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EASTERN EQUINE ENCEPHALITIS IN THE DOMINICAN REPUBLIC, 1978[1]

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An epizootic EEE outbreak, apparently of considerable proportions, occurred in the Dominican Republic in 1978. This article describes measures taken to investigate and control that event.

Introduction

Eastern equine encephalitis (EEE) occurs annually in the eastern United States, usually as sporadic equine cases or small scattered clusters of them, but also occasionally in the form of large epizootics involving both horses and humans (*1*). Even if no equine cases are evident, the virus is transmitted back and forth each summer between mosquitoes and birds in swampy habitats, especially along the Gulf and Atlantic seaboards and up the Mississippi River Valley. These swampy areas appear to be enzootic foci, from which the virus spreads to cause occasional equine epizootics (*2*). It is also true, however, that the virus has been isolated from Canada to Argentina and is widespread throughout the Hemisphere (*3-5*). EEE virus was responsible for outbreaks of eastern equine encephalitis in Cuba in 1969-1972 (*6*) and in Venezuela in 1976 (*7*).

In February 1978 clinical encephalitis cases of unknown etiology were reported in horses of two provinces (María Trinidad Sánchez and Samaná) in the Dominican Republic. Blood and brain specimens from equine animals were sent to the U.S. Department of Agriculture laboratory in Ames, Iowa, for diagnosis. Serologic test results and virus isolations from equine brain tissues indicated that EEE virus was involved in the outbreak. At the request of the Dominican Government, the Pan American Health Organization and the U.S. Center for Disease Control assisted with laboratory and epizoologic investigation of the outbreak. This article reports the results of those investigations.

Briefly, serologic evidence of the virus and viral isolations were obtained, but no epizootic vector was identified. A combined vaccination and vector control campaign appeared to successfully abort the outbreak. These findings are discussed in terms of the enzootic nature of EEE virus in the Dominican Republic and the significance of this outbreak with respect to other parts of the Americas.

Materials and Methods

The Area

As Figure 1 shows, the Dominican Republic occupies the eastern two-thirds of the Island of Hispaniola, has an area of

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Figure 1. Location of the Dominican Republic in relation to other countries and territories of the Caribbean area.

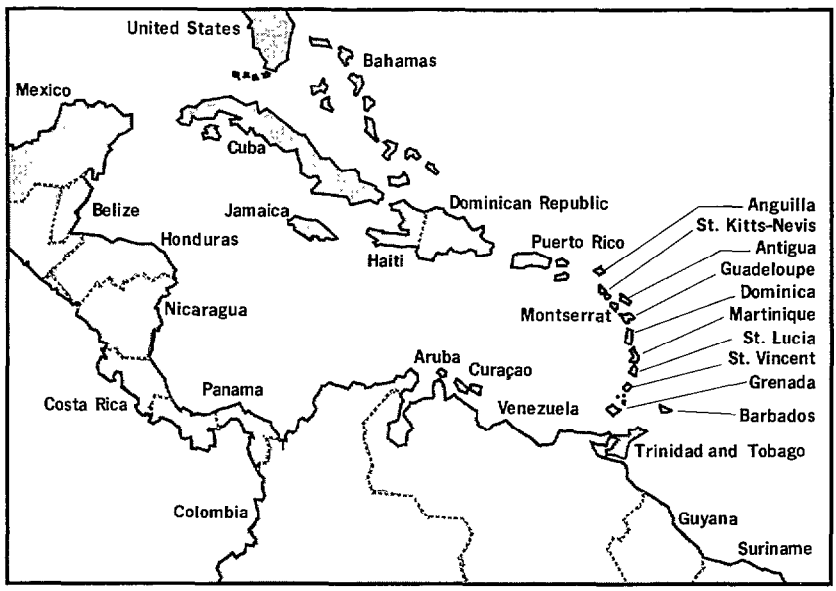
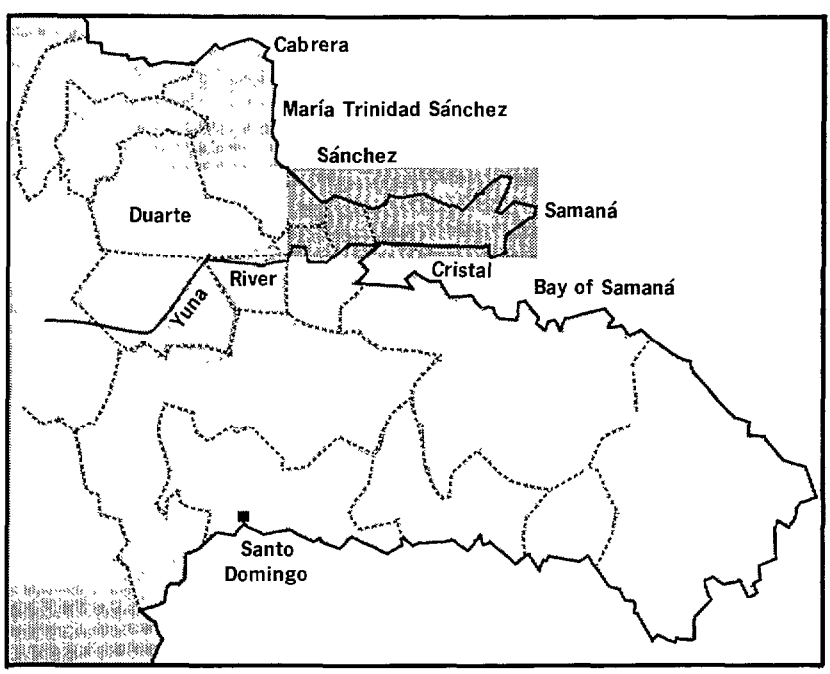


