Intervirology 21: 38–49 (1984)

0300-5526/84/0211-0038$2.75/0

Characterization of the Changuinola Serogroup Viruses (*Reoviridae: Orbivirus*)

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Key Words. Changuinola serogroup · *Orbivirus* · Taxonomy · Serology · Double-stranded RNA

Summary. The antigenic, biological, and chemical properties of 24 selected Changuinola serogroup viruses were examined. The viruses tested were chloroform-resistant, and they were lethal to newborn hamsters after intracerebral inoculation. The prototype Changuinola virus strain (BT-436) replicated in mosquito and sandfly cell cultures. In complement-fixation tests, the viruses were broadly cross-reacting and indistinguishable; but by neutralization test at least 12 distinct serotypes were identified, and by PAGE of double-stranded RNA 22 distinct profiles were found. These data suggest that the Changuinola serogroup may be comprised of a large number of genetically different viruses. A brief review of the natural history of Changuinola serogroup viruses is also given.

The Changuinola serogroup consists of a large number of antigenically related viruses which have been associated with phlebotomine sandflies, mosquitoes, and various species of wild mammals. These viruses have been found only in tropical America, and they are presumed to be arthropod-borne. On the basis of their physicochemical and morphological properties, they are included in the family ***Reoviridae***, genus *Orbivirus* [1–3].

The prototype virus, Changuinola, was first isolated from sandflies (*Lutzomyia* sp.) in Panama in 1960 [4]. A second, antigenically related agent, Irituia virus, was recovered from a rice rat (*Oryzomys* sp.) in the Amazon Basin of Brazil in 1961 [5]. Since then, approximately 176 antigenically similar isolates have been recovered from biting insects and mammals in tropical forested regions of Brazil, Colombia, and Panama [4, 6–9]. These agents are indistinguishable by complement-fixation (CF) test, but a number of different serotypes can be distinguished by neutralization tests [8]. This

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Received: April 19, 1983
Revised: May 26, 1983

Table 1. Changuinola serogroup viruses included in this study

Virus[1]	Strain number	Source	Geographic locality[2]	Date of isolation
Changuinola	BT-436	*Lutzomyia* sp. (sandfly)	Bocas del Toro, Panama	1960
–	BT-104	*Lutzomyia* sp.	Bocas del Toro, Panama	1960
–	BT-766	*Lutzomyia* sp.	Bocas del Toro, Panama	1960
–	BT-2164	*Lutzomyia* sp.	Bocas del Toro, Panama	1961
–	BT-2365	*Lutzomyia* sp.	Bocas del Toro, Panama	1961
–	BT-2380	*Lutzomyia* sp.	Bocas del Toro, Panama	1961
–	VP-19A	*Lutzomyia* sp.	Panama, Panama	1969
–	VP-46F	*Lutzomyia* sp.	Panama, Panama	1969
–	VP-188G	*Lutzomyia trapidoi*	Panama, Panama	1970
–	VP-202A	*Lutzomyia* sp.	Panama, Panama	1970
–	CoAr 2837	*Lutzomyia* sp.	Valle, Colombia	1964
Irituia	BeAn 28873	*Oryzomys* sp. (rice rat)	Para, Brazil	1961
Gurupi	BeAr 35646	*Lutzomyia* sp.	Para, Brazil	1962
Ourem	BeAr 41067	*Lutzomyia* sp.	Para, Brazil	1962
Caninde	BeAr 54342	*Lutzomyia* sp.	Para, Brazil	1963
Jamanxi	BeAr 243090	*Lutzomyia* sp.	Para, Brazil	1973
Altamira	BeAr 264277	*Lutzomyia* sp.	Para, Brazil	1974
Purus	BeAr 361064	*Psorophora albipes* (mosquito)	Acre, Brazil	1977
Jari	BeAn 385199	*Choloepus didactylus* (sloth)	Para, Brazil	1980
Saraca	BeAr 385278	*Lutzomyia* sp.	Para, Brazil	1980
Monte Dourado	BeAn 385401	*Dasypus novemcinctus* (armadillo)	Para, Brazil	1980
Almeirim	BeAr 389709	*Lutzomyia umbratilis*	Para, Brazil	1980
–	BeAr 385274	*Lutzomyia* sp.	Para, Brazil	1980
–	BeAr 385279	*Lutzomyia* sp.	Para, Brazil	1980

1 Hyphen denotes unnamed strain.
2 State province department, country.

paper reports the preliminary characterization of the Changuinola serogroup by comparing the biological, serological and chemical properties of selected members.

