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SURVEILLANCE OF ARBOVIRUS INFECTIONS IN THE ATLANTIC FOREST REGION, STATE OF SÃO PAULO, BRAZIL. I. DETECTION OF HEMAGGLUTINATION-INHIBITING ANTIBODIES IN WILD BIRDS BETWEEN 1978 AND 1990.

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SUMMARY

We report data related to arbovirus antibodies detected in wild birds periodically captured from January 1978 to December 1990 in the counties of Salesópolis (Casa Grande Station), Itapetininga and Ribeira Valley, considering the different capture environments.

Plasmas were examined using hemagglutination-inhibition (HI) tests. Only monotypic reactions were considered, except for two heterotypic reactions in which a significant difference in titer was observed for a determined virus of the same antigenic group.

Among a total of 39,911 birds, 269 birds (0.7%) belonging to 66 species and 22 families were found to have a monotypic reaction for Eastern equine encephalitis (EEE), Venezuelan equine encephalitis (VEE), Western equine encephalitis (WEE), Ilheus (ILH), Rocio (ROC), St. Louis encephalitis (SLE), SP An 71686, or Caraparu (CAR) viruses.

Analysis of the data provided information of epidemiologic interest with respect to these agents. Birds with positive serology were distributed among different habitats, with a predominance of unforested habitats. The greatest diversity of positive reactions was observed among species which concentrate in culture fields.

KEYWORDS: Arbovirus; Ecology, Hosts; Hemagglutination-inhibiting antibodies.

INTRODUCTION

The Section of Arthropod-Transmitted Viruses (S. A. T. V.) of the Adolfo Lutz Institute (A. L. I.), Health Department of the State of São Paulo, has been conducting ecologic and epidemiologic studies concerning to arbovirus infections in the Atlantic Forest region of the State of São Paulo, Brazil, since 1961. The objective of these studies is to identify arbovirus hosts and vectors and the circulation of these viruses in the human population. Some of the activities involve periodic collection of blood samples from wild birds performed in Salesópolis and Itapetininga counties in an attempt to isolate the virus and/or detect arbovirus antibodies. In April 1975, collections were also started in the Ribeira Valley region due to the occurence of an human encephalitis epidemic caused by the Rocio arbovirus, which affected the Southern region of the State of São Paulo in 1975-1977. The data related to this epidemic were published elsewhere [7, 8, 18, 20, 21, 23, 25-27, 30-34]. Information on the isolation and characterization of viruses from this area has also been published [2, 3, 14-20, 22].

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Our objective is to report on data related to hemagglutination-inhibiting (HI) arbovirus antibodies detected in wild birds periodically captured from January 1978 to December 1990 in Casa Grande (Salesópolis), Itapetininga and in various Ribeira Valley counties, considering the different capture environments.

MATERIAL AND METHODS

Studied areas

The field stations used in this study were located in Salesópolis and Itapetininga counties and Ribeira Valley region.

Casa Grande (23° 40'S, 45° 55'W) is located in Salesópolis, 100 km from São Paulo, the state's capital, on the sierra along the Atlantic Coast (Fig. 1). Altitude is approximately 800 meters and the region is covered by an extensive primary forest.

The forested area of Itapetininga (23° 40'S, 48° 55'W) is located 150 km from the State's capital, where the central plateau of Brazil begins (Fig. 1). The region consists of natural fields where rivers and streams are surrounded by gallery forest which acts as a concentration place for the local fauna.

The Ribeira Valley region is occupied by the hydrographic basin of the Ribeira de Iguape river (Fig. 1). The region includes 16 counties. It is located in Southeast of the State of São Paulo (24° 00'S and 25° 16'S and 46° 50'W and 49° 20'W). Most of the region consists of an extensive plain surrounded by mountains. The vegetation consists mainly of primary forests and, to a lesser extent, of secondary forests, crop fields (rice, bananas, guava, tea, passion fruit and vegetables), and pastures.

In the Ribeira Valley region the field work was carried out in the area affected by the epidemics, with priority given to Iguape, Cananéia, and Pariquera-Açú counties and eventually also in Juquiá, Registro, Jacupiranga, Sete Barras, Eldorado Paulista, Barra do Turvo, Itariri and Pedro de Toledo counties. In Iguape, there is an altered natural environment close to the sea coast, with the presence of crop fields and brush side by

