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ARBOVIRUS INVESTIGATIONS IN ARGENTINA, 1977–1980

II. Arthropod Collections and Virus Isolations from Argentine Mosquitoes

C. J. MITCHELL,* T. P. MONATH,* M. S. SABATTINI,† C. B. CROPP,* J. F. DAFFNER,† C. H. CALISHER,* W. L. JAKOB,* AND H. A. CHRISTENSEN‡

*Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, P.O. Box 2087, Fort Collins, Colorado 80522,
†Institute of Virology, Faculty of Medical Science, University of Cordoba, Estafeta 32, Cordoba, Argentina, and
‡Department of Vector Biology, Gorgas Memorial Laboratory, Apartado 6991, Panama 5, Republic of Panama

Abstract. Prospective surveys for arboviruses were carried out in Santa Fe, Corrientes, and Chaco provinces, Argentina, aperiodically during 1977–1980. A total of 313,233 mosquitoes and 598 biting flies other than mosquitoes were collected and tested for virus in 5,197 and 45 pools, respectively. Forty virus strains were isolated, all from mosquitoes, as follows: Santa Fe Province: 4 Gamboa group viruses from *Aedeomyia squamipennis*, 1 strain each of St. Louis encephalitis virus from *Culex pipiens quinquefasciatus* and *Culex* (*Culex*) spp.; Corrientes Province: a single strain of a newly discovered Anopheles A serogroup virus, Las Maloyas, from *Anopheles albitarsis*; and Chaco Province: 4 Gamboa group viruses from *Ad. squamipennis*, 6 strains of new Bunyaviridae (1 Antequera, 1 Barranqueras, and 4 Resistencia) from *Culex* (*Melanoconion*) *delpontei*, 3 strains of a new subtype of western equine encephalitis virus and 1 strain of Para virus from the *Cx.* (*Mel.*) *ocossa* group, 12 strains of a newly discovered subtype (VI) of the Venezuelan equine encephalitis complex from *Cx.* (*Mel.*) *delpontei*, and 1 strain each from *Ad. squamipennis*, *Aedes scapularis*, *Ae.* spp., *Cx.* (*Cux.*) spp., *Cx.* (*Mel.*) *ocossa* group, *Mansonia* spp., and *Psorophora* spp. Bloodmeals from 265 engorged mosquitoes were identified by precipitin test. These data, coupled with data on engorgement rates for 25,995 mosquitoes from bait collections, provide information on the host feeding patterns of several mosquito species. This information is discussed, along with data on relative abundance of mosquito species, within the context of the vector relationships of the species from which viruses were isolated. The association of *Cx.* (*Mel.*) *delpontei* with 18 strains of 4 different viruses in Chaco Province, plus its catholic feeding habits, clearly indicate for the first time the importance of this species as an arbovirus vector.

Prospective surveys for arboviruses were carried out in Santa Fe Province, Argentina, during November and December 1977, May 1978, February 1979, and February 1980, and in Corrientes and Chaco provinces during April 1980. The general ecology of these areas and the collection sites have been described.[1] We report here on the arthropod collections and the virus isolations from mosquitoes.

MATERIALS AND METHODS

Most of the mosquitoes were collected in Centers for Disease Control (CDC) light traps supplemented with approximately 1 kg of dry ice per trap.[2, 3] In addition, mosquitoes were collected in traps baited with horses (Magoon trap), chicken (bait can and Ehrenberg trap), wild bird or hamster (bait can), and mosquitoes were aspirated from natural shelters, human bait, and horse bait. Biting flies other than mosquitoes also were collected in light traps. Following collection, arthropods were anesthetized with CO_2, placed in glass tubes with rubber stoppers, and transported on dry ice to the CDC laboratory in Fort Collins. The collection sites in Santa Fe and Corrientes provinces are representative of predominant habitat types in the rural areas of these provinces. In Chaco Province, collections were restricted to riverine and swamp habitats gen-

