EXPERIMENTAL ST. LOUIS ENCEPHALITIS VIRUS INFECTION OF SLOTHS AND CORMORANTS*

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Abstract. Experimental infection of 11 *Bradypus variegatus* and *Choloepus hoffmanni* sloths with St. Louis encephalitis (SLE) virus produced detectable viremias of seven to 27 (median 13) days duration and maximum titers of 2.7 to 6.5 (median 5.1) $\log_{10}$ median suckling mouse intracranial lethal doses (SMicLD$_{50}$) per ml. Experimental SLE viremia onset was delayed and maximum titer depressed in two sloths concurrently infected with naturally acquired viruses. SLE viremias in four experimentally inoculated cormorants *Phalacrocorax olivaceus* were shorter, and of equal or lower titer, than in sloths. Colonized *Culex pipiens quinquefasciatus* mosquitoes were infected by feeding on sloths circulating at least 4.8 $\log_{10}$ SMicLD$_{50}$ of SLE virus per ml, and subsequently transmitted the infection to mice and chicks. An uninoculated baby *Bradypus* became infected by contact transmission from its mother. The antibody response of sloths to SLE virus was slow, being undetectable until several weeks post-inoculation. However, both sloth species developed high and long-lasting neutralizing and hemagglutination-inhibition antibody titers. The complement-fixation antibody response in *Bradypus* was lower and slower to develop than in *Choloepus*. Sloths with naturally acquired SLE virus antibody did not become detectably viremic after experimental inoculation. Neither sloths nor cormorants become overtly ill from SLE virus infection.

St. Louis encephalitis (SLE) virus, an arthropod-borne flavivirus, occurs from Canada to Argentina. It causes both sporadic and epidemic human disease in North America, where its summer cycle depends on transmission between *Culex* mosquitoes and birds, with mammals as possible important hosts in some areas.[1,2] In the most recent major epidemic, 1,815 documented cases in 1975 in the United States resulted in 142 deaths.[3]

In the tropics, SLE virus is presumed to be enzootic, and probably causes only sporadic human illness,[4] but its natural cycle is unclear. SLE virus has been isolated from a wide variety of tropical birds, and, in South America, from two monkey species, five rodent species, one species of opossum, and from a sloth, *Bradypus tridactylus*.[2]

To clarify the natural cycles of both yellow fever (YF) and SLE viruses in Panamanian forests, the Gorgas Memorial Laboratory surveyed 39 species of wild birds and 28 wild mammal species for antibodies against these two agents and other togaviruses.[5] The highest prevalence of antibody against SLE virus in any group of forest vertebrates was found in sloths, specifically the two-toed sloth *Choloepus hoffmanni* and the three-toed sloth *Bradypus variegatus*. This information, together with the isolation of SLE virus from a Brazilian *B. tridactylus* sloth, suggested that sloths might be important vertebrate hosts in tropical forest cycles of this virus.

To test this hypothesis, we inoculated captive *C. hoffmanni* and *B. variegatus* sloths with SLE virus. These experiments were designed to measure the duration and intensity of experimental viremia in sloths, as indices of their potential as infectious virus sources for vector mosquitoes. A second goal was to measure the humoral immune response of sloths to SLE virus, in order to evaluate the results of the field serologic survey. To compare sloths with a Panamanian bird species which has yielded several isolates of SLE virus,[6] we also inoculated cormorants, *Phalacrocorax olivaceus*. A second reason for the cormorant experiment was to test the pathogenicity of SLE virus for these birds, since the Panamanian isolates have been from moribund birds.

MATERIALS AND METHODS

Viruses

The strain of SLE virus inoculated into sloths and cormorants was isolated in Vero cells from the viscera of a moribund Panamanian cormorant collected in 1973.[6] We inoculated this first Vero cell passage into experimental cormorants. For experimental sloth infections, we inoculated 1-day-old chicks with the first Vero cell passage, and then allowed colonized *Culex pipiens quinquefasciatus* mosquitoes from the Gorgas Memorial Laboratory to feed on these viremic chicks. The infected mosquitoes served as the source of infectious SLE virus for sloth inoculations, as extracts either of whole triturated mosquitoes or of their dissected salivary glands.