Materials and Methods

Insect Cells

Two insect cell lines were used in this study. The mosquito cell line was the C6/36 clone of *Aedes albopictus* cells [10]. The sandfly cell culture was the LL-5 line, started from eggs of *Lutzomyia longipalpis* [11]. Insect cells were maintained at 28°; the media have been described previously [11, 12].

Viruses

Table I lists the Changuinola group viruses included in this study. Stocks of each virus were prepared from infected newborn mouse brain or from infected Vero cells. These virus stocks were used subsequently in the biochemical studies, in the preparation of antigens and immune reagents, in the infection of insect cell cultures, in the determination of virus pathogenicity for animals, and in the neutralization tests. The Indiana serotype of vesicular stomatitis virus and reovirus type 3 (Dearing strain) were also used as controls in two experiments.

Virus Titrations

Virus titrations were done by plaque technique in microplate cultures of Vero cells [13]. Serial 10-fold dilutions of virus suspensions were made in phosphate-buffered saline, pH 7.5, containing 0.5% gelatin. Two

microplate wells were inoculated with each dilution. Virus titers were calculated as the number of PFU/ml.

Immune Reagents

Hyperimmune mouse ascitic fluids were prepared in adult Swiss mice by following established protocols [14]. Briefly, the immunization schedule consisted of 3 or 4 intraperitoneal injections given at 7- to 14-day intervals. Immunizing antigens were prepared from infected newborn mouse brain (10% suspension in phosphate-buffered saline), and they were mixed with equal volumes of Freund's complete adjuvant prior to injection.

Infection of Insect Cells

The growth of Changuinola virus in mosquito and sandfly cell cultures was compared. Tube cultures of each cell line were inoculated with 100 PFU of Changuinola (BT-436) virus, and they were maintained at 28°. One tube culture of each infected cell line was sampled daily for 1 week. Specimens were frozen at −70°, and a thawed sample was titrated in microplate cultures of Vero cells.

Studies of Animal Pathogenicity

The average survival time was determined by intracerebral inoculation of newborn hamsters (2–5 days old). 8 hamsters were inoculated with 0.02 ml of each undiluted virus stock. The animals were examined daily, and their day of death was recorded. The average survival time represents the mean day of death for all hamsters in the litter.

Chloroform Sensitivity

Equal volumes of virus stock and chloroform were mixed, and they were intermittently shaken at ambient temperature for 10 min. The chloroform-virus mixtures, as well as virus controls, were centrifuged for 10 min, the supernatants were assayed for infectivity in microplate cultures of Vero cells, and virus PFU/ml titers were calculated.

Serological Tests

CF tests were done according to a microtechnique modified from *Fulton and Dumbell* [15], using 2 full units of guinea pig complement and antigens made from infected newborn mouse brain and prepared as a 10% crude suspension in borate saline. Mouse neutralization tests (MNT) were done in newborn mice by following the technique described by *Shope and Sather* [16].

Extraction of Double-Stranded RNA (dsRNA)

All steps of the technique (developed by D.L.K.) were carried out in 1.5-ml Eppendorf microfuge tubes, and all centrifugation steps were performed in the Eppendorf model 5412 microfuge at 12,000 *g*. 24-Well cluster plates were seeded with Vero cells, and they were incubated at 37° until confluent. When a complete monolayer was formed, each well was inoculated with 0.2 ml of virus stock. After adsorption for 1 h, maintenance medium was added to the cells. The maintenance medium consisted of MEM with Earle's salts, containing 1% fetal bovine serum, 300 mEq $NaHCO_3$, 1% *L*-glutamine, penicillin (250 units/ml) and streptomycin (200 µg/ml). For in vivo labeling, 0.8 ml of maintenance medium containing 50 µCi of ^{32}P-orthophosphate was added. When pCp labeling was planned, only 0.8 ml of maintenance medium was added. Plates were incubated at 37° until 3–4+ CPE was observed. The wells were harvested, and the cells were pelleted. The supernatant was discarded, and the infected cell pellet was resuspended in 200 µl of 10 m*M* EDTA (pH 7.6) and 6.2 µl of 20% (w/v) SDS. The suspension was gently mixed and incubated at room temperature for 30 min, then 51.6 µl of 5 *M* NaCl was added. The sample was mixed gently and stored at 4° overnight. The precipitate was pelleted by centrifugation for 5 min, the supernatant was removed, and 10 µl of proteinase K (5 mg/ml water) was added to the supernatant. The sample was incubated at 37° for 6 h or overnight. The aqueous sample was extracted twice with 200 µl of phenol equilibrated with 10 m*M* Tris buffer, pH 8.0. The residual phenol was removed from the aqueous phase by a 500-µl ether extraction, and the residual ether was evaporated at 37° for 2 h. The phases were separated in these extraction steps by centrifugation for 15 s. The resulting aqueous phase was adjusted to 2 *M* LiCl, and the suspension was stored at 4° overnight. The precipitate of single-stranded RNA was pelleted by centrifugation for 5 min. The supernatant was removed, and 50 µl of 3 *M* sodium acetate and 1 ml of 100% ethanol were added to the supernatant. This mixture was stored at −70° overnight. The dsRNA was collected by centrifugation for 5 min, the supernatant was discarded, and the pellet was washed with 1 ml of cold 95% ethanol. The final pellet was dried at ambient temperature in vacuo.

