

EXPERIMENTAL TRANSMISSION OF AFRICAN HORSE-SICKNESS BY MEANS OF MOSQUITOES (*)

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SUMMARY

African horse-sickness was transmitted by means of the bites of *Anopheles stephensi* and *Culex pipiens* which had engorged infected horse blood 15 to 22 days previously. Febrile response (40.4 to 40.5 C.) occurred approximately 16 days after the 2 horses used were bitten by infected mosquitoes. Death followed in 9 to 12 days. The cause of death was confirmed as African horse-sickness by postmortem findings and laboratory tests.

Introduction

It has long been known that African horse-sickness is not directly contagious. Many species of arthropods have been suspected as vectors of the disease because horse-sickness is usually most prevalent during warm wet seasons and can be prevented by insect control.

As early as 1912, Schuberg and Kuhn⁹ reported that they had transmitted horse-sickness mechanically by means of *Stomoxys calcitrans*. Williams¹¹ reported *Lyperosia minuta* as a possible transmitter. Van Saceghem¹⁰ suspected ticks and members of the order Diptera. Carpano¹ incriminated the dipterous genera, *Anopheles*, *Aedes*, *Phlebotomus*, and *Simulium* as possible carriers of African horse-sickness virus. The assertions of the latter 3 authors have been made without supporting experiment.

Nieschulz *et al.*,⁵ reported that *Aedes* mosquitoes were not significant vectors, as they could keep horse-sickness virus for only 1 week after experimental infec-

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tion. Furthermore extensive investigations by Nieschultz *et al.*⁶ on mosquitos as potential victors of horse-sickness virus turned out to be negative, and they concluded that mosquitoes were not vectors of horse-sickness.

Du Toit⁶ recovered African horse-sickness virus by inoculating horses with a suspension of *Culicoides* caught in the wild state. It has not been determined how long *Culicoides* may carry the virus or whether *Culicoides* may biologically transmit the disease from horse to horse. Since this report, various species of *Culicoides* have been widely believed to be the major vectors of African horse-sickness as summarized in the recent report by Maurer and McCully.³

In the present experiment, *Anopheles stephensi* Liston and one of the most common mosquitoes, *Culex pipiens*, were used for transmission experiments and horse-sickness was biologically transmitted to horses.

Materials and Methods

Mosquitoes.—*Culex pipiens* were caught in Teheran in April, 1963, and successive breedings were made at the insectarium of the Near East Animal Health Institute, Razi Institute. The 16th generation was used in the experiment.

Anopheles stephensi were also caught in Iran. The colony * had been maintained at the insectarium of the Institute of Parasitology and Malariology, Teheran University. Eight additional successive breedings were made before use in the experiment.

Horses.—Nonvaccinated horses, approximately 12 months old, were obtained from an isolated village in the mountainous area by the Caspian Sea. They were treated with appropriate anthelmintics and kept under close observation for 1 week before the experiment commenced.

Virus.—Horse 20 was infected with African horse-sickness virus by intravenously injecting the 5th mouse passage of Iranian strain 10/60.⁹ The inoculum was 6,300,000 LD50 in suckling mice. Blood obtained from the horse 1 day before it died of horse-sickness (2 wk. after the injection of virus) was defibrinated and stored at 4 C. This blood was used as the material for infecting mosquitoes.

Virus Isolation.—Demonstration of virus from the blood of infected horses and from the tissues of dead horses was carried out by injecting the materials into the brains of 5-week-old mice. Defibrinated blood specimens were diluted with an

* Part of the colony obtained through courtesy of Dr. Modifi, Institute of Parasitology, and Malariology, Teheran University, Teheran, Iran.

equal volume of saline solution. Tissues from infected horses were mixed with an equal volume of saline solution and ground in Ten Broeck tissue grinders. The tissue suspension was centrifuged at 2,000 r.p.m. for 10 minutes, and the supernatant fluids were used as inoculum. Each specimen was injected into 10 mice, each given 0.03 ml. of inoculum. The brains of infected mice in *extremis* were harvested and stored at -25 C.

Neutralization Tests—Antibody levels in blood collected from infected and control horses were determined before and after infection. All the collected serums were heated at 56 C. for 30 minutes and the antibody titers measured by the standard virus dilution method described previously ** The same techniques were employed for identification of the virus isolated, using mice instead of tissue culture in tubes.

Transmission Experiment.—The defibrinated blood of infected horse 20 was obtained on Feb. 25, 1964 (13 days postinoculation).

