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Epidemiological Aspects of Equine Herpes Virus Infection in South Gujarat, India

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ABSTRACT

Background: The equine herpes viruses (EHV) are highly infective pathogens of all members of the Equidae family worldwide. EHV-1 predominantly associated with the abortion, neonatal death, neurological diseases, whereas, EHV-4 is mostly associated with the respiratory disease, generally termed as equine rhinopneumonitis. This present investigation was planned to study sero-prevalence of EHV-1/4 infections in horses of south Gujarat, India.

Methods: A total of 253 horses showing symptoms of respiratory illness or having history were screened by indirect ELISA test. While, blood samples were analysed for various haematological parameters. Effects of risk factors were studied and statistical analysis of data was done.

Result: Over all prevalence of EHV-1/4 infection was 16.60% in South Gujarat, India. Effects of various risk factors (age, sex, breed, and locality) were non-significant. Means of all haematological parameters were in normal range and significant difference was not observed between sero-positive and sero-negative horses. Population of mid cells (monocytes) was found significantly higher ($p<0.05$) in sero-positive horses as compare to sero-negative horses.

Key words: Equine herpes virus, ELISA, Epidemiology, Rhinopeumonitis.

INTRODUCTION

Horses (*Equuscaballus*) have been used since old times by humans as a mean of transportation before invention of mechanical transport vehicles. Efficient performance of equines for transportation and other functions require a sound health and freedom from various ailments including infectious diseases. Respiratory diseases are major cause of morbidity and mortality in foals and adult horses. The equine herpes viruses (EHV) are highly infective pathogens of all members of the *Equidae* family worldwide (Patel and Heldens, 2005). EHV-1 and EHV-4 are respiratory pathogens of domestic horses associated with outbreaks of respiratory disease. EHV-1 predominantly associated with the abortion, neonatal death, neurological diseases, whereas, EHV-4 is mostly associated with the respiratory disease, generally termed as equine rhino-pneumonitis (Matsumara *et al.,* 1992). Both, EHV-1 and 4 are enzootic in most domestic horse populations and majority of horses show serological evidence of exposure to these viruses (van Mannen, 2002). The spread of virus among susceptible population occurs by means of direct contact, inhalation of aerosols, nasal secretion and ingestion of contaminated feed (Garre *et al.,* 2009). Both, EHV-1 and 4 are also capable to establish latent subclinical infections in horses which dynamically act as carriers for lifetime (Foote *et al.,* 2004). Early epidemiological studies were not able to differentiate between EHV-1 and 4 infections as EHV-1 and 4 share most of the external glycoproteins as well as type specific epitopes (Crabb and Studdert, 1993). Development of type specific ELISA gave ability to differentiate antibodies of EHV-1 and EHV-4 and facilitated to diagnose specific infection with these viruses which allowing additional insight in to their epidemiology (Crabb and Studdert, 1995). Nowadays, serological techniques especially ELISA has been demonstrated as an important tool in epidemiological investigations of equine herpes virus infections (Ataseven *et al.,* 2009). Considering the importance of EHV-1 and 4 as important respiratory tract pathogens and under the paucity of epidemiological study in India especially in Gujarat, the present study was carried out to study the sero-prevalence of EHV-1 and EHV-4 infections in horses of south Gujarat, India.

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MATERIALS AND METHODS

Generally equine herpes viruses cause respiratory disorder along with pyrexia, unthriftyness and in some cases abortion

and neurological disorder. Total 253 horses showing any of above symptoms or having history was included in the present study. Sample collection detail is given in (Table 1).

Collection and preservation of samples

The blood samples were collected aseptically by jugular venipucture in vacutainer with clot activator for serum and in EDTA vacutainer for haematological parameters. Blood sample were allowed to clot at room temperature for 15-30 min and centrifuge at 1500 rpm at 4°C for 10 min. The separated serum was carefully harvested and stored at -20°C until further use. The whole blood samples in EDTA vacutainer were immediately subjected to complete blood count in automated blood analyzer (Nihon-Kohden, Japan).

ELISA

A commercial indirect IgG confirmation ELISA assay (IngezimRinoneumonitis 14.HVE.K1, Ingenasa) was used following the manufacturer's instructions to detect antibodies to EHV-1 and/or EHV-4.

Haematological analysis

The whole blood samples in EDTA vacutainer were subject to complete blood count in automated blood analyzer (Nihon-Kohden, Japan). Various haematological parameters *viz.* haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC), differentiate leukocyte count (DLC), platelet count and mean platelet volume (MPV) were estimated.

