

SEROLOGICAL SURVEY ON INFECTION WITH EQUINE RHINOPNEUMONITIS VIRUS AMONG SOLIPED ANIMALS IN IRAN (*)

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SUMMARY — Serum samples from 573 horses, 34 donkeys, and 114 mules collected from various parts of Iran, were tested for neutralising antibody against equine rhinopneumonitis virus.

Antibody was detected in the sera of 88.4 per cent. of the horses, 70.6 per cent. of the donkeys and 98.2 per cent. of the mules.

Horses with high antibody titres were found in all localities. The antibody distribution among horses was found not to be related to sex, but the percentage of positives was higher in the group of animals aged two to 12 years than in younger horses.

The results obtained indicated an extensive dissemination of equine rhinopneumonitis virus, or closely related viruses, among the equine population throughout the country.

Introduction

Equine rhinopneumonitis virus (ERV) infection has been reported from all continents since it was described first by Dimock and Edwards in 1933. The virus has been isolated in several countries and serological surveys indicated that a high percentage of horses over a great part of the world possessed antibodies to the virus (Kawakami *et al.*, 1959; Matumoto *et al.*, 1960; Shimizu *et al.*, 1963; Matumoto *et al.*, 1965; De Boer, 1966a, b; Burrows, 1966; Petzolt *et al.*, 1966; Paulsen, 1966; Brion *et al.*, 1968; Bagust *et al.*, 1972). However, studies on the presence and distribution of ERV infection in donkeys and mules are limited (Matumoto, 1965).

The present communication provides the results of studies undertaken

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to assess the presence and distribution of equine rhinopneumonitis virus infection among horses, mules and donkeys in Iran. The study was performed by testing equine serum specimens collected from different parts of the country for the presence of neutralising antibody against the virus.

Materials and Methods

Cell Cultures.

Monolayer cultures of both primary and secondary calf kidney cells were used for virus propagation and neutralisation tests. Cells were grown in a ELY medium (Earle's balanced salt solution, lactalbumin hydrolysate, yeast extract) containing 10 per cent. inactivated calf serum, 100 units of penicillin and 100 µg. of streptomycin per ml.

Virus

Equine rhinopneumonitis virus, Strain L, (De Boer, 1966a) at its 8th passage level in bovine embryo kidney cells, was supplied by Dr. G. F. de Boer, from the Central Veterinary Institute, Amsterdam.

A few additional passages of the virus were made in primary calf kidney cells before it was used in the experiments. The cultures were inoculated with the virus and incubated at 37° C. for two hours. After this adsorption period the infected cells were overlaid with ELY medium containing 2 per cent. foetal calf serum, and incubated until the cytopathic effect was almost complete. The infected cells were then harvested and intracellular virus was released by freezing and thawing. Cell debris was sedimented by low-speed centrifugation and the supernatant was stored at -70° C. and used as virus stock.

Serum Samples

Blood samples were collected from horses, mules and donkeys of different age groups, from various parts of the country. The animals appeared healthy at the time of bleeding, and no information on the occurrence of any previous epizootic respiratory diseases or of abortion among the equine population in the selected areas was available.

The sera were separated from blood samples and were stored frozen at -20°C., without any preservative, until required. They were heated at 56°C. for 30 minutes prior to testing.

Neutralisation Tests

Sera were tested for neutralising antibody titre against ERV on calf kidney cell cultures. Four-fold dilutions in ELY of the inactivated serum samples were mixed with equal volumes of virus suspension containing 40 to 100 TCID₅₀ of the virus per 0.1 ml. The virus serum mixtures were kept in a refrigerator over-

night, and then each mixture was tested for infectivity by inoculating four cell culture tubes using 0.2 ml. of the mixture as inoculum. For control, non-inoculated cells were treated similarly and incubated. After an adsorption period of two hours, 1.5 ml. of ELY was added to each tube and the inoculated cells were re-incubated at 37°C. The cultures were examined daily for cytopathic effect (CPE) for five days. Serum titres, expressed as the logarithm of the reciprocal of the dilution of serum that would completely inhibit CPE in 50 per cent. of the cell culture tubes, were calculated by the method of Reed and Muench.