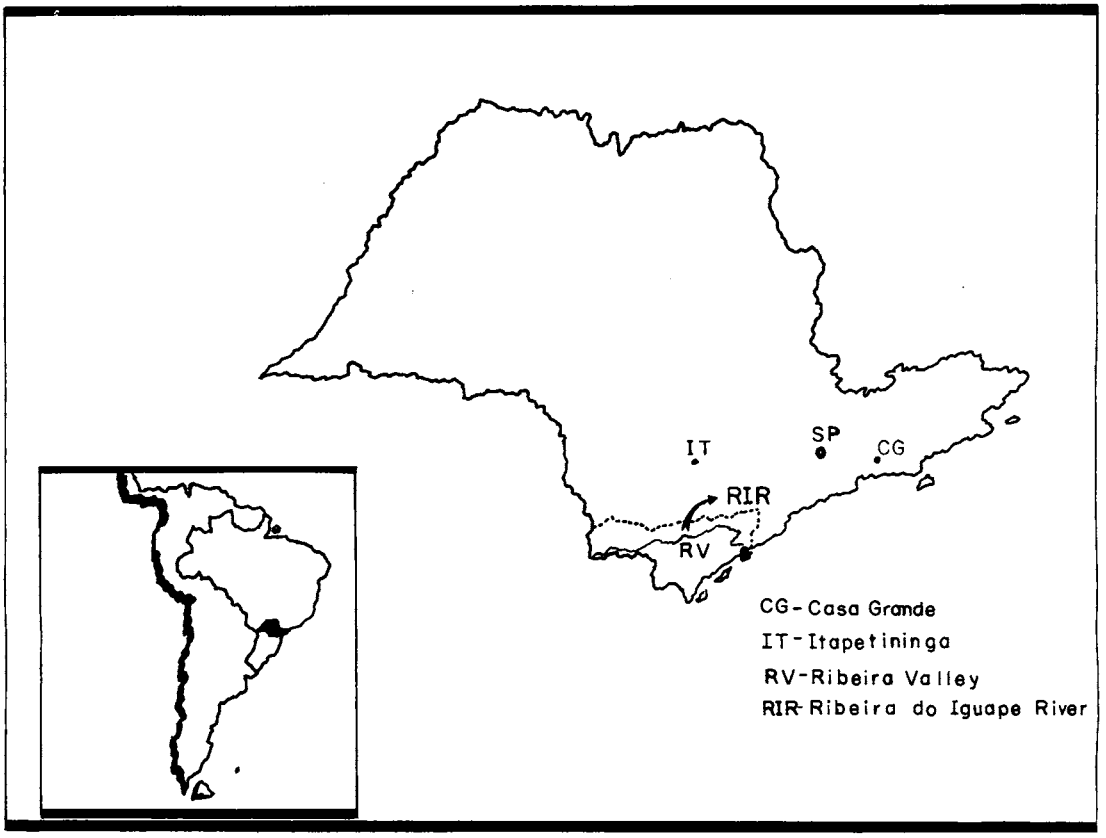

Fig. 1 - Localization of Casa Grande, Itapetininga, Ribeira Valley region and Ribeira do Iguape River, São Paulo state, Brazil.

side with primary or secondary forests; in Cananéia, slightly modified environment in the early stage of deforestation for the formation of crop land and pastures, with the presence of primary forests at both low and high altitudes; in Pariquera-Açú, markedly altered environment with the extensive presence of crop fields and brush, close to residual primary forests.

Bird captures

Wild birds were captured with Japanese mist nets set up in different types of habitat, preferentially at boderline sides between crop fields and primary and secondary forests, crop fields and brush, inside crop fields, brush, primary and secondary forests, and around human dwellings.

The birds were bled by cardiac or jugular puncture, identified, banded and released, for possible future recapture and collection of data related to their population dynamics. The blood collected (0.4ml) into a sterile syringe containing 1.6ml of 0.75% phosphate buffered bovine albumin at pH 7.4 and heparin, was refrigerated and brought to the laboratory. Plasmas were separated by centrifugation at 2,500 rpm for 5 minutes and used for serological testing. Birds were identified by S.A.T.V./A.L.I. under the supervision of Dr. Hélio F. A. Camargo, of the Zoology Museum, University of São Paulo. Captures were conducted weekly, one field station at a time.

It was observed spatial distribution of the birds. There are species that were found only inside forests and others that populated the most different habitats. This observation permitted the classification of captured birds into 7 categories according to site of capture: forest, forest/brush, forest/brush/around human dwellings, forest/brush/cropfields/around human dwellings, brush/crop fields, brush/crop fields/around human dwellings, and crop fields/around human dwellings. There seems to be a gradation of progressive adaptation of these birds to a modified environment. This categorization was used to present serologic results since it permits visualizing a behaviour of these possible arbovirus hosts that might favor proximity to humans.