For serology, we used the strains of SLE, Ilheus, Bussuquara, and YF viruses described elsewhere.[5]

Animals

Bradypus variegatus and *C. hoffmanni* sloths were captured by hand in these central Panamanian localities: Aguacate, Ancón, Arraiján, Chilibre, and Curundu. They were maintained at ambient temperature (25°–28°C) and humidity in screened animal quarters free of biting insects. Sloths were held before inoculation for as little as 1 day or for as long as several weeks. Although all were tested prior to inoculation for pre-existing, naturally acquired flavivirus antibodies, it is theoretically possible that these tests might not have detected low-level, developing antibodies in a very recently naturally infected animal. In captivity, *B. variegatus* fed on fresh *Cecropia* tree leaves, *C. hoffmanni* fed on a variety of fruits and vegetables, differing according to individual tastes.

Cormorants (*Ph. olivaceus*) were captured as nestlings on Pacheca Island, Islas Perlas, Panamá, and maintained in captivity on fish and squid. They were inoculated when all flight and body contour feathers had grown in.

Mosquitoes (*Cx. pipiens quinquefasciatus* and *Haemagogus equinus*) were from established Gorgas Memorial Laboratory colonies. No particular generations were used in transmission and infection experiments.

White mice from the Gorgas Memorial Laboratory colony were used at 0–2 days old for virus isolation and transmission. One-day-old commercial chicks were used in transmission experiments.

Serologic tests

Hemagglutination-inhibition (HI)[7] and complement-fixation (CF)[8] tests followed standard procedures. HI tests were run against 4–8 hemagglutinating units, CF tests were done as block titrations. Both tests used sucrose-acetone extracted SLE virus mouse brain antigen for serum antibody titrations. Crude saline brain extracts were tested against known antisera to identify viral etiology of mouse death.

Plaque reduction neutralization (PRN) tests were done in Vero cells, generally against 50–100 plaque-forming units. Detailed procedures are described elsewhere for tests using flaviviruses[5,9] and various sloth viruses.[10,11]

Virus assays

The SLE virus content of blood and inocula was assayed in 0- to 3-day-old mice to determine the median suckling mouse intracranial lethal dose (SMicLD$_{50}$). Simultaneous titrations were done in Vero cell tube cultures, for cytopathic effect, and Vero monolayers for plaque-forming units.[5,9,10]

No single assay system was consistently the most sensitive. However, at low virus concentrations, suckling mouse titrations gave the most linear results, and are presented here as the standard virus assay system.

A special technique was used to study the simultaneous circulation of SLE virus and a Changuinola (CGL) group virus in *Choloepus* no. 20. To detect and assay SLE virus in this animal, whole sloth blood dilutions were inoculated directly into suckling mice. To neutralize SLE virus which might mask the slower CGL group virus, whole sloth blood or a 1:10 dilution thereof was mixed with an equal volume of high-titered, heat-inactivated mouse ascitic fluid against SLE virus. This mixture was inoculated into suckling mice after incubation for an hour at room temperature. Crude saline CF antigens were prepared from the brains of mice dying after inoculation of the blood-ascitic fluid mixture, as well as after direct inoculation of blood. These antigens were tested for CF reactivity with specific immune reagents against SLE and CGL viruses to calculate viremia titers.

Sloth inoculation and sampling procedures

Preinoculation sloth plasma samples were tested by PRN test against SLE and the three other

known Panamanian sylvatic flaviviruses. Pre-inoculation whole blood samples were taken several days and/or several minutes before inoculation, and were tested in Vero cells for naturally-acquired virus infections.

Four sloths were then infected by the bite of 4, 6, 7 or 19 infected *Cx. p. quinquefasciatus* mosquitoes. The rest were injected subcutaneously in the nose and lips, the areas thought to be most readily attacked by mosquitoes. Needle-and-syringe doses of SLE virus were either 2.6–2.8 $\log_{10}$SMicLD$_{50}$ (triturated whole mosquitoes) or 2.3 $\log_{10}$SMicLD$_{50}$ (triturated mosquito salivary glands).

