American Journal of Epidemiology
Vol. 90, No. 1

Printed in U.S.A.

VESICULAR STOMATITIS VIRUS INFECTIONS IN PANAMANIAN PRIMATES AND OTHER VERTEBRATES[1]

SUNTHORN SRIHONGSE[2]

(Received for publication January 10, 1969)

Srihongse, S. (Gorgas Memorial Laboratory, P.O. Box 2016, Balboa Heights, Canal Zone). Vesicular stomatitis virus infections in Panamanian primates and other vertebrates. *Amer. J. Epid.*, 1969, 90: 69–76.—A large scale survey for antibodies to vesicular stomatitis virus (Indiana type) in monkeys and other wild vertebrates was performed in different areas of Panama. Approximately 75% of 267 monkeys in Darien province were positive in neutralization (NT) tests whereas only 19% positive results were obtained from 383 monkeys collected near Panama City. Spider monkeys (*Ateles* spp.) showed the highest rate of NT antibodies to VSV-Indiana. A high incidence of complement-fixing (CF) antibodies was also demonstrated in Darien monkeys. NT tests in wild vertebrates other than monkeys showed a much higher rate in arboreal mammals than in ground-living animals. Two-toed sloths (*Choloepus hoffmani*) and tropical porcupines (*Coendou rothschildi*) were among arboreal mammals showing evidence of high VSV-Indiana rates. Virus isolation attempts from 1,558 wild vertebrates were negative but a strain of VSV-Indiana was obtained from a worker on a cattle farm in Darien. Four of the sentinel monkeys exposed there showed rises in VSV-Indiana antibody titers, although virus isolation attempts from serial bleedings were negative. The findings above provide supportive evidence that monkeys and other arboreal mammals may be involved in VSV-Indiana cycles.

antibodies; complement fixation tests; monkeys; neutralization tests; vesicular stomatitis virus; virus diseases; viruses

Introduction

For several decades epizootics of vesicular stomatitis virus (VSV) have commonly occurred among cattle, horses and pigs in the United States. Other endemic areas of this livestock disease include Mexico, Guatemala, Panama, Colombia and Venezuela. The virus has also been detected in Brazil and Argentina, and as far from the western hemisphere as France and South Africa (1). However, since VSV is generally considered to be a disease of the New World, the cases in France and South Africa are believed to have been imported from the Americas. Outbreaks of the Indiana type of VSV in North America have been confined largely to the southwestern states, while the other known type, New Jersey, has been prevalent in the east as far north as southern Canada (2). A third member of this group, Cocal virus, has been isolated from wild rodents, mites and mosquitoes in Trinidad and Brazil (3, 4). Recently a fourth member, Alagoas virus, was isolated in Brazil (5).

[1] The author thanks P. Galindo, M. A. Grayson, J. A. Porter, A. Herrer, C. M. Johnson, B. F. Eldridge, D. G. Young, and J. R. Gauld for providing human and animal sera used in this study. Appreciation is extended to G. B. Fairchild and R. E. Shope for their assistance in the preparation of this manuscript. The technical assistance of B. Dutary, N. G. Bright and N. Guardia is gratefully acknowledged.

[2] Gorgas Memorial Laboratory, Panama R. P. (mailing address: P. O. Box 2016, Balboa Heights, Canal Zone).

Federer et al. proposed to classify VSV-Indiana, Cocal and Alagoas viruses, according to their serologic cross reactivities, as Indiana type I, II and III, respectively. VSV has been provisionally grouped taxonomically as a Rhabdovirus by the International Committee on Nomenclature of Viruses.

The mode of transmission of this group of viruses is still not understood. The virus can pass from animal to animal by contact through skin abrasions and mucosae. However, this apparently is not the major means of dissemination as outbreaks have appeared almost simultaneously throughout infected areas (6–8). Although the natural method of spread is not definitely known, VSV is provisionally classified as an arbovirus (1). More recently Casals, at the 1968 meeting of the American Society of Tropical Medicine and Hygiene, recommended the use of "arbovirus" as an ecologic grouping rather than a toxonomic group as it clearly contains viruses which differ in morphology and physical-chemical properties. It is assumed that arthropod vectors may be involved in the natural cycles of VSV since outbreaks occur suddenly during the summer season and disappear with the onset of freezing temperature. However, virus isolation attempts from arthropods rarely have been successful. Phlebotomine sandflies in Panama were found to be infected with a strain closely related to VSV-Indiana (9). A single isolate of this virus type was also obtained from *Aedes* mosquitoes during an outbreak in cattle and horses in New Mexico in 1965 (10).