The data concerning dispersal and environmental distribution of bird species were obtained using the Ringing Program initiated by S.A.T.V. in 1966 [24]. This program has the purpose of determining which species are responsible for the maintenance of arbovirus cycles in nature and which ones, by their migratory habits, could be held responsible for the arboviruses circulation in the American Continent. The aluminum band is put in the right tarsometatarsus of the birds; each one has a recapture control card where the species name, sex and age, the dates of capture and recapture are recorded. The taxonomy recomended by SCHAUENSEE [28] was used to prepare Table 5.

Serological testing

The plasma samples collected were examined by HI test against antigens of the following arboviruses of epidemiologic interest in the region: EEE (SP AN 14723), VEE complex (prototype strain, SP AN 50783 and Mucambo-SP AN 15600), WEE (TR 25717), ILH (prototype strain supplied by the Rockefeller Foundation Virus Laboratories, New York, USA), ROC (SP H 34675), SLE (SP AN 11916), SP AN 71686 (a new Flavivirus isolated in the Ribeira Valley), and CAR (SP AN 26550).

The technique used was that of CLARKE & CASALS [4], adapted to the microtiter method of Takatsy as modified by SEVER [29]. Antigens were made by sucrose-acetone technique for mouse brain, or by two acetone extractions for infected mouse serum. Goose red cells 1:200 were used for hemagglutination. Non specific inhibitors of plasma were acetone extracted by the reference method of CLARKE & CASALS [4]; to remove goose cells agglutinins rehydrated plasmas in the 1:10 dilution were adsorbed with goose cells. The plasmas with titer of 1:20 or greater inhibiting hemagglutination produced by 4 antigen units were considered positive. Starting in 1983, positive plasmas were titrated up to the 1:160 dilution. For analytical purposes and for the sake of precision, we particularly considered monotypic reactions, and only included two heterotypic reactions in which there was a four-fold difference in titer between an antigen and the remaining ones in the same group.

RESULTS

A total of 39,911 plasma samples from wild birds belonging to 162 species and 42 families were examined.

The prevalence of monotypic and heterotypic HI antibodies detected in these plasmas are listed in the table 1 according to the regions where the field stations are located and according to the antigens studied.

TABLE 1

Prevalence of monotypic (M) and heterotypic (H) HI antibodies in the plasma of wild birds captured in the State of São Paulo from 1978 to 1990.

Antigens*	Field Stations						
	Itapetininga		Casa Grande		Ribeira Valley		
	M	H	M	H	M	H	M/H
EEE	1/8856	2/8856	1/4290	-	19/26765	4/26765	3,5
VEE	2/8856	-	-	-	1/26765	-	-
WEE	1/3540	2/3540	-	-	-	-	0,5
ILH	1/8856	12/8856	2/4290	5/4290	41/26765	47/26765	0,7
ROC	4/8856	6/8856	-	-	5/26765	8/26765	0,6
SLE	23/8856	14/8856	13/4290	5/4290	103/26765	50/26765	2,0
SPAN71686	Nt	Nt	Nt	Nt	50/1197	6/1197	8,3
CAR	-	-	-	-	2/11435	-	-

M monotypic reaction, H heterotypic reaction.; * EEE, Eastern equine encephalitis; VEE, Venezuelan equine encephalitis; WEE, Western equine encephalitis; ILH, Ilheus; ROC, Rocio; SLE, St. Louis encephalitis; SP AN 71686, a new Flavivirus isolated in the Ribeira Valley and tested since 1989; CAR, Caraparu, the only representative Bunyavirus.; - Absence of antibodies.; Nt, not tested.

The relative frequency of monotypic and heterotypic antibodies demonstrated that monotypic antibodies predominated for antigens EEE, SLE, and SP AN 71686 (Table 1).

Tables 2, 3 and 4 show the presence of HI antibodies monotypic for EEE, VEE, WEE, ILH, ROC and SLE in Itapetininga, for EEE, ILH and SLE in Casa Grande, and for EEE, VEE, ILH, ROC, SLE, SP AN 71686 and CAR in the Ribeira Valley.

Antibodies monotypic for EEE were more prevalent in the Ribeira Valley region and were detected more regularly during the study (Table 4). The proportion of monotypic and heterotypic reactions for this antigen was 3.5 to 1 (Table 1).

The presence of antibodies monotypic for WEE was only detected in Itapetininga in 1985 in a single bird, which has no migratory habits.

Monotypic antibodies for VEE were detected in Itapetininga in 1978 in two birds and in the Ribeira Valley only in one bird in 1985. Anti-ROC antibodies were first detected in Itapetininga in 1980, in the plasma of 4 birds. The Ribeira Valley presented only one positive plasma in 1978, 2 plasmas in 1985 and 2 plasmas in 1986, these antibodies were not detected again until 1990 (Tables 2 and 4).