Female mosquitoes of each of the 2 species were allotted to 2 groups, A and B; the 4 groups of mosquitoes were kept in separate cages and fed with infected blood on different days (Table 1). Before being fed the blood, all the mos-

TABLE 1—Timetable of Successful Transmission Experiments

Mosquito group		Date fed with infected blood	Date fed on normal horses	Incubation period of virus in mosquitoes* (days)	No. of mosquitoes engorged/No. placed on horses	Horse No.
<i>Anopheles stephensi</i>	(A)	March 1-2	March 18-19	15-18	14/30	TE-3
<i>Anopheles stephensi</i>	(B)	March 1-3				
<i>Culex pipiens</i>	(A)	Feb. 26-27	March 18-19	15-22	2/19	TE-6
<i>Culex pipiens</i>	(B)	March 2-3				

* Minimum and maximum intervals between the 1st mosquito feeding with infected horse blood and the 2nd feeding on the normal horses.

quitoes were starved for more than 8 hours by removing the water and 5% glucose solution from the cages. Then, group A of each species was fed with undiluted infected blood and group B with the blood diluted in an equal volume of physiologic saline solution. To feed the mosquitoes, cotton wool placed in small petri dishes was saturated by adding 12 ml. of either the diluted (B) or undiluted (A) infected horse blood. One petri dish was placed in each cage overnight for about 16 hours, after which the engorged mosquitoes were transferred to separate cages.

** Hazrati, A., and Ozawa, Y.; Serological Studies of African Horse-Sickness Virus—With Emphasis on Neutralization Test in Tissue Culture: Unpublished data, 1964.

These engorged mosquitoes were kept alive by feeding every day with fresh water and 5% glucose solution. They were also fed calf serum twice a week.

The temperature range of the insectarium was 23.0 to 26.5 C., and the range of relative humidity was 62 to 78%.

All horses were maintained in insect-proof rooms throughout the experiment.

At the end of an incubation period of 15 to 22 days, mosquitoes of group A and B of the same species were pooled and placed on a healthy horse by the following method: The mosquitoes were placed in a cage, 15.0 by 8.5 by 7.3 cm., made with a strong metal frame and fabric (organdy). The bottom of the cage was closely attached to the shaved skin of the horse's back. This small cage was covered with another stronger cage of metal with wire mesh. These cages were tightly fixed to horses with belts and springs for about 16 hours. During this period, the cages were covered with thick cloth to keep out the cold.

Two additional horses were placed in the same stable during the experiment. One of them was actually exposed to infected mosquitoes, *C. pipiens*, but none of the mosquitoes engorged the horse's blood. The other horse was kept without any treatment.

Body temperatures of all horses were recorded twice daily throughout the experiment.

Results

African horse-sickness virus was in the defibrinated blood of horse 20 used to feed mosquitoes. The virus was identified as the Asia type virus by serum neutralization tests.

Virus was also found in the blood of horses TE-6, collected when they had fevers above 40 C. and identified as the same Asia type of African horse-sickness virus by neutralization tests in mice.

The course of the disease in infected horses was followed by recording their body temperatures and any abnormal signs. Horses 20, TE-6, and TE-3 had similar temperature responses (Fig. 1), except that the temperature rise appeared severer

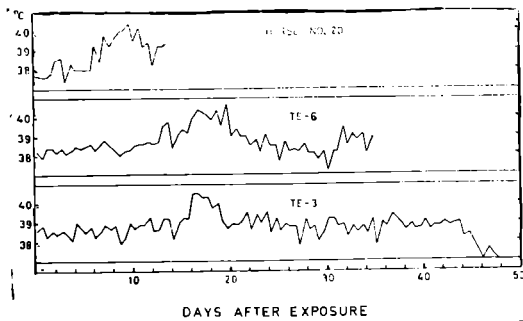


Fig. 1—Postexposure temperatures (C.) of horses 20, TE-6, and TE-3.

in the inoculated horse 20 than in those horses infected by mosquito bites. Also, the disease was much more protracted in the horses infected by mosquito bites, and they lost a considerable amount of weight during the course of the disease. During the last 2 days before death, these 3 horses could not remain standing. The other 2 horses, TE-1 and TE-5, which were not inoculated and on which no

mosquito engorged blood, did not have signs of disease, and their body temperatures remained between 37 and 39 C. throughout the experiment.

Antibody responses were investigated in infected and control horses. Serums were collected from these horses just before infection and 24 days after infection. The results summarized (Table 2) indicate that no significant amount of antibody against Asia type horse-sickness virus had been produced in the infected horses.

TABLE 2—Tests of Antibody Levels in the Serums of the Control and Infected Horses Before and 24 Days After Infection.