Statistical analysis

Data pertaining to sero-diagnosis of equine herpes viruses' infection was analyzed using Chi-square test (probability at 5% and confidence interval at 95% level) using online statistical tool available at https://www.socscistatistics.com. The findings of various haematological parameters of sero-positive and sero-negative were compared by using 't' test.

RESULTS AND DISCUSSION

Overall prevalence of EHV-1/4 infection

Out of 253 serum samples analyzed, 42 serum samples were found positive for presence of antibodies against EHV-1/4, which gave 16.60% overall prevalence among the horses of south Gujarat, India.

Risk factor analysis of EHV-1/4 infection

Various risk factors *viz.* spatial distribution of infected horses, age, sex and breed were statistically analyzed to view any significant importance of these factors in prevalence of disease. The results are shown in below (Table 1).

Spatial distribution of disease

The district-wise sero-prevalence of equine herpes virus infection in Navsari, Surat and other districts of south Gujarat was 15.60% (22/141), 17.48% (18/103) and 22.22% (02/09), respectively (Fig 1). No significant effect of geographical area on sero-positivity was observed.

Sex wise distribution of disease

Prevalence of equine herpes virus infection in males and female was 16.15% (26/161) and 17.39% (16/92), respectively. Similar to the geographical area wise prevalence, effect of sex on sero-prevalence of equine herpes virus infection was non-significant.

Age wise distribution of disease

During present study, sero-prevalence of equine herpes virus infections was 17.07% (7/41) and 16.51% (35/212) in horses aged <2 years and >2 years, respectively. The effect of age on sero-prevalence was also found non-significant.

Breed wise distribution of disease

Sero-prevalence of equine herpes virus infection in Sindhi breed, Kathiawari breed, Marwadi breed and Non-descript breeds was 18.02% (20/111), 16.67% (2/12), 20.69% (6/29) and 13.86% (14/101), respectively. The effect of breeds on sero-prevalence of equine herpes virus infection was also non-significant.

Haematological analysis of sero-positive and sero-negative horses

The mean values of all haematological parameter under study were within the normal range. Similarly, the difference between various haematological parameters of sero-positive and sero-negative horses was non-significant except mid cell percentage which was noted significantly higher ($p<0.05$) in sero-positive horses. The values of various haematological parameters are given in (Table 2).

In this study, sero-prevalence of EHV-1/4 infection was measured in horses using indirect ELISA. The samples were taken largely from unregistered animals in small private ownerships. These types of equids are traditionally used as transport and pack animals in the rural areas or as status symbol and hobby in urban areas. The study revealed overall sero-prevalence of EHV-1/4 infection as 16.60% in region under study. Several epidemiological investigations have been performed for the prevalence of EHV-1 and EHV-4 infections. Previously, 1.64%-38.12% sero-prevalence of EHV-1 was recorded in different states of India (Anonymous, 2019). The sero-prevalence rate of EHV-1 and EHV-4 infection was reported within 8% to 85.2% across various countries of world (Ata *et al.,* 2020; Ataseven *et al.,* 2009; Avci *et al.,* 2019; Mohamed *et al.,* 2017; Singh, 1999; Yildrim *et al.,* 2015). The difference in sero-prevalence may be attributed to many factors including the difference in the epidemiology of EHV-1/4 among different horse populations, the difference in the testing methods, and the antibody titre could be under the detection limit especially in latently infected animals (Dunowska *et al.* 2015). The present findings indicated the presence of natural infection with the

Table 1: Prevalence of EHV-1/4 infection in different age, sex and breed of horses.

Risk factors	Particular	No. of horses covered	No. of positive horses	Prevalence (%)	P value
District	Navsari	141	22	15.60	
	Surat	103	18	17.48	0.834
	Others	009	02	22.22	
	Total	253	42	16.60	
Sex	Male	161	26	16.15	
	Female	092	16	17.39	0.798
	Total	253	42	16.60	
Age	< 2 years	041	07	17.07	
	>2 years	212	35	16.51	0.929
	Total	253	42	16.60	
Breed	Sindhi	111	20	18.02	
	Kathiawadi	012	02	16.67	
	Marwadi	029	06	20.69	0.787
	Non-descript	101	14	13.86	
	Total	253	42	16.60	

Table 2: Comparison of haematological parameters of sero-positive and sero-negative horses.