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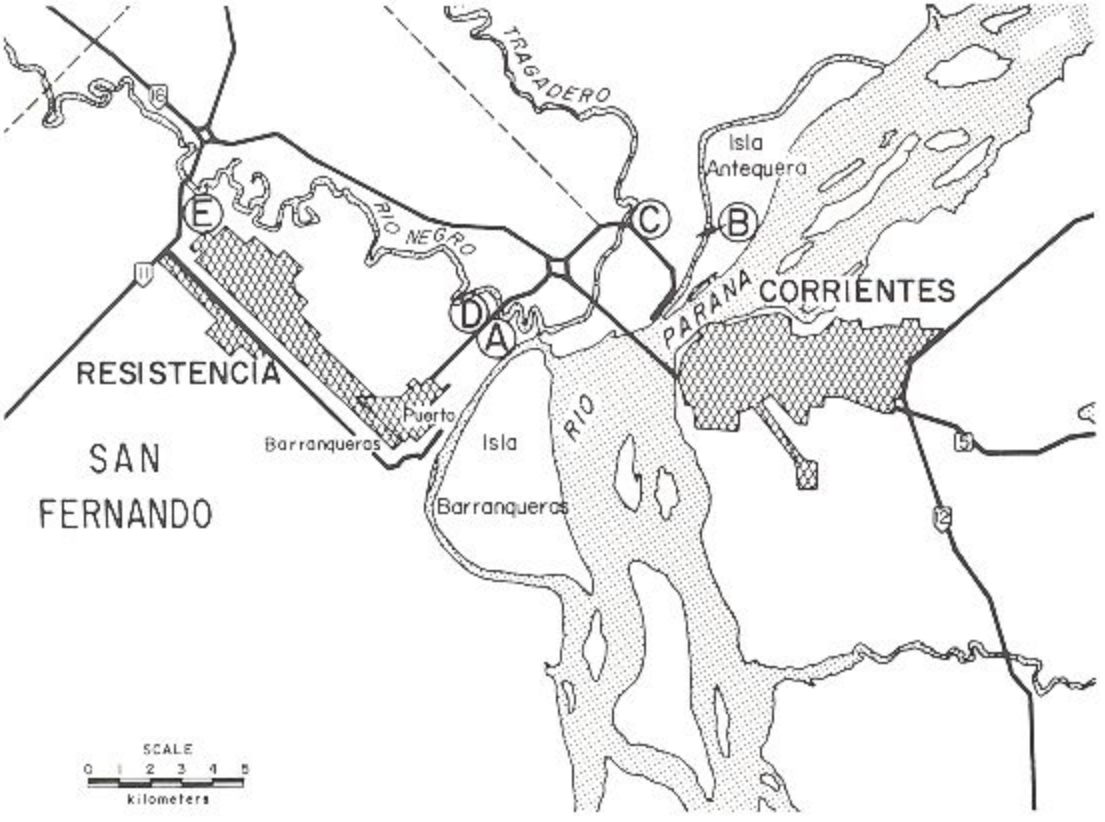

FIGURE 1. Chaco Province collection sites: (A) brick factory near Rio Negro, (B) Antequera Nature Park, (C) Rio Tragadero Swamp, (D) Rio Negro Bridge NE Resistencia, and (E) Rio Negro Bridge NW Resistencia. Note: These sites correspond to sites 9, 10, 8, 11, and 7, respectively, in Table 6.

erally characterized by dense growths of aquatic vegetation, especially water lettuce (*Pistia* spp.). A few collection sites in Santa Fe and Chaco provinces were urban or suburban; nonetheless, those in Chaco Province were always near swampy, forested areas. A map of the collection sites in Chaco Province is provided (Fig. 1) because of the unusual importance of these sites as centers of arbovirus activity.

Mosquitoes were sorted and pooled in lots of 1 to 100. Abdomens were snipped from engorged mosquitoes as they were identified, placed in individual gelatin capsules and shipped frozen to the Gorgas Memorial Laboratory for bloodmeal identification.

All antisera for bloodmeal identification were produced in rabbits except anti-Leporidae, which was prepared in roosters. Class-, order-, and family-specific antisera were harvested after a series of injections into the axillary and inguinal lymph nodes of rabbits with equal quantities of animal plasma or sera and Freund's adjuvant. Titers ranged from 1:10,000 to 1:80,000; those that cross-reacted with sera diluted 1:1,000 from members of heterologous orders or families were absorbed overnight in a refrigerator with undiluted sera of these animals. For additional details see Christensen and Vasquez.[4]

The engorged mosquito abdomens were placed in 12 × 75-mm test tubes containing 0.4 ml of phosphate buffered saline 0.85% (w/v) and refrigerated overnight. The following day the bloodmeals were expressed from the abdomens with applicator sticks and centrifuged at 500 × *g* for 30 min. Antigens in the supernatant were then screened by class-, order-, and family-specific antisera by a microcapillary precipitin method.[5]

Mosquito pools to be tested for virus were triturated in cold mortars containing sterile alundum and 2 ml of M199 + 20% fetal calf serum (FCS). Triturated pools were clarified by centrifugation at 800 × *g* for 30 min. The super-

TABLE 1

Relative abundance of mosquitoes collected in Santa Fe Province, Argentina, 1977–1980 and tested for the presence of arboviruses