Human infections with VSV have been occasionally reported concurrently with outbreaks in livestock, and during the recent epizootic in Colorado and New Mexico, many human cases were detected (11). In Panama, specific neutralizing antibody to VSV-Indiana was demonstrated in a high percentage of humans in native populations near Almirante (see map) where infected sandflies were obtained (9). High antibody rates of VSV-Indiana were also observed in an endemic area (Achiote) of leishmaniasis, a sandfly-transmitted infection. A limited number of wild vertebrates in Panama have been tested by Kuns who demonstrated that the majority of the three-toed sloths and kinkajous collected in the canopy of the tropical moist forests of Panama were immune to the Indiana virus (12).

The present communication describes the results of a large scale survey for VSV-Indiana antibodies in wild-caught Panamanian monkeys and other wild vertebrates. Virus transmission in nature is shown to be detectable by sentinel monkeys. Results of virus isolation attempts from vertebrates and preliminary VSV experimental pathogenesis in monkeys are also presented.

Materials and methods

Serum samples of non-human primates and other vertebrates used in this study were obtained from several projects of the Gorgas Memorial Laboratory (GML). The yellow fever surveillance project, reported in 1967 (13), provided large numbers of monkeys from areas around Tuira and Mono rivers in Darien province and Cerro Azul in Panama province (figure 1). Some monkeys and other wild animal sera were obtained from a preliminary survey in the Darien gap of the Interamerican highway in 1963 (14). Other serum specimens of man and wild vertebrates, collected in Darien province (Santa Fe-Sasardi area) and the northwestern region of Colombia in 1966–1967, were received as part of the cooperative project of GML with the Office of Interoceanic Canal Studies (OICS). OICS also handled the sentinel animals for detection of arbovirus transmission. The overall results of the OICS project will be reported elsewhere. In the province of Panama, monkeys trapped from 1965 to 1968 in the Pacora area and the upper Bayano river

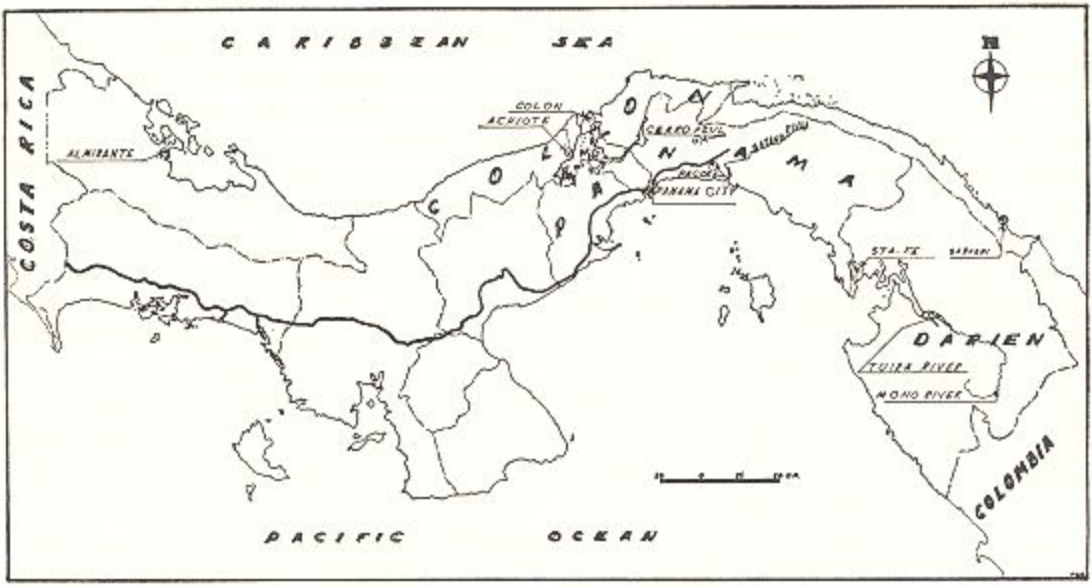

FIGURE 1. Map of the Republic of Panama showing location of study areas.

basin, were purchased by the GML Primate Malaria project which provided serum samples for this study. All other vertebrate sera collected in 1967–1968 were received from the GML leishmaniasis project, which carried on field activities mostly in Colon province (Achiote), Panama.

