

**MOSTICAW WORKSHOP**

5 al 7 de Octubre 2016

# **Ecological and epidemiological aspects of flavivirus and its vectors in Paraguay**

**Bioq., Fatima Cardozo, MSc.**

**Research professor**

**Salud Publica Department**

**Instituto de Investigaciones en Ciencias de la Salud**

**Universidad Nacional de Asunción**

**Paraguay**

# Ecological and epidemiological aspects of flavivirus and its vectors in Paraguay

Cardozo F.<sup>1,2</sup>, Mendoza L.<sup>1,2</sup>, Rojas A.<sup>2</sup>, Páez M.<sup>2</sup>, Guillén Y.<sup>2</sup>, Velilla M.<sup>3</sup>, Lesterhuis A.<sup>3</sup>, Yanosky A.<sup>3</sup>, Martínez N.<sup>4</sup>, Torales M.<sup>4</sup>, Aguayo N.<sup>4</sup>, Ferreira L.<sup>4</sup>, Vallejos M.A.<sup>5</sup>, Herebia L.<sup>5</sup>, Contigiani M.S.<sup>6</sup>, Diaz L.A.<sup>6</sup>, Franco L.<sup>7</sup>

<sup>1</sup> Investigador Principal, <sup>2</sup> Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción (IICS, UNA), <sup>3</sup> Guyra Paraguay, <sup>4</sup> Servicio Nacional de Erradicación del Paludismo (SENEPA), MSP-BS, Paraguay, <sup>5</sup> Instituto de Previsión Social, <sup>6</sup> Universidad Nacional de Córdoba, Argentina, <sup>7</sup> Instituto Carlos III, España.

## Executor:



## Associated Institutions:



Universidad  
Nacional  
de Córdoba



## Funded by:



PROGRAMA PARAGUAYO PARA EL DESARROLLO DE LA CIENCIA Y TECNOLOGÍA

## ARBOVIRUS

- English anagram *arthropod borne virus*.
- 30% of all emerging infectious diseases in the past decade
- They are included in different taxonomic families
- Between them: *Flaviviridae*, *Bunyaviridae*, *Togaviridae*, *Rhabdoviridae*, *Orthomyxoviridae* y *Reoviridae*.

(Jones y col., 2008; Hollidge y col., 2010)

## ARBOVIRUS

- English anagram *arthropod borne virus*.
- 30% of all emerging infectious diseases in the past decade
- They are included in different taxonomic families
- Between them: *Flaviviridae*, *Bunyaviridae*, *Togaviridae*, *Rhabdoviridae*, *Orthomyxoviridae* y *Reoviridae*.

(Jones y col., 2008; Hollidge y col., 2010)