pCp Labeling

The RNA samples were prepared for end-labeling by dissolving dried dsRNA in 40 µl of water. dsRNA from the extraction procedure was end-labeled at the

Table II. CF tests with selected Changuinola group viruses

Antigen	Antiserum											
	CGL	IRI	GUR	OUR	CAN	JAM	ALT	PURUS	JARI	SAR	MD	AMR
Changuinola	32[a]/32	0	8/32	≥256/32	≥256/32	64/32	8/8	128/≥128	128/128	128/128	128/32	≥256/≥128
Irituia	16/8	32/32	64/32	≥256/32	256/32	≥256/32	32/32	128/32	128/128	256/128	256/82	256/32
Gurupi	16/32	32/≥128	64/≥128	≥256/128	≥256/≥128	128/128	16/32	64/128	128/≥128	256/≥128	256/128	128/≥128
Ourem	16/128	8/8	64/128	≥256/≥128	≥256/128	128/128	32/32	≥128/128	256/≥128	256/≥128	≥256/128	256/≥128
Caninde	8/32	32/≥128	64/≥128	≥256/128	512/128	128/128	32/32	32/128	64/≥128	128/128	256/128	128/128
Jamanxi	<16/8	16/32	32/32	256/128	≥256/128	128/128	32/128	32/32	64/≥128	≥256/≥128	≥256/≥128	256/≥128
Altamira	<16/8	8/32	16/32	256/128	≥256/32	64/≥128	32/32	32/32	64/≥128	≥256/≥128	≥256/≥128	256/≥128
Porus	16/32	0	8/8	256/32	128/8	32/32	0	256/32	128/128	64/32	64/32	≥256/32
Jari	8/32	0	8/32	≥256/≥128	256/32	64/32	8/32	64/≥128	256/≥128	128/≥128	128/128	256/≥128
Saraca	<16/8	8/8	32/8	256/32	256/32	32/32	8/8	16/32	64/32	256/128	256/128	64/32
Monte Dourado	<16/8	8/32	32/32	≥256/≥128	≥256/≥128	128/≥128	32/128	64/128	64/≥128	≥256/≥128	≥256/≥128	256/≥128
Almeirim	8/32	8/8	16/≥128	≥256/≥128	256/≥128	64/128	8/32	64/≥128	128/≥128	128/≥128	128/≥128	≥256/≥128

[a] Reciprocal of highest antiserum dilution/reciprocal of highest antigen dilution. 0 = <8/8.

3′ end of the strands of each segment by the addition of [5′-^{32}P]-pCp using T4 RNA ligase as described previously [17]. The reaction was stopped; the labeled RNA was precipitated by adding 150 µl of water, 50 µl of 3 *M* sodium acetate, 1 µg of tRNA, and 1 ml of 100% ethanol. The mixture was stored at −70° overnight, and the precipitate was collected by centrifugation for 5 min. The labeled dsRNA was dissolved in 200 µl of 10 m*M* Tris, pH 8.0, and it was precipitated by adding 50 µl of 3 *M* sodium acetate and 1 ml of 100% ethanol to the solution and storing it at −70° overnight. This precipitation procedure was repeated once. The precipitate was collected by centrifugation for 5 min, and it was washed with 1 ml of cold 95% ethanol. The final pellet was dried in vacuo at ambient temperature.

dsRNA Electrophoresis

The discontinuous PAGE system of *Laemmli* [18] was used. Vacuum-dried, labeled dsRNA was dissolved in 50 µl of Laemmli sample buffer. 10-µl samples were layered onto the gel and electrophoresed at ambient temperature for 20 h at 20 mA. After electrophoresis, the gel was bathed in 7% acetic acid for 1 h, and subsequently in 7% acetic acid containing 1% (v/v) glycerol. It was dried onto filter paper, and dried gels were exposed to Kodak XOMAT-AR film. The film was developed according to the manufacturer's directions.