Sloths were bled from the brachial vein every other day for 2 weeks post-inoculation, beginning on days 1, 2 or 3 post-inoculation. Two blood samples were taken during the 3rd week post-inoculation, followed by two to five samples in the next 4 weeks, depending on the results of viremia tests of preceding samples. For viremia detection and quantification, whole heparinized blood was frozen at −60°C in two aliquots. Titers reported here were measured in samples which had been thawed only once. Plasma was generally separated from a portion of the blood sample taken at approximately weekly intervals after inoculation until day 28, then once during the next 2 weeks. After day 42 plasma samples were taken roughly every 40–50 days.

Cormorant inoculation, bleeding, and explant procedures

Cormorants were tested for naturally acquired PRN antibodies against the four sylvatic flaviviruses. Birds negative to all four viruses were then inoculated subcutaneously in the bare skin of the legs and lores with 200 SMicLD$_{50}$ of SLE virus at the first (Vero cell) passage level. They were bled from wing, leg or jugular vein daily for 10 days after inoculation, again at days 14, 21, and 28, and finally when they were killed at day 80 or 111. Whole heparinized blood samples taken on these days were frozen at −60°C, then assayed for virus content in mice and Vero cell tube cultures. Plasma samples taken before inoculation, and on days 7, 14, 21, 28 and 80 or 111 after inoculation were tested for HI and PRN antibodies.

To detect chronic or latent infections, portions of brain, heart, lung, liver, kidney, spleen, bone marrow and testis or ovary were taken from each cormorant and frozen at −60°C. Ten percent suspensions of these frozen organs in phosphate-buffered saline with 0.5% gelatin were inoculated intracerebrally into suckling mice to detect SLE virus. At the time of necropsy, explant cultures were also made from each of these organs from two antibody-positive cormorants. Explant procedures followed the method of J. Hardy and S. Presser (personal communication, S. Presser, School of Public Health, University of California, Berkeley, CA 94720). Organs were minced into chunks of ⩽1 mm³, rinsed in phosphate-buffered saline, and planted in 60 × 15 mm plastic Falcon petri dishes, in Leibowitz L-15 medium with 30% fetal calf serum plus antibiotics. Cultures were observed for growth and medium was changed every 3 days, at which time 1 ml of medium was frozen, then inoculated into Vero cell tube cultures and suckling mice to detect virus. After 3 weeks the entire culture was frozen, thawed, and inoculated to detect virus.

RESULTS

Sloth viremias

All sloths without pre-existing antibody against SLE virus became viremic. Both genera of sloths responded to SLE virus with remarkably long-lasting viremias of high titer (Tables 1, 2). Differences in route and dose of inoculum did not seem to affect the viremia patterns.

The durations of detectable viremia in five *B. variegatus* ranged from 13–24 days (median 18 days). Viremia duration tended to be shorter in most *C. hoffmanni* than in *B. variegatus*, ranging from 7–27 days (median 11 days).

Maximum observed SLE virus titers in *Bradypus* ranged from 4.0–6.5 (median 5.1) $\log_{10}$SMicLD$_{50}$/ml, and in *Choloepus* from 2.7–6.2 (median 4.9) $\log_{10}$SMicLD$_{50}$/ml. Viremia peaks tended to be rather broad; in sloths with maximum titers of >4.7 $\log_{10}$SMicLD$_{50}$/ml, the average number of days on which viremia titers of at least 4.5 $\log_{10}$SMicLD$_{50}$/ml were detected was 4.1 (range 1–7 days).

Viremia in sloths was generally detectable in the first blood sample taken after inoculation (days 1, 2, or 3). However, in three sloths, SLE viremia onset was delayed. At least two of these three exceptional animals were naturally infected by other viruses at the time of SLE virus infection (Table 2). Both naturally infecting viruses are described in detail elsewhere.[10] *Bradypus* no. 4, infected with