# INTRODUCTION

## *Flaviviridae* family

80 different viruses

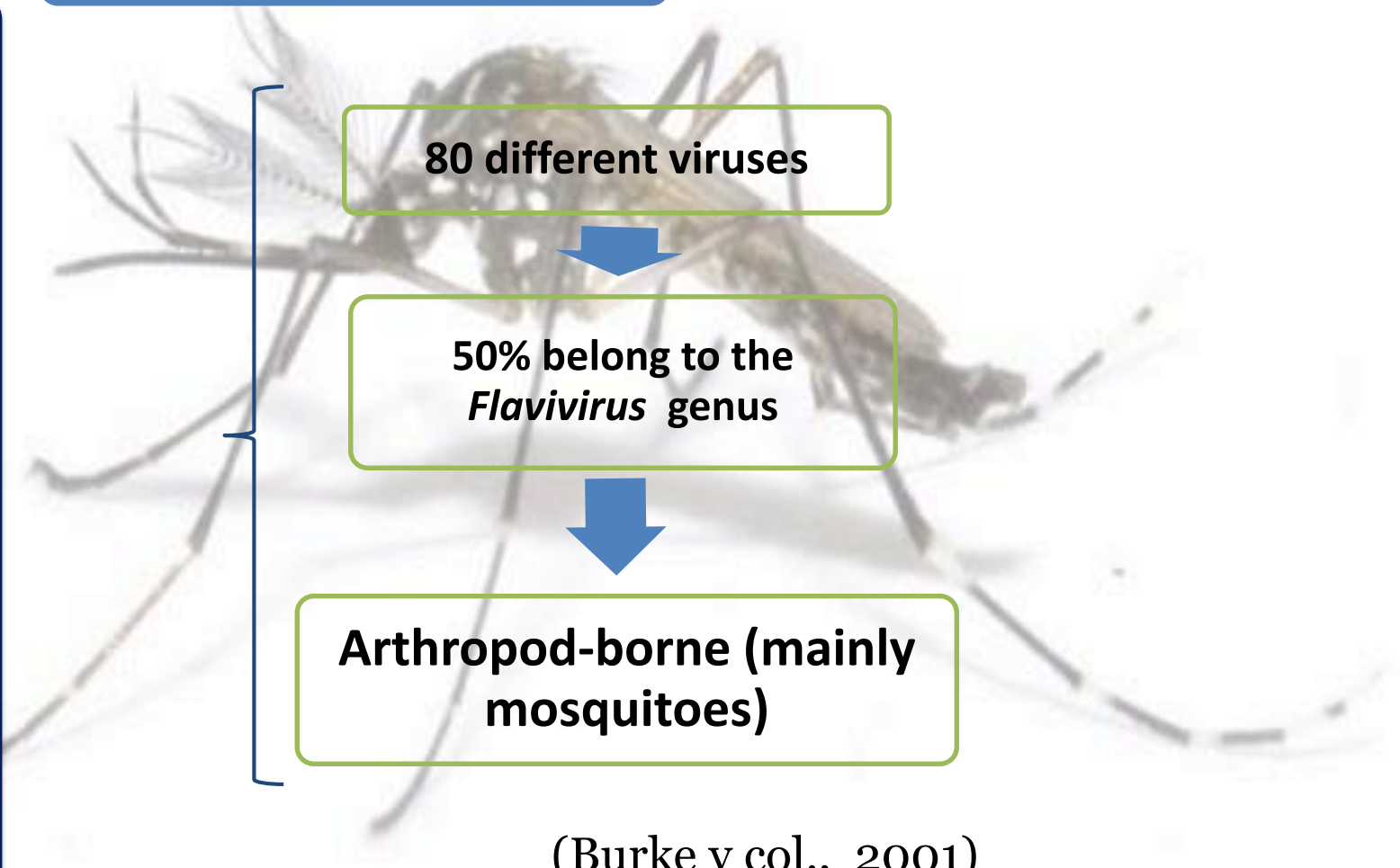


50% belong to the  
*Flavivirus* genus



Arthropod-borne (mainly  
mosquitoes)

