

EASTERN EQUINE ENCEPHALOMYELITIS IN PANAMA: THE EPIDEMIOLOGY OF THE 1973 EPIZOOTIC*

WILLIAM H. DIETZ, JR.,† PEDRO GALINDO, AND KARL M. JOHNSON‡

Gorgas Memorial Institute—Middle America Research Unit, Box 2016, Balboa Heights, Canal Zone

Abstract. In late June 1973, a small outbreak of equine encephalitis caused by eastern equine encephalomyelitis (EEE) virus occurred in the Republic of Panama. At least 100 horses were affected by the disease and 40 died. More than 1,700 human sera were obtained from areas of virus activity but no serological evidence for infection was found. Four isolates of EEE virus were recovered, one of which was from a small pool of *Culex taeniopus* mosquitoes. Serologic studies of infected horses and classification by the short incubation hemagglutination-inhibition test revealed that these isolates were South American strains. Our evidence suggests that another agent virulent for horses was active during this outbreak.

Eastern equine encephalomyelitis (EEE) virus infection was first recognized in Panama in 1936;¹ subsequent recognized outbreaks of the disease have occurred sporadically, most recently in 1958 and 1962 (Murnane, T. G., Galindo, P., Craighead, J. E., Rodaniche, E., Johnson, C., and Shelokov, A., unpublished data). These epizootics have had a common pattern: they involved small numbers of horses, usually occurred in early rainy season (June–July), started and ended suddenly, were recognized late, and were of short duration. As a consequence, little is known about the epidemiology of this infection in Panama.

The chance observation of three dead horses in a small area to the northeast of the city of Panama and the isolation of EEE virus from the brain of one of these animals allowed us to recognize this epizootic at its outset, and permitted a more thorough investigation of its epidemiology.

MATERIALS AND METHODS

Sera and tissue suspensions were tested for virus by intracerebral inoculation of suckling mice (0.02 cc/mouse). Ten percent suspensions of tissue were prepared in Tenbroeck grinders, using phos-

phate buffered saline (pH 7.2) containing 10% fetal calf serum as diluent. Suspensions were clarified at 2,000 × g in a refrigerated centrifuge for 30 min. Titers, calculated by the method of Reed and Muench,² were based on the number of deaths that had occurred by 3 days. Human sera were tested in suckling mice, duplicate tubes (0.1 cc/tube) of African green monkey kidney (Vero) and human diploid fibroblast (WI-38) cell cultures. After adsorption for 1 hour, inocula were washed from cultures and fresh medium containing 2% heated fetal calf serum was added. Cell cultures and mice were observed for 2 weeks. Mosquitoes were triturated in Tenbroeck grinders, using 3 cc of Sorensen's buffer (pH 7.6) containing antibiotics and 25% normal rabbit serum as diluent. The resulting suspension was clarified by centrifugation, and the supernatant was inoculated into suckling mice and duplicate tubes of Vero cell cultures as described.

When deaths occurred in suckling mice, 10% suspensions of suckling mouse brain antigens were prepared and typed by complement-fixation (CF) tests,³ using group- and virus-specific mouse ascitic fluid reagents. The identification of each isolate was reconfirmed by means of a plaque reduction test performed with the same antisera in 96-well Vero panels. For the latter test, tissue culture isolates or tissue passaged virus was used directly. Each isolate was reisolated in suckling mice.

Serum samples were tested for the presence of hemagglutination-inhibition (HI) antibodies by a micromethod,⁴ using a beta-propiolactone treated⁵ South American strain of EEE as antigen. Many sera were also tested for N antibody by a previously described micromethod.⁶ Antibody ti-

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* Address requests for reprints to: Dr. Karl M. Johnson, Center for Disease Control, Atlanta, Georgia 30333.

† Present address: Clinical Research Center, Massachusetts Institute of Technology, 50 Ames Street, Cambridge, Massachusetts 02142.

‡ Present address: Center for Disease Control, Atlanta, Georgia 30333.

