

A Survey to Assess Potential Human Disease Hazards Along Proposed Sea Level Canal Routes in Panamá and Colombia.

V. Arbovirus Infection in Non-human Vertebrates

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THIS paper reports the results of an investigation of arbovirus infections in non-human vertebrates along two proposed sea level canal routes in Panamá and Colombia. The study was designed to demonstrate transmission occurring during the engineering feasibility studies, as well as to produce evidence of past arbovirus infection in vertebrates inhabiting the route areas. To achieve this, various survey techniques were used, including the collection of wild vertebrates and the use of various species of vertebrates as sentinels. Differences in geographic and habitat distribution patterns of the arboviruses were also investigated, as well as correlations with the distribution patterns of known and potential arthropod vectors. These correlations will be dealt with in subsequent papers in this series.

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Researches, National Academy of Sciences—National Research Council.

Materials and Methods

Survey methods and descriptions of the study areas along these two proposed sea level canal routes are described in earlier papers of this series.^{1,2} The principal means of detecting virus activity was by the use of sentinel golden hamsters (*Mesocricetus auratus*). Hamsters were exposed in equal numbers at close to ground level and in forest canopies at heights of 15–20 m. Exposure periods were 30 days, unless the animals which became sick or died during exposure were necropsied in the field and various tissues (heart, liver and brain) were sent to the base laboratory (Gorgas Memorial Laboratory) for virus isolation attempts. Serological tests were performed on the sera of surviving hamsters.

Virus isolation attempts were made by the inoculation of sera or tissue suspensions into suckling mice or into vero cell cultures. This was done routinely with blood samples from wild-caught mammals and from sentinel monkeys, and with tissue specimens from sentinel hamsters and sentinel mice. The techniques used for serological testing of samples and also for attempts to isolate viruses were essentially identical to those used in the handling of human samples, details of which have been given previously.³

Twenty-one antigens prepared from arboviruses previously isolated from Panamá were included in the serological tests of wild vertebrate sera and in antibody conversion studies in sentinels after exposure: Venezuelan equine encephalitis (VEE), eastern equine encephalitis (EEE), Una (UNA), Mayaro (MAY), St. Louis encephalitis (SLE), Yellow fever (YF), Ilheus (ILH), Bussuquara (BSQ), Ossa

TABLE I
DETECTION OF ARBOVIRUS TRANSMISSION ON ROUTE 17 IN EASTERN PANAMA

	No. Tested	No. Positive	Viruses Detected									
			MAY	VEE	UNA	BSQ	MAD	NEP	OSSA	GMA	VSI	CGL
Virus Isolation Attempts:												
Sentinel hamsters	24	14		5			4		4	1		
Sentinel mice	73	1							1			
Sentinel monkeys	13	0										
Wild-caught vertebrates	2029	3		1		1		1				
Subtotal	2139	18		6		1	4	1	5	1		
Antibody Conversions:												
Sentinel hamsters	62	3			2					1		
Sentinel monkeys	13	7	2								4	1
Subtotal	75	10	2		2					1	4	1
Total	2214	28	2	6	2	1	4	1	5	2	4	1

TABLE II
DETECTION OF ARBOVIRUS TRANSMISSION ON ROUTE 25 IN NORTHWESTERN COLOMBIA

	No. Tested	No. Positive	VEE	UNA	SLE	MAD	OSSA	GMA
Virus Isolation Attempts:								
Sentinel hamsters	32	11	4	1	1	3	1	1
Wild-caught vertebrates	492	3				1	1	1
Subtotal	524	14	4	1	1	4	2	2
Antibody Conversions:								
Sentinel hamsters	35	2						
Sentinel monkeys	1	0						
Subtotal	36	2						2
Total	560	16	4	1	1	4	2	4

(OSSA), Madrid (MAD), Nepuyo (NEP), Patois (PAT), Zegla (ZEG), Chagres (CHG), Guama (GMA), Changuinola (CGL), Wyeomyia (WYO), Cache Valley (CV), Mclao (MEL), Guaroa (GRO) and vesicular stomatitis-Indiana (VSI). The principal test used to detect the presence of antibody in blood sera was the hemagglutination-inhibition (HI) test. The seven non-hemagglutinating antigens (GMA, CGL, WYO, CV, MEL, GRO and VSI) were used in the complement-fixation (CF) test. In addition, neutralization tests were performed with some 400 wild vertebrate sera for the presence of VSI antibodies.

Results

Detection of Active Virus Transmission

Of 144 hamsters exposed during 1967 on Route 17, 46 were lost through predation, escape, and other non-specific causes. Of the remaining 98, 30 (31 per cent) sickened or died in the field. From these, 14 virus isolates were obtained (Table I). VEE, MAD and OSSA were the viruses most frequently isolated. GMA was isolated once. Serological tests performed on surviving hamsters showed evidence of

infection with UNA and GMA, but only three of 62 hamsters so tested showed a positive reaction to any of the antigens tested.