Methods of exposing sentinel mice and hamsters were described elsewhere (15, 16). Monkeys were also exposed in the study areas. They were placed in individual cages in the forest canopy. A well balanced meal including fresh fruit was given daily and the animals were checked twice a day for body temperature and signs of illness. Bleedings were performed when high body temperature was detected and upon other occasions during the three- to five-month period of exposure.

Vesicular stomatitis virus strain BT 78, identified as VSV-Indiana (9), isolated from Phlebotomine sandflies in Panama, was kindly supplied by Dr. Pauline Peralta for this study. Virus stocks, in seventh infant mouse brain passages, were prepared as 20 per cent clarified brain suspensions in phosphate buffered saline pH 7.2 containing 0.75 per cent bovine albumin, and were stored in sealed glass ampules at −65 C. Sucrose-acetone extracted antigen (17) was used in the complement-fixation (CF) tests which were done in plastic plates using a modification of the Fulton and Dumbell microtechnique (18). Initial serum dilution of 1:4 was used. Neutralization (NT) testing was performed in monkey kidney (Vero) cell cultures, employing a constant-virus varying-serum dilution technique. The lowest serum dilution tested was 1:8. Serum-virus mixtures were incubated at 4 C overnight prior to inoculation in culture tubes. Samples were considered positive when initial-dilution serum neutralized at least 100 tissue culture infective doses, 50 per cent effective ($TCID_{50}$) of virus in both inoculated tubes. Titration endpoints were calculated by the method of Reed and Muench (19).

Virus isolation attempts from vertebrates were made in Swiss mice two-to-four days old by the intracerebral (I.C.) route of inoculation. In some instances Vero cell cultures were used. Titers of VSV in these cell cultures were similar to those obtained in suckling mice I.C.

Results

Preliminary CF tests of wild vertebrates

All wild vertebrates collected in 1966 and 1967 from the Santa Fe-Sasardi area in Darien province, Panama and from northeastern Colombia were subjected to serologic testing with VSV-Indiana antigen. A total of 2,080 animal sera, including 522 from Colombia, were first tested by the CF technique. Serum samples included a large percentage from wild rodents (40 per cent spiny rats and 16 per cent rice rats). Other specimens were from bats (12 per cent), *Marmosa* opossums (9 per cent), monkeys (2 per cent), other mammals (16 per cent) and birds (5 per cent). Results of these tests showed that only 10 samples of the total were positive; nine of which were from monkeys. A single non-monkey positive was an opossum. The positive results in monkeys represented 32 per cent of all monkeys tested. No positive CF reactions were detected from Colombian samples, including six monkeys.

VSV antibodies in monkeys

Since CF antibody titers are ephemeral while neutralizing antibodies usually persist in viral infections, as shown for VSV in cattle (20) and in man (21), it is probable that monkeys and other wild vertebrates may also react similarly. A large number of monkeys collected in the study area and from other areas were then tested by NT in an attempt to determine the true prevalence of previous VSV infection in this non-human primate population.