Mosquitoes	Nov–Dec 1977		May 1978		Feb 1979		Feb 1980	
	No.	%	No.	%	No.	%	No.	%
Aedeomyia squamipennis	359	0.8	171	0.7	435	0.6	256	0.8
Aedes albifasciatus	4,246	9.6	1,054	4.2	4,290	6.1	224	0.7
scapularis	911	2.0	4	—*	543	0.8	32	0.1
stigmaticus	21	—						
spp.	127	0.3	31	0.1	433	0.6	171	0.5
Anopheles albitarsis	3,861	8.7	1,323	5.2			42	0.1
evansi	100	0.2						
pseudopunctipennis	23	0.1						
punctimacula	36	0.1						
triannulatus							48	0.1
spp.	590	1.3	3,743	14.8	1,840	2.6	248	0.7
Coquillettidia venezuelensis	59	0.1					3	—
Culex delponei							19	0.1
p. quinquefasciatus			3,293	13.0	3,425	4.9	8,261	24.3
(*Cux.*) spp.	14,753	33.2	12,889	51.0	21,201	30.2	9,493	27.9
(*Mel.*) spp.	21	—	47	0.2	102	0.1	5	—
Mansonia titillans	1,044	2.3	162	0.6				
spp.	12,564	28.3	2,377	9.4	34,744	49.5	15,132	44.5
Phoniomyia spp.	46	0.1						
Psorophora albipes			1	—				
ciliata	44	0.1	1	—	8	—		
confinnis	294	0.7	6	—	1,796	2.6	2	—
cyanescens	88	0.2			70	0.1		
dimidiata					1	—	7	—
discrucians	5	—			636	0.9		
ferox	69	0.2						
pallescens	123	0.3			4	—		
paulli	3,233	7.3	41	0.2				
varinervis	1,170	2.6	75	0.3	401	0.6	2	—
varipes	518	1.2						
spp.	21	—	1	—	227	0.3	8	—
Uranotaenia spp.	138	0.3	76	0.3	55	0.1	50	0.1
Total mosquitoes	44,464		25,295		70,211		34,003	

* — less than 0.1%.

natant was poured into individual screw-cap vials and frozen at −70°C.

Specimens were tested for virus in Vero cell culture and in primary duck embryo (DE) cell culture grown in 25-cm² flasks. Specimens were inoculated in 0.2-ml quantities into each cell culture, adsorbed for 1 hr at 37°C, then the cells were overlayed with 1% Noble agar in M199 + 2% FCS, 2.0 g of $NaHCO_3$, 150 g/ml of DEAE-dextran and 1:40,000 neutral red plus 50 g/ml of Gentamicin and 1 g/ml of Fungizone. Cell cultures were incubated at 37°C and examined for 12 days for plaques. Positive cell cultures were harvested in 2 ml of M199 + 20% FCS and frozen at −70°C until passed into suckling mice by intracranial (ic) inoculation and into fluid cultures of Vero and DE cells. Identification and characterization of virus isolates is described elsewhere.[6]

To facilitate comparison of virus infection rates in particular mosquito species and at specific sites, minimum infection rates (MIR) were calculated as follows: MIR = number of virus isolations by species/total number of that species tested from that site × 1,000.

RESULTS

A total of 313,233 mosquitoes were collected. In addition to mosquitoes, 485 Ceratopogoni-

TABLE 2

Relative abundance of mosquitoes collected in Corrientes and Chaco provinces, Argentina, during April 1980 and tested for the presence of arboviruses

Mosquitoes	Corrientes Province		Chaco Province	
	No.	%	No.	%
Aedeomyia squamipennis	179	0.5	2,181	2.1
Aedes albifasciatus			1	—*
scapularis	740	2.2	3,074	2.9
serratus	308	0.9	22	—
stigmaticus			16	—
spp.	207	0.6	944	0.9
Anopheles albitarsis	1,749	5.2	565	0.5
triannulatus	1,388	4.1	139	0.1
spp.	6,569	19.5	17,056	16.1
Coquillettidia chrysonotum	2	—	1,065	1.0
venezuelensis	777	2.3	793	0.7
Culex amazonensis	11	—	1	—
delpontei	32	0.1	8,081	7.6
ocossa group	144	0.4	22,969	21.7
p. quinquefasciatus	1,313	3.9	109	0.1
(*Cux.*) spp.	1,121	3.3	13,428	12.7
(*Mel.*) spp.	304	0.9	1,266	1.2
Haemagogus spp.	2	—	1	—
Mansonia flaveola	4	—	5	—
spp.	10,049	29.9	31,487	29.8
Phoniomyia spp.	2	—		
Psorophora albipes	5	—	1	—
ciliata	45	0.1	33	—
confinnis	3,479	10.3	195	0.2
cyanescens	2,352	7.0	24	—
dimidiata	225	0.7	4	—
discrucians	533	1.6	546	0.5
ferox	2	—	70	0.1
pallescens	527	1.6	10	—
paulli	103	0.3		
varinervis	484	1.4	78	0.1
varipes	14	—		
spp.	901	2.7	267	0.3
Uranotaenia spp.	54	0.2	1,204	1.1
Total mosquitoes	33,625		105,635	

* — less than 0.1%.

dae, 104 Simuliidae, and 9 Psychodidae were collected in the light traps and were pooled and tested for virus.