Figure 2. A map of the Dominican Republic showing localities involved in the 1978 EEE outbreak. Shading shows the area that was quarantined.



48,734 km^2, and is situated about 960 km southeast of Florida, between 17° and 20° north latitude. The climate is tropical, with rainfall averaging 150 to 200 cm per year in the northern coastal regions; the southern part of the country is drier.

On the Samaná Peninsula (Figure 2), where the outbreak occurred, 138 cm of rainfall were recorded in 1976 and 229 cm in 1977. While the heaviest rains ordinarily occur from July to October, 57 cm fell in April and May of 1977, more than three times the total recorded during the same months of 1976. The mean monthly temperature on the peninsula was 78.4°F (25.8°C) in 1976 and 79.2°F (26.2°C) in 1977, but the first four months of 1977 averaged 2.9°F higher than the same months in 1976. Approximately 5 million people live in the Dominican Republic, and most earn their living from agriculture. Sugar cane, coffee, and cacao beans are the principal cash crops. Rice is grown throughout the country; coconut plantations are common in the northeast.

The Samaná Peninsula is a continuation of the northern mountain range (*Cordillera Septentrional*) that parallels the north coast for 200 km. The central portion of the peninsula is hilly, with elevations of more than 500 m. The Gran Estero Swamp, about 15 km wide, interrupts the cordillera at the base of the peninsula and extends from the Atlantic Ocean on the north to Samaná Bay on the southeast.

Soon after the presence of EEE virus was clinically confirmed, a vaccination campaign was initiated in the affected area of the Samaná Peninsula. By 1 March 1978 approximately 20,000 doses of bivalent EEE-western equine encephalitis (WEE) vaccine (Encephalomyelitis Vaccine,[8] Colorado Serum Co., Denver, Colorado 80215, U.S.A.) had been received and vaccination had begun.

The vaccine was diluted in the field and administered in 1-ml intradermal doses by teams of two to four trained individuals from the Livestock Division of the Dominican Ministry of Agriculture. When first vaccinated, each equine animal was marked on the left foreleg with a horizontal brand easily visible from a reasonable distance. After a three-week interval a second dose was given and the animal was again marked, this time with a vertical brand passing through the first to form a cross. Overall, it appears that approximately 8,000 animals received two doses of vaccine and another 4,000 received one dose.