Table III. Biological studies with selected Changuinola group viruses

Virus or strain	Passage history[a]	Virus titer, control[b]	Virus titer after chloroform treatment	Average survival time[c]
Changuinola	SM_{13}, $Vero_3$	5.3	5.6	7.3
BT-766	SM_{10}, $Vero_2$	6.4	6.2	9.8
BT-2380	SM_8, $Vero_3$	5.9	6.0	7.8
VP-19A	$Vero_3$	6.2	6.6	10.4
VP-46F	$Vero_3$	6.6	6.2	6.6
VP-188G	$Vero_2$	5.9	6.0	12.0[d]
VP-202A	$Vero_3$	5.4	5.7	8.7
Irituia	SM?, $Vero_1$	6.1	5.7	3.9
Gurupi	SM_{11}, $Vero_2$	6.4	6.5	3.7
Caninde	SM_8, $Vero_1$	6.3	5.8	3.8
Vesicular stomatitis – Indiana	$Vero_5$	7.3	3.2	–

[a] SM = Suckling mouse; Vero = Vero cell cultures. Subscripts represent the number of passages.
[b] Titer given as $\log_{10}$ PFU/ml. Inoculum for newborn hamsters was 0.02 ml of virus stock.
[c] Average survival time (days) of inoculated newborn hamsters.
[d] 2 of 12 inoculated newborn hamsters died on day 12 postinoculation; the remainder survived.

Results

Table I lists the Changuinola serogroup virus strains that were used in this study. The strains are listed by their country of origin and year of isolation. The lack of species identification of the *Lutzomyia* from which many of these viruses have been isolated is due to inherent difficulties in their identification [19].

Since the CF test has been the basis for serologic grouping of orbiviruses, this procedure was done to establish that each of the isolates was antigenically related. Results of the cross CF tests with 12 Changuinola group virus antigens and immune sera are given in table II. In general, these agents were broadly cross-reactive, with most of the viruses being indistinguishable.

Table III summarizes the results of pathogenicity studies with 10 selected Changuinola group viruses. The passage history, titer and average survival time of each virus are shown. Although these agents were practically indistinguishable by CF tests, biological differences were detected among them. The average survival time of baby hamsters inoculated with

Table IV. Growth of Changuinola virus (BT-436) in *L. longipalpis* (LL-5) and *Ae. albopictus* (C6/36) cells

Day postinoculation	Virus titer[1]	
	LL-5	C6/36
1	1.0	0.7
2	3.5	3.9
3	5.5	5.2
4	6.0	6.2
5	6.2	6.4
6	5.9	6.6
7	6.4	6.7

[1] Titer expressed as $\log_{10}$ PFU/ml of frozen cell harvest.

Table V. MNT with selected Changuinola group viruses

Virus	Antiserum											
	CGL	IRI	GUR	OUR	CAN	JAM	ALT	PURUS	JARI	SAR	MD	AMR
Changuinola	*2.1*[a]	0	≤1.1	0	0	≤1.1	0	0	0	0	≤1.1	0
Irituia	0	*2.4*	0	0	1.2	0	0	0	0	0	0	0
Gurupi	0	0	*2.6*	0	0	0	0	1.2	0	0	0	0
Ourem	0	0	0	*3.7*	0	0	0	1.2	1.2	0	0	0
Caninde	0	0	0	0	*3.0*	0	0	0	0	1.9	0	0
Jamanxi	0	0	0	0	0	*3.1*	0	0	0	0	0	0
Altamira	0	1.1	1.2	0	0	0	*3.6*	1.2	0	0	0	0
Purus	0	0	0	0	0	0	0	*2.5*	0	0	0	0
Jari	0	0	0	0	0	0	0	0	*1.7*	0	0	0
Saraca	1.2	0	0	0	1.7	0	0	1.1	0	*3.0*	0	≤1.1
Monte Dourado	0	0	0	0	1.4	0	0	1.2	0	0	*3.0*	0
Almeirim	0	0	0	0	0	0	0	0	0	0	0	*2.4*

[a] $\log_{10}$ neutralization index. 0 = ≤1.0. Italics indicate reciprocal titers.