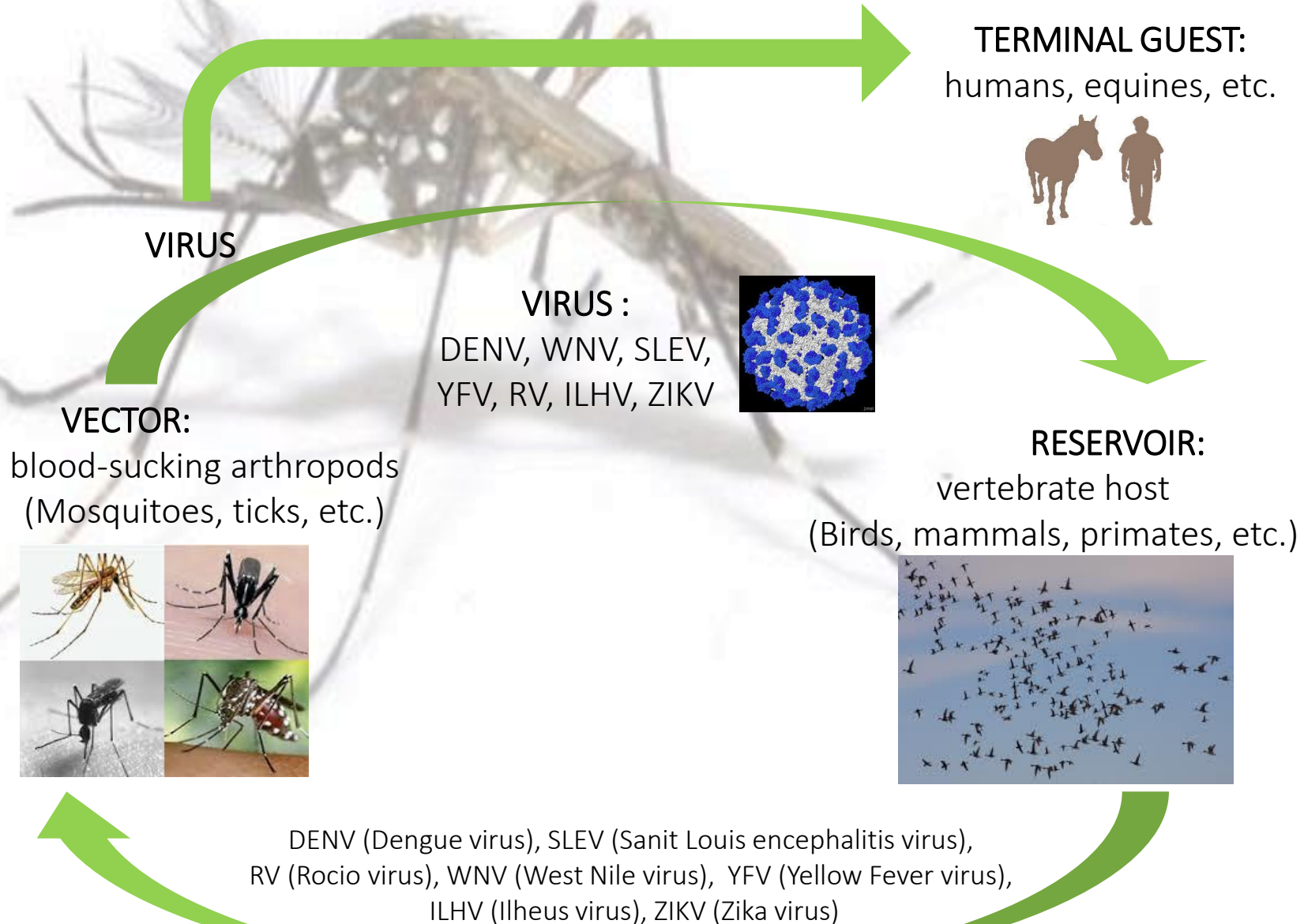
(Burke y col., 2001)





# INTRODUCTION

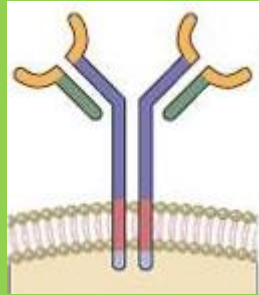
## Transmission cycle



# INTRODUCTION

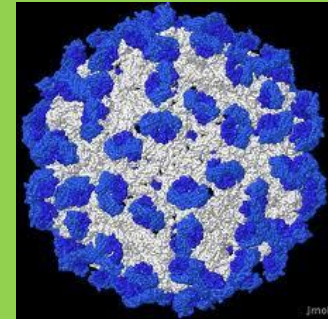
## Diagnosis

### INDIRECT TECHNIQUES



- A week after showing symptoms
- Eg.: ELISA, IFI, IH, NT, etc.

### DIRECT TECHNIQUES



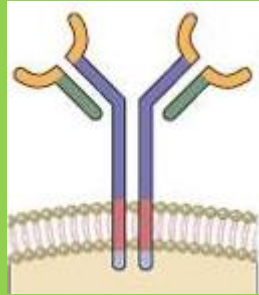
- A few days of evolution.
- Eg.: *RT-nested PCR*, real time PCR, etc.

