Epizootic eastern equine encephalitis in the Bragança region of Pará, Brazil*

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During the past several years reports have reached the Belém Virus Laboratory in March or April that horses in the Bragança region of the state of Para had died of Mal de roda or circling illness, following the 1st rains of the season. In order to be on the spot to attempt isolation of the causative agent or agents, an exploratory team was sent out in mid-January 1960. At this time the pastures were still denuded of grass, dry and dusty, there having been no appreciable rain in this area for several months. The common pasture on which the animals graze is a belt of natural grassland, between the wooded estuaries of the seacoast and the higher land of the terras firmes, much of it in cultivation or secondary growth, but with some climax forest still remaining. Because of the low elevation of the area, in seasons of heavy rainfall the land is poorly drained by the meandering streams, and the islands of wooded growth on the higher margins are often swampy in the center. At the end of the dry season the grass is parched, and the stream beds are dry, except for the rare deeper depressions that retain water. These have the appearance of the pans in Africa. Within a couple of weeks after the 1st rains in January or February the entire area is covered with lush grass. Concurrently with the sprouting grass, clouds of Aedes taeniorhynchus often appear in and around the margin of the

wooded areas. Searches for the larvae have failed to reveal the breeding foci. It is characteristic of this species to breed in brackish swamps and marshes. Following spring tides in the temperate zones and the 1st rains of the wet season in the tropics, these mosquitoes appear in enormous numbers and may invade the surrounding country to a distance of 10 to 20 miles. The clouds of mosquitoes observed in the Bragança pastures may have represented one such invasion from the coastal salt marshes. The ranchers associate the illness in the horses with the 1st appearance of grass rather than with mosquitoes that emerge at the same time.

On February 22 a messenger from one of the ranchers reported sick and dying horses near Tracuateua. Seven field trips, involving a total of 43 days, were made to the Bragança region between February 23 and April 18. The expedition of February 23 to 24 was devoted to collecting material for virus isolation from the sick and sacrificed horse at two fazendas, obtaining sera from well animals for antibody studies, and catching mosquitoes in the bushes near the sick and dead animals. Upon arrival at the Fazenda São Francisco, which had notified the Laboratory, it was learned that horses had been dying for two weeks in the region to the east, but that the majority of cases at the farm had occurred during the three days prior to the arrival of the investigators. Showers during the previous two or three weeks had promoted the growth of grass, especially in the low areas. The pastures were littered with sick and dying animals, while black clouds of vultures circled overhead. There were an estimated 500 or more horses in the area with about five percent fatalities. In addition to the horses there were 700 to 1,000 cattle grazing on the common pastures, and a few sheep, pigs, chickens, and dogs were present in the region, but none of these were known to become ill. One of the ranchers volunteered the information that when his horses were dying the only other deaths observed were in birds. He had found several Anu (Crotophaga ani) and a swallow dead near his corral. Human encephalitis was neither seen nor reported, although the one year old son of the manager at the Fazenda was said to have just recovered from a two weeks’ febrile illness.
At the *Fazenda São Francisco* three of the sick horses examined were on their sides, unable to rise and demonstrating purposeless movements of the legs, and a 4th was staggering. No horses were seen circling, although this sign is said to have been present in other animals. There was no glanular enlargement or other physical abnormality and no evidence of bites by vampire bats. These horses had been ill for from one to three days and some of the stricken ones were reported to die within three days after showing illness. Rectal temperatures of sick horses were all above normal. However, most animals were lying in the midday sun.

Autopsies performed on five horses at this ranch revealed generally normal organs, excepting for brain and meninges. One horse, two hours post-mortem, had petechiae in the pericardium and degenerative changes of liver and heart (possibly terminal ante-mortem changes). Brains of all animals, three dead and two sacrificed, revealed marked edema and swelling, vascular congestion, and petechial hemorrhages throughout. Direct examination of blood for Trypanosomiasis was negative. Peritoneal worms were present in most of the animals.

Field inoculation of baby mice was done with sera, spinal fluid, and brain material of autopsied animals. In addition, nine animals (one sick, others well) were bled, and an aliquot transported on wet ice for inoculation in Belém. Autopsy material was preserved in formalin and in glycerin for later study.

Similar investigation was made at *Fazenda Sta. Teresa* where material was obtained from six sick and sacrificed horses.

The clinical picture of CNS disease, autopsy finding of cerebral edema and petechiae, and the fact that only young horses were involved pointed to Equine Encephalitis virus as the cause of the epizootic. The abundance of mosquitoes lent added evidence to this presumptive diagnosis.
The 2nd expedition, on February 29 to March 1, was limited to collection of mosquitoes and birds for attempted virus isolation.

The 3rd expedition, on March 3 and 4, was directed principally toward obtaining material from horses in an early phase of illness. *Fazenda Piramucal* reported that none of their animals were sick. However, when tested in the morning, rectal temperatures of several were above normal and these animals were bled for virus isolation. One of them, a colt six months of age, died four days later of encephalitis.

A return visit to the *Fazenda São Francisco* at this time revealed no new cases of illness among the horses. Birds and mosquitoes were again collected here for virus isolation. Sera were obtained from 23 men who had been long-time residents of the area and who were exposed with sick horses to mosquitoes in the pastures.

A 4th expedition, on March 7 to 9 was made to areas previously visited and to neighboring *fazendas*. The epizootic now was definitely on the wane as indicated by the scarcity of sick horses, although mosquitoes were still fairly abundant. Mosquitoes, birds, and sera from horses were collected.

