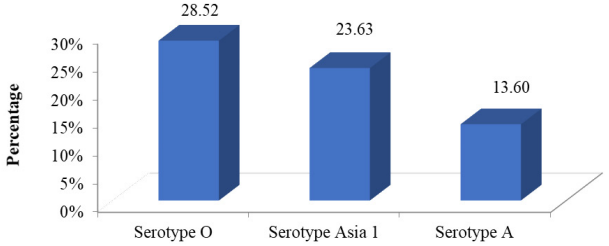

Figure 2: *Overall seroprevalence of FMD serotype Asia 1, O and A distribution in bovine of District Bahawalpur.*

Tehsil wise Distribution of serotype Asia 1, O and A of FMDv prevalent in bovine in district of Bahawalpur

Tehsil wise multiple serotypes carrying more than one serotype specific antibodies titer in bovine was studied and it was recorded that maximum of sera that carry out multiple serotypes was found 36.64% in tehsil Ahmedpur East followed by 27.58% in tehsil Bahawalpur, 21.21% in tehsil Khairpur Tamewali, 12.41% in tehsil Hasilpur and 10.45% in tehsil Yazman as indicated in Table 1.

Cow population was carrying maximum seroprevalence of FMD type O (60.55%) in tehsil Ahmedpur East followed by 40% in tehsil Khairpur Tamewali. The cow population was carrying maximum seroprevalence of type Asia 1 (48.62%) in tehsil Ahmedpur followed by 31.63% in tehsil Bahawalpur. Similarly, cow population was carrying maximum seroprevalence of type A (29.35%) in tehsil Ahmedpur East followed by 22.44% in tehsil Bahawalpur as detailed in Table 1. However, it was recorded that buffalo population was carrying maximum seroprevalence of type O (36.58%) in tehsil Ahmedpur East followed by 22.41% in tehsil Khairpur Tamewali. Buffalo population was carrying maximum seroprevalence of type Asia 1 (31.57%) in Bahawalpur followed by 21.95% in tehsil Ahmedpur East. Buffalo population was carrying maximum seroprevalence of type A (21.05%) in tehsil Bahawalpur followed by 9.75% in tehsil Ahmedpur East as indicated in Table 1.

Tehsil wise percentage of sera that carrying multiple serotypes was further segregated into cows and buffaloes. It was recorded that maximum percentage of multiple carriers of serotypes in cows was found 48.62% in tehsil Ahmedpur East followed by 31.63% in tehsil Bahawalpur as indicated in table 1. Maximum percentage of multi serotype carrier in buffaloes was found with 22.36% in tehsil of Bahawalpur followed by 18.96% in tehsil of Khairpur Tamewali as indicated in Table 1.

Age wise seroprevalence of FMDv serotype carrier distributed in bovine of district Bahawalpur

Age wise significantly higher seroprevalence of type O (38.46%) was recorded in adult bovine population having more than 4 years of age followed by 33.33% in bovine of 2-4 years. Maximum seroprevalence of type Asia 1 (27.35%) was recorded in adult bovine population having more than 4 years of age followed by 27.16% in bovine of 2-4 years. Maximum seroprevalence of type A (20.99%) was noted in adult bovine population having more than 2-4 years of age followed by 15.38% in bovine of 4 years as indicated in Table 2.

Table 1: *Tehsil wise Distribution of serotype Asia 1, O and A of foot and mouth disease virus prevalent in bovine in District of Bahawalpur through application of SPC-ELISA test.*

Tehsil in District of Bahawalpur	Bovine species	Total number of sera tested	Number and %age of sera positive cases			Number of sera that carry out multiple serotypes	Number of sera that test negative for all serotypes
			Sero-Asia 1	Sero-O	Sero-A		
Ahmed Pur East	Cows	109	48.62	60.55	29.35	48.62	39.44
	Buffalo	82	21.95	36.58	9.75	21.95	65.90
Bahawalpur	Cows	98	31.63	37.75	22.44	31.63	63.26
	Buffalo	76	31.57	22.36	21.05	22.36	68.42
Yazman	Cows	96	16.66	11.45	8.33	11.45	83.33
	Buffalo	127	11.02	9.44	5.51	9.44	88.97
Hasilpur	Cows	67	23.88	29.85	16.41	23.88	70.14
	Buffalo	78	2.56	8.97	00	2.56	91.02
Khairpur Tamewali	Cow	50	20	40	14	20	76
	Buffalo	58	18.96	22.41	3.44	18.96	63.79

p-value ≤ 0.05 indicated that prevalence is statistically significant.