Cristal, a settlement where we found and sacrificed a sick horse, lies southeast of several thousand hectares of rice fields. It is located on the banks of the Barracote River and north of Los Haitises, a series of forested limestone cone karst formations. The area around Cristal contains pastureland for horses.

No human deaths were attributed to infection with EEE virus. No patients with encephalitis or aseptic meningitis were seen in local hospitals while the work reported here was being done.

View of the settlement Cristal, where a sick horse was found and sacrificed.

[8]Use of trade names is for identification only and does not constitute endorsement by the U.S. Public Health Service or the Department of Health, Education, and Welfare.

Serosurvey, Virus Isolation, and Virus Identification Techniques

A serosurvey was conducted in the affected area during the last week of March and first week of April 1978. Two hundred eighty-eight humans and 370 equine animals were bled. Whole bloods were allowed to clot at ambient temperatures (approximately 27°C). They were then centrifuged, and the resulting sera were separated, vialed, labeled, and held at 4°C until shipment on dry ice to Fort Collins, Colorado.

In addition, both brain and blood specimens were obtained from a sick horse at Cristal. This horse was found prostrate and obviously moribund in the early evening of 3 April, and was sacrificed at that time.

At Fort Collins, the sera were inactivated at 56°C for 30 minutes and tested by a serum-dilution, plaque-reduction neutralization (N) technique in accord with previously described methods (*8*). All the sera were screened at a single 1:10 dilution in primary duck embryo cells against the following prototype viruses: EEE virus, strain NJO; WEE virus, strain Fleming; Venezuelan equine encephalitis (VEE) virus, strain TC-83; and St. Louis encephalitis (SLE) virus, strain TBH-28. All sera that caused 90 per cent or greater reduction of approximately 100 plaque-forming units (pfu), as compared to the controls, were considered positive. Sera positive in N tests were also tested by complement-fixation (CF), using a microtiter method (*9*).

Virus isolation was attempted by intracerebral inoculation of suckling mice 2 to 4 days old with 0.02 ml of suspensions of the following: macerated mosquitoes (*10*), clarified 10 per cent V/V suspension of brain sections, undiluted serum, or whole blood. The mice were examined daily for signs of illness; those obviously ill were collected for further viral passage as well as identification and production of seed viruses for use in N tests. Viruses were first identified by means of CF tests (*11*), and the identification was then confirmed with N tests in cell cultures employing 100 pfu and reference immune reagents for controls. Short-incubation hemagglutination-inhibition (HI) tests, involving incubation for one hour and performance in accord with a previously published method, were used for subtyping (*5*).

Brain tissues from another horse sent to the U.S. Department of Agriculture in Ames, Iowa, by the Dominican veterinary authorities were also found to contain a virus (designated strain 78-10365) tentatively identified as EEE. The tissues were taken from a dying horse found near the western end of the Samaná Peninsula during mid-February 1978.

Birds, caught in Japanese mist nets or shot during field studies in March, were bled for purposes of virus isolation and serologic testing.

Arthropods were collected with battery-operated CDC light traps (*12*) supplemented by approximately 1 kg of dry ice per trap (*13*). Collection sites were selected on the basis of recent or concurrent equine encephalitis cases occurring in the area. These sites have already been described (*14*). Using the techniques of Sudia and Chamberlain (*10*), arthropods were removed from the traps, put in appropriately labeled and sealed glass tubes, and placed on dry ice for transport to the Fort Collins laboratory.

Results

Virus Isolation

EEE viral isolates (identified by N tests) were obtained in suckling mice from the cerebellum and hippocampus—but not from the left or right cortex, midbrain, serum, or whole blood—of the horse sacrificed at Cristal on 3 April. All 16 suckling mice inoculated with cerebellum tissue, and 8 of 15 suckling mice inoculated with hippocampus tissue, showed signs of illness

(paralysis, prostration, anoxia, or moribundity), were cannibalized, or were found dead within 48 hours of inoculation. An additional passage in suckling mice reduced the survival time to 18-24 hours. The cerebellar isolate (R-22361) and the isolate obtained by the U.S. Department of Agriculture from Dominican horse brain tissue (78-10365) were tested with four prototype strains of EEE by means of short-incubation HI tests for determination of subtype. Table 1 shows that both equine isolates from the Dominican Republic reacted more with North American Strains (NJO, New Jersey; and Arth-167, Louisiana) than with South American strains (Tr-24443, Trinidad; and BeAn-5122, Brazil). It was therefore concluded that the Dominican strains were more closely related to the North American subtypes.