(Morales y col., 2008)

# INTRODUCTION

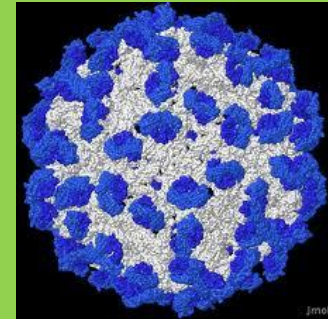
## Diagnosis

### INDIRECT TECHNIQUES



- A week after present the symptoms
- Eg.: ELISA, IFI, IH, NT, etc.

### DIRECT TECHNIQUES



- A few days of evolution.
- Eg.: *RT-nested PCR*, real time PCR, etc.

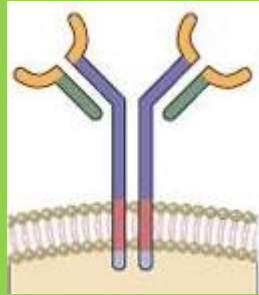
(Morales y col., 2008)



# INTRODUCTION

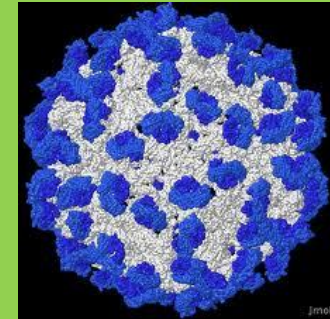
## Diagnosis

### INDIRECT TECHNIQUES



- A week after present the symptoms
- Eg.: ELISA, IFI, IH, NT, etc.

### DIRECT TECHNIQUES



- A few days of evolution.
- Eg.: *RT-nested PCR*, real time PCR, etc.

(Morales y col., 2008)

# INTRODUCTION

## Flavivirus known in Paraguay:



(DGVS MSPyBS)

# INTRODUCTION

## Flavivirus known in Paraguay:



(DGVS MSPyBS)

**There are still no reports of other flavivirus circulation in our country.**

# INTRODUCTION

## Flavivirus of importance in the region:

Virus	Natural Host	Signs/ Symptoms	Distribution
Yellow fever virus (YFV)	Mosquitoes (urban areas: <i>Aedes aegypti</i> , jungle areas: <i>Haemagogus</i> and <i>Sabethes</i> mosquitoes).	Viral hemorrhagic fever, jaundice due to liver damage	Three countries had reported jungle yellow fever: Brazil, Colombia, and Peru The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela.
Dengue fever virus (DENV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Viral hemorrhagic fever	Public health problem in the Americas
West Nile virus (WNV)	Mosquitoes ( <i>Culex</i> , in particular <i>Cx. pipiens</i> ).	Fever, headache, chills, fatigue, joint pain, excessive sweating, swollen lymph nodes.	Africa, Europe, the Middle East, North America, and West Asia.
Zika virus (ZIKV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Not very high fever, rash, conjunctivitis, muscle and joint pain, discomfort or headaches. There is a scientific consensus on the causal relationship between Zika virus and microcephaly and Guillain-Barre syndrome. Also they are investigating the relationships with other neurological complications.	47 countries and territories in the Americas have confirmed autochthonous
Saint Louis Encephalitis virus (SLEV)	Mosquitoes ( <i>Culex tarsalis</i> , <i>C quinquefasciatus</i> , <i>C pipiens</i> )	fever, headaches, nausea until signs of infection in the central nervous system, coma and death.	widely distributed from Canada to Argentina.. Sporadic cases have occurred in South America and the Caribbean.