Because of transportation problems, the use of sentinel mice proved discouraging. Only a single virus isolation (OSSA) was obtained from a total of 73 families exposed from April to September 1967; many of the families became sick in the field but died before they could be returned to the base laboratory. Sentinel mice were not used on Route 25, which was even more distant from the base laboratory.

Virus isolation attempts from sera of sentinel monkeys were negative after exposure periods ranging from a few weeks to almost a year. Rises in body temperature were occasionally observed among 13 rhesus, black spider and red spider monkeys used, but febrile blood specimens invariably tested negative for virus. Post-exposure serological tests, however, were positive in seven out of 13 monkeys exposed on Route 17. Viruses detected were VSI, MAY and CGL. VSI was detected in four monkeys. This was the first time evidence of transmission of this cattle disease to non-human primates in nature had been demonstrated.⁴ MAY and CGL, have both been isolated from man elsewhere.

TABLE III
ARBOVIRUS ANTIBODIES IN WILD-CAUGHT VERTEBRATES ROUTE 17, PANAMA

Vertebrates	HI								CF								
	No. Tested	Pos. Individ.	No. Positive						No. Tested	Pos. Individ.	No. Positive						
			Gr A	Gr B	Gr C	Gr Pat	Gr Phl	Total			GMA	CV	WYO	VSI	CGL	MEL	Total
<i>Proechimys</i>	705	148	68	85	50	14	9	226	570	4	1	1		2		4	
<i>Oryzomys</i>	377	27	16	7	4	1	1	29	263	5				5		5	
Other rodents	101	5	1	3			1	5	65	1				1		1	
<i>Marmosa</i>	216	26	15	3	9	2		29	160	1			1			1	
Other opossums	146	25	19	5	3		1	28	134								
Rabbit	24	3	2	1				3	14	1					1	1	
Monkeys	26	5	5		1			6	21	10			9	3		12	
Bird	118	4	1	4				5	96								
Bat	356	6		3			3	6	212								
Reptile	8								3								
Other	36	3		1			2	3	17	1		1				1	
Total	2113	252	127	112	67	17	17	340	1538	23	1	1	1	10	11	1	25

Three arboviruses (VEE, BSQ and NEP) were isolated from a total of 2,029 sera or plasmas collected from wild-caught vertebrates. A single isolate of VEE was from a mouse opossum (*Marmosa* spp.); isolates of BSQ and NEP were from spiny rats (*Proechimys semispinosus*). Neither of these viruses was detected from sentinel animals, although hamsters are known to be susceptible.⁹

On Route 25, 109 hamsters were exposed, of which 39 sickened or died during exposure. Eleven virus isolates were obtained (Table II) and evidence of six more infections was obtained by pathological examination.¹¹ VEE and MAD were most frequently isolated, and UNA, SLE, OSSA, and GMA were each isolated once. Of 35 surviving hamsters, two were found to have antibodies against GMA.

From 630 wild vertebrates collected on Route 25, sera from only 492 were available for virus isolation attempts. The remainder were inactivated in the field prior to shipment, in accordance with measures established to prevent introduction of foot and mouth disease into Panamá from northwestern Colombia where, unfortunately, an epizootic occurred in late 1967. This affected collections mainly from Río Atrato (Area 8), an area found to be highly endemic for arboviruses. Three samples from *Proechimys semispinosus* yielded viruses: OSSA, MAD, and GMA.

Only one sentinel monkey was exposed on Route 25 (Alto Curiche, Area 6). This exposure was negative in all respects.

Antibody Detection

The results of HI and CF tests of sera from wild-caught vertebrates on Route 17 is shown in Table III. Spiny rats made up nearly a third of all vertebrates collected, and about a fifth of these had virus antibodies. Mouse opossums (*Marmosa*) and rice rats (*Oryzomys*) also were collected in substantial numbers, but had lower antibody rates. Flying animals (birds and bats), although tested in relatively large numbers, showed antibody rates of less than two per cent.

Of a total of 2,113 samples tested by HI, 252 were positive to one or more groups of antigens (Table III). Group A positive sera probably contained antibodies to VEE, since this virus is highly endemic in Panamá. Two other Group

A viruses, MAY and UNA, however, are also known to occur in the area, although no isolations of these two viruses were made from Route 17. Group B positive sera could have had antibodies to SLE, ILH, and/or BSQ, all of which are endemic in Panamá. Samples positive for Group C probably resulted from OSSA, MAD and/or NEP viruses. Of the Patois group, only PAT and ZEG are presently known from Panamá.