A higher prevalence of the SLE antibodies was detected in the 3 regions studied, but periods of absence of these antibodies were observed at regular intervals in Casa Grande and Itapetininga (Tables 2 and 3). In the Ribeira Valley, these intervals were no longer observed after 1985 when the presence of antibodies became constant, with variations in the prevalence (Table 4).

For the ILH antigen, a single plasma sample with a monotypic reaction was observed in Itapetininga in

TABLE 2

Prevalence of monotypic HI antibodies in the plasma of birds from the Itapetininga region, State of São Paulo, captures from 1978 to 1985.

Antigens**	Years*				
	1978	1980	1982	1985	Total
EEE	0/599	0/1434	0/1004	1/752	1/8856
VEE	2/599	0/1434	0/1004	0/752	2/8856
WEE	Nt***	Nt	0/1004	1/752	1/3540
ILH	1/599	0/1434	0/1004	0/752	1/8856
ROC	0/599	4/1434	0/1004	0/752	4/8856
SLE	4/599	1/1434	9/1004	9/752	23/8856

* A total of 5067 sera were tested in 1979, 1981, 1983 and 1984, with no detection of monotypic antibodies against the antigens tested.; ** Only antigens to which there was a positive response are listed.; *** Nt, not tested for this antigen

TABLE 3

Prevalence of monotypic HI antibodies in the plasma of the birds from the Casa Grande region, State of São Paulo, during the period from 1978 to 1988.

Antigens**	Years*			
	1978	1980	1983	Total
EEE	0/625	0/743	1/362	1/4290
ILH	1/625	0/743	1/362	2/4290
SLE	10/625	2/743	1/362	13/4290

* A total of 2560 sera were tested in 1979, 1981, 1982, 1984, 1985, 1986, 1987 and 1988, with no detection of monotypic antibodies against the antigens tested.; ** Only antigens to which there was a positive response are listed.

TABLE 4

Prevalence of HI antibodies in the plasma of wild birds from the Ribeira Valley region, State of São Paulo, from 1978 to 1990.

Antigens	Years*											
	1978	1979	1980	1982	1983	1985	1986	1987	1988	1989	1990**	Total
EEE	2/5119	5/3560	3/1936	1/2695	1/1885	1/2107	0/1832	0/1459	3/580	0/591	3/606	19/26765
VEE***	0/5190	0/3560	0/1936	0/2695	0/1885	1/2107	0/1832	0/1459	0/580	0/591	0/606	1/26765
ILH	40/5119	0/3560	1/1936	0/2695	0/1885	0/2107	0/1832	0/1459	0/580	0/591	0/606	41/26765
ROC	1/5119	0/3560	0/1936	0/2695	0/1885	2/2107	2/1832	0/1459	0/580	0/591	0/606	5/26765
SLE	37/5119	0/3560	0/1936	35/2695	0/1885	14/2107	2/1832	4/1459	2/580	1/591	8/606	103/26765
SPAN71686	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	13/591	37/606	50/1197
CAR****	Nt	Nt	Nt	Nt	1/1885	0/2107	1/1832	0/1459	0/580	0/591	0/606	2/11435

* A total of 4395 sera were tested in 1981 and 1984, with no detection of monotypic antibodies against the antigens tested.; ** Two heterotypic reactions which presented 4-fold higher titers for EEE and SLE viruses were included.; *** The prototype strain was used from 1978 to 1982, and the SP AN 50783 strain, isolated in the region, started to be used in 1983.; **** Antigen tested from 1983 on.

1978, in Casa Grande one positive plasma sample was observed in 1978 and another in 1983 (Tables 2 and 3). In the Ribeira Valley, a larger number of positive plasma samples was detected in 1978, but the presence of monotypic anti-ILH antibodies was no longer detected after 1980 (Table 4).

Anti-SP AN 71686 antibodies were observed in the Ribeira Valley at the 8.3 to 1 proportion for monotypic and heterotypic reactions (table 1). Two positive plasmas for the CAR antigen were also detected in the Ribeira Valley after this antigen started to be studied in 1983.

Table 5 shows the distribution of bird species which presented monotypic antibodies according to virus and to the extent of bird dispersal in the Atlantic Forest region. HI antibodies (monotypic reaction) were observed in 269 birds belonging to 66 species of 22 families; several species presented a reaction to more than one arbovirus. The largest number of positive tests was obtained for plasmas of birds belonging to the family Fringillidae (93/269) and the family Tyrannidae had the largest number of species (11/66) with positive serology (Table 5).

Table 6 and figure 2 summarize the variation in frequency of the antibodies studied according to site of capture. Birds whose habitats involve unforested environments presented a larger number of positive reactions (177/269) than did birds living in environments which include forests (92/269).