The relative abundance of mosquito species collected and tested for virus is summarized in Tables 1 and 2. *Culex* mosquitoes that could not be identified to species were grouped according to subgenus where possible because of important differences in the biology and vector relationships of *Culex* (*Culex*) and *Culex* (*Melanoconion*) mosquitoes. Mosquitoes that are listed in the tables and referred to subsequently in the text as *Cx.* (*Mel.*) *ocossa* group were initially identified as mixed pools of *Cx.* (*Mel.*) *ocossa* and *Cx.* (*Mel.*) *paracrybda*. However, there is doubt about whether the latter species occurs in Argentina. Therefore, mosquitoes referred to as the *Cx.* (*Mel.*) *ocossa* group in the following discussion probably represent 2 or more species.

The mosquitoes collected in Santa Fe Province were predominantly *Cx.* (*Cux.*) (33.2%) and *Mansonia* (30.6%) in November–December 1977, *Cx.* (*Cux*) (64%) and *Anopheles* (20%) during May 1978, *Ma.* (49.5%) and *Cx.* (*Cux.*) (35.1%) during February 1979, and *Cx.* (*Cux.*) (52.2%) and *Ma.* (44.5%) during February 1980.

TABLE 3

*Identification of bloodmeals from engorged mosquitoes collected in Corrientes and Chaco provinces during April 1980**

Bloodmeal source	Province	*An. albitarsis*	*An. triannulatus*	*An. (Nys.)* spp.	*Ae. scapularis*	*Ae.* spp.	*Cq. chrysonotum*	*Cq. venezuelensis*	*Cx. (Cux.)* spp.	*Cx. (Mel.) delpontei*	*Cx. (Mel.) ocossa*	*Ma.* spp.	*Ps. confinnis*	*Ps. cyanescens*	*Ps. discrucians*
Avian	Chaco										1				
Ardeidae	Chaco										2				
Momotidae	Corrientes											1			
	Chaco				1										
Passeriformes	Chaco										2				
Mammalian	Corrientes	1												1	
	Chaco									2					
Didelphidae	Chaco				1										
Leporidae	Corrientes											2			
	Chaco									1					
Rodentia	Corrientes							1			1				
	Chaco									6		1			
Hydrochoeridae	Corrientes							5				10			
	Chaco							3		4	2	2			
Sciuridae	Chaco									2					
Felidae	Chaco				1										
Canidae	Corrientes													1	
	Chaco							1	1						1
Perissodactyla	Corrientes				11							13		5	
	Chaco					1	1	1	1	1	3	13			1
Artiodactyla	Corrientes													3	
Bovidae	Corrientes	1	1	1	5			1				23	6	26	1
	Chaco			1	3							1			
Cervidae	Corrientes				1										
Hominidae	Chaco				1										

* In addition, in Chaco Province, 1 *Cx. (Mel.)* sp. had fed on an amphibian, and 1 *Ma.* sp. contained a mixed bloodmeal from a Perissodactyla and a bovid.

These time periods during 1977, 1978, and 1979–1980 fall within the antipodean seasons of spring, autumn, and summer, respectively.

The collections in Corrientes Province during April 1980 (early autumn) were composed principally of *Ma.* (29.9%), *An.* (28.8%) and *Psorophora* (25.8%). *Culex* mosquitoes made up a relatively small segment (8.7%) of the collection, and these were predominantly *Cx. (Cux.)* (7.2%). In contrast, the collections in Chaco Province, made during the same time period, were predominantly *Cx.* (43.4%), and *Cx. (Mel.)* mosquitoes made up 30.6% of the total collection from that province. *Mansonia* (29.8%) and *An.* (16.8%) also were prevalent in the Chaco collections.

Bloodmeals from 80 engorged mosquitoes collected in Santa Fe Province during May 1978 and February 1979 were identified by precipitin test. Sixty-six bloodmeals (82.5%) were of bovine origin, and these came from the following mosquitoes: 1 *An. (Nyssorhynchus)* sp., 16 *Ae. albifasciatus*, 4 *Ae. scapularis*, 22 *Cx. (Cux.)* spp., 18 *Ma.* spp., and 5 *Ps.* spp. In addition, 10 *Cx. (Cux.)* spp. had fed on 1 Perissodactyla, 4 Ardeidae, 1 Strigidae, 3 Coraciiformes, and 1 Thraupidae. Three *Ma.* spp. had fed on 2 Perissodactyla and 1 Hominidae, and 1 *Ae. albifasciatus* contained a mixture of bloods from Leporidae and Hominidae.

The results of bloodmeal identifications from engorged mosquitoes collected in Corrientes and Chaco provinces during April 1980 are summarized in Table 3. Only 1 of 109 engorged mosquitoes collected in Corrientes Province had fed on an avian host. The remainder had fed on mammals, and 86 of these (80%) had fed on large ungulates, i.e., Perissodactyla and Artiodactyla, including Bovidae and Cervidae. Engorged *Cx. (Mel.)* were not represented in the Corrientes

TABLE 4

Relative abundance of mosquitoes in bait collections, Santa Fe Province, Argentina, 1977–1980