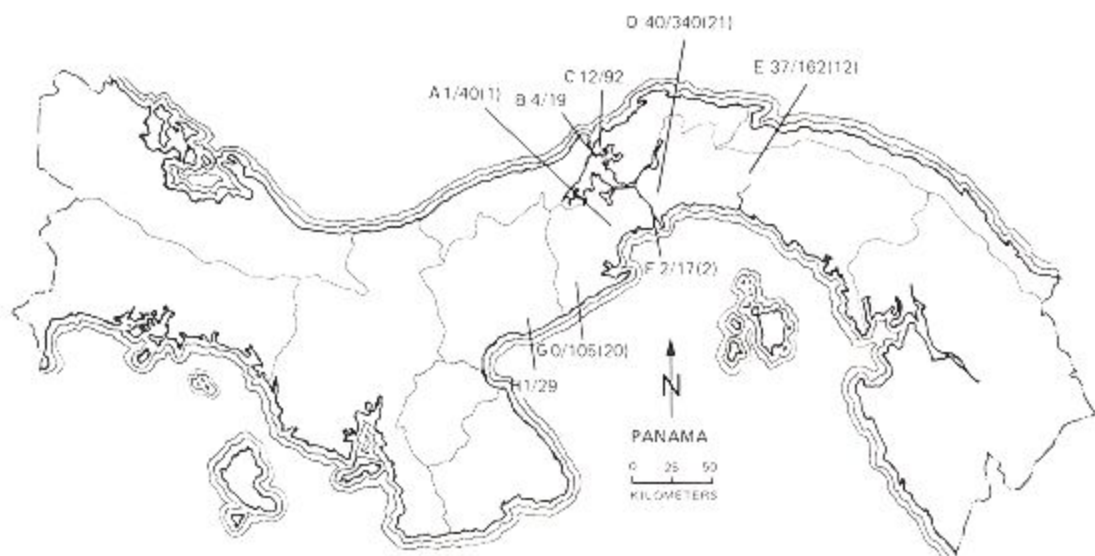


FIGURE 1. Map of Panama showing the regions studied and the number of animals affected during the 1973 Eastern equine encephalomyelitis virus epizootic. Regions are indicated by letters: A: Chorrera; B: Canal Zone, Pacific Side; C: Colon; D: Panama City, racetrack, and Club de Equitacion; E: Chepo; F: Canal Zone, Atlantic Side; G: Capira and Ollas Arriba; H: Aguadulce. Fraction indicates number of animals infected/number tested. Parenthesis shows number of horse deaths in each area.

ter was defined as the dilution of serum giving 80% reduction of 30–150 plaque-forming units of virus. Both North and South American strains of EEE virus were incubated under gum tragacanth⁷ for 24 hours prior to the second overlay. St. Louis encephalitis virus (SLE) and California virus required 48 hours of incubation under agar prior to the overlay containing neutral red.

The prototype strain of North American EEE virus was horse brain isolate 64A 11 provided in 1965 by Dr. Martin Goldfield of the New Jersey Department of Health. This strain had been passed twice in chicks and twice in suckling mice in our laboratory. The prototype strain of South American EEE virus was a horse brain isolate from Panama obtained by Dr. Thomas Murnane in 1958. This strain had been passed seven times in suckling mice.

Data comparing rates of infection or seroconversion to EEE virus were tested for significance by Chi-square, using a 2×2 contingency table.

Description of the outbreak and study design

The outbreak was first recognized in mid-June 1973, when one of the authors observed three dead horses in a relatively small geographic area

to the northeast of the city of Panama. After EEE virus was isolated from the brain of one of these animals, the Ministry of Health was notified and an intensive surveillance program was initiated. When horse illnesses or deaths were reported, blood was collected from horses and humans living in or adjacent to the area.

During the 6-week period from mid-June to mid-July, we received reports of approximately 60 horse deaths. Most of these cases were in the area to the east of the capital (Fig. 1). This area is sparsely populated rolling savannah with gallery forests. Numerous rivers flow out of the mountains into the lowlands; during heavy rains, these rivers often overflow their channels, creating large numbers of ox-bow lakes and fresh water swamps. Near the Pacific Ocean, there is significant tidal flow, and the banks are lined with mangroves.

Reports of horse deaths occurred for the last 3 weeks of June and ceased prior to the last week in July, shortly before an equine vaccination campaign was begun using a combined formalin inactivated EEE-Western equine encephalitis (WEE) virus vaccine (Fort Dodge, Lot #17013A).

