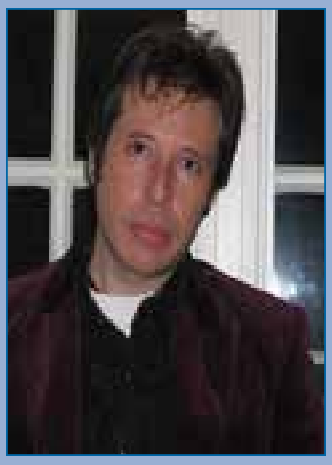

Carlos Machain Williams

Dr. Carlos Machain Williams obtained his degree in Veterinary Medicine in 2000 from the School of Veterinary Medicine at the Universidad Autonoma de Yucatan, Mexico. He then collaborated as a visitor researcher at the Swedish University of Agricultural Scineces in several projects related to animal health. In 2002 Dr. Machain-Williams started his Ph.D under the supervision of Dr. Carol D. Blair and Barry J. Beaty at Colorado State University USA, in the laboratory of artrophod-borne and infectious diseases laboratory. The main area of research was the pathogenesis of flaviviruses related to mosquito saliva. Currently Dr. Machain Williams is working in the Universidad Autonoma de Yucatan at the arbovirology laboratory at the Hideyo Noguchi Research Center. His main interests are vector-borne emerging viruses, zoonosis and urban cycles of arboviruses. In addition some new research is dedicated to the study of enteroviruses. Some of his time is spent in tropical diseases colaborations and lecturing at the School of Medicine in the Universidad de Yucatan. Universidad Autonoma de Yucatan at the arbovirology laboratory at the Hideyo Noguchi research center.

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ISID Grant Report

Isolation and characterization of arthropod-borne viruses from rodents in Merida city, Mexico

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Introduction

Arboviruses are a group of viruses transmitted between susceptible hosts by the bite of blood-sucking arthropods. It is composed of seven families hosting virusesof medical and veterinary importance, (ICTV 2011). These viruses cause mildclinical signs including fever, but in some cases severe complications such asencephalitis and hemorrhagic manifestations occur, therefore; becoming fatal.Currently, arboviruses represent 23% of emerging infectious diseases. In turn, 75% of emerging diseases are of zoonotic nature (PAHO 2013). It has been demonstrated experimentally the infection caused by arboviruses in rodents (Davisand Hardy 1974, Adams et al. 2013), however, there are no studies on naturallyinfection of flaviviruses in urban rodents in Mexico. The aim of this study was to detect the circulation of arbovirus, restricted to the family *Flaviviridae, Togaviridae* and *Bunyaviridae* in domiciliary rodents captured in the city of Merida, Yucatan, Mexico.

Materials and Methods

Rodent Trapping

Rodents were captured in the city of Mérida, Yucatán, México, using Sherman traps inside home areas (bedrooms and kitchens). Sampling was carried out for three consecutive nights in each house. Rodents were identified with keys of Emmons (1990) and Reid (2009). Captured rodents were asleep by saturation of CO2 according to the recommendations of the AAMV (2007). From all rodents, blood samples, liver, spleen, kidney, lung, heart, urine, feces and skin were taken. All samples were stored at -70°C, and analyzed for identification of arboviruses.

RNA extraction, tissue processing and PCR

Total RNA extraction was performed from collected tissue using the Trizol (Invitrogen) technique. RNA was stored at -70°C before performing reverse transcription. Reverse transcription was done using Promega® Improm II Kit following the manufacturer's instructions with hexamer primers. For detection of arbovirus by PCR, the following primers were used: family *Flaviviridae*, CFD2, MAMD and FS778 (Scaramozzino et al, 2001); Family *Bunyaviridae* BCS332V and BCS82 (Kuno et al, 1996) and Family *Togaviridae* VIR966 and VIR966c (Eshoo et al, 2007).

Serology analysis

After blood collection, sera was acquired for serology testing: Blocking ELISA was used for the detection of anti-WNV antibodies. For this test, WNV specific monoclonal antibody (MAb) 3.1112G® (Millipore Bioscience Research Reagents, Temecula, California USA) was used following the protocol previously reported by Blitvich et al, (2003).

Plaque reduction neutralization test (PRNT)

Aliquoted sera samples were sent to Iowa State University (BSL 3) for PRNT analysis. All sera were assayed by PRNT, using viral prototypes WNV (strain NY99-35261-11), DENV-4 (strain 241), MODV (strain M544). Sera is still under testing for Sal Vieja virus and other

continued on next page

related flaviviruses.

Results and Discussion

In total, 195 rodents were captured inside houses in the city. The most abundant species was *Mus musculus* (58.46%), followed by *Rattus. rattus* (41.53%). The greater abundance of rodents was observed in the months of April, May and September (Table 1). The ratio male:female was similar in *R. rattus*, while in *M.musculus*, males percentage was higher than females (Table 2).

Table 1. Abundance of rodent species captured per month

Species	Jan	Feb	Mar	Apr	My	Jun	Jul	Aug	Spt	Oct	Nov	Dec	Total per specie
Mus musculus	5	3	9	21	9	3	8	7	12	17	12	8	114
Ratus rattus	15	7	3	13	4	2	6	5	6	10	9	1	81
Total/mo.	20	10	12	34	13	5	14	12	18	27	21	9	
Total													**195**

All blood and liver samples were determined as negative for the family *Bunyaviridae* and *Togaviridae* by PCR. By PCR, *Flaviviridae* family was detected in five liver samples, of these, three were from *R. rattus* and two of *M. musculus*, all of them were adults. These samples were directly purified and sent to sequencing for viral identification. The rodent´s sera tested positive for antibodies for different arboviruses. We observed a higher seroprevalence in *R. rattus* that in *M.musculus* (table 2).

Table 2. Rodents' sera analyzed by epitope-blocking ELISA and PRNT for Flavivirus detection

Especie	Blocking ELISA	PRNT			
	WNV	WNV	DENV-4	MODV	APOIV
R. rattus	n=3 (3.5%)	n=23 (12.1%)	n=2 (1.1%)	n=34 (17.9%)	n=3 (1.6%)
M. musculus	n=2 (2.4%)	n=1 (0.5%)	n=3 (1.6%)	n=1 (0.5%)	n=4 (2.1%)

WNV= West Nile Virus, **DENV**=Dengue virus 4, **MODV**=Modock virus, and; **APOIV**= Apoi virus

Discussion and Conclusion

Our results confirmed the circulation and infection of *M. musculus* and *R. rattus* with arboviruses most of the year, particularly of the *Flaviviridae* family; specifically the identification of WNV, DENV, MODV and APOIV. Previous studies in naturally infected rodents, mentioned that voles develop a persistent infection of tick-borne encephalitis (TBE) without clinical signs (Achazi et al. 2011). In our study, all animals observed were healthy and for the first time it was found that domiciliary *M.musculus* and *R. rattus* participate in the flavivirus circulation in urban settings. This extended the range of flavivirus presence in the Yucatan Peninsula. Our study provides a first evidence of activity of domiciliary rodents in the urban cycle of arboviruses. It is likely that these rodents will perform an increasingly important role in the ecology of zoonotic diseases. This is the basis for subsequent studies and will help to clarify the role it perform in the maintenance and circulation of arboviruses as well as its

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implication in public health arena. It is important to determine the mechanism of infection and determine the potential vectors that could serve as bridge between the domiciliary rodents and humans.

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