Mosquitoes	Magoon trap with horse			Aspirated from horse			Aspirated from human			Bait can with chicken		
	D*	E	G	D	E	G	D	E	G	D	E	G
Ad. squamipennis	1		1							11-	54	1
Ae. albifasciatus	1	22	3	26	2	3	1			13	1	6
scapularis		1										
serratus										5	4	
spp.				1	2	1						
An. albitarsis				106	3		24					
triannulatus										1		
spp.	9	10	2	32	2		8			23	2	
Cx. delpontei				1								
p. quinquefasciatus	654	199	22		7		2			1,999	6,051	157
(*Cux.*) spp.	184	565	17	295	24	33	2	1		1,140	8,834	51
Ma. titillans		1	1	2								
spp.		19	5	326	2	33	57	1		881	47	43
Ps. confinnis										1		
discrucians										6	4	
varinervis							3					
spp.										5		1
Number trap-nights		20									132	

* D = deplete; E = engorged; G = gravid.

collections. In the Chaco collections, 6 mosquitoes (8%) had fed on avian hosts, 1 on an amphibian, and 69 (91%) on mammalian hosts. Among the latter, 39 (57%) had fed on ungulates. The *Cx.* (*Mel.*) had fed on a variety of birds and mammals, as well as 1 amphibian.

With 2 exceptions, the relative abundance of deplete, engorged, and gravid mosquitoes in bait collections is summarized in Tables 4 and 5. The exceptions are: 1) a single collection from a trap baited with a wild bird that yielded 2 deplete *Ma.* spp., and 2) chicken-baited Ehrenberg trap collections. The latter trap is designed to separate mosquitoes from the host and thus prevent them from feeding. Mosquitoes that were attracted to horses and chickens in Santa Fe Province, and that subsequently fed on these animals, were predominantly *Cx.* (*Cux.*). *Aedeomyia squamipennis* was collected in chicken-baited traps in Santa Fe and Chaco provinces, and significant proportions (82%–100%) subsequently engorged. Most of the mosquitoes attracted to bait animals in Corrientes Province were species of *An.*, *Ma.*, and *Ps.*; few *Cx.* were collected. In Chaco Province, *Cx.* made up 85.7% of the mosquitoes collected in chicken-baited traps, and 75.6% of those collected in hamster-baited traps. The *Cx.* from the chicken-baited traps were predominantly *Cx.* (*Cux.*). (75.7%), whereas the collections from the hamster-baited traps were composed of comparable numbers of *Cx.* (*Cux.*) (51.6%) and *Cx.* (*Mel.*) (48.4%). Nonetheless, on a per trap-night basis, the chicken-baited trap yielded 6 times as many *Cx.* (*Mel.*) (168/trap-night) as did the hamster-baited trap (28/trap-night) in Chaco Province.

The 313,233 mosquitoes were tested for virus in 5,197 pools. The number of pools yielding virus per number tested by province and year are as follows: Santa Fe Province 1977 (3/666), 1978 (1/590), 1979 (2/1,230), 1980 (0/656); Corrientes Province 1980 (1/585); and Chaco Province 1980 (33/1,470). A total of 598 biting flies other than mosquitoes was tested for virus in 45 pools with negative results.

Virus isolations and MIR by mosquito species and collection site are summarized in Table 6. Eight Gamboa group viruses were isolated exclusively from *Ad. squamipennis* in Santa Fe (4 strains) and Chaco (4 strains) provinces. One strain of St. Louis encephalitis (SLE) virus was isolated from *Cx. p. quinquefasciatus* in 1978 and 1 from *Cx.* (*Cux.*) spp. in 1979, both from Santa Fe Province. A single strain of a newly

TABLE 5

Relative abundance of mosquitoes in bait collections, Corrientes and Chaco provinces, Argentina, 1980

	Corrientes Province									Chaco Province					
	Aspirated from horse			Aspirated from human			Bait can with chicken			Bait can with chicken			Bait can with hamster		
Mosquitoes	D*	E	G	D	E	G	D	E	G	D	E	G	D	E	G
Ad. squamipennis											19				
Ae. scapularis											14		4	1	1
serratus						1									
spp.											1				
An. albitarsis	1	3	1					1							
triannulatus													2		
spp.								300							
Cq. chrysonotum											6		1		
venezuelensis											1			1	
Cx. delpontei										420	28		59	78	7
ocossa group										1	27		10	1	
(*Cux.*) spp.							38			24	1,544		142	33	4
(*Mel.*) spp.										9	11	8	13		
Ma. spp.							393	1	6	6	294		70	24	7
Ps. ciliata	1	1	1												
confinnis				2											
cyanescens				100	4										
discrucians														1	
pallescens		1													
spp.	37	58	5	88	12			10							
Number trap-nights								3			3			6	

* D = deplete; E = engorged; G = gravid.

discovered Anopheles A serogroup virus, Las Maloyas, was isolated from *An. albitarsis* collected in Corrientes Province during 1980.