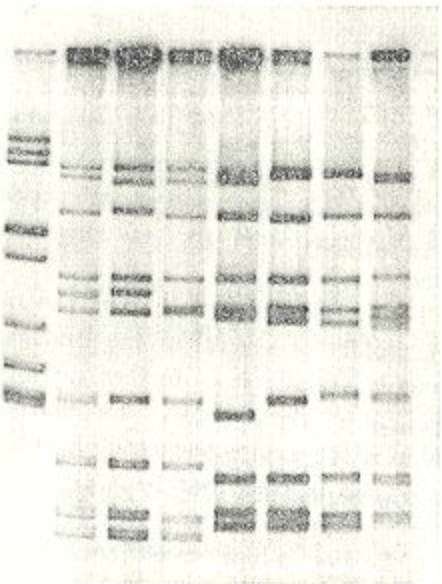
these 10 viruses ranged from 3.7 to greater than 12 days, indicating marked variation in their animal pathogenicity. The least pathogenic virus was VP-188G; only 2 of 12 hamsters inoculated with this agent died. There was no significant correlation between hamster pathogenicity, virus titer, or passage history.

Table III also indicates the effect of chloroform treatment on the same 10 Changuinola group viruses. Titers of the control and chloroform-treated specimens were almost identical, indicating that the viruses were resistant to lipid solvents. In contrast, the titer of vesicular stomatitis virus (family ***Rhabdoviridae***) dropped more than 10^4 PFU after chloroform treatment.

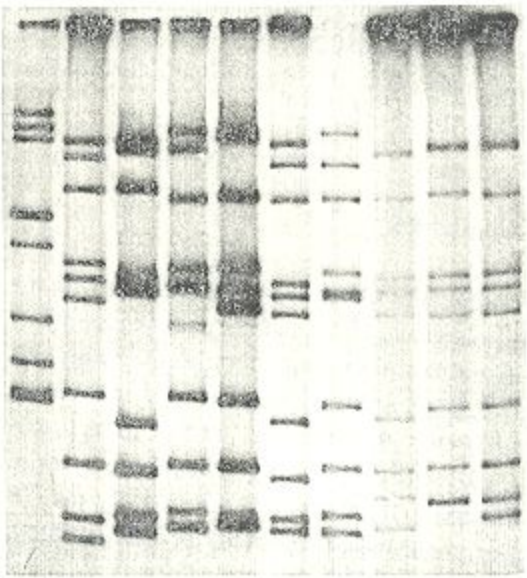
The comparative growth of the prototype strain of Changuinola virus (BT-436) in sandfly (LL-5) and mosquito (C6/36) cell cultures is summarized in table IV. Virus replication occurred in both cell lines with comparable rates of growth. Virus CPE was observed in the infected LL-5 cells between the 7th and 9th days, when many of the cells began to detach from the glass surface and to lyse. However, new cells appeared within 10–14 days and a new monolayer was formed. The infected cells were subsequently subcultured at weekly intervals for 6 weeks. Samples of the resulting cell suspensions were frozen and later titrated. The weekly samples continued to yield 10^4–10^5 PFU of Changuinola virus per ml, indicating that a persistent infection may have been established in the sandfly cells. In contrast, the C6/36 cells did not show CPE, and they were not tested for persistent infection.

Table V shows the MNT results with 12 Changuinola group viruses and antisera. Since each of the viruses could be differentiated by this technique and was antigenically distinct, the Changuinola serogroup is comprised of at least 12 distinct serotypes.