Table 2: *Age wise seroprevalence of FMD Virus serotype carrier distributed in bovine of District Bahawalpur using SPC -ELISA test.*

Calves and adults	Age	Serotypes	Total no of samples tested	Total no of positive samples	Prevalence (%)	Chi-square ≥ 5 <5*	p-value ≤ 0.05 > 0.05*
Bovine calves	<3 Months	Sero-Asia 1	105	15	14.29	18.33	0.03
		Sero O		18	17.14	28.12	0.04
		Sero A		8	7.62	39.34	0.02
	3-6 Months	Sero-Asia 1	154	27	17.53	23.03	0.04
		Sero O		30	19.48	11.98	0.04
		Sero A		15	9.74	25.34	0.03
	7-12 Months	Sero-Asia 1	74	22	29.73	17.28	0.02
		Sero O		24	32.43	21.03	0.03
		Sero A		8	10.81	21.90	0.01
Bovine adults	1Y-2Y	Sero-Asia 1	226	60	26.55	33.03	0.01
		Sero O		68	30.09	10.36	0.03
		Sero A		31	13.72	11.99	0.04
	2Y-4Y	Sero-Asia 1	162	44	27.16	14.09	0.02
		Sero O		54	33.33	20.34	0.03
		Sero A		34	20.99	23.09	0.02
	>4 Years	Sero-Asia 1	117	32	27.35	18.34	0.02
		Sero O		45	38.46	31.34	0.03
		Sero A		18	15.38	9.99	0.02

p-value ≤ 0.05 indicated that prevalence is statistically significant.

Within bovine calves' population, seroprevalence of type O was noted maximum (32.43%) in 7-12 months age group followed by 19.48% in 3-6 months age group. Seroprevalence of serotype Asia 1 was noted maximum (29.73%) in 7-12 months followed by 17.53% in 3-6 months of age calves. Seroprevalence of type A was noted maximum (10.81%) in 7-12 months followed by 9.74% in 3-6 months of age calves as detailed in Table 2.

Seroprevalence of FMD serotypes Asia 1, O and A among vaccinated and non-vaccinated bovine in the district of Bahawalpur

In vaccinated bovine seroprevalence of FMD serotypes Asia 1, O and A was variably distributed in the district of Bahawalpur and it was recorded that seropositive cases of serotype O, Asia 1 and A were significantly higher in vaccinated bovine with (42.88%), (36.69%) and (21.33%) compared to the

non-vaccinated population of bovine with (12.93%), (9.45%) and (5.22%), respectively across the district as shown in Figure 3.

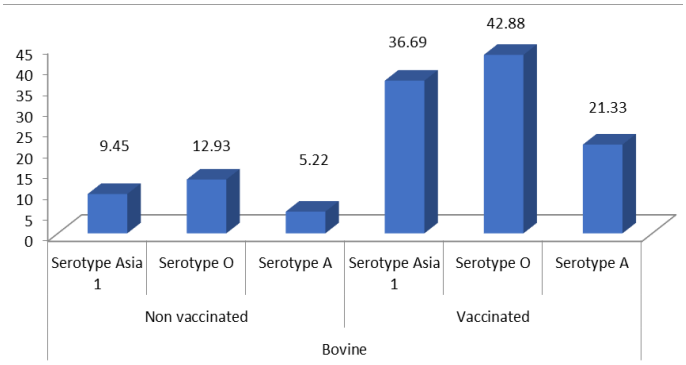

Figure 3: *Seroprevalence of FMD serotypes Asia 1, O and A among vaccinated and non-vaccinated bovine in the Districts of Bahawalpur (2.48%) across the district as indicated in* Figure 4.