Although six CDC light traps supplemented with dry ice were set in the affected areas for six consecutive nights (29 March-3 April), no virus was isolated from the mosquitoes and *Culicoides* spp. collected. Macerated suspensions of totals of 6,752 mosquitoes and 10,948 *Culicoides* spp. were tested in suckling mice. The arthropod collection data have been summarized elsewhere (*14*). Briefly, *Culex* (*Cx.*) *nigripalpus* comprised 72 per cent of the mosquito collection; the remainder was composed of *Cx. childesteri, Cx. secutor, Cx* (*Melanoconion*) *atratus, Cx.* (*Mel.*) *opisthopus, Aedes* (*Ae.*) *hemisurus, Ae. pertinax, Psorophora jamaicensis, Mansonia dyari, Uranotaenia socialis,* and *Dinocerites cancer.* The *Culicoides* species collected were *insignis* (99 per cent), *foxi, pusillus,* and *furens.*

Serologic Survey

The first N tests of sera from 370 equine animals in the affected area were done with prototype EEE (NJO strain) virus. Table 2 shows the results in terms of the subjects' known history of vaccination. More than 90 per cent of the vaccinated animals had antibody to EEE virus, a figure that markedly surpasses that for unvaccinated animals and hence demonstrates the success of the vaccination campaign. Nevertheless, 11 (35.5 per cent) of the unvaccinated animals and 5 (33.3 per cent) of the very recently vaccinated animals also had N antibody to EEE virus; this finding suggests that EEE virus was widespread in the affected area and that a considerable proportion of the equine population was naturally infected.

Dominican EEE virus (strain R-22361) and WEE virus (Fleming strain) were used in all subsequent tests. Analysis with regard to the numbers of doses of bivalent vaccine given (Table 3) showed that 157 (48.8 per cent) of the vaccinated animals had monotypic antibody to EEE virus, and that 135 (41.9 per cent) had antibody to both EEE and WEE viruses; these 292 represented 90.7

Table 1. The number of units of indicated eastern equine encephalitis (EEE) virus antigen inhibited by optimum antibody dilution in short-incubation HI tests.

Strain	Antibody units	Antigen units of two North American and two South American virus strains			
		NJO	Arth-167	Tr-24443	BeAn-5122
78-10365	8	≥ 64	≥ 64	16	16
R-22361	8	≥ 128	64	8	16
NJO	4	*16*	16	2	1
Arth-167	2	8	*16*	1	1
Tr-24443	2	< 1	1	≥*32*	16
BeAn-5122	4	1	4	32	*32*

Table 2. Equine N antibody to EEE virus, strain NJO, detected in vaccinated and unvaccinated animals in the Dominican Republic, 1978.

	Positive response (titer ≥ 10)		Negative response (titer < 10)		Total	
	No.	%	No.	%	No.	%
Animals vaccinated > 5 days before bleeding	292	90.7	30	9.3	322	100
Animals receiving only one vaccine dose < 5 days before bleeding	5	33.3	10	66.7	15	100
Unvaccinated animals	11	35.5	20	64.5	31	100

Table 3. N antibody to EEE and WEE viruses resulting from administration of EEE/WEE bivalent vaccine; Dominican Republic, 1978.

