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Research Paper

Seroprevalence of Equine herpes virus (EHV1) in equidae Using serum neutralization test

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Equine rhinopneumonitis is a term , which describes a constellation of several disease entities of horses, which includes, respiratory disease, abortion, neonatal foal pneumonitis and myelo-encephalopathy. Sharma *et al.* (1965) incriminated EHV-1 as the cause of equine abortions on the basis of histopathology of tissues from aborted fetuses. Equine herpesvirus -1 and 4 (EHV-1 and 4) are enzootic in all countries and are ubiquitous in horse population. Both viruses can cause acute febrile respiratory disease.

I. MATERIALS AND METHODS

A total of 162 sera sample were collected from horses, mules and donkeys with various clinical symptoms from different parts of Tamilnadu. 20 nasal swabs and vaginal swabs were collected from horses and mules from different parts of Tamilnadu. All the sera (162) were subjected to Serum neutralization test (SNT).

The negative EHV-1 serum, Standard EHV-1 positive serum with merthiolate and freeze dried live cell culture adapted EHV1 antigen was supplied by Dr.Cecilia Monica Galosi, Investigator, Faculty of Virology, La Plata, Bs As, Argentina was used for SNT.

Madin Darby Bovine Kidney cell line (MDBK) obtained from National Centre for Cell Sciences; Pune was used for virus propagation and virus isolation attempts. Serum neutralization test was conducted in MDBK as described by Shankar and Yadav (1986).

The micro method of serum neutralization test was followed to assess neutralizing antibody level against EHV 1 in horses. The test was performed in microtitre plate – 96 wells flat bottomed (Nunc, Denmark). 50µl maintenance medium was added to all test wells (50µl/ well), 50µl of test serum was added to the first well. Then it is serially diluted tenfold times. After dilution of serum, 50µl of 100 $TCID_{50}$ of virus was added to all test wells. Plates were vibrated to mix thoroughly the contents in each well and incubated at 37^0C for 45 min. Then 50µl MDBK cells were dispensed in all wells. Controls with known positive serum, known negative serum and test serum were incorporated. Cell control and antigen control were also incorporated. Then the plate was closed with lid, made airtight by applying cello tape around the plate and incubated at 37^0 C for five days. Every day the plates were examined under microscope and observations were recorded. On completion of five days of incubation the final reading was taken.

II. RESULTS AND DISCUSSION

A total of 162 sera sample were subjected to serum neutralization test using MDBK cell line adapted EHV-1 antigen and 53 (32.71 per cent) were found to be positive for EHV-1 antibody. This test was used to detect the seroprevalence as well as the highest antibody titre in the seropositive animals.

In the present study the serum neutralization test titres ranged from 1:10 to 1:1000. The prevalence of EHV-1 antibody were 32 per cent in horses attending Madras Veterinary College Hospital, 47.05 per cent in

Kancheepuram, 28.57 per cent in Guindy, 13.33 per cent in St.Thomas Mount, 28.57 per cent in Egmore and 28.57 per cent in Chettinad stud farm respectively. Kancheepuram recorded the highest (47.05 per cent) seroprevalence and St.Thomas Mount recorded the lowest (13.33 per cent). The high prevalence was seen in horses, mules and donkeys where all the animals are kept in the rehabilitation shelter.

Overall prevalence of EHV-1 antibody was 32.71 per cent. The frequency of occurrence of 1:100 titre in serum neutralization test was maximum (48.89 per cent) followed by 1:10 (41.51 per cent), 1:1000 (9.43 per cent). The entire 162 sera sample collected from different places in Tamilnadu where subjected for SNT, 53 (32.71 per cent) showed positive titres against EHV-1 antigen. The serum neutralization indices were ranging from 1.0 - 3.0.

Out of 162 sera screened by SNT, highest prevalence (50 per cent) observed in mules followed by donkeys (33.33 per cent) and in horses (32 per cent).Sex wise analysis showed 28.41 per cent of males, 37.84 per cent of females were seropositive by SNT. Among the total seropositives males contributed 53.66 per cent while females 48.78 per cent. Analysis of data revealed that the prevalence of EHV-1 antibody showed no significant difference between sexes.