DISCUSSION

According to the available data and keeping in mind the limitations of the HI test, the present results suggest the circulation of the arboviruses EEE, VEE, WEE, ROC, SLE and CAR, pathogenic to man, and of the Flavivirus SP AN 71686, of unknown human pathogenicity, among wild birds of the Atlantic Forest region in the State of São Paulo.

In the interpretation of the results for each antigen, we usually considered the monotypic reactions; the majority of the titers obtained in the monotypic reactions were low ($\leq$ 40), a justifiable occurrence in primary reactions.

In all 3 regions studied, the most prevalent antibodies were those against antigens SLE (139/39,911), ILH (44/39,911) and EEE (21/39,911). In the Ribeira Valley there was a marked prevalence of antibodies against SP AN 71686 (59/1197), a new virus isolated in this region in 1979. Antibodies with a monotypic reaction to SLE, ILH and EEE were not uniformly detected in the 1978-1990 period. The variation in number of birds captured in different years is not sufficient to explain the absence of these antibodies in certain years. Thus, it is reasonable to assume that there were periods of low virus circulation among wild birds. In support of this hypothesis is the fact that in the years when monotypic reactions were absent, heterotypic reactions were also absent or present in very small number. In general, the disappearance of antibodies involved antigens of the same antigenic group.

The following information of epidemiologic interest should also be commented:

- Antibodies against the EEE virus were present in the birds studied since 1983 in Casa Grande and in 1985 in Itapetininga. Among the species with anti-

TABLE 5

Wild bird species with naturally acquired HI antibodies to arboviruses according to virus and extent of bird dispersal in the Atlantic Forest region, São Paulo, Brazil, 1978-1990.

Family	Species	Virus EEE	VEE	WEE	ILH	ROC	SLE	SPAN71686	CAR	Total
Rallidae	*Porzana albicollis*	1R	-	-	-	-	-	-	-	1
Columbidae	*Columbina talpacoti*	2RM	-	-	1RM	-	11RM	10RM	-	24
	Leptotila rufaxilla	-	-	-	-	-	1R	-	-	1
Cuculidae	*Crotophaga ani*	-	-	-	1RM	-	-	-	-	1
	Guira guira	1RM	-	-	-	-	-	-	-	1
Trochilidae	*Melanotrochilus fuscus*	-	-	-	-	-	1M	-	-	1
	Anthracothorax nigricollis	-	-	-	-	-	1M	-	-	1
	Thalurania glaucopis	-	-	-	-	-	1M	-	-	1
	Amazilia versicolor	-	-	-	1M	-	1M	-	-	2
	Amazilia fimbriata	-	-	-	-	-	1M	-	-	1
Bucconidae	*Nystalus chacuru*	-	-	-	-	-	1M	-	-	1
Picidae	*Picumnus temminckii*	-	-	-	-	-	4R	-	-	4
	Celeus flavescens	-	-	-	-	-	1RM	-	-	1
Furnaridae	*Synallaxis ruficapilla*	-	-	-	-	-	1R	2R	-	3
	Synallaxis spixi	1R	-	-	-	-	-	-	-	1
	Syndactyla rufosuperciliata	-	1R	-	-	-	1R	-	-	2
	Anabacerthia amaurotis	1R	-	-	-	-	-	-	-	1
	Automolus leucophthalmus	-	-	-	-	1R	1R	-	-	2
	Sclerurus scansor	-	-	-	-	-	2R	-	-	2
Formicariidae	*Thamnophilus caerulescens*	-	-	-	-	-	-	1R	-	1
	Dysithamnus mentalis	-	-	-	1R	-	1R	-	-	2
	Pyriglena leucoptera	-	-	-	-	-	1R	-	-	1
	Myrmeciza squamosa	-	-	-	-	-	1R	-	-	1
Conopophagidae	*Conopophaga lineata*	-	-	-	-	-	1R	1R	-	2
Cotingidae	*Attila rufus*	-	-	-	-	-	1R	-	-	1
Piiridae	*Chiroxiphia caudata*	-	-	-	-	-	2R	1R	-	3
	Ilicura militaris	-	-	-	-	-	1R	-	-	1
	Manacus manacus	-	-	-	-	-	2R	4R	1R	7
	Schiffornis virescens	-	-	-	-	-	2R	-	-	2
Tyrannidae	*Machetornis rixosus*	1M	-	-	-	-	-	-	-	1
	Legatus leucophaius	-	-	-	-	-	1M	-	-	1
	Myiarchus swainsoni	-	-	-	-	-	-	1M	-	1
	Myiophobus fasciatus	-	-	-	2RM	-	1RM	-	-	3
	Platyrinchus mystaceus	-	-	-	-	-	4RM	-	-	4
	Elaenia parvirostris	-	-	-	-	-	2RM	-	-	2
	Elaenia chiriquensis	-	-	-	1RM	-	-	1RM	-	2
	Camptostoma obsoletum	-	-	-	-	1NI	-	-	-	1
	Phyllomyias fasciatus	-	-	-	1NI	-	-	-	-	1
	Leptopogon amaurocephalus	-	-	-	1NI	-	-	-	-	1
	Pipromorpha rufiventris	-	-	-	1NI	-	2NI	3NI	-	6
Hirundinidae	*Stelgidopteryx ruficollis*	-	-	-	-	-	-	1NI	-	1
Troglodytidae	*Thryothorus longirostris*	-	1RM	-	1RM	-	-	-	-	2
	Troglodytes aedon	1R	-	-	1R	-	1R	1R	-	4
Turdidae	*Platycichla flavipes*	-	-	-	-	-	1RM	2RM	-	3
	Turdus rufiventris	-	-	-	1RM	-	2RM	1RM	-	4
	Turdus amaurochalinus	-	-	-	-	1RM	3RM	-	-	4
	Turdus albicollis	-	-	-	1R	-	1R	-	-	2
Motacillidae	*Anthus lutescens*	-	-	-	-	-	2R	1R	-	3
Plodeidae	*Passer domesticus*	1R	-	-	3R	2R	8R	2R	1R	17
Vireonidae	*Vireo olivaceus*	-	-	-	-	-	5M	1M	-	6
Icteridae	*Molothrus bonariensis*	-	-	-	-	-	-	5R	-	5
Parulidae	*Geothlypis aequinoctialis*	1R	-	-	1R	1R	3R	1R	-	7
	Basileuterus culicivorus	-	-	-	-	1R	-	1R	-	2
Thraupidae	*Tanagra pectoralis*	-	-	-	-	-	-	1RM	-	1
	Tangara desmaresti	-	-	-	-	-	1RM	-	-	1
	Thraupis sayaca	1R	-	-	1R	-	2RM	-	-	4
	Thraupis palmarum	1RM	-	-	-	-	1RM	-	-	2
	Ramphocelus bresilius	-	-	-	2R	1R	2R	2R	-	7
	Tachyphonus coronatus	1R	-	-	2R	-	2R	-	-	5
	Trichothraupis melanops	-	-	-	-	-	1R	-	-	1
Fringillidae	*Volatinia jacarina*	-	-	-	-	-	12RM	2RM	-	14
	Tiaris fuliginosa	-	-	-	-	-	1R	1R	-	2
	Sporophila lineola	-	-	-	-	-	1RM	-	-	1
	Sporophila caerulescens	1RM	-	-	11RM	1RM	18RM	3RM	-	34
	Sicalis flaveola	-	-	-	-	-	2RM	-	-	2
	Zonotrichia capensis	7R	1R	1R	10R	-	20R	1R	-	40
Total		21	3	1	44	9	139	50	2	269

