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ARBOVIRUS SURVEILLANCE IN SOUTH CAROLINA, 1996–98

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ABSTRACT. Arboviruses isolated and identified from mosquitoes in South Carolina (USA) are described, including new state records for eastern equine encephalitis virus (EEE), St. Louis encephalitis virus (SLE), Flanders virus, Tensaw virus (TEN), and a variant of Jamestown Canyon virus (JC). Mosquitoes were collected at 52 locations in 30 of 46 South Carolina counties beginning in June 1996, and ending in October 1998, and tested for arboviruses. Of 1,329 mosquito pools tested by virus isolation (85,806 mosquitoes representing 34 mosquito species or complexes), 15 pools were positive. Virus isolations included EEE from 1 pool each of *Anopheles crucians* complex and *Culex erraticus*; a variant of JC from 1 pool of *An. crucians* complex; a California serogroup virus from 1 pool of *Aedes atlanticus/tormentor*; TEN from 5 pools of *An. crucians* complex and 1 pool each of *Culex salinarius* and *Psorophora ciliata*; Flanders virus from 1 pool of *Culiseta melanura*; and Potosi virus from 1 pool each of *Aedes vexans, Coquillettidia perturbans,* and *Psorophora columbiae.* Of 300 mosquito pools tested by antigen-capture assay for EEE and SLE (14,303 mosquitoes representing 16 mosquito species or complexes), 21 were positive for EEE and 1 was positive for SLE. Positive EEE mosquito pools by antigen-capture assay included *An. crucians* complex (14 pools), *Anopheles punctipennis* (1 pool), *Anopheles quadrimaculatus* (1 pool), *Cq. perturbans* (4 pools), and *Cs. melanura* (1 pool). One pool of *Cx. salinarius* was positive for SLE by antigen-capture assay. Arbovirus-positive mosquito pools were identified from 12 South Carolina counties, all located in the Atlantic Coastal Plain, and from 4 of 8 Carolina bays surveyed.

KEY WORDS Mosquitoes, arboviruses, *Anopheles crucians,* eastern equine encephalitis virus, South Carolina

INTRODUCTION

Infections caused by eastern equine encephalitis virus (EEE) occur annually in South Carolina (USA), most notably in animals of commercial importance, such as horses, donkeys, emus, pheasants, and quail. Historically, most of these animal cases have occurred in the Atlantic Coastal Plain area of South Carolina (Tidwell et al. 1984, Wright 1993). Cases of human disease caused by EEE also occur in South Carolina, but are infrequent. Two human cases were confirmed, including 1 fatal case in 1997, 1 nonfatal case was confirmed in 1996, and no cases were confirmed for the years 1991–95 and 1998 (South Carolina Department of Health and Environmental Control, reportable disease case reports). All 3 of the 1996 and 1997 human cases were residents of counties located in the Atlantic Coastal Plain, specifically 1 case in Horry County in 1996, and 2 cases in Charleston County in 1997.

Although EEE is enzootic in South Carolina, natural history studies of arboviral diseases and mosquito vectors in South Carolina have been limited to a few field sites and relatively small sample sizes. For example, Durden et al. (1997) collected 121 avian sera at 1 South Carolina coastal site and found EEE-neutralizing antibodies in 4 sera and St. Louis encephalitis virus (SLE)-neutralizing antibodies in 2 sera. In another study, Tidwell et al. (1984) collected mosquitoes at 2 South Carolina coastal sites and reported no arbovirus isolations from 41 mosquito pools comprising 1,913 mosquitoes and representing 11 species. Before 1996, when we began our study, no statewide mosquito collections had been performed to determine arbovirus activity in South Carolina. The purposes of our study were to identify the mosquito vectors of arboviral diseases of public health importance and to identify naturally occurring endemic foci of arbovirus activity in South Carolina.

MATERIALS AND METHODS

Field sites: Fifty-two field sites were located in 30 of 46 South Carolina counties, including all 6 Atlantic Coast counties (Beaufort, Charleston, Colleton, Georgetown, Horry, and Jasper), 18 inland Atlantic Coastal Plain counties (Aiken, Allendale, Bamberg, Barnwell, Berkeley, Calhoun, Chesterfield, Clarendon, Darlington, Dillon, Dorchester, Hampton, Kershaw, Lee, Marion, Orangeburg, Richland, and Sumter), 3 Piedmont counties (Fairfield, Lancaster, and McCormick), and 3 counties that extend from the Piedmont into the Blue Ridge geologic province (Greenville, Oconee, and Pickens) (Shimer 1972).