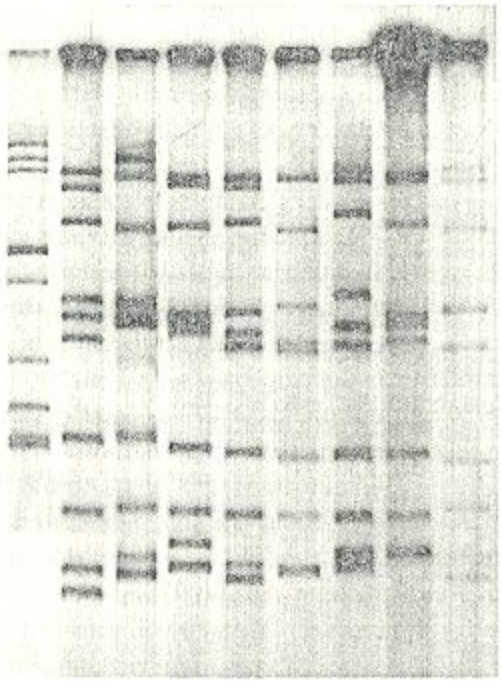
When the dsRNA of Changuinola serogroup viruses was analyzed by PAGE, distinctive profiles of the 10 dsRNA segments were observed for most of the isolates (fig. 1–3). The apparent molecular weights of the dsRNA segments were calculated using the Dearing strain



1



2



3

Fig. 1–3. Autoradiograms depicting the resolution of the segmented dsRNA genome of selected members of the Changuinola serogroup by electrophoresis of 3′ end-labeled dsRNA through Tris-glycine buffered 10% polyacrylamide gel. The viruses are from left to right for fig. 1: reovirus, BT-104, Changuinola (BT-436), BT-2380, VP-19A, VP-46F, VP-188G, and VP-202A; for fig. 2: reovirus, Changuinola, CoAr 2837, Irituia, Gurupi, Ourem, Caninde, Jamanxi, BeAr 385274, BeAr 385279; and for fig. 3: reovirus, Changuinola, Irituia, Altamira, Purus, Jari, Saraca, Monte Dourado, Almeirim.

of reovirus type 3 as a molecular weight standard in linear regression analyses. These data are presented in table VI to allow comparisons of dsRNA profiles analyzed under different conditions.

22 unique dsRNA profiles were found for the 24 isolates that were examined. Strains BT-436 (Changuinola) and BT-766 were indistinguishable by PAGE. Likewise, the mobilities of the dsRNA segments of BT-2164 and

BT-2365 were identical. 7 of the 10 segments of BT-104 were identical to BT-436 and BT-766, but minor electrophoretic variations were seen consistently in segments 2, 3, and 5 (fig. 1, table VI). The dsRNA profiles for six of the viruses also exhibited additional minor molar species (fig. 1–3).

Discussion

Results of CF tests indicate that the Changuinola group viruses are broadly cross-reacting and that they share common CF antigens (table II). Since the isolates from Panama had been tested previously and shown to be cross-reactive with the prototype Changuinola strain, BT-436 [8], they were not retested. By the CF method, the viruses could not be differentiated. In contrast, 12 distinct virus serotypes were identified by MNT (table V). When these 12 serotypes were examined by PAGE, each also exhibited a unique dsRNA profile. Although neutralization tests were not performed for all of the strains (table I), each of these agents was examined by PAGE. By this technique, 10 additional virus strains with distinct dsRNA profiles were detected. Perhaps, these 10 viruses may also represent additional serotypes. Although similarity in dsRNA segment profiles does not imply identity between viral isolates, an overall PAGE pattern of similarity of the 10 genes may reflect a degree of relatedness [20]. In this regard, it is noteworthy that Panama virus isolates BT-436 and BT-766 had identical dsRNA profiles, as did viruses BT-2164 and BT-2365. In plaque reduction neutralization tests, these two virus pairs were also indistinguishable [21]. Thus, these data suggest that there may be a correlation between neutralization results and the dsRNA profiles among the Changuinola serogroup viruses and that the dsRNA profile of the viral genome may be a useful diagnostic tool.

Four of the Panamanian virus isolates (VP-19A, VP-46F, VP-188G, and VP-202A) were recovered from sandflies collected around a single large fig tree (*Ficus* sp.) between May 1969 and January 1970 [8]. In fact, two of the isolates were obtained just 5 days apart. The recovery of these four distinct viruses from the same collection site within a 10-month period suggests that more than one type may have circulated simultaneously.

The finding of minor molar bands in the dsRNA profiles of several of the Brazilian Changuinola virus isolates further supports this hypothesis. The presence of a dsRNA segment in minor molar concentrations in the dsRNA profile indicates that the viral stock is a mixture of types. Three of the Brazilian samples showing minor molar bands were isolated from pools of sandflies. If the mixtures were not the result of laboratory contamination, then the sandfly pools probably contained two or more infected insects with different viral types. In view of the high field infection rates with Changuinola viruses among wild-caught sandflies [22], this hypothesis is plausible. The presence of sandflies infected with two different Changuinola types in the same insect pool also implies that both agents were active in nature simultaneously.