R - Resident species.; RM - Resident-migratory species.; M - Strictly-migratory species.; NI - Bird dispersal not identified.

TABLE 6

Frequency of monotypic HI antibodies to nine arboviruses in birds according to site of capture, 1978-1990.

Virus	Site of capture							Total
	Including forests				Unforested			
	F	F/B	F/B/HD	F/B/CF/HD	B/CF	B/CF/HD	CF/HD	
EEE	3	1	0	0	1	14	2	21
VEE	1	1	1	0	0	0	0	3
WEE	0	0	0	0	0	1	0	1
ILH	6	6	1	1	1	26	3	44
ROC	2	0	1	0	1	3	2	9
SLE	28	10	0	10	10	73	8	139
SPAN71686	16	2	0	1	4	25	2	50
CAR	1	0	0	0	0	0	1	2
Total	57	20	3	12	17	142	18	269

F, Forest; B, brush; CF, crop field; HD, around human dwellings

EEE antibodies captured in the Ribeira Valley are resident species which have also migratory habits (*Thraupis palmarum, Columbina talpacoti* and *Guira guira*) and one migratory species (*Machetornis rixosus*), which explains the dispersal of the virus from this site to another or vice versa. This information about the circulation of the virus updates data reported in other studies in which the EEE virus was isolated 9 times at these sites: in 1969 and 1970 in Casa Grande (from the marsupial *Didelphis marsupialis* (1), from the rodent *Oryzomys nigripes* (2) and from *Grallaria varia* (1), a bird considered to be resident); in 1969 and 1970 in Itapetininga (2 isolations from the rodent *Oryzomys sp* and 3 isolations from birds considered to be resident, *Chiroxiphia caudata* (2) and *Pipromorpha rufiventris* (1)) [16], and in 1976 in the Ribeira Valley (5 isolations from the mosquito *Culex* (*Melanoconion*) *sp* [1].