Statistical analysis revealed significantly higher prevalence (P<0.01) in the order of Kancheepuram, horses attending Madras Veterinary College Hospital, Chennai, Guindy, Egmore, Chettinad stud farm and St.Thomas Mount.

Table 1: Prevalence percentage of EHV-1 antibody by serum neutralization test (SNT) among the equine population

S.No	Location	No. of samples	SNT	Per cent positive
1	Madras Veterinary College Hospital	25	8	32.00**
2	Kancheepuram	17	8	47.05**
3	Guindy	21	6	28.57**
4	St.Thomas Mount	15	2	13.33**
5	Egmore	14	4	28.57**
6	Chettinad stud farm	70	25	35.71**
Total		162	53	

** Highly significant (P<0.01)

Table 2: Frequency distribution of serum neutralizing antibody for EHV-1

S. No.	SNT titre	No. of sample	Per cent positive
1	1:10	22	41.51 ^{NS}
2	1:100	26	48.89 ^{NS}
3	1:1000	5	9.43 ^{NS}
Total		53	

NS – Not significant

Table 3: Species-wise prevalence of EHV-1 antibody by Serum neutralization test (SNT)

S.No	Species	No. of sample	SNT positive	Per cent positive
1	Mule	6	3	50.00**
2	Donkey	6	2	33.33**
3	Horse	150	48	32.00**
Total		162	53	

** Highly significant (P<0.01)

Shankar *et al.* (1988) reported that 27.7 per cent of the horses and mules sera screened were positive for EHV-1 antibodies with serum neutralization test. Dunowska *et al.* (2002) reported that serum neutralization titre of > 1:2 were regarded as positive and a fourfold increase in SNT titre of foals indicates recent infection with EHV-1.

Garg *et al.* (1977) reported the prevalence of EHV -1 on serological basis using serum neutralization test. In India, the association of EHV- 1 in abortion (Jain *et al.*, 1976), nervous disease (Shankar and Yadav, loc.cit) and respiratory disease (Uppal *et al.*, 1991) in equines have been documented.

A higher sero-positivity of 53.45 per cent was recorded in aborted mares belonging to 3 farms located in Haryana where as in Uttar Pradesh 7.85 per cent to 13.24 per cent sero-positivity of EHV-1 in apparently healthy horses and foals under 2 years of age. In horses peak incidence of about 57.69 per cent of abortion due to EHV-1 was noticed between 9^{th} month to 10^{th} month of gestation (Singh *et al.*, 1992).

Similar prevalence rates were observed by Shankar *et al.* (loc.cit) in Haryana, Bihar and Uttar Pradesh. Out of 135 sera tested, 27.7 per cent of the horses and mules were positive for EHV-1 serum neutralizing antibodies, with Serum neutralization indices ranging from 1.0 to 3.0.

Dunowska *et al.* (loc.cit) reported, out of the 82 sera tested 59 (70 per cent) sera showed neutralization antibodies to EHV-1 and among horses in NewZealand. The frequency distribution of serum neutralization antibody titres to EHV-1 and 4 ranged from 1:2 to1:512. Sera were regarded as positive to EHV-1, if they showed a titre > 1:2.

III. SUMMARY

Out of 162 sera screened by SNT, highest prevalence (50 per cent) observed in mules followed by donkeys (33.33 per cent) and in horses (32 per cent).Sex wise analysis showed 28.41 per cent of males, 37.84 per cent of females were seropositive by SNT. Among the total seropositives males contributed 53.66 per cent while females 48.78 per cent. Analysis of data revealed that the prevalence of EHV-1 antibody showed no significant difference between sexes. Over all seroprevalence detected by SNT was 32.71 per cent in Tamilnadu. The maximum seroprevalence was at Kancheepuram (47.05 per cent) and lowest was at St.Thomas Mount (13.33 per cent).

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