Doses of vaccine administered	Virus or viruses (EEE or WEE) eliciting a positive N antibody response*									
	None		EEE		EEE + WEE		WEE		Total	
	No. of sera	(%)	No. of sera	(%)	No. of sera	(%)	No. of sera	(%)	No. of sera	(%)
0	20	(64.5)	10	(32.3)	1	(3.2)	0	(0)	31	(100)
1	7	(11.9)	29	(49.1)	21	(35.6)	2	(3.4)	59	(100)
2	13	(4.9)	128	(48.7)	114	(43.3)	8	(3.0)	263	(100)

*The presence of antibody was determined at a single (1:10) serum dilution. Thus positive titers are ≥ 10.

per cent of the 322 animals that received the EEE-WEE bivalent vaccine. Only 10 (3.1 per cent) of the vaccinated equine animals had monotypic N antibody to WEE virus, and only 30 (9.3 per cent) had no N antibody to EEE virus. It should also be noted, however, that 11 (35.5 per cent) of the unvaccinated animals with monotypic N antibody to EEE virus also had CF antibody (≥ 1:16) to EEE virus, indicating fairly recent infection.

None of the 21 wild-caught birds had N antibody to either EEE or WEE virus.

The formalin-inactivated EEE-WEE bivalent vaccine employed stimulates production of N antibody in virtually all the equine animals vaccinated, while stimulating production of CF antibody in only about 10 per cent (*15*). A similar prevalence (about 50 per cent) of CF antibody in both vaccinated and unvaccinated equines suggested that the CF antibodies were due to recent natural infections (Table 4).

Of the 288 people tested, only 11 (six males and five females) were found to have N antibody to EEE virus. A total of 162 people in this sample came from rural areas, and this rural group included all 11 of those with N antibodies to EEE. Of the remaining 126 not classified as rural dwellers, 125 came from the town of Sánchez; the residence of one was unknown. None of the 11 with N antibody showed concomitant CF antibody to EEE virus, a finding that indicates their infections with EEE virus had occurred sometime in the remote past.

Three of these people were born between 1957 and 1967, four between 1947 and 1957, three between 1937 and 1947, and one before 1926.

In addition to EEE and WEE testing, the equine, human, and bird sera involved were N-tested with VEE (strain TC-83) and SLE (strain TBH-83) viruses. As the summary in Table 5 shows, 88.9 per cent of the equine animals, 68.1 per cent of the people, and 4.8 per cent of the birds (one bird) were found to have N antibody to SLE virus, whereas only two of 370 (0.5 per cent) of the equine animals—and no humans or birds—exhibited N antibody to VEE virus.

Considering sex and residence, the results

Table 4. CF antibody to EEE virus in vaccinated and unvaccinated equines, by neutralizing (N) antibody status; Dominican Republic, 1978.

CF titers	N antibody in:			
	Unvaccinated animals		Vaccinated animals	
	Yes (titer ≥ 10)	No (titer < 10)	Yes (titer ≥ 10)	No (titer < 10)
< 8 (negative response)	4	20	142	35
8	2	0	72	2
16	1	0	21	1
32	1	0	24	0
≥ 64	2	0	20	0
Total No. tested*	10	20	279	38
% with CF antibody	60%	0%	49.1%	7.9%

*Total tested differ from those of Table 2 due to exhaustion of certain specimens.

Table 5. N antibody to EEE, WEE, VEE, and SLE viruses in equine animals, humans, and birds; Dominican Republic, 1978.

	No. of sera tested	Number with N antibody (titer ≥ 10) to:			
		EEE	WEE	VEE	SLE
Equine animals	370	309	142	2	329
Humans	288	11	3	0	196
Birds	21	0	0	0	1

Table 6. N antibody to SLE virus (titer ≥ 10) detected in human sera, by age group of subject; Dominican Republic, 1978.

Age group	No. of subjects tested	No. with antibody (titer ≥ 10)	% positive
0-5	24	10	41.7
6-10	51	27	52.9
11-20	142	98	69.0
21-30	31	27	87.1
31-40	19	16	84.2
41-50	16	14	87.5
> 50	4	4	100.0
Unknown	1	0	0
Total	288	196	68.1

of antibody tests for SLE virus among humans were remarkably uniform. The antibody prevalence observed was 69 per cent (100/145) in males and 67.1 per cent (96/143) in females; similarly, 70.4 per cent (88/125) of the people from the more urban area (Sánchez)—as compared with 66.3 per cent (108/163) from more rural areas—showed N antibody to SLE virus. Table 6 presents a summary of the N antibody test results by age group. As expected, the lowest prevalence rate occurred in the youngest age group, but over 40 per cent of the children tested had N antibodies to SLE by age 5. The youngest child found to have N antibody to SLE was 15 months old.