TABLE 1

Viremia profiles of nine sloths experimentally infected with St. Louis encephalitis virus*

| Sloth no. | Route of inoculation (dose) | Duration of detectable viremia (days)† | Maximum viremia | | No. of days on which titer ≥ 4.5 log$_{10}$SMicLD$_{50}$/ml‡ |
			Peak titer	Day post inoculation	
Bradypus 1	Subcutaneous (A)§	24	6.5	11	7
Bradypus 3	Subcutaneous (A)	19	5.1	11	1
Bradypus 13	Mosquito bite (4)	16	5.3	8	5
Bradypus 15	Mosquito bite (7)	13	4.6	8	1
Choloepus 7	Mosquito bite (19)	9	6.2	5	5
Choloepus 8	Mosquito bite (6)	11	5.0	6	5
Choloepus 11	Subcutaneous (B)	27**	2.7	17	0
Choloepus 17	Subcutaneous (B)	7	4.8	6	3
Choloepus 19	Subcutaneous (A)	11	5.6	5	3

* Includes only animals in which no heterologous viremia from a naturally infecting virus was detected during the course of experimental St. Louis encephalitis virus infections.
† Unless otherwise indicated viremia was detected in the first blood sample taken, on day 1, 2, or 3 after inoculation.
‡ SMicLD$_{50}$. Median suckling mouse intracranial lethal doses. Numbers include days on which sloths were not bled but which fell between days on which viremic titers were ≥4.5 log/ml.
§ A: 2.5–2.8 log$_{10}$SMicLD$_{50}$, whole triturated mosquitoes. B: 2.3 log$_{10}$SMicLD$_{50}$, triturated mosquito salivary glands. Numbers: Number of infected mosquitoes engorging.
Log$_{10}$SMicLD$_{50}$/ml whole blood.
** Viremia not detected until day 6 post inoculation.

Bradypus-4 virus at the time of SLE Virus inoculation, did not develop detectable SLE viremia until 15 days after inoculation. In *Choloepus* no. 20, infected with an agent related to Changuinola virus, SLE viremia was detected on day 3 but not day 1 post-inoculation. In both animals, the peak SLE viremia titer was well below most (*Choloepus*) or all (*Bradypus*) of the peak titers in other sloths.

This pattern of a delayed viremia of depressed titer was also observed in a third sloth, *Choloepus* no. 11 (Table 1), suggesting that this animal too was naturally infected by another virus at the time of SLE virus inoculation. However, no such virus was detected in pre- or post-inoculation blood samples. Furthermore, sequential plasmas from *Choloepus* no. 11 did not show a rise in antibody titer against Bradypus-4 virus nor against two Changuinola group viruses, one phlebotomus fever group virus, and one virus from group Simbu, all isolated from Panamanian sloths.[10]

Detectable PRN antibody against the naturally-infecting virus developed in both *Bradypus* no. 4 and *Choloepus* no. 20 before detectable PRN antibody against SLE virus (Table 2). This observation suggests that productive infection of the natural virus ended before that of SLE virus. However, in *Choloepus* no. 20, the Changuinola group virus circulated simultaneously with SLE virus for 13 days and the maximum detected viremia titer for both viruses occurred at roughly the same time.

TABLE 2

Viremia profiles and appearance of neutralizing antibody in two sloths shown to be infected with naturally acquired viruses when inoculated subcutaneously with 66–200 plaque forming units of St. Louis encephalitis (SLE) virus

| Sloth | Virus | DPI* of detected viremias | | Maximum viremia | | DPI of first detected neutralizing antibody° |
		Initial	Final	Peak titer (assay system)	DPI of occurrence	
Bradypus No. 4	Bradypus—4	−4	3	0.9 (Vero)‡	−4	41
	SLE	17	34	4.0 (SMic)	25	53
Choloepus No. 20	Changuinola group	0	≥28	≥2.5 (Vero)	5, 7, 9, 13	13
	SLE	3	15	3.8 (SMic)	7	21

* DPI: day post inoculation. A negative value specifies a given day before inoculation, 0 specifies the day of inoculation, immediately prior to inoculation itself.
° Plaque reduction neutralization, with 90% reduction by plasma 1:8 considered positive.
‡ Titers expressed as log$_{10}$ effective doses per ml whole blood. Vero: tissue culture median infectious doses. SMic: Suckling mouse intracranial median lethal doses.