Ten of the field sites were located on private land, including 7 farms, 2 barrier island communities, and 1 coastal residential subdivision. Mosquito collections were made at these 10 sites at the invitation of the property owners or their community representatives, and with the cooperation of lo-

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cal mosquito control personnel, all of whom wanted to learn more about the mosquitoes in their area. Forty-two field sites were state-owned or state-managed lands, for example, parks, wildlife management areas, public utility company lands, Heritage Preserves, and university field research centers. These 42 sites were selected for their favorable mosquito breeding habitats and histories of low pesticide pressure. Eight Carolina bays in 7 counties were included as field sites: Cathedral Bay (Bamberg), Santee Coastal Reserve Carolina bay complex (Charleston), Dingle Pond (Clarendon), Little Pee Dee Bay (Dillon), Cartwheel Bay (Horry), Lewis Ocean Bay complex (Horry), Savage Bay (Kershaw), and Woods Bay (Sumter). Six coastal marsh areas were also included as field sites.

Mosquito collections: Mosquitoes were collected using CO_2-baited, Centers for Disease Control miniature light traps. Collections began in June 1996, and continued through October 1998. All of the collections were made during the months of March through October. Five to 13 mosquito traps were used at each field site for 1–4 nights for a total of 1,431 trap nights. Although mosquitoes were collected at some of the field sites once or twice annually, no established time intervals were used to determine when a particular field site was revisited. The trap nets were collected at dawn, and placed in chests containing dry ice to kill the mosquitoes. The dead mosquitoes were placed in labeled, 150 × 15-mm polystyrene petri dishes, taped shut, and stored and transported to our laboratory at −70°C using dry ice.

Laboratory analyses: Female mosquitoes from each field site were identified and pooled by species in 100 × 15-mm polystyrene petri dishes. During the identification and pooling process, petri dishes were placed in small styrofoam boxes containing dry ice to permit sorting at −70°C while using a dissecting microscope to identify the mosquitoes to species. Virus isolations, and, for EEE and SLE, antigen-capture assays were performed with the species-specific mosquito pools following previously published methods (Tsai et al. 1987, Day and Stark 1996). Mosquito pools for virus isolation consisted of 1–135 mosquitoes. Mosquito pools for the viral antigen-capture assays consisted of 20–68 mosquitoes; one half of the homogenized volume of each pool was used for the EEE antigen-capture assay and other half was used for the SLE antigen-capture assay. Vero cell cultures demonstrating cytopathic effect were confirmed by virus neutralization testing, initially using a panel of mouse hyperimmune ascitic fluids (MHIAFs) containing antibodies against EEE, SLE, California encephalitis virus (CE), western equine encephalitis virus, and Highlands J virus. Mosquito pools testing positive by viral antigen-capture assay were confirmed by antigen-capture inhibition testing using MHIAFs containing antibodies against EEE and SLE (Tsai et al. 1987, Day and Stark 1996). The California group viral isolate was initially identified as being a member of the California (CAL) serogroup by positive neutralization using mouse ascitic fluid containing antibody to CE. Flanders virus was identified by an indirect immunofluorescent antibody (IFA) test using MHIAF to prototype Flanders virus. The Jamestown Canyon virus (JC) variant, Potosi virus, and Tensaw virus (TEN) isolates were placed into antigenic groups initially by IFA using National Institutes of Health immune grouping fluids. Potosi virus and TEN isolates were then identified by 1-way neutralization tests with pretitered MHIAF for the 6 prototype domestic virus members of the Bunyamwera serogroup. These included Cache Valley, Tensaw, Potosi, Main Drain, Lokern, and Northway viruses. After placement of the JC isolate in the CAL serogroup, the isolate was identified in 1-way neutralization assays using pretitered single-injection antibodies for prototype California encephalitis, Keystone, Jamestown Canyon, LaCrosse, San Angelo, and snowshoe hare viruses. A MHIAF for trivittatus virus was also employed in 1-way neutralization tests. Single-injection immune serum to the Jamestown Canyon–like viral isolate was prepared in adult mice and the virus and its antibody were compared in cross-neutralization tests with prototype JC and its homologous single-injection antibody. Both the variant and prototype JCs also were tested against a monoclonal antibody to JC that had neutralizing activity (Artsob et al. 1992).