Evidence of infection by *Phlebotomus* fever group agents in mammals was indicated by HI test results. Although Chagres virus has caused human infections in Panamá, CF tests using this antigen failed to produce any positive reactions among sera positive to the group by HI. CF antibodies to CGL, GMA, MEL, CV, WYO, and VSI were demonstrated in several samples. Most of the VSI positives were from wild-caught monkeys, which was consistent with infections seen in sentinel monkeys.

Neutralization tests with VSI virus were performed with randomly selected sera from vertebrates collected on Route 17. Forty-eight of 419 tested were found positive. Of these, 83 per cent were from arboreal animals.

Results of antibody tests on sera from vertebrates collected on Route 25 are shown in Table IV. Fewer vertebrates were collected, but overall species composition was in about the same proportion as for Route 17, and the overall antibody rate was nearly the same.

Geographic Distribution of Arboviruses on the Routes

The presence of arboviruses, as detected by sentinel animals and wild-caught vertebrates, was found to be distributed unevenly on the routes (Table V). On Route 17, Sasardi (Area 5) was found to be a highly endemic area for most viruses occurring on the route. Only two viruses, MAY and CGL, were detected in the Santa Fé Area (1), and only UNA and VSI in the Morti Hydro Area (3). The results of the screening HI tests showed a similar pattern. From the Sasardi area, 25.5 per cent of all vertebrate blood specimens showed an HI positive reaction; the percentages for the Santa Fé and Morti Hydro areas were 8.3 per cent and 4.9 per cent, respectively.

On Route 25, Teresita (Area 2) was found to be endemic

TABLE IV
ARBOVIRUS ANTIBODIES IN WILD-CAUGHT VERTEBRATES ROUTE 25, COLOMBIA

Vertebrates	HI								CF					
	No. Tested	Pos. Ind.	No. Positive					No. Tested	Pos. Individ.	No. Positive				
			Gr A	Gr B	Gr C	Gr Pat.	Gr Phil.			Total	GMA	CGL	MEL	Total
<i>Proechimys</i>	325	49	25	15	11	3		54	271	4	1		3	4
<i>Oryzomys</i>	81	3	2	2				4	67	0				0
Other rodent	31	0						0	30	1		1		1
<i>Didelphis</i>	43	7	4	2	1		4	11	40	0				0
<i>Metachinus</i>	37	4	2	2				4	37	0				0
<i>Marmosa</i>	32	1			1			1	32	0				0
Monkey	6	3	3					3	4	0				0
Bat	71	1	1					1	41	0				0
Others	4	0												
Total	630	60	37	21	13	3	4	78	522	5	1	1	3	5

TABLE V
GEOGRAPHICAL DISTRIBUTION OF ARBOVIRUSES DETECTED BY SENTINEL ANIMALS OR ISOLATED
FROM WILD-CAUGHT VERTEBRATES

Route	Collecting Area	Viruses											Total
		MAY	VEE	UNA	BSQ	SLE	MAD	NEP	OSSA	GMA	VSI	CGL	
17	1—Santa Fé	1										1	2
	3—Morti Hydro			2								2	4
	5—Sasardi	1	6		1		4	1	5	2	2		22
	Route Total	2	6	2	1		4	1	5	2	4	1	28
25	2—Teresita		3	1			2		2	1			9
	4—Curiche		1										1
	6—Alto Curiche												0
	8—Río Atrato					1	2			3			6
	Route Total		4	1		1	4		2	4			16
Total		2	10	3	1	1	8	1	7	6	4	1	44

for VEE, UNA, OSSA, and MAD viruses. In the Río Atrato Area (8), MAD, SLE, and GMA viruses were present. The percentage of positive HI reactions among specimens collected in the four collecting areas of Route 25 were as follows: Río Atrato (Area 8), 12.5 per cent; Teresita (Area 2), 11.8 per cent; Curiche (Area 4), 8.1 per cent; and Alto Curiche (Area 6), 6.9 per cent.

The overall percentage of positive HI tests for all blood specimens tested were: Route 17, 11.9 per cent; Route 25, 10.8 per cent.

Habitat Distribution of Arboviruses

Table VI lists the vertical distribution of arboviruses detected in sentinel animals and virus isolations from wild-caught vertebrates from both routes. Less than a third of the detections were from animals exposed or captured in the forest canopy. MAY, CGL, and VSI viruses were only detected in the canopy, however. VEE, UNA, OSSA, and MAD viruses were detected in the canopy. BSQ, SLE, NEP, and GMA were detected only at ground level.

An analysis was made of the number of arbovirus antibody detections by HI, CF, and neutralization test according to the habitat from which the host vertebrate was collected. Most positive specimens came from vertebrates trapped in forests, but there was no significant difference in the proportion of positive reactors among vertebrates collected in forests, villages, grasslands, mangrove swamps, or abandoned farmlands covered with wild banana (*Heliconia* spp.)