<div align="center">TABLE 3</div>

Appearance of hemagglutination-inhibition (HI), neutralizing (PRN), and complement-fixing (CF) antibody in sloths experimentally infected with St. Louis encephalitis virus

Species	No. of animals	Median (range) day post inoculation of earliest detected antibody*			Median (range) day post viremia of earliest detected antibody		
		HI	PRN	CF	HI	PRN	CF
Bradypus variegatus	5	21 (13–41)	31 (21–53)	53 (28–90)	6 (−12–7)[†]	10 (6–19)	28 (11–69)
Choloepus hoffmanni	6	14.5 (11–21)	23 (21–50)	21 (21–28)	5 (−21–6)	14.5 (6–18)	9.5 (−4–14)

* Lowest antibody titers considered positive: HI—1:10 against 4–8 hemagglutinating units; N—1:8, 90% reduction of 40–120 plaque forming units; CF—1:4, in block titration.
† Negative values indicate appearance of detectable antibody before the end of detectable viremia. Antibody was detected in samples taken during detectable viremia in only one sloth of each species.

Antibody response of sloths

All infected sloths produced specific HI, PRN and CF antibodies against SLE virus. The times of appearance of each type of antibody are shown in Table 3. The humoral immune response in sloths against replicating SLE virus antigen is slow; in general, antibody is first detectable several weeks after inoculation. This period is somewhat shortened if the appearance of detectable antibody is considered relative to the end of detectable viremia. HI antibody was detected in two sloths while they were still viremic, and never more than a week after the last day of detected viremia. Since plasma was collected only at weekly intervals, it is likely that several other animals were actually circulating HI antibody with detectable virus. PRN antibody appeared somewhat later than HI antibody (21–53 days after inoculation, 6–19 days after detectable viremia). In *Choloepus*, CF antibody appeared at the same time or earlier than PRN antibody, but the first detectable CF response in *Bradypus* was distinctly delayed (up to 90 days post-inoculation and 69 days post-viremia).

Although the humoral antibody response was slow, the maximum observed titers were high by all three serologic tests used (Table 4). In five *B. variegatus* and four *C. hoffmanni* observed for 78–189 days after inoculation, maximum observed HI and PRN titers were not lower than 1:320 and 1:128 respectively. The HI and PRN responses of these nine animals were long-lasting as well as high, since the lowest observed HI and PRN antibody titers at 78–189 days post-inoculation were 1:80 and 1:64, respectively. The maximum CF antibody response was consistently lower in *Bradypus* than *Choloepus*, corresponding with the slower appearance of CF antibody in *Bradypus*. The highest CF titer observed in a *Bradypus* was 1:16, which equalled the lowest peak titer observed in any *Choloepus*; the one *Choloepus* with a maximum observed CF titer of 1:16 was released only 28 days after inoculation, while its CF titer was still rising.

Sloth neutralizing antibodies were quite specific against SLE virus. Two plasmas from each of three experimental *C. hoffmanni* and four *B. variegatus* were tested at 1:8 and weaker dilutions for neutralizing antibodies against YF, Ilheus, and Bussuquara flaviviruses. These plasmas had been taken at various intervals between 35 and 189 days post-inoculation. Homologous titers against SLE virus ranged from 1:64 to ≥1:512. None of the 14 plasmas reacted with any of the other three flaviviruses, except one *Bradypus* plasma taken day 41 post-inoculation. This plasma inhibited Ilheus virus at a titer of 1:8 only, and was negative against YF and Bussuquara viruses; the homologous titer against SLE virus was >1:512.

Challenge inoculation of sloths

Three *C. hoffmanni* with pre-existing naturally acquired PRN antibody against SLE virus were inoculated subcutaneously with 2.0–2.7 $\log_{10}$SMicLD$_{50}$ of SLE virus. In one animal with a pre-existing PRN titer of 1:256, no virus was detected in seven blood samples taken at 2 to 3-day intervals during the first 3 weeks after inoculation. No evidence of a secondary PRN antibody response was seen in six plasma samples taken from this animal during this period, including days 2, 5 and 7 post-inoculation.