Table 1

VSV-Indiana neutralizing antibodies in Panamanian monkeys collected between 1962 and 1968

Species	Darien Province		Panama Province	
	No. Tested	% Positive	No. Tested	% Positive
Black Spider (*Ateles fusciceps*)	90	86.7	62	43.5
Red Spider (*Ateles geoffroyi*)	35	80.0	18	44.4
White Face (*Cebus capucinus*)	58	81.0	39	25.6
Marmoset (*Saguinus geoffroyi*)	12	75.0	96	9.4
Howler (*Alouatta palliata*)	72	51.4	39	28.2
Night Monkey (*Aotus trivirgatus*)	0	—	129	7.0
Total	267	74.5	383	19.3

A total of 650 monkeys, collected in Darien and Panama provinces from 1962 to 1968, were tested by NT with VSV-Indiana. Samples from 28 monkeys tested by CF above were also included in the tests. Results shown in table 1 demonstrated that almost half of these monkeys had neutralizing antibodies to VSV-Indiana. In monkeys from Darien province, 75 per cent of 267 samples tested were positive, whereas only 19 per cent antibody rate was detected in 383 monkeys from the areas closer to Panama City. The different species of monkeys included in the tests are shown in this table. All species tested were positive by NT tests. In Darien province, Black Spider monkey (*Ateles fusciceps*) showed the highest rate (87 per cent) of previous infection. White Face and Red Spider monkeys, and marmosets also had high rates of antibodies. Serum neutralizing antibody titers in some of the positive samples were as high as or higher than 1:1,280 when tested with 100 $TCID_{50}$ of virus. A group of nine squirrel monkeys (*Saimiri sciureus*) shipped from Peru was also tested with VSV-Indiana. No NT antibodies were found in any of these monkeys.

In order to determine the occurrence of recent transmission of this virus, CF tests were then performed with this group of monkeys. Since many of these sera were anticomplementary, only 353 tested samples were subjected to the analysis. As can be seen in table 2, 14 per cent of the total monkeys tested were positive, representing one-third of those positive in NT tests. CF reactors in Darien monkeys were four

TABLE 2

VSV-Indiana complement-fixing antibodies in Panamanian monkeys collected between 1962 and 1968

Species	Darien Province		Panama Province	
	No. Tested	No. Positive	No. Tested	No. Positive
Black Spider (*Ateles fusciceps*)	61	12	46	8
Red Spider (*Ateles geoffroyi*)	4	2	19	3
White Face (*Cebus capucinus*)	18	2	25	2
Marmoset (*Saguinus geoffroyi*)	10	6	63	0
Howler (*Alouatta palliata*)	26	11	23	2
Night Monkey (*Aotus trivirgatus*)	0	—	58	1
Total	119	33 (=27.7%)	234	16 (=6.8%)

times higher than those from Panama province. It should be noted that most of the positive samples from the latter area were collected from a single locality near the headwaters of the Pacora River called Cerro Azul (figure 1).

VSV antibodies in wild vertebrates other than monkeys

A total of 511 non-primate vertebrates collected from 1966 to 1968 were tested by NT. Table 3 demonstrates the results of NT tests in non-primate animals from two study areas. Vertebrates in Darien province were collected from areas where high VSV-Indiana antibody rates in monkeys were detected. On the other hand, more than 90 per cent of the animals in Panama and Colon provinces were collected from areas where no monkeys have been trapped. However, an area in Colon province (Achiote) provided a large number of arboreal mammals. This is a known endemic area of leishmaniasis, where a high antibody rate of VSV-Indiana also was shown in man (9).

Results shown in table 3 demonstrate that arboreal vertebrates experienced higher rates of VSV-Indiana infections than terrestrial animals. In the two regions combined, 30 per cent of 214 arboreal mammals showed neutralizing substances to VSV-Indiana whereas only 5 per cent of 213 terrestrial animals tested were positive. Among arboreal mammals, the two-toed sloth (*Choloepus hoffmani*) and the

TABLE 3

VSV-Indiana neutralizing antibodies in wild vertebrates other than monkeys collected between 1966 and 1968