Sera were collected from 803 horses in areas where equine death or encephalitis had occurred. Prior to this epizootic, EEE virus vaccination was

not practiced routinely in the Republic of Panama, but vaccination histories were always sought. An additional group of 68 horses was bled before and 1 month after vaccination with two intradermal injections of 1 cc of the EEE-WEE virus vaccine.

Efforts were made to collect brain tissue and serum for virus isolation from all dead equines, and serum from those with symptoms. Brain tissue from 16 animals was available for study. Sera for virus isolation were collected from a sample of 35 pasturemates of sick animals.

Sera from over 500 chickens on farms in close proximity to areas of recognized EEE activity in horses were collected for serologic investigation. Because dead and dying Cormorants (*Phalacrocorax olivaceus*) were observed throughout the epizootic area during the outbreak, four sick or recently expired Cormorants were collected from Pacheca, an island seabird rookery located in the Perlas Islands approximately 25 miles off the southern coast of Panama. One additional ill cormorant was collected in the city of Panama. Serum, brain, and a pool of visceral organs (spleen, liver, kidney) from each bird was tested separately for virus. Sera from 25 fledgling Frigate Birds (*Fregata magnificens*) and 20 domestic ducks also were obtained from the Perlas Islands.

Single sera for antibody tests were obtained from over 1,700 persons living in or close to areas where equine encephalitis or death occurred. Acute sera, throat and rectal swabs, and occasionally urine and spinal fluids, were collected for virus isolation from all human cases of encephalitis or aseptic meningitis in either the Canal Zone or the Republic of Panama during the outbreak. Convalescent sera collected 2 weeks after the initial sera were available from most patients.

For 8 nights during the peak of the epizootic, mosquitoes were collected in Magoon stable traps baited with horses negative for neutralizing (N) antibodies to five group A arboviruses (EEE, WEE, Venezuelan Equine Encephalomyelitis (VEE), Una and Mayaro). Smaller numbers of mosquitoes were collected from a similar trap baited with chickens negative for HI antibodies to EEE virus. Both traps were set on a ranch where several horses had died of EEE virus infection. Mosquitoes were transported live to the laboratory where they were held at room temperature for 24-48 hours, then chilled, sorted, and frozen until they were processed for virus isolation.

RESULTS

Hemagglutination-inhibition antibody titers from 68 equines vaccinated 1 month previously against EEE virus indicated that less than 5% had EEE-HI titers equal to or greater than 1:80. One hundred of 803 animals sampled, most without a history of vaccination, had HI titers \geq 1:80 suggesting an attack rate of 12%. Although the prevalence of significant HI antibody titers (\geq 1:80) ranged from 3-23% in the districts shown (Fig. 1), it was unusual for more than several cases to occur on the same ranch, regardless of the number of horses present.

Approximately 60 horse deaths were reported. We attributed 36 of these deaths to EEE on the basis of virus isolation or high HI antibody in the serum of the infected animal or its pasturemates. Since 100 animals were infected, the case-fatality rate of this infection in the equine population of Panama was approximately 40%.

Horse deaths occurred in two areas where we were unable to find HI or N antibody to EEE virus in sick horses or stable or pasturemates of sick animals. At the Panama race track, three cases of equine encephalitis were observed, two of which were fatal. One animal had a sudden onset of hyperactivity similar to that occasionally observed in VEE virus infections. Histopathologic examination of the brain of one animal was consistent with the diagnosis of arboviral encephalitis. The second area in which horse deaths could not be explained by our criteria was in Ollas Arriba, a village located in an area of grassy savannah to the west of the Panama Canal (Fig. 1). In this town, approximately 20 horses died of an encephalitis-like illness within a 2-week period. No virus could be isolated from either the serum or brain tissue of one moribund animal that was killed. Histopathologic examination of the brain of this animal was negative for Negri bodies by light and fluorescent antibody microscopy. Serum from this case and over 100 additional animals from this town and two neighboring villages failed to reveal any HI antibody to EEE.