Between 1960 and 1980, a total of 178 Changuinola group virus strains were isolated from various geographic localities in Brazil, Colombia and Panama [6–9, 16; *P. H. Peralta* and *A. P. A. Travassos da Rosa*, unpublished data]. In the present study, we selected 24 viruses from this total for examination. 12 of the viruses were distinct by neutralization tests and PAGE; 10 others had unique dsRNA profiles. If this sample is representative, then a great many more Changuinola serotypes may exist.

Table VI. Changuinola serogroup viruses: apparent molecular weight of the dsRNA segments

Virus[a]	Strain	dsRNA segment and total genome molecular weights										
		1	2	3	4	5	6	7	8	9	10	Sum
Changuinola	BT-436	2.28[b]	2.12	1.80	1.25	1.15	1.04	0.65	0.47	0.36	0.32	11.44
		0.04[c]	0.03	0.02	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.16
–	BT-104	2.29	2.17	1.78	1.24	1.12	1.03	0.64	0.47	0.37	0.33	11.42
		0.06	0.06	0.04	0.03	0.02	0.02	0.01	0.02	0.02	0.02	0.25
–	BT-766	2.29	2.12	1.79	1.23	1.11	1.02	0.64	0.46	0.36	0.32	11.34
		0.03	0.02	0.02	0.05	0.06	0.05	0.02	0.01	0.00	0.00	0.19
–	BT-2164	2.29	2.17	1.73	1.19	0.99	0.99	0.62	0.45	0.35	0.32	11.11
		0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.02
–	BT-2365	2.30	2.18	1.73	1.19	0.99	0.99	0.62	0.45	0.34	0.32	11.14
		0.04	0.03	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.03
–	BT-2380	2.41	2.15	1.75	1.25	1.05	1.05	0.65	0.46	0.34	0.32	11.46
		0.15	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.13
–	VP-19A	2.19	2.10	1.72	1.22	1.00	0.99	0.58	0.43	0.36	0.33	10.93
		0.03	0.04	0.03	0.05	0.04	0.04	0.02	0.01	0.01	0.01	0.21
–	VP-46F	2.20	2.20	1.70	1.21	1.03	0.96	0.64	0.43	0.36	0.33	11.06
		0.03	0.03	0.03	0.05	0.05	0.04	0.03	0.02	0.01	0.01	0.25
–	VP-188G	2.22	2.19	1.76	1.26	1.07	1.00	0.69	0.45	0.36	0.35	11.37
		0.03	0.06	0.03	0.04	0.04	0.03	0.03	0.02	0.02	0.01	0.29
–	VP-202A	2.20	2.11	1.75	1.29	1.09	1.04	0.98	0.69	0.45	0.37	11.96
		0.05	0.04	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.02	0.33
–	CoAr 2837	2.29	2.18	1.79	1.14	1.06	1.06	0.55	0.45	0.35	0.33	11.21
		0.03	0.02	0.01	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.08
Irituia	BeAn 28873	2.41	2.22	1.73	1.21	1.11	1.11	0.65	0.47	0.38	0.35	11.65
		0.04	0.04	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.26
Gurupi	BeAr 35646	2.39	2.31	1.74	1.21	1.09	0.99	0.99	0.63	0.45	0.34	12.17
		0.04	0.04	0.01	0.04	0.02	0.02	0.02	0.01	0.02	0.01	0.23
Ourem	BeAr 41067	2.27	2.06	1.73	1.14	1.07	0.98	0.58	0.45	0.37	0.34	11.00
		0.05	0.05	0.04	0.05	0.04	0.04	0.02	0.02	0.02	0.01	0.31

In forested areas of tropical America, where sandflies are abundant, Changuinola group viruses appear to be extremely common. For example, 120 isolations of Changuinola group viruses were made from wild-caught sandflies during a 26-month study of arbovirus activity at a single locality in Panama [8]. The minimum field infection rate among female sandflies collected in this study was 1:1,346. In view of their abundance and of the well-known ability of orbiviruses to form recombinants [23], new Changuinola serotypes may continue to evolve. In this case, the number of potential serotypes may be high.