Area	Species	No. Tested	No. Positive	% Positive
Darien Province	Arboreal vertebrates	*45**	*4*	*9.3*
	Brown-masked Opossums (*Metachirus nudicaudatus*)	7	1	
	Two-toed sloths (*Choloepus hoffmani*)	4	1	
	Wooly Opossums (*Caluromys derbianus*)	3	1	
	Other arboreal vertebrates	29	1	
	Ground and arboreal vertebrates (*Didelphis marsupialis*)	*24*	*4*	*16.7*
	Bats and Birds	*44*	*0*	*0*
	Ground vertebrates	*182*	*10*	*5.5*
	Spiny rats (*Proechimys semispinosus*)	106	4	
	Rice rats (*Oryzomys talamancae*)	33	4	
	Other ground vertebrates	43	2	
	Sub-total	293	18	6.1
Panama and Colon Provinces	Arboreal vertebrates	*171*	*61*	*35.7*
	Two-toed sloths (*Choloepus hoffmani*)	90	33	
	Three-toed sloths (*Bradypus griseus*)	40	6	
	Porcupines (*Coendou rothschildi*)	27	17	
	Other arboreal vertebrates	14	5	
	Ground and arboreal vertebrates (*Didelphis marsupialis*)	*16*	*3*	*18.8*
	Ground vertebrates	*31*	*1*	*3.2*
	Spiny rats (*Proechimys semispinosus*)	25	0	
	Spiny rats (*Hoplomys gymnurus*)	2	1	
	Other ground vertebrates	4	0	
	Sub-total	218	65	29.8
	Total	511	83	16.2

* Italicized numbers represent totals of indicated groups of vertebrates.

Porcupine (*Coendou rothschildi*) were more frequently reactive than others. CF tests were also performed with 159 sera from arboreal animals. Only two positives were detected, both in two-toed sloths from which 42 samples were tested.

Virus isolation attempts

Blood samples of 1,558 vertebrates from Darien province (Santa Fe-Sasardi area) were used for virus isolation attempts. No VSV was obtained. In addition, 199 monkey sera collected from Darien and Panama provinces were tested without recovering virus. Ninety of these monkeys were later found to be immune to VSV-Indiana. Liver, spleen and kidney suspensions from three monkeys, 32 arboreal and 40 ground mammals were also inoculated in mice. No VSV isolates were obtained.

During a 12-month period of field studies in Darien province in 1966-1967, sera of human fever cases in that area were also tested for virus. From 31 fever cases examined, one isolate of VSV-Indiana was obtained. This was a 20-year-old male worker on a cattle farm in Darien province. At the time of his febrile illness, he experienced chilly sensation, headache and diarrhea. No vesicular lesions were observed. We were unable to secure convalescent-phase serum of this positive case for serologic studies. Reisolation attempts from acute-phase serum after two months storage at −65 C were unsuccessful. Since validity of this isolate was not confirmed, the isolation cannot be substantiated. However, in asymptomatic subjects from the same area, neutralizing antibodies were detected in 29 of 132 samples tested (22 per cent).

Detection of VSV infection in sentinel monkeys

During the same one-year period of field study, seven Black Spider monkeys, three rhesus monkeys, two White Face and one Red Spider monkey were exposed in Darien province. Virus isolation attempts from all serum samples of periodic bleedings of these monkeys were negative. However, VSV-Indiana antibody conversions were detected by CF and NT tests in four sentinel monkeys exposed in the forest canopy in two different localities. The positive sentinels were two Black Spider monkeys, one White Face and one rhesus monkey. CF titers of the two positives were higher than 1:32, the highest dilution tested. Neutralizing antibody titer determinations in post-exposure samples of all four monkeys showed conversion to more than 1:1,024 when tested with 100 $TCID_{50}$ of VSV-Indiana.

Sentinels other than monkeys were also exposed in the same area; these included 73 litters of mice and 86 hamsters. The latter animal has been shown to be susceptible to VSV-Indiana (22). No isolates of this virus were obtained from all sentinel mice or from 22 hamsters found dead after exposure. Likewise, no positive VSV antibody conversions were demonstrated in 64 surviving hamsters (some of which were placed in the canopy) after a one-month period of exposure.

Experimental pathogenesis of VSV in monkeys

Two young adult spider monkeys were inoculated subcutaneously with 6 Log_{10} $TCID_{50}$ of VSV-Indiana virus. Both animals were shown to be free of neutralizing antibodies to VSV-Indiana before inoculation. Bleedings were done daily for a period of one week after inoculation. Serum samples (1:5) of each bleeding were inoculated in suckling mice intracerebrally. Viremia was not detectable and no vesicular lesions were observed in these monkeys during the period of these experiments. No attempts were made to isolate virus from specimens other than blood. High CF and neutralizing antibody titers developed in both monkeys, tested at one month post-

inoculation. However, persistence of these antibodies has not been determined.