Testing of chickens was not fruitful; only four of the 426 chickens bled proved to have HI antibody to EEE, and these birds were all located on the same chicken farm. Testing of the Pacheca birds revealed high titers of N and HI antibody to EEE virus in two of 25 fledgling frigate bird sera. Hemagglutination-inhibition antibody was present in two of 15 domestic duck sera, but only

TABLE 1

Virus isolates from mosquitoes trapped during the 1973 eastern equine encephalomyelitis (EEE) virus outbreak in Panama

Locality	Species	Total number trapped	Number of pools	Virus isolate
Pacora	<i>Culex (Culex) nigripalpus</i>	1,228	16	St. Louis
	<i>Aedes taeniorhynchus</i>	882	13	—
	<i>Culex (Melanoconion) spp.</i>	799	14	—
	<i>Culex (Culex) declarator</i>	688	13	—
	<i>Aedes angustivittatus</i>	244	9	California-group
	<i>Culex taeniopus</i>	24	5	EEE
	Various (17)	1,172	78	—
Racetrack	<i>Culex (Culex) declarator</i>	878	13	—
	<i>Anopheles albimanus</i>	625	9	—
	<i>Aedes taeniorhynchus</i>	75	2	—
	Various (6)	157	10	—

one of the two sera contained N antibody. Sera from five Muscovy Ducks and five Cormorants contained no HI or N antibody to EEE virus.

Two of 1,700 human sera collected during the outbreak contained HI but no N antibody to EEE virus. In both sera, the HI antibody titer was low (1:10 and 1:20). No isolates of EEE virus were recovered from any of the clinical specimens from hospitalized patients, and none of their sera contained HI or N antibody to EEE virus.

Virus isolation

Eastern equine encephalomyelitis virus was isolated from brain tissue of three of 16 (19%) horses tested. Isolation attempts from the sera of 35 pasturemates of these horses were unsuccessful. Likewise, no isolates were obtained from the acute sera of any symptomatic animals.

The isolation results from mosquitoes are shown in Table 1. St. Louis encephalitis virus was recovered from a single pool containing 56 *Culex (C.) nigripalpus* mosquitoes. A California-group encephalitis virus was obtained from one pool of 25 *Aedes angustivittatus* and EEE virus was isolated from one pool of eight *Culex (M.) taeniopus* mosquitoes.

Three isolates of St. Louis encephalitis virus also were made in suckling mice from the tissue of three of the five sick or dead Cormorants tested. The virus was present in the brain and viscera of one Cormorant, serum and viscera of one Cormorant and serum only of one Cormorant.

No virus was recovered from any of the human material tested.

Serologic investigations for evidence of California and SLE virus infection

Screening of horses from the race track, Ollas Arriba, the birds from Pacheca, and the hospitalized patients for N antibody to SLE virus and the California-group virus isolate failed to reveal any serologic positives. Three human sera from hospitalized patients contained low titer N antibody to the California-group virus isolate and one of these also contained low titer CF antibody. No seroconversions or rising titers could be demonstrated in any human patient.

Serologic studies of equines

Since the capacity of the inactivated EEE virus vaccine to produce N antibody is unknown, N antibody responses to vaccination were studied in 68 horses from the race track and from a riding club. Seroconversion occurred in 75% of the vaccinated animals when tested against a North American strain of EEE virus, but in only 33% of the horses when tested against a South American strain. In both cases, the antibody titer was low. As Table 2 indicates, seroconversion to the North American strain of EEE virus occurred with a significantly greater frequency (Chi-square) in horses with pre-existing neutralizing antibody to Venezuelan equine encephalitis (VEE) virus.

Sera from 168 horses from the epizootic area were studied to determine whether prior VEE virus antibody interfered with EEE virus infection. Since only 4% of the horses in the vaccine study developed South American EEE virus neutraliz-

TABLE 2

Relationship of eastern equine encephalomyelitis (EEE) virus vaccination and infection to prior Venezuelan equine encephalomyelitis (VEE) virus antibody in Panamanian horses

Group	VEE antibody	Number with EEE Virus Antibody	
		North American	South American
Vaccinated	40 positive*	34† (85%)	15 (38%)
	28 negative	.05 > P > .01‡ P > .05	8 (28%)
Infected§	97 positive	Not tested	15 (15%) P > .05
	65 negative	Not tested	14 (22%)

* Includes only horses with 100% plaque reduction to VEE vaccine virus in lowest serum dilution.