RESULTS

A total of 1,329 mosquito pools were tested by virus isolation. These pools represented 34 mosquito species or complexes and 85,806 mosquitoes. Viral antigen-capture assays for EEE and SLE were performed on 300 mosquito pools representing 16 mosquito species or complexes and 14,303 mosquitoes (Table 1). Thirty-seven (37) mosquito pools from 14 field sites in 12 counties, representing 11 mosquito species or complexes, tested positive for arboviruses by isolation or antigen-capture assay (Table 2). All 14 field sites were located in the Atlantic Coastal Plain. Four of these 14 field sites were Carolina bays, including Cathedral Bay in Bamberg County, Dingle Pond in Clarendon County, Little Pee Dee Bay in Dillon County, and Woods Bay in Sumter County. All 37 positive mosquito pools were collected during the months of March through September. Eastern equine encephalitis virus (*Togaviridae*: *Alphavirus*) was most frequently identified, with 2 positive virus isolations and 21 positive antigen-capture assays from 12 field sites.

Anopheles crucians complex was most frequently found positive for EEE, with 1 positive isolation and 14 positive antigen-capture assays from 8 field sites, including 3 Carolina bays. Two Carolina bays, Dingle Pond and Woods Bay, yielded EEE-

Table 1. Mosquito species from South Carolina tested for arboviruses, 1996–98.

Mosquito species[1]	No. arbovirus-positive pools/no. pools tested (total number of mosquitoes tested)			
	Virus culture		Viral antigen-capture assay	
Ae. albopictus	0/2	(40)	NT[2]	
Ae. atlanticus/tormentor	1/27	(1,460)	0/4	(202)
Ae. atropalpus	0/1	(5)	NT	
Ae. canadensis	0/88	(5,744)	0/20	(982)
Ae. fulvus pallens	0/6	(71)	NT	
Ae. hendersoni	0/3	(300)	NT	
Ae. infirmatus	0/30	(2,421)	0/3	(147)
Ae. mitchellae	0/2	(44)	NT	
Ae. sollicitans	0/32	(1,803)	0/10	(439)
Ae. sticticus	0/2	(67)	NT	
Ae. taeniorhynchus	0/99	(7,887)	0/25	(1,299)
Ae. thibaulti	0/2	(3)	NT	
Ae. triseriseriatus	0/2	(40)	NT	
Ae. vexans	1/50	(3,377)	0/14	(671)
An. barberi	0/1	(2)	NT	
An. crucians complex	7/364	(25,388)	14/98	(4,636)
An. punctipennis	0/16	(648)	1/3	(150)
An. quadrimaculatus	0/38	(1,799)	1/6	(284)
An. walkeri	0/1	(1)	NT	
Cq. perturbans	1/111	(6,618)	4/26	(1,222)
Cx. erraticus	1/76	(3,757)	0/12	(584)
Cx. nigripalpus	0/15	(721)	NT	
Cx. pipiens/quinquefasciatus	0/4	(222)	0/1	(50)
Cx. restuans	0/12	(332)	NT	
Cx. salinarius	1/230	(18,071)	1/59	(2,789)
Cx. territans	0/2	(47)	NT	
Cs. melanura	1/53	(3,354)	1/15	(678)
Or. signifera	0/1	(1)	NT	
Ps. ciliata	1/16	(117)	NT	
Ps. columbiae	1/18	(814)	0/1	(50)
Ps. cyanescens	0/1	(2)	NT	
Ps. ferox	0/10	(217)	NT	
Ps. howardii	0/3	(23)	NT	
Ur. sapphirina	0/11	(412)	0/3	(120)
Totals	15/1,329	(85,806)	22/300	(14,303)

[1] *Ae., Aedes*; *An., Anopheles*; *Cq., Coquillettidia*; *Cx., Culex*; *Cs., Culiseta*; *Or., Orthopodomyia*; *Ps., Psorophora*; *Ur., Uranotaenia.*
[2] NT, not tested.

infected *An. crucians* complex mosquitoes in both the springtime (March–April) and late summer (August–September). One other pool of *An. crucians* complex was isolation positive for a neutralization variant of JC (*Bunyaviridae*: *Bunyavirus*). In addition to the JC isolate, discussed above, a 2nd CAL serogroup virus was isolated from a pool of *Aedes atlanticus/tormentor.* However, this isolate became nonviable during passage and could not be further identified.

