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Serological survey on the presence and distribution of equine infectious anaemia in Iran.

By

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Summary

A total of 1100 horse serum samples were tested for the presence of precipitating antibody against equine infectious anaemia antigen. 28 samples were proved to contain antibody, and therefore, the presence of infection with equine infectious anaemia virus among Iranian horses was confirmed.

Introduction

Equine infectious anaemia, EIA, is caused by a virus which contains RNA and belongs to retrovirus group. The disease is generally being recognized as one of the most important viral infection of horses. The infection, if death does not occur, usually terminates in persistent infection accompanied by recurrent fever, anaemia and viraemia at various intervals for life.

Animals in the chronic phase of infectious anaemia are usually true reservoir of the virus in nature and may serve as a source of infection to the susceptible horses. Therefore, it seems important to investigate the presence of the disease in every country.

In this communication we report the results of an investigation which, for the first time, shows the presence and distribution of equine infectious anaemia among horse population in Iran. The survey was performed by testing horse serum specimens collected from various parts of the country for the presence of precipitating antibody against the virus antigen.

Materials and Methods

Serum samples. - Blood samples were collected from horses of various parts of the country. The animals appeared healthy at the time of bleeding and no information on the occurrence of cases related to equine infectious anaemia among equine population in the area was available.

The sera were separated from blood samples and were stored frozen at - 20 C. until required.

Antigen and antiserum. - The precipitating antigen, consisting of crushed spleen of a horse experimentally infected with a virulent strain of equine infectious anaemia, was supplied by Professor Toma, Ecole National Vétérinaire, Alfort, France.

Positive reference serum was also supplied by Professor Toma. The serum was collected from a horse during the chronic phase of equine infectious anaemia. (4,5)

The antigen and antiserum were received in freeze dried form and each was reconstituted in distilled water to a predetermined optimal concentration before use. The reagents produced a distinct precipitin line when tested against each other by gel diffusion test.

Agar precipitation test. - A medium consisting of 1 to 1.25 percent of Nobles' special agar in borate buffer with a pH value of approximately 8.6 (2 g. NaOH and 9 g. H3BO3 in 1 litre distilled water) was used.

Immediately after preparation the medium was distributed, in 25 ml. amount, into flat bottmed Petri dishes of 90 mm. diametre.

The agar gel was allowed to solidify. The plates were kept inverted at room temperature overnight, and then in a 4°C. refrigerator for a period not longer than 2 weeks, before use.

Wells were punched using a template with 6 circular cutter 7 mm in diametre and spaced 3 mm. from a central cutter of the same diametre.

The central well, in each set, was filled to capacity with the antigen. The reference positive serum was placed in two of the peripheral wells and the remaining 4 wells were filled each with one of the samples to be tested (Figs. 1 and 2).

Plates were kept at room temperature (25°C.) in a closed moist chamber and were examined daily (3 days) for the appearance of precipitin lines, in a darkened room over an indirect light from a microscope lamp reflected by a concave mirror.

Precipitin lines between wells containing reference serum and that with antigen were usually formed within 48 hours after incubation (control lines).

The test serum was considered positive when a precipitin line appeared between the serum and antigen joining the control line to form a continuous line of precipitin, (Fig. 1,2).

If no distinct line was produced between the test serum and antigen, but the control line bent slightly toward the antigen well or ended about half way across its normal position, the test serum was considered doubtful. In doubtful reactions the test was repeated using dilutions of the suspect samples. (1,2)

Results

The results of agar double-diffusion precipitation tests on 1100 serum samples, collected from horses of several parts of Iran, against equine infectious anaemia viral antigen, are summarized in Table 1. From total serum samples tested 28 (2.54%) had the precipitating antibody. The percentage of positives varied, however, from O to 6.87 percent.

Table 1. Distribution of precipitin antibody against equine infectious anaemia virus among horses in Iran.

Localities	No. of sera tested	Positives	Percentage of Positives
Varamin	262	18	
Kermanshah	43	0	
Sanandaj	67	3	
Tehran	100	0	
Esfahan	17	1	
Ardabil, Rezayieh	80	3	
Maragheh	84	1	
Shiraz	32	0	
Ahwaz	15	0	
Guilan	42	0	
Mazendaran	79	0	
Zabol	4	0	
Other places	275	2	
Total	1100	28	2.54

Discussion

Various experiments have indicated that blood sucking arthropods such as Stomoxys calcitrans, tabanidae and even mosquito play an important role in natural transmission of equine infectious anaemia (3). These arthropods ap-

parently transmit the EIA virus mechanically from infected to susceptible individuals. In addition to diseased animals with clinical symptoms, horses in chronic or subclinical infections, also supply a potnet source of virus for spreading of equine infectious anaemia. Therefore, in controling or eradicating of EIA in an area, diagnosis of the infection in such animals is of absolute necessity.

Among several tests, so far developed, demonstration of the presence of precipitating antibody in serum of horse, by Coggins' agar precipitation method, has been shown to be the most practical and reliable test for detection of EIA infection. This test has been extensively used since first introduced in 1970 (1,2,4,5,6).

Coggins' agar diffusion test was used to investigate the presence and distribution of EIA among Iranian horses. From 1100 serum samples tested 28 sera (2.54%) had the precipitin antibody. The percentage of positives varied in various parts but. due to limited number of animals tested in some area under study, it was difficult to give a real picture of the dessimination of the infection in the country. The sera with a higher percentage of positives, however, had been collected from animals in horse breeding centres (Varamin) where horses were kept close together and under climatic conditions which favour the activities of the arthropods.

This is the first report on the presence and distribution of infection with EIA virus among horse population in Iran.