The other animals, with respective SLE virus PRN antibody titers of 1:32 and 1:256 were bled

TABLE 4

Duration and maximum titer of hemagglutination-inhibition (HI), neutralization (PRN), and complement-fixation (CF) antibodies against St. Louis encephalitis virus in experimentally infected sloths

Species	No. of animals	Days of observation		Median (ranges) of maximum observed titers*			Median ranges of final observed titers		
		Days post inoculation	Days post last viremic sample	HI	PRN	CF	HI	PRN	CF
Bradypus variegatus	5	122–189	105–164	640† (320–1,280)	256 (128–≥512)	16 (8–16)	320 (80–640)	128 (64–≥512)	4 (<4–8)
Choloepus hoffmanni	4	78–122	61–110	320 (320–640)	128 (128–512)	32 (32–128)	320 (160–640)	128 (128–512)	32 (16–64)
	2	28–49	17–34	160 (80–320)	24 (16–32)	24 (16–32)	160 (80–320)	24 (16–32)	12 (<8–32)

* HI titer against 4–8 hemagglutinating units; 90% plaque-reduction PRN titer against 40–250 plaque-forming units; maximal CF titer in block titration.
† Reciprocal of plasma dilution.

from 2–6 days post-inoculation, a period when 9/11 antibody-negative sloths were detectably viremic. A total of five blood samples from these two sloths were negative for virus. Their plasmas were not tested for secondary immune response.

Mosquito infection and transmission

All *Bradypus* and *Choloepus* sloths bitten by SLE virus-infected *Cx. p. quinquefasciatus* mosquitoes became infected (Table 1).

The SLE virus infection rates of two species of colonized mosquitoes feeding on viremic sloths are shown in Table 5. The 50% infection threshold for *Cx. p. quinquefasciatus* feeding on both sloth species fell between 5.1 and 6.2 $\log_{10}$SMicLD$_{50}$/ml of sloth blood, and the 0–10% infection threshold was roughly 4.5–5.0 logs. Equivalent values for mosquitoes of this colony feeding on viremic mice and chicks were approximately 4.1–5.0 (50% threshold) and 3.0–4.0 (10% threshold) $\log_{10}$SMicLD$_{50}$ per ml chick or mouse blood.

No *Hg. equinus* became infected after feeding on viremic *Bradypus* sloths circulating up to 5.1–6.2 $\log_{10}$SMicLD$_{50}$ per ml. The 50% infection threshold for these colonized mosquitoes feeding on viremic mice and chicks was 5.1–5.7 logs of SLE virus per ml blood.

Of the *Cx. p. quinquefasciatus* infected by feeding on sloths, 80% were able to transmit SLE virus to chicks or mice after 14–27 days extrinsic incubation. A chick-mosquito-sloth-mosquito-chick SLE virus transmission cycle was completed in the laboratory using a *B. variegatus* and *Cx. p. quinquefasciatus* mosquitoes.

Non-arthropod transmission between sloths

An uninoculated baby *B. variegatus*, housed with its experimentally infected mother, developed HI antibody against SLE virus. The mother was not lactating, but the baby was in close contact with her, sharing her food and clinging to her, as is typical of *Bradypus* sloths less than 6 months old.

The mother was viremic from at least as early as day 3 post-inoculation until day 25. HI antibody against SLE virus was first detected in her at 13 days post-inoculation, and PRN antibody at 31 days. Nineteen days after its mother's inoculation the baby's plasma was negative for SLE virus antibody by both HI and PRN test, as was its plasma taken before the mother's inoculation.

TABLE 5

Infection rates of colonized Culex pipiens quinquefasciatus *and* Haemagogus equinus *mosquitos feeding on* Choloepus hoffmanni *and* Bradypus variegatus *sloths experimentally infected with St. Louis encephalitis virus*

Blood meal titer	C. p. quinquefasciatus		Haemagogus equinus	
	Choloepus	Bradypus	Choloepus	Bradypus
Trace–4.0*	NT†	1/56 (2%)‡	NT	0/69
4.1–4.6	0/24	0/9	0/3	NT
4.8–5.0	5/28 (18%)	0/6	0/21	0/8
5.1–6.2	65/128 (51%)	11/18 (61%)	NT	0/17

* $Log_{10}SMicLD_{50}$ St. Louis encephalitis virus per ml of sloth whole blood.
† NT—not tested.
‡ Number of mosquitos positive/number tested (percent positive).

On day 68, the baby's HI titer was 1:160 against SLE virus antigen. This plasma was negative by HI test against Venezuelan encephalitis virus, a togavirus antigenically unrelated to SLE virus, supporting the specificity of the HI seroconversion against SLE virus. The day 68 sample was the last plasma taken from the baby, and the volume was insufficient for other serologic tests.