# INTRODUCTION

## Flavivirus of importance in the region:

Virus	Natural Host	Signs/ Symptoms	Distribution
Yellow fever virus (YFV)	Mosquitoes (urban areas: <i>Aedes aegypti</i> , jungle areas: <i>Haemagogus</i> and <i>Sabethes</i> mosquitoes).	Viral hemorrhagic fever, jaundice due to liver damage	Three countries had reported jungle yellow fever: Brazil, Colombia, and Peru The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela.
Dengue fever virus (DENV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Viral hemorrhagic fever	Public health problem in the Americas
West Nile virus (WNV)	Mosquitoes ( <i>Culex</i> , in particular <i>Cx. pipiens</i> ).	Fever, headache, chills, fatigue, joint pain, excessive sweating, swollen lymph nodes.	Africa, Europe, the Middle East, North America, and West Asia.
Zika virus (ZIKV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Not very high fever, rash, conjunctivitis, muscle and joint pain, discomfort or headaches. There is a scientific consensus on the causal relationship between Zika virus and microcephaly and Guillain-Barre syndrome. Also they are investigating the relationships with other neurological complications.	47 countries and territories in the Americas have confirmed autochthonous
Saint Louis Encephalitis virus (SLEV)	Mosquitoes ( <i>Culex tarsalis</i> , <i>C quinquefasciatus</i> , <i>C pipiens</i> )	fever, headaches, nausea until signs of infection in the central nervous system, coma and death.	widely distributed from Canada to Argentina.. Sporadic cases have occurred in South America and the Caribbean.



# INTRODUCTION

## Flavivirus of importance in the region:

Virus	Natural Host	Signs/ Symptoms	Distribution
Yellow fever virus (YFV)	Mosquitoes (urban areas: <i>Aedes aegypti</i> , jungle areas: <i>Haemagogus</i> and <i>Sabethes</i> mosquitoes).	Viral hemorrhagic fever, jaundice due to liver damage	Three countries had reported jungle yellow fever: Brazil, Colombia, and Peru The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela.
<b>Dengue fever virus (DENV)</b>	<b>Mosquitoes (<i>Aedes</i> mosquitoes)</b>	<b>Viral hemorrhagic fever</b>	<b>Public health problem in the Americas</b>
West Nile virus (WNV)	Mosquitoes ( <i>Culex</i> , in particular <i>Cx. pipiens</i> ).	Fever, headache, chills, fatigue, joint pain, excessive sweating, swollen lymph nodes.	Africa, Europe, the Middle East, North America, and West Asia.
Zika virus (ZIKV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Not very high fever, rash, conjunctivitis, muscle and joint pain, discomfort or headaches. There is a scientific consensus on the causal relationship between Zika virus and microcephaly and Guillain-Barre syndrome. Also they are investigating the relationships with other neurological complications.	47 countries and territories in the Americas have confirmed autochthonous
Saint Louis Encephalitis virus (SLEV)	Mosquitoes ( <i>Culex tarsalis</i> , <i>C quinquefasciatus</i> , <i>C pipiens</i> )	fever, headaches, nausea until signs of infection in the central nervous system, coma and death.	widely distributed from Canada to Argentina.. Sporadic cases have occurred in South America and the Caribbean.

# INTRODUCTION

## Flavivirus of importance in the region:

Virus	Natural Host	Signs/ Symptoms	Distribution
Yellow fever virus (YFV)	Mosquitoes (urban areas: <i>Aedes aegypti</i> , jungle areas: <i>Haemagogus</i> and <i>Sabethes</i> mosquitoes).	Viral hemorrhagic fever, jaundice due to liver damage	Three countries had reported jungle yellow fever: Brazil, Colombia, and Peru The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela.
Dengue fever virus (DENV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Viral hemorrhagic fever	Public health problem in the Americas
<b>West Nile virus (WNV)</b>	<b>Mosquitoes (<i>Culex</i>, in particular <i>Cx. pipiens</i>).</b>	<b>Fever, headache, chills, fatigue, joint pain, excessive sweating, swollen lymph nodes.</b>	<b>Africa, Europe, the Middle East, North America, and West Asia.</b>
Zika virus (ZIKV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Not very high fever, rash, conjunctivitis, muscle and joint pain, discomfort or headaches. There is a scientific consensus on the causal relationship between Zika virus and microcephaly and Guillain-Barre syndrome. Also they are investigating the relationships with other neurological complications.	47 countries and territories in the Americas have confirmed autochthonous
Saint Louis Encephalitis virus (SLEV)	Mosquitoes ( <i>Culex tarsalis</i> , <i>C quinquefasciatus</i> , <i>C pipiens</i> )	fever, headaches, nausea until signs of infection in the central nervous system, coma and death.	widely distributed from Canada to Argentina.. Sporadic cases have occurred in South America and the Caribbean.