Discussion

The method of exposing sentinel hamsters to demonstrate transmission of arboviruses, developed by Scherer *et al*⁶ and successfully used by Srihongse *et al*,⁷ was found to be a highly productive technique to detect activity of various viruses along the two proposed sea level canal routes. Ten per cent of 253 hamsters exposed along these two study areas yielded several viruses, namely VEE, UNA, SLE, OSSA, MAD and GMA. Although SLE and GMA viruses have been shown to be non-pathogenic to the adult hamster by experimental inoculation,⁵ these two viruses were acci-

dentally isolated from the sick hamsters removed from exposure in the field.

The exposure of sentinel monkeys revealed the presence of additional viruses not previously isolated from the area. Although no virus isolates were obtained, antibody conversions to MAY, VSI and CGL antigens proved that these viruses were active during the study period. The presence of VSI antibodies in monkeys is consistent with recent evidence of arboreal transmission of the disease by phlebotomine sand flies.^{8,9}

In remote areas, as in the present study, exposure of sentinel mice may not be as effective as in regions where there is less difficulty in shipping exposed mouse groups to a central laboratory. Some of the mice which died during the delayed transits could have been positive for viruses. However, the animals arrived too late for isolation attempts. There may have been differences in efficiency of virus detections between hamsters and mice. Furthermore, the attractiveness to insect vectors of these two hosts may differ.

Results of virus isolation attempts and the detection of antibody conversions in this study, combined with the studies in man,³ demonstrated that at least 12 arboviruses were actively transmitted in nature during the study period in 1967. These viruses included VEE, UNA, MAY in group A; BSQ, ILH, SLE in group B; OSSA, MAD, NEP in group C and GMA, CGL, VSI in miscellaneous groups. In addition, serological findings in man and wild vertebrates indicated that these viruses were widely spread along these two proposed sea level canal routes.

A comparison of virus isolations, antibody conversion rates, and the percentage of positive reacting sera for the various collecting areas on the two routes indicates that arbovirus activity was greater on the Atlantic slope of Route 17 than on the Pacific slope. This agrees with the fact that most previous virus isolates from Panamá have been from areas near the Atlantic coast, especially Almirante.¹⁰ Serological surveys in human residents of the Pacific towns in the western part of Panamá and the Azuero Peninsula also show much lower antibody rates to arboviruses than areas along the Atlantic coast. A factor contributing to this is probably the greater precipitation on the Atlantic coast of the Panamá,¹ including Route 17. Along Route 25, the

TABLE VI
VERTICAL DISTRIBUTION OF VIRUS TRANSMISSIONS DETECTED

	Ground	Canopy	Total
Source			
Sentinel hamster	26	4	30
Sentinel mice	1	0	1
Sentinel monkeys	0	7	7
Wild-caught vertebrates	6	0	6
Total	33	11	44
Viruses			
Group A VEE	9	1	10
UNA	2	1	3
MAY	0	2	2
Group B BSQ	1	0	1
SLE	1	0	1
Group C OSSA	6	1	7
MAD	7	1	8
NEP	1	0	1
Others GMA	6	0	6
VSI	0	4	4
CGL	0	1	1

geographical differences were less pronounced, and antibody rates were roughly comparable in all areas. Teresita and Curiche, the areas receiving the most rainfall, did show the highest antibody rates among wild-caught vertebrates.

The predominance of MAY, CGL, and VSI viruses in the forest canopy implies a transmission cycle involving arboreal vertebrates and insects. This is certainly true of VSI.^{4,8,9}

Failure to show significant differences in antibody rates among vertebrates collected in various ecological habitats is indicative of the mobility of vertebrate animals, even ground inhabiting mammals from which the greatest number of antibody detections were made.

Summary

A study of arbovirus infections in non-human vertebrates along two proposed sea level canal routes in Panamá and Colombia showed that VEE, Una, Mayaro, SLE, Ossa, Madrid, Guama, Changuinola and VSV-Indiana viruses were active in the study area and that they are detectable by the use of sentinel animals. Additional strains of VEE, Ossa, Madrid, Guama, Nepuyo and Bussuquara viruses

were obtained from wild vertebrate sera. The last two agents were not detected in sentinels.

Virus transmission was shown to be more prevalent along the Atlantic coast than on the Pacific side of Route 17. On Route 25, transmission rates were about the same in all areas. Detection of viruses from different levels of the forest demonstrated that at least three viruses, Mayaro, Changuinola and VSV-Indiana, were more prevalent in the canopy.

Analysis of habitat distribution of wild-caught vertebrates failed to show any significant differences in arbovirus antibody rates among animals captured in forests, grasslands, villages, mangrove swamps, and abandoned, wild-banana covered farmland.

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