Discussion

This large scale survey for VSV-Indiana antibodies in wild vertebrates clearly demonstrated that a high percentage of monkeys and other arboreal mammals in certain areas of Panama were being infected with this virus. The results suggest that besides the normal cycle of infection in domestic livestock, the virus may maintain itself in a wild animal cycle in the forest canopy. Although insect vectors for this virus were not definitely proved, phlebotomine sandflies, which have been shown to harbour the virus (9), are present in the forest canopy. As indicated in the results of surveys in areas where leishmaniasis was endemic, a high percentage of VSV antibodies in wild vertebrates was also demonstrated. As has been shown in the past, the low titer viremia characteristic of VSV infected cattle is unlikely to constitute a source of infection for arthropod vectors. If blood is the source of infection, animal reservoirs with higher titer viremias might play an important role in maintaining the virus cycles; the existence of such wild hosts is still unproven. Thus far, the source of virus for sandflies is unknown but unlike mosquitoes, these flies may occasionally feed on wounds and body secretions. Thus, they may obtain viruses from lesions of infected cattle. Hanson pointed out that virus could be introduced by these flies into wounds caused by plant spines or sharp metal on uninfected animals (23). This is highly speculative since although sandflies may feed on wounds on occasion, there is little evidence that they do so to any notable extent. Therefore, transmission of the virus among monkeys and other arboreal vertebrates by direct contact cannot be excluded. Jonkers recently presented several objections to the theory of virus transmission by vectors (2). He also proposed a working hypothesis, of a non-vector-borne nature, for further investigation of transmission of this group of viruses.

Additional supportive evidence that monkeys and biting insects may be involved in the VSV cycle was shown in this study when four sentinel monkeys became positive for VSV antibodies after varying periods of exposure. Unfortunately, systematic bleedings were not done during this period. Thus, failure of virus isolation attempts from blood samples drawn occasionally did not necessarily mean that viremia did not occur in infected monkeys. However, our preliminary experiments in monkeys inoculated with high dosage of seventh passage virus showed that viremia was undetectable in one of the species tested. Since these sentinels were individually kept in cages in the canopy, they could have been infected by insect vectors. No attempts were made to isolate virus from biting insects collected in these areas during the period of exposure.

From the same study areas where high rates of VSV-Indiana antibodies in monkeys were found, more than a thousand wild vertebrate sera were used for virus isolation attempts without success. However, most of these were terrestrial wild vertebrates. In the naturally infected monkeys or arboreal vertebrates, vesicular lesions similar to those in cattle were not found. Likewise, the search for virus in these wild animals was not systematically performed. Perhaps various tissues including salivary gland, buccal mucosa, lung, or kidney may be the lodging place for VSV. Urine and fecal material from naturally infected animals should also be investigated for virus.

Although Cocal virus, a member of the same VSV group, has been shown to affect ground rodents (4), VSV-Indiana infection was proved to be uncommon among rodents in Panama. In another study area of northwestern Panama where VSV-Indiana

has been isolated from sandflies (9), no CF positive reactions for VSV were found among 459 ground rodents tested (24).

As previously mentioned, animal reservoir hosts with high titer viremias of VSV have not been found. The present study suggests that monkeys and other arboreal vertebrates might be worth further investigation in this regard. Although preliminary experiments with VSV-Indiana in certain species of monkeys showed absence of viremia, further trials with various other species of arboreal mammals and birds of different ages are indicated.