Coquillettidia perturbans (Walker) was collected at 29 field sites and 111 pools (6,618 mosquitoes) were tested by virus isolation and 26 pools (1,022 mosquitoes) were tested by antigen-capture assay. At 1 field site in Lee County, 4 of 4 pools (200 mosquitoes) of *Cq. perturbans* were positive for EEE by antigen-capture assay. *Coquillettidia perturbans* was the 2nd most frequently found EEE-positive mosquito species. Four additional mosquito species were identified as positive for EEE, including *Anopheles punctipennis* (Say), *Anopheles quadrimaculatus* Say, *Culex erraticus* (Dyar and Knab), and *Culiseta melanura* (Coquillett).

Potosi virus (*Bunyaviridae*: *Bunyavirus*) was isolated from a pool of *Cq. perturbans* and from a pool of *Psorophora columbiae* (Dyar and Knab). A Potosi-like virus was also isolated from a pool of *Aedes vexans* (Meigen). All 3 pools were collected alongside the Waccamaw River in Horry County.

Three other viruses were also isolated. Flanders virus, a mosquito-borne member of the *Rhabdoviridae,* was isolated from a pool of *Cs. melanura* collected at Little Pee Dee Bay in Dillon County. Tensaw virus (*Bunyaviridae*: *Bunyavirus*) was isolated from 5 pools of *An. crucians* complex and from 1 pool each of *Culex salinarius* Coquillett and *Pso-*

Table 2. Arbovirus-positive mosquito pools from South Carolina, 1996–98.

Mosquito species[1]	Arbovirus[2]	Lab method	Collection date (month-year)	County; habitat; no. positive pools/total pools (no. mosquitoes)
Ae. atlanticus/tormentor	CAL	Culture	8-96	Sumter; Carolina bay; 1/5 (317)
Ae. vexans	Potosi-like	Culture	9-98	Horry; riverside; 1/9 (500)
An. crucians	EEE	Ag[3]	6-97	Allendale; mixed pine–hardwood wetland; 1/4 (180)
An. crucians	EEE	Ag	4-97	Bamberg; Carolina bay; 1/4 (195)
An. crucians	JC variant	Culture	6-96	Barnwell; man-made ponds, mixed pine–hardwood; 1/4 (200)
An. crucians	EEE	Ag	5-98	Beaufort; coastal subdivision; 1/4 (194)
An. crucians	EEE	Ag	9-96	Clarendon; Carolina bay; 1/1 (40)
An. crucians	EEE	Ag	4-97	Clarendon; Carolina bay; 1/5 (250)
An. crucians	EEE	Ag	4-98	Clarendon; Carolina bay; 2/3 (132)
An. crucians	EEE	Ag	3-98	Colleton; swamp; 2/3 (135)
An. crucians	EEE	Culture	7-6	Dillon; Carolina bay; 1/1 (66)
An. crucians	EEE	Ag	4-98	Horry; riverside; 1/1 (45)
An. crucians	TEN	Culture	9-98	Horry; riverside; 5/42 (2100)
An. crucians	EEE	Ag	8-96	Sumter; Carolina bay; 1/14 (696)
An. crucians	EEE	Ag	3-97	Sumter; Carolina bay; 3/5 (250)
An. punctipennis	EEE	Ag	4-97	Hampton; mixed pine–hardwood wetland; 1/3 (150)
An. quadrimaculatus	EEE	Ag	6-96	Charleston; coastal marsh; 1/3 (149)
Cq. perturbans	Potosi	Culture	9-98	Horry; riverside; 1/1 (50)
Cq. perturbans	EEE	Ag	5-97	Lee; riverside/swamp; 4/4 (200)
Cx. erraticus	EEE	Culture	7-96	Dillon; Carolina bay; 1/3 (195)
Cx. salinarius	SLE	Ag	4-98	Clarendon; Carolina bay; 1/1 (45)
Cx. salinarius	TEN	Culture	9-98	Horry; riverside; 1/10 (500)
Cs. melanura	EEE	Ag	4-98	Charleston; coastal rice field, marsh; 1/2 (149)
Cx. melanura	Flanders	Culture	7-96	Dillon; Carolina bay; 1/1 (58)
Ps. columbiae	Potosi	Culture	9-98	Horry; riverside; 1/2 (78)
Ps. ciliata	TEN	Culture	9-98	Horry; riverside; 1/2 (20)