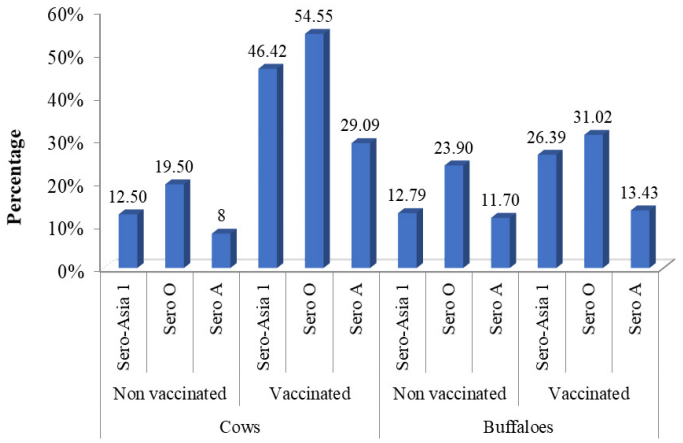

Figure 4: *Seroprevalence of foot and mouth disease virus distributed among vaccinated and non-vaccinated bovine in District Bahawalpur.*

Seroprevalence of FMDv distributed among vaccinated and non-vaccinated bovine

More specifically, seropositive cases of type O were significantly higher in vaccinated cows (54.55%) and buffaloes (31.2%) compared to the nonvaccinated population of cows and buffaloes (19.50%) and (6.44%) across the district. Seropositive cases of type Asia 1 were significantly higher in vaccinated cows (46.82%) and buffaloes (26.39%) compared to the nonvaccinated population of cows (12.50%) and buffaloes (6.44%) across the district. Seropositive cases of type A were significantly higher in vaccinated cows (29.09%) and buffaloes (13.43%) compared to the nonvaccinated population of cows and buffaloes (8.00%).

Gender wise seroprevalence of serotype Asia 1, O and A of FMDv distributed among bovines

Irrespective of the age and vaccination status, gender wise, the antibody-based prevalence was lower against type O (30.11%), Asia 1 (26.34%), and type A (13.98%) of FMDv in bulls, compared with type O (44.02%), Asia 1 (33.76%) and type A (23.08%) in cows. Similar trend was noted in seroprevalence of type O (16.85%), Asia 1 (15.76%) and serotype A (7.61%) in ox population, compared with type O (20.94%), Asia 1 (17.52%) and serotype A (8.55%) in buffaloes as mentioned in Table 3. Chi-square values ≥ 5 indicated that there was a high correlation between two sets of data. A p-value ≤ 0.05 indicated that prevalence was statistically significant.

Table 3: *Gender wise seroprevalence of serotype Asia 1, O and A of FMDV distributed among bovine in District Bahawalpur.*

Groups	Gender	Serotype	Total no of samples tested	Total no of positive sample	% Prevalence	Chi-square ≥ 5	p-value ≤ 0.05
Bovines	Bull	Sero-Asia 1	186	49	26.34	5.92	0.03
		Sero-O		56	30.11	7.55	0.04
		Sero-A		26	13.98	9.65	0.02
	Cow	Sero-Asia 1	234	79	33.76	15.90	0.10
		Sero-O		103	44.02	18.65	0.02
		Sero-A		54	23.08	13.90	0.02
	Ox	Sero-Asia 1	184	29	15.76	26.90	0.03
		Sero-O		31	16.85	12.77	0.02
		Sero-A		14	7.61	14.76	0.01
	Buffalo	Sero Asia 1	234	41	17.52	24.76	0.02
		Sero-O		49	20.94	18.87	0.02
		Sero-A		20	8.55	15.87	0.01

p-value ≤ 0.05 indicated that prevalence is statistically significant.