References

1. Casals, J. and Clarke, D. H. Arboviruses other than groups A and B. *In* Viral and Rickettsial Infections of Man. Horsfall, F. L., and Tamm, I., eds., Philadelphia, Lippincott, 1965.
2. Jonkers, A. H. The epizootiology of the vesicular stomatitis virsues: A reappraisal. Amer. J. Epid., 1967, *86:* 286–291.
3. Jonkers, A. H., Shope, R. E., Aitken, T. H. G. and Spence, L. Cocal virus, a new agent in Trinidad related to vesicular stomatitis virus, type Indiana. Amer. J. Vet. Res., 1964, *25:* 236–242.
4. Jonkers, A. H., Spence, L. and Aitken, T. H. G. Cocal virus epizootiology in Bush Bush forest and the Nariva swamp, Trinidad, W. I.: Further studies. Amer. J. Vet. Res., 1965, *26:* 758–763.
5. Federer, K. E., Burrows, R. and Brooksby, J. B. Vesicular stomatitis virus—the relationship between some strains of the Indiana serotype. Res. Vet. Sci., 1967, *8:* 103–117.
6. Meyer, N. L., Moulton, W. M., Jenney, E. W. and Rodgers, R. F. Outbreaks of vesicular stomatitis in Oklahoma and Texas. Proc. U.S. Livestock San. Ass'n., 1958, *62:* 1–9.
7. Acree, J. A., Hodgson, D. R. and Page, R. W. Epizootic Indiana vesicular stomatitis in southwestern U.S. Proc. U.S. Livestock San Ass'n., 1964, *68:* 375–379.
8. Heiny, E. Vesicular stomatitis in cattle and horses in Colorado. North Amer. Vet., 1945, *26:* 726–730.
9. Shelokov, A. and Peralta P. H. Vesicular stomatitis virus, Indiana type: An arbovirus infection of tropical sandflies and humans? Amer. J. Epid., 1967, *86:* 149–157.
10. Sudia, W. D., Fields, B. N., and Calisher, C. H. The isolation of vesicular stomatitis virus (Indiana strain) and other viruses from mosquitoes in New Mexico. Amer. J. Epid., 1967, *86:* 598–602.
11. Fields, B. N. and Hawkins, K. H. Human infection with the virus of vesicular stomatitis, Indiana strain, during the 1965 epizootic in Colorado and New Mexico. New Eng. J. Med., 1967, *277:* 989–994.
12. Kuns, M. E. VSV-Indiana. *In* Catalogue of Arthropod-borne Viruses of the World. Compiled by R. M. Taylor. U.S. Government Printing Office, Washington, D.C. 1967, pp. 641–644.
13. Galindo, P. and Srihongse, S. Evidence of recent jungle yellow-fever activity in eastern Panama. Bull. W.H.O., 1967, *36:* 151–161.
14. Johnson, C. M. Gorgas Memorial Laboratory, Panama. Personal communication, 1963.
15. Galindo, P., Srihongse, S., Rodaniche, E. de and Grayson, M. A. An ecological survey for arboviruses in Almirante, Panama, 1959–1962. Amer. J. Trop. Med. & Hyg., 1966, *15:* 385–400.
16. Srihongse, S., Scherer, W. F. and Galindo, P. Detection of arboviruses by sentinel hamsters during the low period of transmission. Amer. J. Trop. Med. & Hyg., 1967, *16:* 519–524.
17. Clarke, D. H. and Casals, J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. & Hyg., 1958, *7:* 561–573.
18. Fulton, F., and Dumbell, K. R. The serological comparison of strains of influenza virus. J. Gen. Microbiol., 1949, *3:* 97–111.
19. Reed, L. J. and Muench, H. A simple method of estimating fifty per cent endpoints. Amer. J. Hyg., 1938, *27:* 493–497.
20. Casteneda, J. and Hanson, R. P. Complement fixing antibodies as a measure of immunity of cattle to the virus of vesicular stomatitis New Jersey. Amer. J. Vet. Res., 1966, *27:* 963–969.
21. Johnson, K. M. and Peralta, P. H. Clinical and serological response to laboratory-acquired human infection by Indiana type vesicular stomatitis virus (VSV). Amer. J. Trop. Med. & Hyg., 1966, *15:* 244–246.
22. Srihongse, S. and Johnson, K. M. Hemagglutinin production and infectivity patterns in adult hamsters inoculated with group C and other New World arboviruses. Amer. J. Trop. Med. & Hyg., 1969, *18:* 273–279.
23. Hanson, R. P. Discussion of the natural history of vesicular stomatitis. Amer. J. Epid., 1968, *87:* 264–266.
24. Srihongse, S. and Galindo, P. Gorgas Memorial Laboratory. Unpublished data.