In addition to the 4 strains of Gamboa group viruses referred to above, 29 strains of other viruses were isolated from mosquitoes collected in Chaco Province during 1980. These can be grouped as follows: 6 strains of new Bunyaviridae (1 Antequera, 1 Barranqueras, and 4 Resistencia) from *Cx.* (*Mel.*) *delpontei*, 3 strains of a new subtype of western equine encephalitis (WEE) virus and 1 strain of Para virus from the *Cx.* (*Mel.*) *ocossa* group. 12 strains of a newly discovered member of the Venezuelan equine encephalitis (VEE) complex, designated subtype VI, from *Cx.* (*Mel.*) *delpontei*, and 1 strain each from *Ad. squamipennis*, *Ae. scapularis*, *Ae.* spp., *Cx.* (*Cux.*) spp., *Cx.* (*Mel.*) *ocossa* group, *Ma.* spp., and *Ps.* spp. The serologic relationships and biological characterization of these new viruses are described by Calisher et al.[6]

Eleven of the 40 mosquito pools from which viruses were isolated contained some specimens engorged with blood. Four of these pools (AG80-222, 226, 239, and 517) were composed of mosquitoes collected in chicken-baited traps (Table 6), which probably explains the source of their bloodmeals. The remaining 7 pools were made up of engorged, gravid and deplete mosquitoes, respectively, as follows: 79V-2533 (1, 2, 96), AG80-504 (1, 8, 56), AG80-728 (1, 4, 95), AG80-903 (3, 5, 89), AG80-912 (1, 2, 95), AG80-1657 (2, 5, 93), and AG80-1726 (2, 2, 86).

DISCUSSION

In Santa Fe Province the 2 predominant groups of mosquitoes in each of the 4 annual collections were *Cx.* (*Cux.*) and *Ma.*, with the exception of the May 1978 collections in which *An.* (20%) were more abundant than *Ma.* (10%) (Table 1). The aquatic stages of all these groups characteristically reside in permanent bodies of water. Floodwater species of *Ae.* and *Ps.* were relatively scarce in the Santa Fe collections. Also, only 194 specimens of *Cx.* (*Mel.*) were collected in Santa Fe Province. With rare exceptions, mosquitoes in the subgenus *Melanoconion* are restricted in distribution to the tropics and subtropics. Species in this subgenus probably reach the southern lim-

TABLE 6

Virus isolations and minimum infection rates (MIR) by species and site in mosquitoes collected in Argentina, 1977–1980

Mosquito species or group by province	Collection site*	Total no. tested		MIR	No. virus isolations	Strain designations†
		Pools	Specimens			
Santa Fe Province:						
Ad. squamipennis	1	6	34	29.4	1 Gamboa group	78V-2514
	2	5	322	6.2	2 Gamboa group	78V-2441 and 3016
	3	2	117	8.5	1 Gamboa group	79V-2481
Cx. p. quinquefasciatus	4	83	2,881	0.4	1 SLE	78V-6507
Cx. (Cux.) spp.	5	11	621	1.6	1 SLE	79V-2533
Corrientes Province:						
An. albitarsis	6	9	730	1.4	1 Las Maloyas	AG80-24
Chaco Province:						
Ad. squamipennis	7	6	422	2.4	1 Gamboa group	AG80-1670
	8	4	215	9.3	2 Gamboa group	AG80-1403 and 1404
	9	16	1,213	0.8	1 Gamboa group	AG80-821
	9	16	1,213	0.8	1 VEE VI	AG80-911
Cx. (Mel.) ocossa group	9	53	4,860	0.2	1 Para	AG80-934
	9	53	4,860	0.6	3 WEE	AG80-463, 621 and 646
	10	8	634	1.6	1 VEE VI	AG80-1801
Ae. scapularis	10	1	10	100.0	1 VEE VI	AG80-239
Ae. spp.	9	9	578	1.7	1 VEE VI	AG80-953
Ma. spp.	10	19	1,655	0.6	1 VEE VI	AG80-222
Ps. spp.	9	8	171	5.8	1 VEE VI	AG80-1028
Cx. (Cux.) spp.	9	74	6,027	0.2	1 VEE VI	AG80-1026
Cx. (Mel.) delponiei	9	28	2,403	2.5	6 VEE VI	AG80-663, 727, 728, 903, 912, and 937
	7	34	2,866	1.4	4 VEE VI	AG80-1568, 1619, 1657, and 1726
	11	10	541	1.8	1 VEE VI	AG80-1985
	10	9	791	1.3	1 VEE VI	AG80-1477
	10	9	791	1.3	1 Antequera	AG80-226
	11	10	541	1.8	1 Barranqueras	AG80-381
	11	10	541	3.7	2 Resistencia	AG80-504 and 517
	9	28	2,403	0.4	1 Resistencia	AG80-785
	7	34	2,866	0.3	1 Resistencia	AG80-1545

* 1 = Las Gamas, 2 = Valla California, 3 = near Rio Salado, 4 = Esperanza Facultad, 5 = Rio Salado/La Nicolina Ranch, 6 = Rincon de Vances, 7 = Rio Negro Bridge NW Resistencia, 8 = Rio Tragadero Swamp, 9 = brick factory near Rio Negro, 10 = Antequera Nature Park, 11 = Rio Negro Bridge NE Resistencia.