The purpose of current cross-sectional study was also to determine the prevalence of FMDv serotypes Asia 1, O and A in bovine of district Bahawalpur of Punjab Province, Pakistan. Efforts have been taken to control and eradicate FMD from this region so that the district may be declared as FMD free zone by FAO and OIE. Our data indicated that overall bovine population was carrying maximum seroprevalence of serotype O (28.52%) followed by serotype Asia 1 (23.63%) and serotype A (13.6%) in district Bahawalpur, which coincided with the results of the study conducted In Punjab, Pakistan which indicated that prevalence of serotype O was maximum (45.83 %), followed by Asia-1 (29.17%) and A (13.89%) (Ali *et al*., 2022). Results of a previous study conducted in India indicated that prevalence of serotype O was maximum (80 %), followed by Asia-1 (12 %) and A (8 %) (Subramaniam *et al*., 2013). A study conducted in the southern provinces of Cambodia indicated that the seroprevalence of serotype O was 28.5% followed by Asia 1 (19.3%) and A (9.5%) (Tum *et al*., 2015). In a previous study, it was indicated that serotype O (65.52 %) was having maximum seroprevalence, followed by serotype A (24.14 %) and serotype Asia-1 (10.35 %) in Pakistan (Abubakar *et al*., 2015). An epidemiological investigation conducted in Punjab, Pakistan revealed that type O (45.83%) was the highly prevalent serotype, followed by Asia1 (29.17%) and A (13.89%) (Ali *et al*., 2022).

The cow population was indicated as more susceptible to type O in two tehsils of Bahawalpur including tehsil Ahmedpur East (60.55%) followed by (40%) in tehsil Khairpur Tamewali whereas, the Buffalo population in these tehsils were carrying similar pattern of serotype-specific prevalence with proportionately lower rate of type O (36.58%) in tehsil Ahmedpur East followed by (22.41%) in tehsil Khairpur Tamewali which may reflect comparatively resistant status of Buffalo population to FMDv. Our findings coincide with the results of a previous study conducted in Pakistan that showed the prevalence of FMDv in cattle was 37.1 %, higher than in buffalo (28.7 %) (Abubakar *et al*., 2012).

Maximum seroprevalence of type O was of (38.46%) in adult bovine population having more than 4 years of age followed by 33.33% and 30.09% in bovine of 2-4 years and 1-2 years of age respectively. Findings of a study conducted at Savar in Bangladesh indicated that 6 months to 3 years aged infected cattle showed higher seroprevalence of serotype O and A in animals of 6 months to 3 years of age as compared to animals of more than 4 years of age which showed 82.14% seroprevalence of type-O followed by type-A (78.57%) (Jannat *et al*., 2019). This difference in seroprevalence of FMD may be due to different management and environmental conditions and an overall exposure to the virus (Jamil *et al*., 2015; Awel *et al*., 2021). There was significantly higher prevalence of serotype O recorded among vaccinated (42.88%), and non-vaccinated (12.93%) bovine population in district Bahawalpur. Such variation may indicate that the natural challenge of FMDv in district Bahawalpur remained high.

Conclusions and Recommendations

Our study showed updated seroprevalence data in district Bahawalpur. It was concluded that comparing the seroprevalence of FMD specific serotypes, it was found that maximum exposure of serotype O was evident in adult bovine followed by serotype Asia 1 and serotype A. A similar pattern of serotype specific prevalence was also noted in non-vaccinated bovine population which might be an indication of predominantly prevalent serotype of FMD type O existing in bovine population of Bahawalpur district. It is reasonable to expect that decreasing the circulation of all three serotypes considerably serotype O through regular immunization with trivalent vaccine of higher efficacy can help in substantially reducing the overall circulation of FMDv serotypes initially in the district and ultimately throughout the country.

Acknowledgments

Authors acknowledge the technical support of NVL/FAO/Laboratories, Islamabad and the office of the Research Innovation and commercialization, UAF. Authors also acknowledge Dr. Muhammad Afzal, FAO, Islamabad for his continuous guidance throughout the study.

Novelty Statement

This is a unique study providing the information about Prevalent serotypes of FMD in Bahawalpur. It will provide a new insight on serotype prevalence of foot and mouth disease and will help assess the vaccine and immunization strategies particularly in Bahawalpur and generally in Pakistan.

Author's Contribution

Muhammad Mohsin Abbas: Collection and processing of samples.
Sajjad Ur Rahman: Execution of research in the molecular diagnostic laboratory.
Muhammad Abubakar: Execution of research.
Muhammad Imran Arshad: Provided continuous technical support and interpretation of results.
Khurram Ashfaq: Contributed his outreach and helped out in sample collection.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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.

Conflict of interest
The authors have declared no conflict of interest.

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