[1] *Ae., Aedes*; *An., Anopheles*; *Cq., Coquillettidia*; *Cx., Culex*; *Cs., Culiseta*; *Ps., Psorophora.*

[2] CAL, California serogroup virus; EEE, eastern equine encephalitis virus; JC variant, Jamestown Canyon virus variant; TEN, Tensaw virus; SLE, St. Louis encephalitis virus.

[3] Ag, antigen-capture assay.

rophora ciliata (Fabricius). All 7 TEN-positive pools were collected alongside the Waccamaw River in Horry County. One pool of *Cx. salinarius* was positive for SLE (*Flaviviridae*: *Flavivirus*) by antigen-capture assay. The SLE-positive pool was collected at a Carolina bay, Dingle Pond in Clarendon County.

DISCUSSION

In 1954, Chamberlain et al. reported laboratory studies indicating that *Anopheles crucians* Wiedemann was not an efficient vector for EEE. Shortly afterwards, Kissling et al. (1955) isolated EEE from *An. crucians* collected in Georgia. Because vector competence for virus transmission by mosquitoes and viral infection in mosquitoes are not synonymous, *An. crucians* received little further consideration as a vector for EEE until recently when Day and Stark (1996) studied arboviral infections in emus at a Florida ranch and isolated EEE from *An. crucians, Cx. erraticus,* and *Culex nigripalpus* Theobald. They suggested that each species might be an epidemic vector species in Florida. Our results include isolating EEE from mosquitoes for the 1st time in South Carolina. Our identification of EEE-infected *An. crucians* complex mosquitoes from 8 South Carolina counties also suggests that *An. crucians* complex may be a significant vector for EEE in South Carolina. Three anopheline species comprise the *An. crucians* complex: *An. crucians, Anopheles georgianus* King, and *Anopheles bradleyi* King. The adult stages of each are indistinguishable, and their natural ranges overlap in South Carolina (Darsie and Ward 1981). *Anopheles crucians* complex has been reported from every South Carolina county except York (Weathersbee and Arnold 1947). The preferred breeding sites of mosquitoes of this complex are in acidic waters, such as those found in the Carolina bays. Elliptical in shape and aligned along a northwest–southeast axis, Carolina bays are shallow and poorly drained inland basins found in the Atlantic Coastal Plain, particularly in Virginia and the Carolinas, as single bays or in clusters (Shimer 1972). We found EEE-infected *An. crucians* complex mosquitoes at 4 of

the 8 Carolina bays included in our study, suggesting that Carolina bays may be enzootic foci for EEE in South Carolina. Additionally, the flight range for *An. crucians* may exceed 1 mi (1.6 km), these mosquitoes will feed on birds, and these mosqutioes will enter shelters and feed on humans (Horsfall 1955). Sabrosky (1946) has also reported that 47.3% of the *An. crucians* complex mosquitoes in 1 South Carolina study had fed on equine blood. These characteristics and descriptions provide additional support for considering the members of the *An. crucians* complex as possible vectors for EEE, and perhaps other arboviruses, such as JC and TEN, in South Carolina and in the southeastern United States. Our findings strongly suggest that *An. crucians* complex mosquitoes in the southeastern United States should be reexamined for their competency to serve as arbovirus vectors.

Indeed, our findings include isolating a variant JC from an *An. crucians* complex pool for the 1st time in South Carolina. The JC isolate was identified as a neutralization variant. It was neutralized significantly less effectively by homologous and heterologous prototype JC antibodies, although it was neutralized by a JC monoclonal antibody at least as well as the prototype virus was.