# INTRODUCTION

## Flavivirus of importance in the region:

Virus	Natural Host	Signs/ Symptoms	Distribution
Yellow fever virus (YFV)	Mosquitoes (urban areas: <i>Aedes aegypti</i> , jungle areas: <i>Haemagogus</i> and <i>Sabethes</i> mosquitoes).	Viral hemorrhagic fever, jaundice due to liver damage	Three countries had reported jungle yellow fever: Brazil, Colombia, and Peru The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela.
Dengue fever virus (DENV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Viral hemorrhagic fever	Public health problem in the Americas
West Nile virus (WNV)	Mosquitoes ( <i>Culex</i> , in particular <i>Cx. pipiens</i> ).	Fever, headache, chills, fatigue, joint pain, excessive sweating, swollen lymph nodes.	Africa, Europe, the Middle East, North America, and West Asia.
<b>Zika virus (ZIKV)</b>	<b>Mosquitoes (<i>Aedes</i> mosquitoes)</b>	<b>Not very high fever, rash, conjunctivitis, muscle and joint pain, discomfort or headaches. There is a scientific consensus on the causal relationship between Zika virus and microcephaly and Guillain-Barre syndrome. Also they are investigating the relationships with other neurological complications.</b>	<b>47 countries and territories in the Americas have confirmed autochthonous circulation</b>
Saint Louis Encephalitis virus (SLEV)	Mosquitoes ( <i>Culex tarsalis</i> , <i>C quinquefasciatus</i> , <i>C pipiens</i> )	fever, headaches, nausea until signs of infection in the central nervous system, coma and death.	widely distributed from Canada to Argentina.. Sporadic cases have occurred in South America and the Caribbean.