† Numbers represent numbers of animals with antibody to indicated virus strain. Criterion for seroconversion to EEE strain was 80-100% plaque reduction in lowest serum dilution in vaccinated horses.

‡ P values determined by Chi-square using 2 x 2 contingency table.

§ Horses with neutralizing antibody titer \geq 1:256 to South American EEE were considered to have been infected.

ing antibody titers equal to or greater than 1:256, and since the vaccination history for many of these animals was unknown, only horses with N antibody equal to or greater than 1:256 were considered to have been infected. Only horses with 100% plaque reduction of the VEE vaccine virus (TC-83) in the lowest serum dilution were considered to have VEE virus antibody. As shown in Table 2, the prevalence of EEE virus infection was the same (Chi-square), regardless of the presence or absence of VEE antibody.

In a third investigation, sera from horses with high HI ($>$ 1:80) and N antibody titers ($>$ 1:256) to EEE virus were tested for their ability to distinguish South from North American strains of EEE virus by plaque neutralization. All of the horses included either had a history of recent encephalitis or were pasturemates of encephalitic animals. In every case, serum N antibody titers were reproducibly higher against the South American strain of EEE virus (Table 3). Such differences could not be demonstrated in the sera of animals with low titers ($<$ 1:256) of N antibody to the South American strain, nor was any animal found with titers \geq 1:256 to the North American strain. These data suggested that the EEE virus strain responsible for this epizootic was a South American strain. Classification by the short incubation hemagglutination-inhibition test (courtesy of Dr. Charles Calisher, CDC Vector-Borne Diseases Division, Fort Collins, Colorado) indi-

TABLE 3

Reciprocal serum neutralizing antibody titers* against North and South American strains of eastern equine encephalomyelitis (EEE) virus in 11 Panamanian horses infected with EEE virus

Horse number	EEE virus strain	
	North American	South American
1	16	1024
2	16	\geq 4095
3	64	1024
4	16	256
5	64	256
6	64	1024
7	64	1024
8	64	256
9	64	1024
10	64	256
11	64	256

* Titers based on 80% plaque reduction of 50-150 pfus.

cated that all four of our isolates from this outbreak were South American strains of EEE virus.

Finally, sera from nine animals with observed encephalitic symptoms were tested for N antibodies to VEE virus. Eight of the nine tested showed no evidence of VEE virus antibody.

DISCUSSION

All evidence indicates that EEE virus infection of equines and man is an end-stage phenomenon correlated to the primary cycle of natural virus amplification. Central to this concept are data from experimental¹⁶ and natural¹⁹ infection of equines which indicate that viremia rarely reaches the levels necessary for mosquito infection. High viremia also has not been found in human EEE virus infection.

These observations appear to explain the small number of mosquito isolates of EEE virus during previous epizootics in Panama, Brazil,^{10,11} and Trinidad,^{11,22} and may account for the low rates of equine disease observed in these outbreaks. The 1947 EEE equine epizootic in Louisiana in which an estimated 5,000 horses died is one of the few EEE virus outbreaks which does not follow this general pattern.^{13,14}

The epidemiology of EEE virus contrasts with that of the closely related VEE virus. High equine viremias,¹⁵ high infection rates of multiple mosquito species,¹⁶⁻¹⁸ and widespread human infections^{16,19,20} occur in VEE epizootics. Equine viremia appears to be the major determinant of the

amplification and dissemination of such outbreaks.²¹

Eastern equine encephalomyelitis virus infection of man has been rare in epizootics in Central and South America, whereas a low but significant incidence of human disease is seen frequently during similar outbreaks in North America. These differences may be attributable to the habits of the respective proven or presumptive arthropod vectors. *Aedes sollicitans*, which are frequently infected during North American epizootics, feed voraciously on humans and are diurnally active. *Culex (M.) taeniopus*, previously incriminated in EEE virus transmission in Trinidad,¹² Brazil,^{10,11} and Panama (P. Peralta, personal communication), and possibly one of the vectors of the 1973 outbreak, differs in its activity. Although this mosquito has a broad host range,²² it is largely sylvan, breeds in swamps, has a crepuscular activity cycle, and apparently does not enter houses.^{23,24} Despite the frequent EEE virus isolations made from this species, its behavioral characteristics favor its role as an enzootic vector.¹¹