Results

The results of neutralisation tests on 721 serum samples collected from soliped animals from several parts of Iran, against Strain L of equine rhinopneumonitis virus are summarised in Table I. Neutralising antibody titres equal to or greater than 0.6 were taken as positives, and on this basis the percentage of positive sera was calculated.

TABLE I
Distribution of Neutralising Antibody to Equine Rhinopneumonitis Virus among Soliped Animals in Iran

Localities	No. of sera tested	No. of sera with indicated antibody titre					Percentage of positives
		<0.6*	0.6-1.2	1.5-1.8	2.1-2.4	>2.4	
Maraghah	86 H	0	19	32	31	4	100
Varamin	175 H	5	60	46	54	10	97.1
Sanandage	40 H	2	12	15	11	0	95
Ardabil	26 H	10	12	4	0	0	61.5
Zabol	8 H	4	2	2	0	0	50
Ahwaz	40 H	22	16	2	0	0	45
Other localities	198 H	23	87	45	33	10	88.3
	34 D	10	10	14	0	0	70.6
	114 M	2	24	60	22	6	98.2
Total	573 H	66	204	146	129	24	88.4
	34 D	10	10	14	0	0	70.6
	114 M	2	24	60	22	6	98.2

* = Serum antibody titre (Log.10)

H = Horse

D = Donkey

M = Mule

TABLE II

Distribution of Neutralising Antibody against Equine Rhinopneumonitis Virus among 198 Horses of Different Sex and Age

Age (years)	Sex	No. of sera tested	No. of sera with indicated antibody titre					Positive per cent.	
			<0.6*	0.6-1.2	1.5-1.8	2.1-2.4	>2.4	Sex	Age
1-2	M	37	9	13	11	4	0	75.6	75.3
	F	36	9	14	9	4	0	75.0	
2-12	M	60	3	25	17	10	5	95.0	96.0
	F	65	2	35	8	15	5	96.9	

* = Serum antibody titre (Log.10)

M = Male

F = Female

From 573 horses tested 88.4 per cent. had neutralising antibody against ERV. The percentage of animals having neutralising antibody varied from 45 per cent. in Ahwaz, to 100 per cent. in Maraghah. In addition, 70.6 per cent. of 34 donkeys' and 98.2 per cent. of 114 mules' sera were found to have neutralising antibody against the virus.

The distribution of antibody in 198 horses of different sex and age is shown in Table II. The percentage of positives was found not to be related to the sex of the horses. The incidence of neutralising antibody was 75.6 per cent. in males and 75 per cent. in females, in animals younger than two years; 95.0 per cent. in males and 96.9 per cent. in females in animals two to 12 years old. A difference was observed in the distribution of neutralising antibody titre among horses of different age groups. The percentage of positives in a group aged two to 12 years and that of those younger than two years were found to be 96 per cent. and 75.3 per cent. respectively.

Discussion

Equine rhinopneumonitis virus has been known to cause both frank and inapparent infections of horses. In many countries the virus has been isolated from aborted foetuses in natural cases of equine abortion and from the nasal secretion of young horses suffering from acute respiratory infection.

In Iran, the presence of equine rhinopneumonitis virus infection has not been investigated, and no evidence of the distribution of the infection has so far been reported. The data presented in the present survey, however, indicate that the infection is widespread among the equine population of the country.

The difference between the incidence of neutralising antibody in the ser:

of horses from different localities is probably due to differing forms of management rather than to geographical distribution. The sera, with a very high percentage of positives, had been collected from animals in horse breeding centres where the horses are kept close together in large barns under conditions which favour the distribution of any respiratory infection, while the sera from localities having rather low percentages of positives had been obtained from farm animals widely dispersed over a large area.

Previous studies of ERV infection in other species of soliped animals are confined to the report by Matumoto (1965) who showed the presence of neutralising antibody in all 10 donkey sera from Egypt. The results of the present investigation confirmed Matumoto's finding and showed that, in addition to a high percentage (70.6 per cent.) of donkeys having neutralising antibody to ERV, a large number of mules (98.2 per cent.) also had neutralising antibody to the virus. From these data it seems that equine rhinopneumonitis virus could spread among all species of domestic solipeds and produce frank or inapparent infection.

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