Although antibodies to the ROC virus have been detected infrequently in birds regularly captured in the Ribeira Valley since 1978, after the encephalitis epidemic caused by this virus, there is evidence of the circulation of the virus in these hosts at least until 1986, in agreement with the identification of recent human infection in 1983 and 1987 [10, 11] among residents of Iguape county, the site where the birds were captured. Four birds, considered to be resident, with ROC antibodies, monotypic reaction, (*Passer domesticus, Basileuterus culicivorus,*

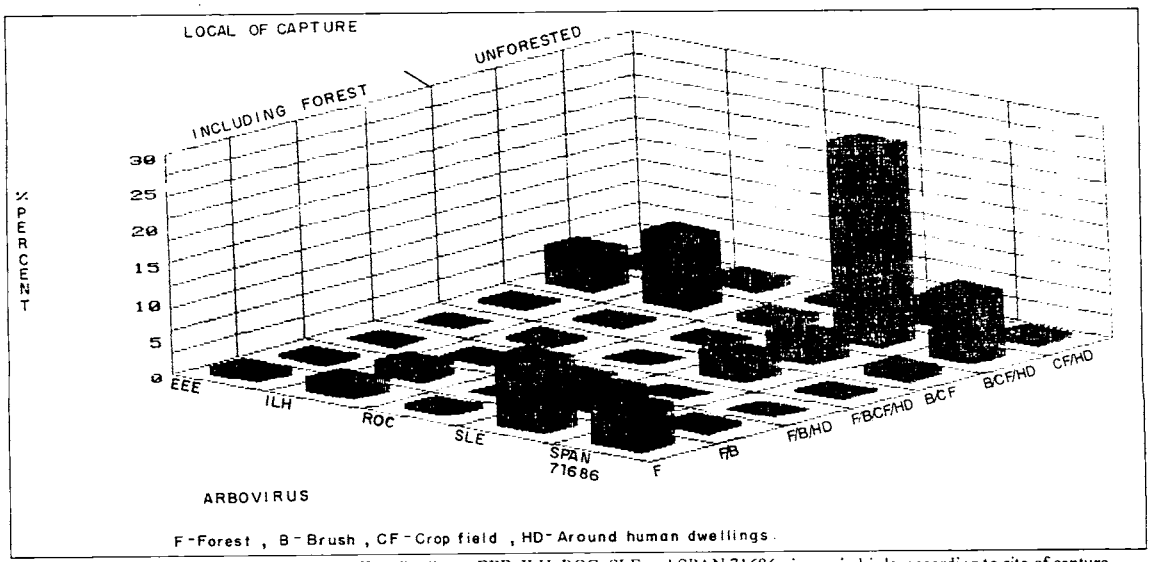

Fig. 2 - Percentual distribution of monotypic HI antibodies to EEE, ILH, ROC, SLE and SPAN 71686 viruses in birds, according to site of capture.

Automolus leucophthalmus and *Camptostoma obsoletum*) were captured in 1980 in Itapetininga, a county close to the Ribeira Valley located on the plateau. The virus was apparently introduced in Itapetininga by birds of migratory habits originating from the Ribeira Valley. In this respect, it should be pointed out that anti-ROC antibodies were detected in the present study in bird species with migratory habits (*Sporophila caerulescens* and *Turdus amaurochalinus*) (Table 5). It is reasonable to assume that the ROC virus may have been dispersed to other areas in the State of São Paulo or to other Brazilian states by migratory birds. The identification of a *Sporophila caerulescens* specimen with monotypic anti-ROC antibodies in the rural area of Ribeirão Preto, in 1985 [6], reinforces this hypothesis, as also does a recent report of ROC antibodies in birds from the Amazon region [5]. The detection of anti-ROC antibodies in Itapetininga birds is of broad epidemiologic interest if we consider the high pathogenicity and virulence of this virus. In the Ringing Program of A.L.I., a bird ringed in Iguape on October 17, 1984 (*Platycichla flavipes*) was recaptured on May 28, 1988 in São Miguel Arcanjo, a town near Itapetininga.