Growth of Changuinola virus BT-436 occurred in both the sandfly and mosquito cell cultures (table III). The growth of Changuinola virus in the C6/36 cell line was not unexpected, because the replication of this virus has been demonstrated previously in cell cultures of the

Table VI, (continued)

Virus[a]	Strain	dsRNA segment and total genome molecular weights										
		1	2	3	4	5	6	7	8	9	10	Sum
Caninde	BeAr 54342	2.39	2.06	1.73	1.21	1.10	1.07	0.63	0.47	0.37	0.34	11.39
		0.04	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.26
Jamanxi	BeAr 243090	2.15	2.15	1.72	1.16	1.08	0.98	0.57	0.44	0.39	0.33	11.01
		0.00	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.09
Altamira	BeAr 264277	2.24	2.18	1.79	1.18	1.14	1.09	0.63	0.47	0.41	0.37	11.51
		0.04	0.04	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.16
Purus	BeAr 361064	2.21	2.12	1.80	1.18	1.07	1.01	0.62	0.46	0.37	0.35	11.22
		0.01	0.01	0.03	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01
Jari	BeAn 385199	2.22	2.22	1.74	1.22	1.04	1.00	0.61	0.47	0.36	0.36	11.24
		0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.05
Saraca	BeAr 385278	2.30	2.19	1.88	1.28	1.10	1.01	0.60	0.45	0.38	0.36	11.58
		0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.12
Monte Dourado	BeAn 385401	2.25	2.17	1.77	1.16	1.11	1.04	0.61	0.46	0.39	0.39	11.35
		0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.19
Almeirim	BeAr 389709	2.26	2.14	1.72	1.19	1.18	0.99	0.58	0.47	0.38	0.35	11.26
		0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.14
–	BeAr 385274	2.21	2.20	1.76	1.17	1.10	0.96	0.60	0.45	0.38	0.38	11.24
		0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.05
–	BeAr 385279	2.22	2.22	1.75	1.19	1.10	0.97	0.61	0.46	0.38	0.35	11.29
		0.01	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.08
Reovirus 3	Dearing	2.60	2.42	2.29	1.57	1.57	1.35	0.93	0.75	0.65	0.63	14.76
		0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02

[a] Hyphen denotes unnamed strain. Reovirus 3 included as the molecular weight standard in the calculations.
[b] Molecular weight $\times 10^{-6}$.
[c] Standard deviation.

mosquito, *Toxorhynchites amboinensis* [24]. In addition, *Buckley* [25] has reported that Irituia virus replicates in Singh's *Aedes aegypti* and *Ae. albopictus* cells. The C6/36 cells were derived from a clone of the original Singh's *Ae. albopictus* line [10]. Despite the growth of Changuinola group viruses in mosquito cells and their isolation from wild-caught mosquitoes, these insects probably do not play a significant role in the natural history of this group of viruses. Of the 178 known Changuinola group virus isolates, 158 have come from phlebotomine sandflies, 5 from mosquitoes, and 15 from mammals. Thus, sandflies appear to be the major arthropod vector of these agents.

The role of vertebrates in the ecology of Changuinola group viruses is less clear. The 15 vertebrate isolates have been made from a variety of mammalian species (rice rat, ar-

madillo, sloth, and human). The human isolation of Changuinola was made from a febrile patient. 12 of the vertebrate recoveries have come from sloths [26; *Peralta*, unpublished data]. These arboreal mammals are extremely abundant in neotropical forests [27], and they are the preferred host of a number of sandfly species [22]. The limited data suggest that sloths and possibly other edentates are involved in the natural cycle of Changuinola group viruses. It is noteworthy that edentates (sloths, armadillos and anteaters) have also been implicated in the New World as important reservoirs of *Leishmania brasiliensis*, a sandfly-borne protozoan which causes cutaneous leishmaniasis [28–30]. However, the actual role of mammals in the natural history of this biologically interesting group of orbiviruses remains obscure.

Acknowledgments

This work was supported in part by grants from the Brazilian Government ('Programa dos Polos Agropecuarios da Amazonia, Superintendencia de Desenvolvimento da Amazonia, Ministerio do Interior'), from the National Institutes of Health (POA1-11132 and AI-10984), and from the U.S. Army Medical Research and Development Command (DAMD 17-81-C-1121 and DAMD-17-80-C-0178).

A.P.A.Travassos da Rosa and *J.S.Travassos da Rosa* were recipients of fellowships from the Pan American Health Organization during part of this study. The expert technical assistance of *J.G.Anderson* and *L.G.Preli* is acknowledged.

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