Parameters	Reference range#	Total (n=253)	Sero-Positive (n=42)	Sero-negative (n=211)	p value
Haemoglobin (gram/dl)	11.0-19.0	11.13±2.29	10.93±2.04	11.17±2.33	0.340
Packed cell volume (%)	32-53	33.00±6.84	32.11±6.13	33.17±6.95	0.263
Total erythrocyte count (× 10^6 cells/cmm)	6.8-12.9	7.25±1.49	7.17±1.37	7.26±1.51	0.390
Mean corpuscular volume (fl)	37-59	45.37±3.66	45.17±3.32	45.41±3.72	0.382
Mean corpuscular haemoglobin (pg)	12.3-19.7	15.39±0.94	15.43±0.96	15.38±0.94	0.401
Mean corpuscular haemoglobin conc. (%)	31-38.6	34.11±1.27	34.08±1.15	34.11±1.30	0.457
Total leukocyte count (× 10^3 cells/cmm)	5.4-14.3	9.77±2.37	9.49±2.32	9.83±2.40	0.292
Lymphocyte (%)	21-42	31.10±10.79	30.05±10.44	31.31±10.85	0.292
Mid cells (%)	2-9	7.35±2.16	8.12±1.61	7.19±2.23	**0.027***
Granulocyte (%)	52-70	61.44±11.56	61.66±11.35	61.39±11.59	0.463
Total platelet count (× 10^3 cells/cmm)	100-350	102.43±16.58	104.29±15.54	102.06±16.79	0.294
Mean platelet volume (fl)	5.0-6.5	5.72±0.27	5.73±0.22	5.72±0.28	0.405

*Significant at P<0.05 #Reference range (Constable *et al.* 2017).

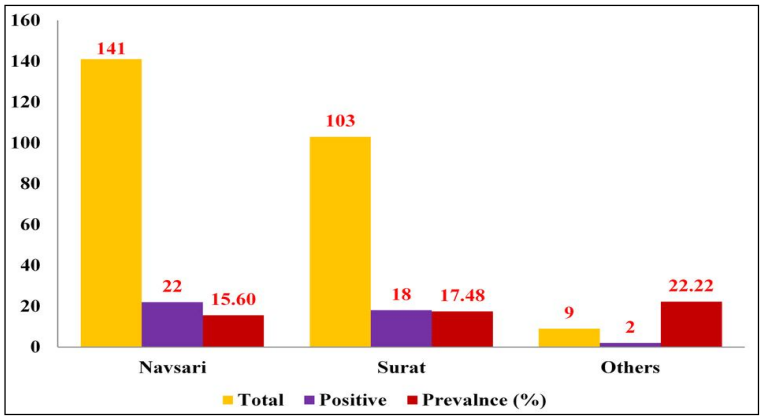

Fig 1: Spatial distribution of cases.

virus and/or latent cases reactivation as vaccination against EHVs in the study region is not in practice.

As per findings of present study, effect of risk factors *viz.* geographical location, age, sex and breed on occurrence of disease were non-significant. Sex may not considered as an influential factor on occurrence of disease (Hafshejani *et al.,* 2015). On contrary to findings of present study, in certain studies higher sero-prevalence of EHV-1 was found in females than in males (Ata *et al.,* 2020; Senthil and Parameswaran, 2014). This might be due to unequal gender wise sample collection. Moreover, in urban areas people kept mostly male horses as status symbol and hobby purpose. Similarly, on contrary to present study, some studies found age as an important factor that can influence the occurrence of disease (Ata *et al.,* 2020; Hafshejani *et al.,* 2015). Standardbred and thoroughbred horses have higher susceptibility than local Arabian and Turkoman breeds (Hafshejani *et al.,* 2015). Certain horse breeds have slight higher susceptibility to equine herpes virus infection than others, but variation in immune status and rate of infection should be taken into consideration before establishing the facts (Lunn *et al.,* 2009). Presently, in region under study, most of reared horses are lineage of Arabian horses and non-descript horses are mostly cross over off-springs in between this breeds. This might be the reason behind equal distribution of disease among different breeds under study.

Haematological as well as serum biochemical examinations are neither specific nor diagnostically significant, as major detectable variations in both examinations are not present in EHV-1 and 4 infected horses (Constable *et al.,* 2017). In agreement to this, mean values of all haematological parameters under study were within the normal range. The mean values of mid cells in sero-positive horses were found significantly higher than the values of sero-negative horses. Similarly, two studies found increased monocyte count in EHV-1 infected working horses (Mason *et al.,* 1989; Fararh *et al.,* 2016). Correct timing of sample collection during specific phase of disease is immensely important, even though it only indicates non specific viral infection (Slater, 2014).

CONCLUSION

EHV-1/4 infection is endemic among horse population of south Gujarat, India and constituteshealth risk factors for other healthy horses. Further structured serological or molecular investigations are required to study the epidemiological aspect of disease like role of mules and donkey as well as other unknown live carriers. These surveys could also look for different risk factors for spreading of disease among different populations.

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Conflict of interest

No competing interest by authors.

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