- Since the isolation of the SLE virus from sentinel rodents, birds and mice in three sites in the State of São Paulo, including Itapetininga and Casa Grande, the surveillance developed by S.A.T.V. for arbovirus vertebrate hosts has constantly detected HI antibodies (monotypic reactions) against the SLE virus in birds, although no isolations have been made since 1969 [22]. The data of these studies indicate the presence of 49 bird species belonging to 19 families with monotypic anti-SLE antibodies, many of them of migratory habits. In surveys carried out on resident people in Itapetininga and Casa Grande, about 5% presented SLE-neutralizing antibodies [22]. In the Ribeira Valley, the proportion was 6.8% with HI antibodies [9]. However, there are no reports of human disease despite the circulation of the virus among birds and humans in these areas, suggesting a lower pathogenicity and virulence of the circulating strain as compared to other countries, and/or the presence of undiagnosed disease.
- Monotypic anti-VEE (prototype strain) antibodies were identified in only 2 bird species (*Zonotrichia capensis* and *Syndactyla rufosuperciliata*) captured in Itapetininga in 1978. Antibodies against SP AN 50783 subtype I of the VEE Complex, isolated in 1977 from a bat in the Ribeira Valley [2], were observed in a bird of migratory habit captured in the Ribeira Valley (*Thryothorus longirostris*). The surveillance for human disease caused by this virus has been of great interest since the occurrence of febrile disease in 1990 among military personnel camping in a forested area in the Ribeira Valley [13]. This was the only human naturally acquired disease caused by the virus detected in Brazil. The circulation of the virus in the human population of the Ribeira Valley seems to be intense, as indicated by the high prevalence of neutralizing antibodies (26% and 60%) in human sera respectively collected in 1983 and 1987 [10, 12, 35]. The low prevalence of antibodies in the birds studied suggests that they may not be the primary vertebrate host of the virus.
- Anti-SP AN 71686 antibodies were detected in 25 species belonging to 16 families, 8 of these species being of migratory habits. This finding suggests a significant participation of birds in the cycle of virus maintenance and dispersal to other sites.
- An ecologic distribution of the arboviruses studied can be traced according to the type of habitat of wild birds. A spatial distribution pattern of the captured birds was observed. While some species tend to stay in certain habitats, others occupy different sites. In general, many more birds were captured in unforested environments, although the nets were distributed homogeneously in the soil. Thus, 65.5% of the birds with positive serology, belonging to 25 species, were captured in unforested environments. This fact has obvious repercussions on the distribution of arboviruses which infect them and on the access of vectors to these viruses. A distribution of arboviruses according to these environments may be suggested (Table 6), whereby EEE, ILH, SLE, ROC and SP AN 71686 may circulate among birds in the forest, brush, crop field and areas around human dwellings; VEE may circulate among birds in the forest, brush, and around human dwellings, and CAR in the forest, crop field and around human dwellings.

These observations explain, for example, the existence of disease induced by ROC virus among people who did not leave the area around their homes. According to the present data, this finding would not be accounted for only by the presence of wild vectors infected in the area around human dwellings.

It was also demonstrated that there are species with a greater diversity of infection by arboviruses. It is interesting to note that this always occurs in resident species which temporarily concentrate in flocks at times of greater food availability in crop lands. This is the case for *Passer domesticus*, *Zonotrichia capensis* (the only bird from which the ROC virus was isolated and in which anti-WEE antibodies were detected), *Sporophila caerulescens*, *Columbina talpacoti* and *Troglodytes aedon*.

Meanwhile the smaller number of birds with positive serology captured in environments that includes the forest (34.5%) belongs to a larger number of species (41 species).

RESUMO

Vigilância de infecções por arbovírus na Região da Mata Atlântica, Estado de São Paulo, Brasil. I. Detecção de anticorpos inibidores de hemaglutinação em aves silvestres entre 1978 e 1990.

Apresentam-se os resultados referentes a anticorpos para arbovírus em aves silvestres capturadas, periodicamente, de janeiro de 1978 a dezembro de 1990, nos municípios de Salesópolis (Estação de Casa Grande); Itapetininga e municípios do Vale do Ribeira, considerando-se os diferentes ambientes de captura.

Plasmas foram examinados, por testes de Inibição de Hemaglutinação (IH). Considerou-se apenas as reações monotípicas, com exceção de duas reações heterotípicas, onde ocorreu uma diferença de título significativa para um determinado vírus de um mesmo grupo antigênico.

Em um total de 39.911 aves, foram encontradas 269 aves pertencentes a 66 espécies e 22 famílias, com reação monotípica para os vírus Encefalite Equina do Leste (EEE), Encefalite Equina Venezuelana (VEE), Encefalite Equina do Oeste (WEE), Ilhéus (ILH), Rocio (ROC), Encefalite São Luis (SLE), SP AN 71686, ou Caraparu (CAR).

A análise dos resultados contribue com informações de interesse epidemiológico em relação àqueles agentes. Observou-se distribuição das aves com sorologia positiva em diferentes habitats, predominando os que excluem mata e a presença de maior diversidade de reações positivas nas espécies que se concentram em campos de cultura.

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