Horse No.	Preinfection	Postinfection	NI
TE-1	106.3*	106.3	0
TE-5	106.2	106.2	0
TE-3	106.5	106.2	0.3
TE-6	106.2	105.5	0.7

* Titers of virus-serum mixtures (TCID₅₀/ml. of the mixture).

The postmortem examinations of the 3 dead horses were made with Dr. Sohrab, who had conducted numerous necropsies on animals affected with African horse-sickness in Iran. The major gross lesions are summarized.

Horse 20.—There was subcutaneous yellow gelatinous infiltrate around the eyes and ears and in the neck, especially around the site of injection over the shoulder, and it was pronounced along the jugular furrow. The pericardial sac contained an excess of reddish yellow pericardial fluid. The fat around the base of the heart was orange-yellow and gelatinous, and there were numerous petechiae on the heart surface. On section, hemorrhages were found in the myocardium. There were small focal areas of subendocardial hemorrhage. The valves were thick and edematous. One of the lungs was slightly swollen and heavier than the other. On cross section, the lung exuded a small amount of frothy serous fluid. The frothy fluid in the lungs extended into the trachea. The gastric mucosa was hemorrhagic and acutely inflamed. The fat covering the kidneys was edematous. On cross section, the corticomedullary area was extensively congested.

Horse TE-6.—Yellow, gelatinous, edematous infiltrate was mostly confined to the ventral muscles and hindlegs. The yellow gelatinous infiltrate in the ventral muscles was most pronounced in an area below the site where the mosquito cage was placed. Acute hemorrhage and necrotic inflammation were found in the intestine. The fat covering the kidney nearest the site of mosquito bites was gelatinous, and the corticomedullary area was severely congested. The changes in the heart and lungs were similar to those in horse 20, except the lungs were more

edematous and filled with frothy fluid, and some yellowish serous fluid was in the bronchioles.

Horse TE-3.—Yellow gelatinous infiltrate was not particularly pronounced at the site of the mosquito bites but occurred mostly in the ventral muscles and legs. The eyelids also were edematous and a slight amount of yellow gelatinous infiltrate occurred around the eyes, in the region of the trachea, and along the lumbar vertebra. The lungs contained less frothy fluid than those in the other horses, but the other horses, but the interlobular septums stood out rather prominently in some parts of the lungs. There was white froth in the bronchial tubes. There were few spots of subendocardial hemorrhage in the heart. Other changes were generally the same as in the other 2 horses.

These 3 horses are considered to have died of African horse-sickness.

Discussion

Horses TE-3 and TE-6 died of African horse-sickness as determined by clinical signs, postmortem changes, and virus recovery.

That the disease was transmitted by the infected mosquitoes is indicated by: (1) Colony-bred mosquitoes were used to avoid presence of virus in the insects prior to the experiment, (2) the stable in which the horses were maintained provided strict insect-proof isolation, (3) the characteristic pathologic changes of horse-sickness developed in the infected horses, and (4) the intervals between the time of mosquito bites and the 1st temperature responses are within a reasonable period of incubation to indicate that the disease was transmitted by the mosquito bites.

The incubation periods of disease in the 2 horses infected by mosquito bites were longer than those in artificially infected horse 20, possibly because of individual differences in susceptibility and also because of a difference in the amount of virus introduced. The latter may also explain the differences in the course of the disease.

Gelatinous infiltration of the fat was most prevalent in the hindquarters of infected horses and close to the sites of mosquito bites. The experiment also indicates that 1 or 2 mosquito bites are sufficient to transmit the disease.

It is known that certain mosquitoes which are capable of transmitting disease do not always transmit the disease even after engorging infective materials. A delicate relationship exists between the viral agent and the mosquito as intermediate host, and the viral agent will multiply in mosquitoes only if its requirements are met. The amount of virus engorged and the ambient temperature and humidity are critical during the survival period of the infected mosquitoes. This may account

for negative results previously obtained by other workers utilizing mosquitoes.⁶ As in the case with Japanese B encephalitis virus,⁴ many transmission experiments were unsuccessful before positive results were obtained with the same species of mosquito.

According to Nieschulz and Du Toit's experiment,⁶ the virus administered to mosquitoes was detectable only for the first 7 days. This is one reason why an interval of more than 15 days was chosen before permitting the infected mosquitoes to feed on normal horses in the present experiment.

Several factors remain to be clarified as a result of this transmission of African horse-sickness by mosquitoes. It appears that: (1) The mosquitoes acted as biologic hosts rather than mechanical transmitters; (2) virus ingested by the mosquitoes would have been either inactivated or excreted together with the water and glucose solution taken daily for more than 2 weeks, thus eliminating residual virus from their salivary glands; and (3) African horse-sickness virus multiples in mosquitoes when they are kept under favorable conditions.

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