One explanation for the baby's seroconversion is contact transmission of SLE virus shed by the non-lactating mother. To determine if sloths can shed SLE virus, throat swabs were taken from an infected *Choloepus* every other day for 3 weeks after inoculation. SLE virus was detected only in the throat swab taken on day 13, although the animal was viremic days 3–15 post inoculation. The titer of SLE virus in the day 13 oropharyngeal secretion was at least 10-fold greater than the trace viremia detected that day, eliminating occult blood as a possible virus source.

Cormorants

Three of the four cormorants inoculated developed detectable SLE viremia and both HI and PRN antibodies. The fourth bird developed neither detectable viremia nor antibody, and apparently was uninfected.

In two birds, detectable viremia began on day 2 post inoculation, and lasted 4–5 days, with maximum titers of 3.5 and 4.0 $log_{10}SMicLD_{50}$/ml. HI and PRN antibodies were detectable in these birds in the first post inoculation plasma sample, taken day 7.

A third cormorant was apparently sick at the time of SLE virus inoculation; signs of illness included weakness and head-shaking. Detectable SLE viremia was delayed until day 5, and continued through at least day 10, but not day 14. The maximum titer was 5.9 $log_{10}SMicLD_{50}$/ml. Plasma

taken on day 7, during viremia, was negative for antibodies by both HI and PRN tests; day 14 plasma was positive by both tests.

Hemagglutination-inhibition and PRN antibodies persisted in infected birds until days 80–111, when they were killed. Maximum HI titers of 1:320–1:2,560 were reached by day 14 post-inoculation, but had declined to 1:20–1:80 by the end of the experiment. Maximum PRN antibody titers of 1:32–1:128 persisted without decline.

Organ suspensions of all four birds were negative for virus by direct inoculation of suckling mice. Culture fluids of explant cultures of the organs of two infected birds were consistently negative for virus in Vero cells and suckling mice, as were the explant cultures themselves at 21 days post seeding. In these cultures, growth of spleen, lung, testes, and bone marrow was moderate to heavy, but brain, liver and pancreas explants grew only slightly.

No cormorant showed any signs of illness attributable to SLE virus infection. In fact, the weakness and head-shaking of the bird which was ill at the time of inoculation began to disappear during the period of SLE viremia; by 2 weeks after inoculation the bird had apparently recovered completely.

DISCUSSION

High, long-lasting viremias infected for mosquitoes qualify both *Bradypus* and *Choloepus* sloths as potentially important hosts of tropical SLE virus. Arbovirus field studies provide data which support this conclusion, because they show that specific SLE antibody is exceptionally prevalent in Panamanian sloths, and because the virus itself has been isolated from a Brazilian *Bradypus tridactylus* (Pinheiro, F.P., unpublished data cited by McLean and Bowen[2]). The abundance of

sloths in Neotropical forests, reviewed elsewhere,[3] underlines their potential as SLE virus hosts; this abundance is little recognized, perhaps because of sloths' natural camouflage.

The long high SLE viremia in *B. variegatus* was not surprising. Experimental infections of this species with two other togaviruses, Venezuelan encephalitis (VE)[12] and YF[13] viruses, have yielded similar results. It is curious that experimental viremias in *B. tridactylus* following infection with Oropouche virus were not exceptionally long or of high titer, particularly since this species has yielded the only four isolates of Oropouche virus from a wild vertebrate source.[14]

Experimental SLE viremias in *C. hoffmanni* were as high as in *Bradypus*, and also remarkably long, though generally somewhat shorter than in *Bradypus*. This result contrasts with observation of minimal or undetectable viremias in *C. hoffmanni* and *C. didactylus* following infection with YF and VE viruses.[12, 13, 15]

Sloth experimental SLE viremias were as high or higher than those of birds, including the cormorants in these studies and the many species reviewed elsewhere.[2] The weeks-long durations of sloth SLE viremias are remarkable by any standards.