No sick animals were encountered after this 4th expedition. At this time some pastures already were flooded by the heavy rains, and the crossing of them was impossible even by jeep. Until and including this trip, mosquitoes came to human bait in great numbers at stations in the upland bushy growth. They appeared to be scarce on the open pasture, at least during the daylight hours when captures were made. Collection of mosquitoes, birds, and wild animals was continued until April 18, in three expeditions on March 12 to 16, March 19 to 22, and March 25 to April 18.
ISOLATION OF VIRUS

The virus of Eastern Equine Encephalitis (EEE) was isolated, by intracerebral injection of infant mice, from three of 27 pools composed of 1,308 Aedes taeniorhynchus, collected at Fazenda São Francisco on February 23 and March 4. The positive pools contained a total of 154 specimens. Identification of the virus was made by CF testing with hyperimmune mouse sera. The virus reacted with EEE and not with Venezuelan Equine Encephalitis or AR 10315 immune sera. None of the 75 pools containing 606 other Aedes, 17 Culex, 328 Sabethini and 46 miscellaneous mosquitoes yielded EEE virus. Virus was not isolated from the mosquitoes caught at Fazenda São Francisco after horses ceased to be ill. Blood taken from surviving horses at this Fazenda showed that they were all immune to EEE.

The virus of EEE was isolated from two of the sick horses at neighboring fazendas. One of these was a moribund six month old colt bled and sacrificed on February 24 at Fazenda Sta. Teresa. Blood, liver, spleen, kidney, brain cortex, and cerebellum were inoculated into infant mice, one group for each specimen, with negative results for EEE virus. However, on reinoculation of the original brain material preserved in glycerin in the field, EEE virus was isolated from this specimen. This animal had a high titer by HI for EEE antibodies in the serum obtained at to time of death.

The other isolation of EEE virus from a horse in this epizootic was obtained from the colt bled on March 4 at Fazenda Piramucal before signs of encephalitis were evident, and which died four days later. This animal had no HI antibodies for EEE at the time the sample was obtained. Serum was inoculated i. c. into infant mice at a dilution of 1-10, both in the field and on return to the Laboratory, 12 hours later, and EEE virus was obtained from both mouse groups.

An effort to isolate EEE from other local was unsuccessful. Totals of 215 birds and 64 wild animals were examined for virus with negative results.
A 2nd viral agent, Encephalomyocarditis (EMC) virus, was isolated from two horses during this epizootic. A moribund six month old colt was sacrificed, and blood, liver, spleen, kidney, brain cortex and cerebellum were inoculated into infant mice. EMC virus was obtained from mice inoculated with the liver and cerebellum. Reinoculation of cerebellum preserved in glycerine yielded isolations of both EEE and EMC virus. EMC was isolated also from two year old colt.

Trypanosoma equinum was demonstrated in one of the sick horses at Fazenda Pery. This animal had a temperature of 103°F. at the time blood was taken. Injection of serum i. c. into infant mice produced fatal infection in all mice inoculated both in the field and in the Laboratory and trypanosomes were demonstrated by darkfield and by Giemsa stain in blood and peritoneal fluid of these mice. The serum of this horse contained antibodies for EEE and EMC viruses. Trypanosomiasis is a serious illness of horses on Marajó island and might have been responsible for some of the illness in the epizootic at Bragança.

SEROLOGICAL TESTING

The sera collected in the Bragança area from man, horse, cow, bird, opossum, rat, sloth, lizard, and frog were tested for HI antibodies to group A viruses isolated in the Belém region. These are EEE, VEE, Mayaro and two new group A viruses, AR 10315 and AR 13136.

Thirty eight of 25 horse sera examined, or sixty nine percent, had EEE antibody. Six of the horses had VEE antibody in high titer. Antibody for AR 10315, AR 13136 or Mayaro was detectable in some horses in low titer but its significance can no be assessed.

Sera of two of the cattlemen tested had EEE antibody. The 23 men examined had been in contact with sick horses as well as mosquitoes in the same area where a high rate of mosquito infection was demonstrated. It is evident that as far as HI antibodies can show 21 of these men are fully susceptible. The blood was collected after the
epidemic had essentially ceased in horses and the mosquito population was waning.

Three of the 19 *Jacana jacana*, the commonly encountered water bird in the area, were found to have EEE antibodies. Also tested were a few swallows and doves which were negative.

Cows which graze with the horses, and also enter the bushes with them were negative and must be assumed to be insusceptible to EEE.

One among 20 opossums had EEE antibody, while all of the six rodents, one sloth, three lizards and two frogs examined were negative for EEE antibody by HI testing.

SUMÁRIO

Uma epizootia de vírus EEE, entre cavalos nas pastagens de Bragança foi estudada em 1960. O vírus foi isolado do *Aedes taeniorhynchus* de cavalos jovens. Outros animais domésticos, pássaros e animais silvestres foram negativos para o vírus. Anticorpos hemaglutinantes para EEE estavam presentes em cavalos (69%), pássaros (10%), homem (9%) e mucura (5%)

*Trypanosoma equinum* foi isolado de um dos cavalos doentes e vírus de Encephalomyocarditis foi obtido de dois outros.

SUMMARY

An epizootic of EEE virus among horses on the pastures of Bragança was studied in 1960. The virus was isolated from *Aedes taeniorhynchus* and from young horses. Other domestic animals, birds, and beasts were negative for virus. HI antibodies for EEE were present in horses (69%), birds (10%), man (9%), and opossum (5%)

*Trypanosoma equinum* was isolated from one of the sick horses, and Encephalomyocarditis virus was obtained from two others.