The virus strain responsible for the 1973 epizootic belonged to the antigenic subgroup of South American EEE virus. All previous EEE isolates from Panama also have been South American strains²⁵ (P. Peralta, personal communication). The 1973 epizootic, as well as those in 1958 and 1962, occurred shortly after the annual south to north migration of northern birds. Whether these outbreaks resulted from the introduction of EEE virus by migrating birds, or whether South American EEE virus strains are enzootic in Panama, is not clear. However, it is of interest that several recent epizootics of EEE virus on islands in the Caribbean took place in the fall and were of the North American subtype (C. Calisher, personal communication), suggesting that the virus may have been introduced and amplified during the southward migration of northern birds.

The duration of the 1973 epizootic bore little relation to the vaccination program. Conversion rates and antibody titers following vaccination were low, especially to the South American EEE virus subtype. The level of N antibody required to protect equines from lethal infection is unknown, and appropriate challenge experiments have not been performed. Horse deaths had ended by the start of the vaccination program and, since horse infections have little to do with the main-

tenance of the epizootic, factors other than vaccination were probably responsible.

We also investigated the possibility that VEE virus antibody in animals that had been vaccinated or infected with enzootic VEE virus strains might have interfered with EEE virus infection or vaccination. Field observations during the VEE virus epizootic in the United States suggested that prior experience with WEE or EEE viruses had a significant effect on conversion rates to the VEE virus vaccine.²⁶ In addition, prior VEE virus infection may have interfered with subsequent experimental EEE virus infection in burros.²⁷ We were unable to demonstrate any adverse effect of VEE virus immunity on either conversion rates after vaccination or natural infection with EEE virus. Rates of seroconversion to North American strains of EEE virus were significantly greater in horses with VEE virus antibody. However, increased seroconversion rates in these animals may only represent a secondary response in animals previously infected or immunized with the antigenically-related VEE virus, or even with EEE virus itself. Although VEE virus antibody had no apparent effect on infection rates, our observation that eight of nine symptomatic animals lacked such antibody suggests that VEE virus antibody may affect the clinical course of South American EEE virus infection.

Our data from the Panama race track and from Ollas Arriba suggest that another agent virulent for horses was active during this epizootic. This hypothesis is supported by the following: 1) in other areas reporting equine illness or death, serologic investigation consistently revealed high titers of N or HI antibody in either symptomatic horses, pasturemates or both; no such evidence was obtained from these two localities despite significant numbers of horse deaths and adequate sampling of equines; 2) the absence of CF titers to WEE or VEE viruses suggests that infection with these viruses was unlikely; in addition, all the race horses had been vaccinated against VEE virus prior to the epizootic; 3) in 1973-1974, similar small outbreaks of equine encephalitis occurred in Guatemala and Salvador in which no serologic evidence could be obtained for VEE, WEE or EEE virus infection (W. H. Dietz, unpublished data); 4) the brain histopathology of the only race track horse available for study was consistent with arboviral encephalitis. Since it has been shown that SLE²⁸ and California^{29,30} viruses cause

only small viremias and no clinical disease after experimental inoculation of equines, it is unlikely that these agents were responsible for the deaths. Likewise, all four ill or moribund horses from these areas had neither CF nor N antibody to these agents.

Although at least 500 horse deaths were attributed to an EEE virus epizootic in 1947, most outbreaks in Panama have involved small numbers of animals and have been of short duration. The equine population in Panama in 1970 was greater than 170,000,²¹ and most of these animals were in rural areas. Since the current recommendations are that animals be revaccinated annually against EEE virus, the expense and logistical difficulties are considerable when compared to the potential loss of unvaccinated animals. In addition, the effectiveness and duration of immunity conferred by the North American EEE virus vaccine strain against infection with South American EEE virus is unknown. The absence of antibody to South American EEE virus in vaccinated animals, as well as the low titers of N antibody following vaccination, have been demonstrated in this study. These data emphasize the need for a single dose vaccine capable of producing documented, long-lasting immunity against both South American and North American strains of EEE virus.

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