Discussion

These results indicate not only that EEE virus was present in the Dominican Republic as late as April 1978, but also that an epizootic of considerable proportions occurred, at least on the Samaná Peninsula and in contiguous areas. A recent but uncertified census of equine animals indicated the presence of about 400,000 such animals in the Dominican Republic—including 183,000 horses, 126,000 donkeys, and 93,000 mules. Nationwide, there is no basis for accurately determining EEE attack rates in either equine or human populations. In the affected area of the Samaná Peninsula, however, at least 76 equine animals died and 45 were euthanized. Since the equine population was estimated at 12,500 in María T. Sánchez Province and 4,500 in Samaná Province, and since 35.5 per cent of the unvaccinated equines tested had N antibody (Table 2) and 60 per cent of those tested had CF antibody (Table 4), we assume that nearly 6,000 equines had experienced natural infection with EEE virus at some time in the past—and that perhaps 3,600 of those infections were recent. Lumping together the figures for dead and euthanized equine animals, and dividing by 3,600, suggests an infection-fatality rate on the order of 34 per thousand. This is similar to the equine case-fatality rate of 22 per thousand reported for Samaná during the 1949 EEE outbreak by Eklund et al. (*16*).

In 1978, administration of nearly 20,000 doses of bivalent vaccine halted the epizootic. No animals with a history of receiving two vaccinations were found sick. The epizootic ended approximately 40 days after the vaccination campaign began.

Because equine animals are considered nonamplifying hosts for EEE virus, protecting these animals should have had little effect on spillover from the EEE virus cycle in nature. However, since we isolated no virus from arthropods and found no antibody to EEE virus in the few birds captured, we could not ascertain involvement of arthropods or birds in the country's natural EEE cycle.

Eklund et al. (*16*) studied an epizootic of EEE that lasted from October 1948 to February 1949 in Monti Cristi Province—on the northwest coast near the Haitian border. Later, in March 1949, they investigated a similar outbreak in Samaná Province, on the border of the same Gran Estero Swamp where the study reported here was made (*16*). The 1949 outbreak in this area was first reported on 1 March of that year and appeared to have terminated by 1 April, a pattern similar to that of the 1978 epizootic. Eklund et al. (*16*) could not isolate EEE virus from more than 9,000 mosquitoes belonging to 10 species of six genera. Their results, like those reported here, do not reflect either the distribution of vector species or the relative abundance of potential vectors present during the epizootic.

The 1949 outbreak in Samaná Province may have been an extension of the Monti Cristi epizootic. However, EEE has been unknown in the Dominican Republic since 1960, suggesting either that the 1978 outbreak arose *de novo* from an indigenous source, or else that EEE virus was reintroduced quite recently. In this regard, it may not be coincidental that the amount and

seasonal distribution of rainfall in 1977 were strikingly dissimilar to those of the previous year, or that the 1978 outbreak began too early in the year to be explained by introduction of the virus by migrating birds.

The paucity of antibody-positive human subjects (3.8 per cent) and the absence of large numbers of recognized human infections could indicate minimal human exposure to an undetermined arthropod vector of EEE virus in the Samaná Peninsula. However, extensive equine involvement in the epizootic area is apparent from the following observations: (1) a positive N antibody response in 35.5 per cent of the equine sera tested, and (2) detection of CF antibody to EEE virus in unvaccinated equine animals. This CF antibody in vaccinated equines probably indicates a booster response to previous (and possibly recent) infections, since the vaccine used does not stimulate production of CF antibody in most equines (*15*).

Although the vaccination campaign may have taken place late in the ostensibly short-lived epizootic, it was obviously successful. All equine animals in the area were vaccinated and approximately 55 per cent gained protection, as indicated by the observed difference in antibody prevalences (35.5 per cent and 90.7 per cent, respectively) among unvaccinated and vaccinated animals.