Acknowledgement

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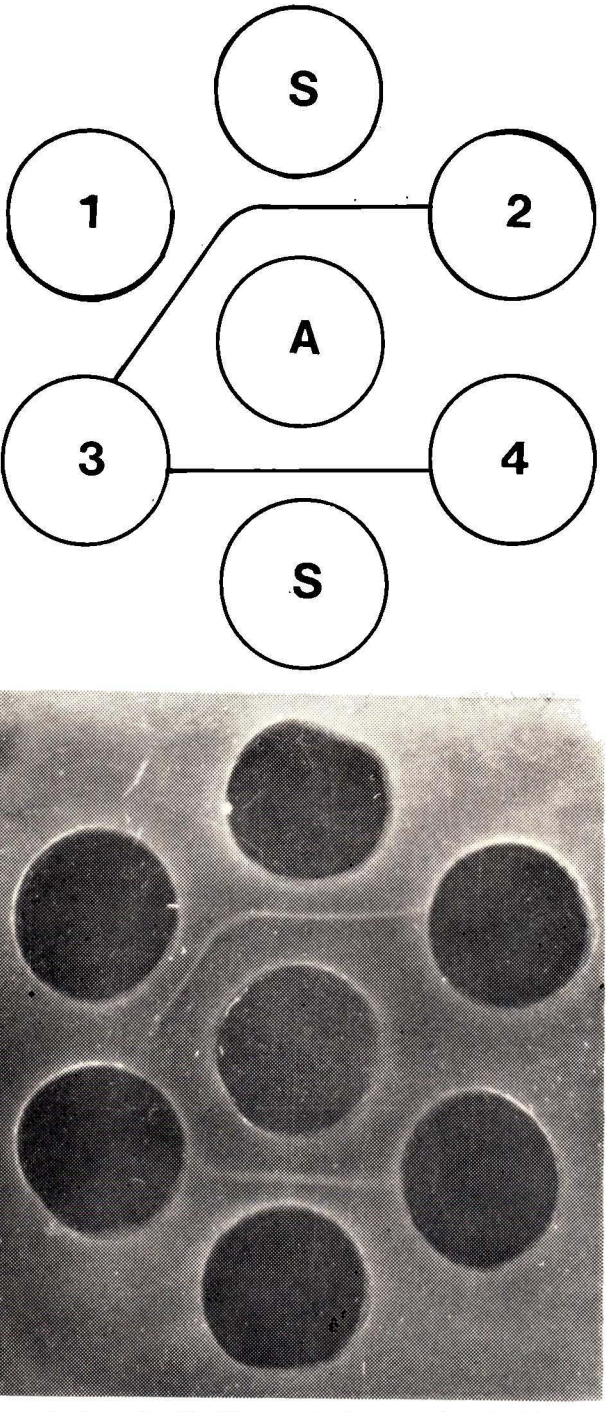

Fig.1. A = Antigen S = Positive control serum 1 = Positive sample 2,3,4 = Negative samples

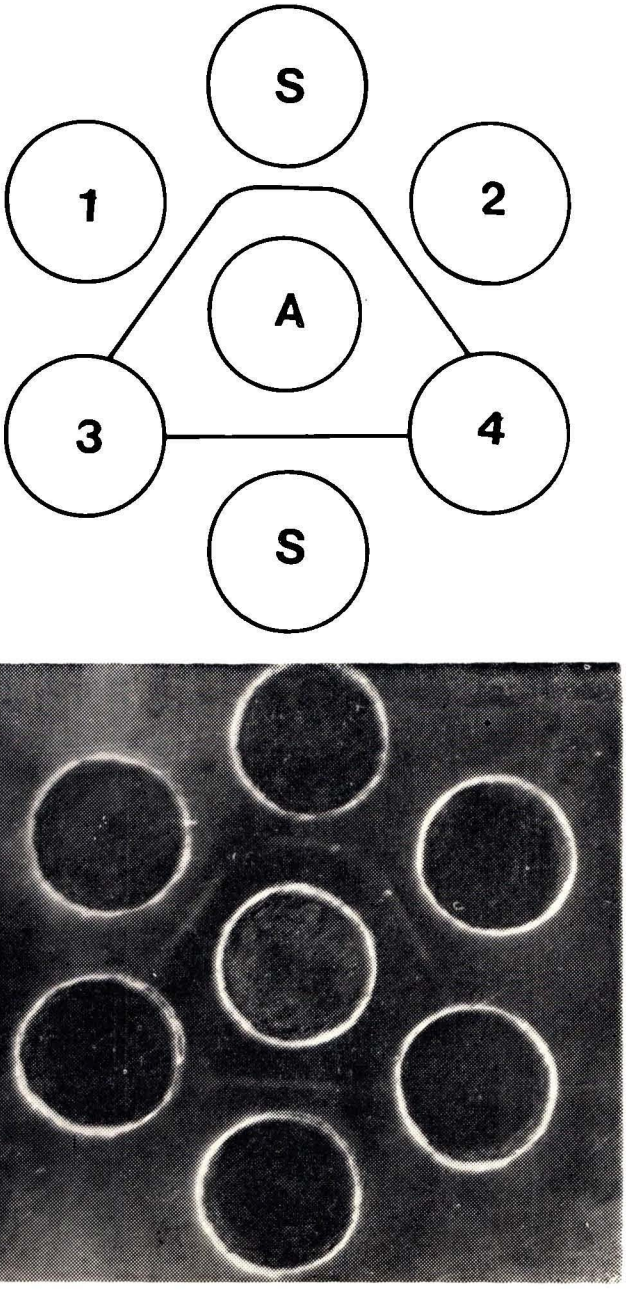

Fig.2. A=Antigen S=Positive conntrol serum 1,2=Positive samples 3,4=Negative samples