† Year of collection indicated by prefix in all cases except 78V-2514, 2441, and 3016 which were collected during 1977. Strains AG80-222, 226, 239, and 517 are from mosquitoes collected in chicken-baited traps; all others are from mosquitoes collected in CDC light traps supplemented with dry ice.

its of their range in Argentina somewhere in northern Buenos Aires Province.

The composition of the mosquito fauna in Santa Fe Province during the time collections were made may explain the pattern of virus isolations. The 4 isolates of Gamboa group viruses came from *Ad. squamipennis,* a species that breeds in permanent water with dense growths of aquatic vegetation. Subtyping of 1 Gamboa group viral strain (78V-2441) indicated that it is identical or closely related to San Juan virus.[7] The high MIR of Gamboa group viruses in *Ad. squamipennis* (Table 6) may indicate transovarial transmission. Gamboa group viruses have been isolated from *Ad. squamipennis* collected in Panama, Ecuador, and Argentina.[7] and Galindo et al.[8] reported isolations from larvae and adult male mosquitoes as well as from adult female mosquitoes in Panama. Tests of sera from birds and mammals collected in 1978 in Santa Fe Province showed a high prevalence rate of neutralization (N) antibody to strain 78V-2441 in birds, whereas few mammals were found to have antibody.[7] Our data (Tables 4 and 5) suggest

that *Ad. squamipennis* feeds primarily on birds in the study areas sampled. This species has been shown to be attracted to birds in Venezuela and to be a natural vector of strains of plasmodia that infect non-passerine birds in that country.[9,10]

The isolation of SLE virus from *Cx. p. quinquefasciatus* in 1978 and from *Cx.* (*Cux.*) spp. in 1979 is not surprising in view of the known vector relationships of this virus in northern temperate areas.[11] *Cx. p. quinquefasciatus*, collected at the same time and from the same site as those from which SLE viral strain 78V-6507 was isolated, was colonized and shown to be an efficient vector of both the Argentine and United States SLE viral strains.[12] Strain 79V-2533 also readily infects Argentinian *Cx. p. quinquefasciatus* by the oral route.[13] The prevalence of engorged *Cx.* (*Cux.*) spp., including *Cx. p. quinquefasciatus*, in horse-baited Magoon trap collections (Table 4) provides a plausible explanation for the high prevalence of flavivirus antibodies in equines in Santa Fe Province.[14]

The mosquito collections in Corrientes Province contained comparable percentages of floodwater *Ps.* and permanent water *Ma.* and *An.*, but relatively few *Cx.* (Table 2). A single viral strain, AG80-24, was isolated from *An. albitarsis*. This strain, registered as Las Maloyas virus, represents a new member of the Anopheles A group of viruses in the family Bunyaviridae. *Anopheles albitarsis* is assigned to the subgenus *Nyssorhynchus* which is restricted to the neotropical region. Three additional arboviruses have been isolated from other species in this subgenus, i.e., Tlacotalpan virus (Bunyaviridae) isolated from *An.* (*Nys.*) *albimanus* in Mexico, VEE and Tonate viruses (Togaviridae, genus alphavirus) isolated from *An.* (*Nys.*) *aquasalis* in Venezuela and *An.* (*Nys.*) *brasiliensis* in French Guiana, respectively.[15]

Viral strain AG80-934 is identical or closely related to Para virus, an ungrouped virus registered by Drs. F. Pinheiro and A. P. A. Travassos da Rosa. Para virus has been isolated from the brain and liver of a single sentinel mouse exposed in a tropical rain forest in Belem, Para, Brazil in 1975. The isolation of AG80-934 in Argentina from mosquitoes (*Cx. (Mel.) ocossa* group) that are known to feed on rodents (Table 5) is consistent with the isolation of Para virus from a sentinel mouse in Brazil. Strain AG80-934 grew to high titer in parenterally-infected *Cx. p. quinquefasciatus* from Argentina; however, the same species of mosquito failed to become infected by the oral route following ingestion of a blood/virus/sugar suspension containing $10^{5.7}$ Vero cell plaque forming units/ml (C. J. Mitchell, personal communication). This information suggests that AG80-934 is indeed an arbovirus, but that the strain of *Cx. p. quinquefasciatus* tested is not a vector in nature.

Three viral strains isolated from *Cx.* (*Mel.*) *ocossa* group mosquitoes collected at a single fresh water swamp habitat in Chaco Province have been identified as WEE virus (Table 6); however, a one-way antigenic difference from the Fleming strain of WEE virus has been found in N tests.[6] Serologic evidence indicates that the 3 strains also differ from a strain of WEE virus (Cba 87) isolated in 1958 from a horse in Cordoba Province. The virulence characteristics of the strains from Chaco Province are unknown; they may represent a variant of WEE virus sequestered in an enzootic transmission cycle and not involved in equine encephalitis outbreaks.