A study of antibody to a flavivirus, SLE virus, in the same human and equine populations showed remarkably high prevalences of SLE antibody, even in the youngest age groups tested. SLE virus was isolated in Haiti from the green heron (*Butorides virescens*) in 1957 (*17*); before then, preliminary data had caused its presence to be suspected but unconfirmed.

It is also possible, however, that the SLE antibody detected represents a heterotypic response to one or more dengue viruses. If that is the case, then the prevalence indicated by testing with SLE virus is probably an underestimate of the true prevalence of dengue antibody. Dengue is known to have occurred in the Dominican Republic in both the distant and very recent past (*18*), and the prevalence of SLE virus antibody is remarkably similar to that reported for dengue antibody in Haiti (*9*). Further studies of dengue and other arboviruses in the area of the Samaná Peninsula may yield useful information concerning dengue virus hyperendemicity and its role with regard to the severity of clinical illness.

The question of how EEE outbreaks originate in the Dominican Republic is intriguing. Casals found that the "Sánchez" strain, isolated from the brain of a donkey found sick during the 1949 Samaná outbreak, is a North American subtype, as is the strain isolated from a horse found dead in a 1962 Jamaica outbreak (*3*). Calisher (*20*) has shown that two EEE virus strains collected in 1971 from horses in Cuba are North American subtypes. In addition, the work reported here has identified two more Dominican strains as North American subtypes. Stamm and Newman (*21*) and Lord and Calisher (*22*) have described the autumnal movement of EEE virus in southbound migratory birds on their way to winter residences. Thus, conceivably, North American subtypes of EEE virus could "seed" the Caribbean islands. It is also true that EEE virus strains obtained from northbound migrating birds captured on the Mississippi Delta in Louisiana have been identified as South American virus subtypes. But although strains of South American EEE virus subtypes may be introduced into the United States, they apparently do not initiate cycles of infection in local vector and avian populations, and therefore do not become established in enzootic foci.

None of this rules out the possibility that foci of North American EEE virus subtypes could be established in areas of the Caribbean where favorable conditions exist. On the contrary, fall conditions in the Caribbean, when birds migrate southward, favor initiation of virus circulation much more

than spring conditions in the United States, when birds migrate northward. Nevertheless, documented EEE outbreaks in Cuba, the Dominican Republic, and Jamaica have preceded and roughly coincided with EEE outbreaks in the southeastern United States. We do not know if this is attributable to coincident amplification or to actual movement of the virus.

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SUMMARY

Clinical equine cases of encephalitis were reported in the Dominican Republic in February 1978. In response, an extensive equine vaccination and vector control program was carried out. The outbreak ended in early April 1978. Eastern equine encephalitis (EEE) virus was subsequently isolated from the brain of a moribund horse found in the affected area, the Samaná Peninsula in the northeastern part of the country.

During the last week of March and the first week of April, a serosurvey of humans and equines was carried out in the affected area. No viral isolates were obtained from 6,752 mosquitoes or 10,948 *Culicoides* spp. collected in the region. Although serologic studies indicated numerous recent equine infections caused by EEE virus, only 3.8 per cent of 288 human sera tested showed EEE antibody, none of it due to recent infections.

The estimated equine infection-fatality ratio of 34 per 1,000 animals was similar to ratios observed during past epizootics in the same area. The EEE virus strain isolated during the outbreak was shown to be a North American subtype by the short-incubation hemagglutination-inhibition technique. The source of this outbreak could not be determined, but the possible influence of coincident amplification in other Caribbean and circum-Caribbean areas, as well as possible dissemination of the virus by migrating birds, is discussed.

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INTERNATIONAL COURSE ON THE IMMUNOLOGY OF BACTERIAL INFECTIONS

The Bacteriological Institute of Chile scheduled a course (in English and Spanish) on the immunology of bacterial infections, designed to meet the special needs of laboratory scientists interested in applying their skill in diagnosis and in research in this field. The course was held from 5-16 November 1979 in Santiago.