Although infectable by mosquito bite and infectious for feeding *Culex*, sloths were surprisingly inefficient infectious virus sources for the mosquito species tested, especially when compared with chicks and mice. These results must be interpreted with these considerations:

1. The two mosquito species used have not been implicated as tropical SLE virus vectors;
2. Both mosquito species were colonized;
3. Parallel sloth and mouse/chick mosquito feeding experiments did not use the same mosquito generation, for practical reasons; and
4. Far from being natural hosts, mice and chicks are laboratory and commercial artifacts.

Definitive experiments would have to include sloths, wild tropical birds, and either uncolonized or F_1 mosquitoes of as yet unidentified enzootic forest vector species. Until such experiments have been done, the chick-mosquito-sloth-mosquito-chick laboratory transmission cycle is strong evidence for a role for sloths in tropical SLE virus cycles.

The humoral immune response of sloths to SLE virus was remarkably slow, especially considering that circulating SLE virus was detectable within

3 days of inoculation in 10/11 animals. The slow antibody response may be a function of low sloth body temperature; the rectal temperatures of five *Bradypus* and five *Choloepus* used in our SLE virus infection experiments ranged from 31.2° to 35.5°C during 10 days of observation. Little is known of the immune system of sloths, or for that matter of any other xenarthrans, a group of mammals including armadillos in which low body temperatures are common. The immune functions of the nine-banded armadillo *Dasypus novemcinctus* have been studied, because of this animal's importance as a leprosy model. Although there are no gross or microscopic deficiencies or abnormality in the immunologic components of *D. novemcinctus* and some other armadillos,[16, 17] an in vitro study of *D. novemcinctus* lymphocytes showed that cooler temperatures do reduce transformation in this species, as well as in humans.[18] An in vivo study of armadillo humoral and cellular immune response to non-replicating antigens showed no abnoramlities, but was not designed to measure slowness in response.[19] Following inoculation with foot-and-mouth disease virus, four armadillos showed a clinical response by 3 days, but only two of these animals had developed precipitating antibodies against a viral group antigen by day 14 post-inoculation.[20] In contrast, these antibodies are detectable in cattle 7 days after inoculation.[21]

The hypothesis that low body temperature in sloths delays antibody development relative to homothermic mammals could be tested by warming infected sloths to 37°C. Also, experimental inoculations of sloths with non-replicating antigens would determine whether sloths are simply slow in developing antibodies, or are insensitive to foreign antigens, needing a prolonged stimulus for an immune response. Finally, SLE virus harvested from infected sloths should be tested for changes in biological character, similar to changes observed for this virus persistently infecting turtle cells at 31°C.[22]

Whatever its cause, the delayed antibody response may explain the relatively long viremia durations in sloths. Regardless of slow development, the antibody levels attained and persistently maintained in both sloth species are high, and apparently functional, since challenged immune sloths did not become viremic.

The specificity of the neutralizing antibody response for SLE virus is useful for evaluating field surveys in areas where other flaviviruses are active. High antibody titers in experimental sloths,

against SLE virus, compared with very low titers or negative results against YF, Ilheus, and Bus-suquara viruses, are similar to observations on naturally acquired immunity in Panamanian sloths.[5]

The small number of experimentally infected cormorants precludes generalizations on viremia or antibody response. However, SLE virus infection clearly does not cause disease in this species, unless synergistically with some unknown agent. During 1973, SLE virus had been isolated from three of five moribund cormorants tested, suggesting an epizootic in these birds.[6] But none of three cormorants became ill when experimentally infected with an SLE virus strain isolated from a moribund cormorant in 1973 and passed once in Vero cells. The isolation of several SLE virus strains from this species in 1973 might otherwise be explained by recrudescence of a past, latent infection in stressed birds; however, no virus was recovered from organ explants of two birds known to have been infected with SLE virus, nor from frozen organs of these and the two other inoculated cormorants.

The apparent contact transmission of SLE virus from an infected mother *Bradypus* to her baby might be dismissed as a laboratory curiosity. However, a remarkably similar seroconversion pattern occurred in nature in a non-lactating mother-baby *Choloepus* pair.[5] Mother-baby transmission, possibly through salivary contamination of shared leaves, could double the period of vertebrate virus circulation and availability for vector mosquitoes. This increased effective viremia duration would be especially important during a period of vector scarcity such as the dry season.

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