The collection sites in Chaco Province (Table 6, Fig. 1) are located within a radius of 9 km of each other. Para and WEE viruses were isolated from *Cx.* (*Mel.*) *ocossa* group mosquitoes collected at a single site (brick factory near Rio Negro) despite the testing of 18,119 additional mosquitoes similarly identified from the other 4 sites. Either this virus was very localized geographically, or the pools contained a mixture of species with different vector relationships. In a previous publication on the taxonomy and distribution of mosquitoes from our collections, Sirivanakarn and Jakob[16] stated that "several isolates of western equine encephalomyelitis (WEE) virus have been obtained from this species [*Cx.* (*Mel.*) *ocossa*] from Chaco and Corrientes provinces." This statement should be revised, since 1) the mosquito identifications for *ocossa* were imprecise and more than 1 species may have been included in the pools, and 2) all of the virus isolations from this group came from mosquitoes collected in Chaco Province.

Nineteen strains of a member of the VEE virus complex, designated subtype VI,[6] were isolated from mosquitoes collected in Chaco Province (Table 6). Twelve of the strains are from *Cx.* (*Mel.*) *delpontei*. *Culex* mosquitoes of the subgenus *Melanoconion* are the principal vectors for enzootic transmission of VEE viruses.[17,18] These mosquitoes feed on various kinds of wild rodents and are mainly restricted to forested, swampy

habitats. Twelve of 16 engorged *Cx.* (*Mel.*) *delpontei* from Chaco light trap collections had fed on rodents (Table 3), and significant numbers (54%) fed on hamsters following entry into baited traps (Table 5). These findings support the view that subtype VI is an enzootic VEE virus. This hypothesis also is supported by the finding of N antibodies to VEE subtype VI virus in rodents from the Chaco collection sites.[14] However, the isolations from ornithophilic mosquitoes such as *Ad. squamipennis* and *Cx.* (*Cux.*) spp. and from infected mosquitoes collected in chicken-baited traps (*Ae. scapularis, Ma.* spp.) suggest that secondary natural transmission chains may exist. In Panama, herons have been shown to circulate enzootic VEE virus at sufficiently high titers to infect efficient mosquito vectors such as members of the *Cx.* (*Mel.*) *ocossa* group.[18] In view of the catholic feeding habits of the *Cx.* (*Mel.*) mosquitoes collected from the Chaco sites (Tables 3 and 5), search for transmission chains other than usual rodent-mosquito-rodent cycles would seem warranted.

The VEE virus subtype VI was isolated from *Cx.* (*Mel.*) *delpontei* at each site sampled in Chaco Province except Rio Tragedero Swamp (1,480 tested from this site). Therefore, there appears to have been a rather large focus (minimal radius of ≈9 km) of enzootic VEE virus activity in the area sampled during April 1980. Galindo[18] has speculated that dislodgement and movement of *Pistia* plants by floodwaters could result in the spread of VEE virus from highly localized centers of intense virus activity to localities downstream by transporting infected gravid female *Cx.* (*Mel.*). Undoubtedly, the extensive flooding that occurred along the Rio Parana from the Paraguayan border south to Santa Fe City during December 1982 and the first half of 1983 provided ample opportunity for such spread. VEE viruses were isolated for the first time in Santa Fe Province from *Cx.* (*Mel.*) *delpontei* collected during December 1982 (C. J. Mitchell et al., personal communication).

Cx. (*Mel.*) *delpontei* from Chaco Province was also host to 6 strains of new Bunyaviridae that have been named Antequera, Barranqueras, and Resistencia viruses (Table 6).[6] The apparent feeding preference of the vector, and serologic data, suggest that these strains may also be cycling between rodents and mosquitoes.

Eighteen of the 40 viral isolates (45%) obtained during these investigations were from *Cx.* (*Mel.*) *delpontei*, despite the fact that this species made up only 7.4% of the total mosquitoes tested. To our knowledge, this is the first report of *Cx.* (*Mel.*) *delpontei* as an arbovirus vector. This species is obviously a very important vector and a great deal remains to be done in defining its ecology, distribution, and vector competence. *Cx.* (*Mel.*) *delpontei* is presently known from northern Argentina, Paraguay, and southern Brazil.

Problems encountered in identifying and processing more than 300,000 mosquitoes for virus isolation were formidable. In the present case, a great deal of information was lost because of these problems; for example, 23.3% of the 313,233 mosquitoes were identified only as *Cx.* (*Cux.*) spp. Obviously, adult females must be identified to species in order to obtain reliable information on habitat associations, seasonal abundance, and feeding habits as well as on specific vector relationships. Morphological and coloration characteristics of adult females in the subgenus *Culex* are extremely difficult to differentiate, and keys using adult female characters may serve only to approximate species. The problem of accurate identification of adult female *Cx.* (*Cux.*) in Argentina must be solved by concentrated taxonomic studies that include rearing larvae individually and associating immature life history stages with adult male and female specimens.

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