Two other EEE-infected anopheline species were identified in our study, *An. punctipennis* and *An. quadrimaculatus.* Considering that both species will feed on humans, and the latter's past role as a malaria vector in the southeastern United States (Horsfall 1955), neither species can be ignored as arbovirus vectors for human disease.

The possible role of *Cx. erraticus* as a vector for EEE in Florida has been mentioned above (Day and Stark 1996). We also isolated EEE from *Cx. erraticus* collected at Little Pee Dee Bay, a Carolina bay, in Dillon County. Additionally, we identified 4 EEE antigen-positive pools of *Cq. perturbans* that were collected alongside Lynches River in Lee County. The roles of *Cq. perturbans* and *Cx. erraticus* as enzootic or epidemic vectors of EEE in South Carolina are unclear and require further study.

Culiseta melanura is frequently cited as a principle enzootic vector of EEE in the eastern United States, especially north of the Carolinas (Scott and Weaver 1989). We collected *Cs. melanura* at 16 field sites and tested 53 pools (3,354 mosquitoes) by virus isolation and 15 pools (678 mosquitoes) by antigen-capture assay, but identified only 1 EEE-positive *Cs. melanura* pool by antigen-capture assay. The role of *Cs. melanura* as a vector of EEE in South Carolina, and perhaps southwards as well, seems to be diminished when compared to that of the *An. crucians* complex. The EEE-positive *Cs. melanura* pool was collected in an abandoned rice field on the coast in Charleston County. Flanders virus, a nonhuman pathogen and member of the *Rhabdoviridae,* was also isolated from a pool of *Cs. melanura* for the 1st time in South Carolina. This pool was collected at Little Pee Dee Bay in Dillon County.

Tensaw virus was 1st described by Sudia et al. in 1968. Chamberlain et al. (1969) collected mosquitoes in Georgia, Alabama, and Florida, and reported isolating TEN primarily from pools of *An. crucians* and *Ps. columbiae,* and, to a much lesser extent, from pools of *An. quadrimaculatus, Aedes atlanticus* Dyar and Knab, *Aedes mitchellae* (Dyar), *Cx. nigripalpus,* and *Cq. perturbans.* Since then, TEN has also been isolated from mosquitoes collected in Mississippi, Louisiana, and east Texas, and *Aedes taeniorhynchus* (Wiedemann), *Cx. salinarius,* and *Cq. perturbans* have also yielded TEN isolates (Calisher et al. 1986). We demonstrated the presence of TEN in mosquitoes for the 1st time in South Carolina by isolating the virus from 5 pools of *An. crucians* complex and from 1 pool each of *Cx. salinarius* and *Ps. ciliata.* All 7 pools were collected at 1 site, alongside the Waccamaw River in Horry County. Antibodies to TEN have been detected in rodents, gray foxes, raccoons, dogs, cattle, and humans, but not in wild birds or sentinel chickens (Calisher et al. 1986). Calisher and Sever (1995) discussed the possible role of TEN as an etiologic agent of human congenital defects of the central nervous system, and additional virology and epidemiology studies seem to be indicated.

Mitchell et al. (1996) reported isolating Potosi virus from a pool of *Ps. columbiae* collected in Charleston, SC, in 1991. We also isolated Potosi virus from a pool of *Ps. columbiae,* as well as from 1 pool of *Cq. perturbans.* A Potosi-like virus was also isolated from a pool of *Ae. vexans.* All 3 Potosi virus–positive pools were collected from the same site where the TEN-positive pools were collected, alongside the Waccamaw River in Horry County. Potosi virus and TEN are distinct species but are serologically related (McLean et al. 1996). The role of Potosi virus as a human pathogen is not yet clear.

Using an antigen-capture assay, we identified SLE virus for the 1st time in South Carolina in a pool of *Cx. salinarius* collected at Dingle Pond, a Carolina bay, in Clarendon County. The SLE-positive antigen-capture assay was confirmed by an SLE antigen-capture inhibition assay. However, since then, West Nile (WN) virus has been found in the New York City metropolitan area (CDC 1999). West Nile viral antigens and antibodies may cross-react with some SLE enzyme immunoassays, yielding false-positive SLE test results. We were unable to perform retrospective testing for WN on the SLE-positive *Cx. salinarius* pool, because this pool had been consumed in the SLE testing.

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