# INTRODUCTION

## Flavivirus of importance in the region:

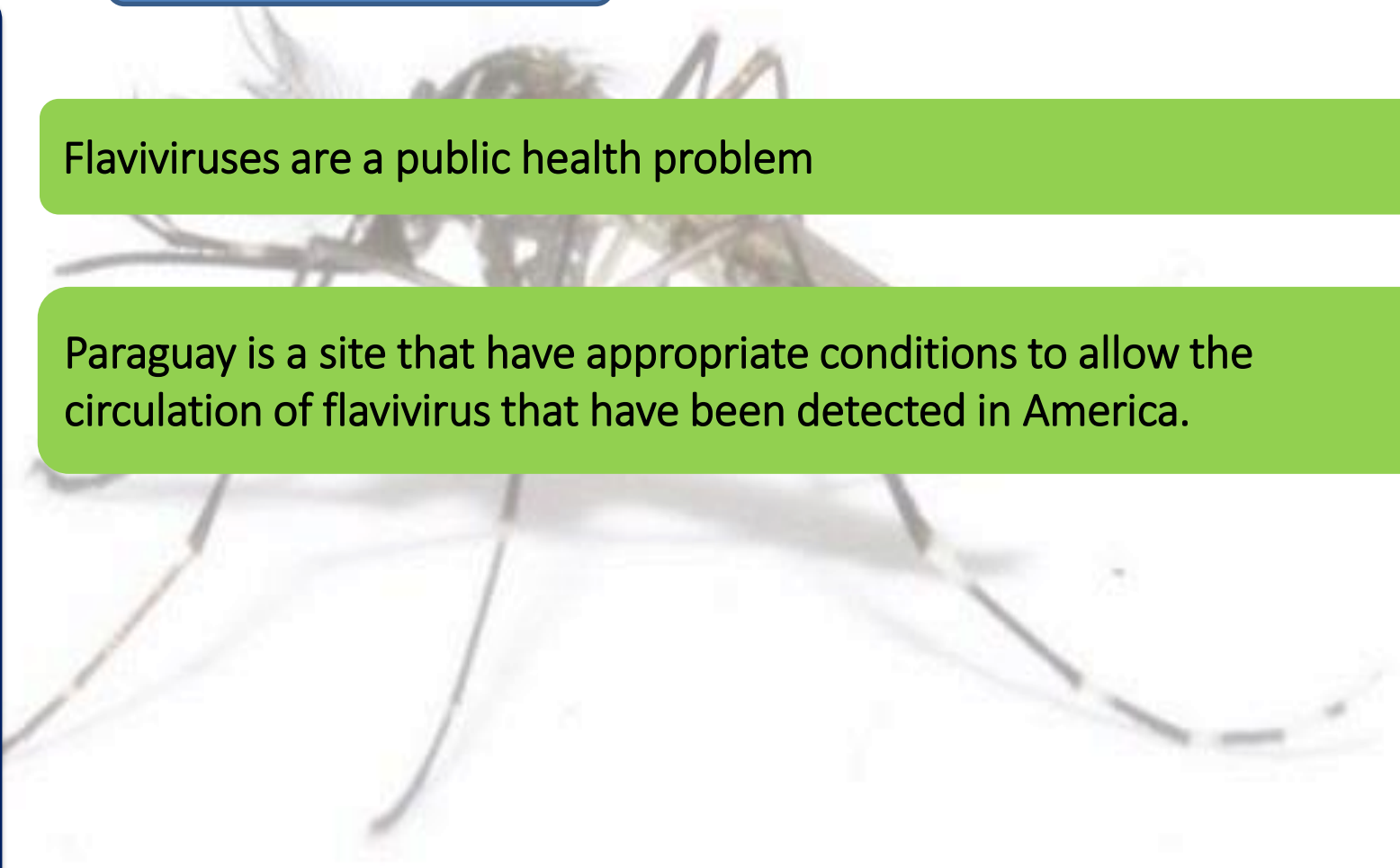
Virus	Natural Host	Signs/ Symptoms	Distribution
Yellow fever virus (YFV)	Mosquitoes (urban areas: <i>Aedes aegypti</i> , jungle areas: <i>Haemagogus</i> and <i>Sabethes</i> mosquitoes).	Viral hemorrhagic fever, jaundice due to liver damage	Three countries had reported jungle yellow fever: Brazil, Colombia, and Peru The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela.
Dengue fever virus (DENV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Viral hemorrhagic fever	Public health problem in the Americas
West Nile virus (WNV)	Mosquitoes ( <i>Culex</i> , in particular <i>Cx. pipiens</i> ).	Fever, headache, chills, fatigue, joint pain, excessive sweating, swollen lymph nodes.	Africa, Europe, the Middle East, North America, and West Asia.
Zika virus (ZIKV)	Mosquitoes ( <i>Aedes</i> mosquitoes)	Not very high fever, rash, conjunctivitis, muscle and joint pain, discomfort or headaches. There is a scientific consensus on the causal relationship between Zika virus and microcephaly and Guillain-Barre syndrome. Also they are investigating the relationships with other neurological complications.	47 countries and territories in the Americas have confirmed autochthonous
<b>Saint Louis Encephalitis virus (SLEV)</b>	<b>Mosquitoes (<i>Culex tarsalis</i>, <i>C. quinquefasciatus</i>, <i>C. pipiens</i>)</b>	<b>fever, headaches, nausea until signs of infection in the central nervous system, coma and death.</b>	<b>Widely distributed from Canada to Argentina.. Sporadic cases have occurred in South America and the Caribbean.</b>

# INTRODUCTION

## JUSTIFICATION

Flaviviruses are a public health problem

Paraguay is a site that have appropriate conditions to allow the circulation of flavivirus that have been detected in America.





## JUSTIFICATION

Flaviviruses are a public health problem

Paraguay is a site that have appropriate conditions to allow the circulation of flavivirus that have been detected in America.



### VECTORS

**90 species of mosquitoes**  
(Dpto. Entomología, SENEPA)



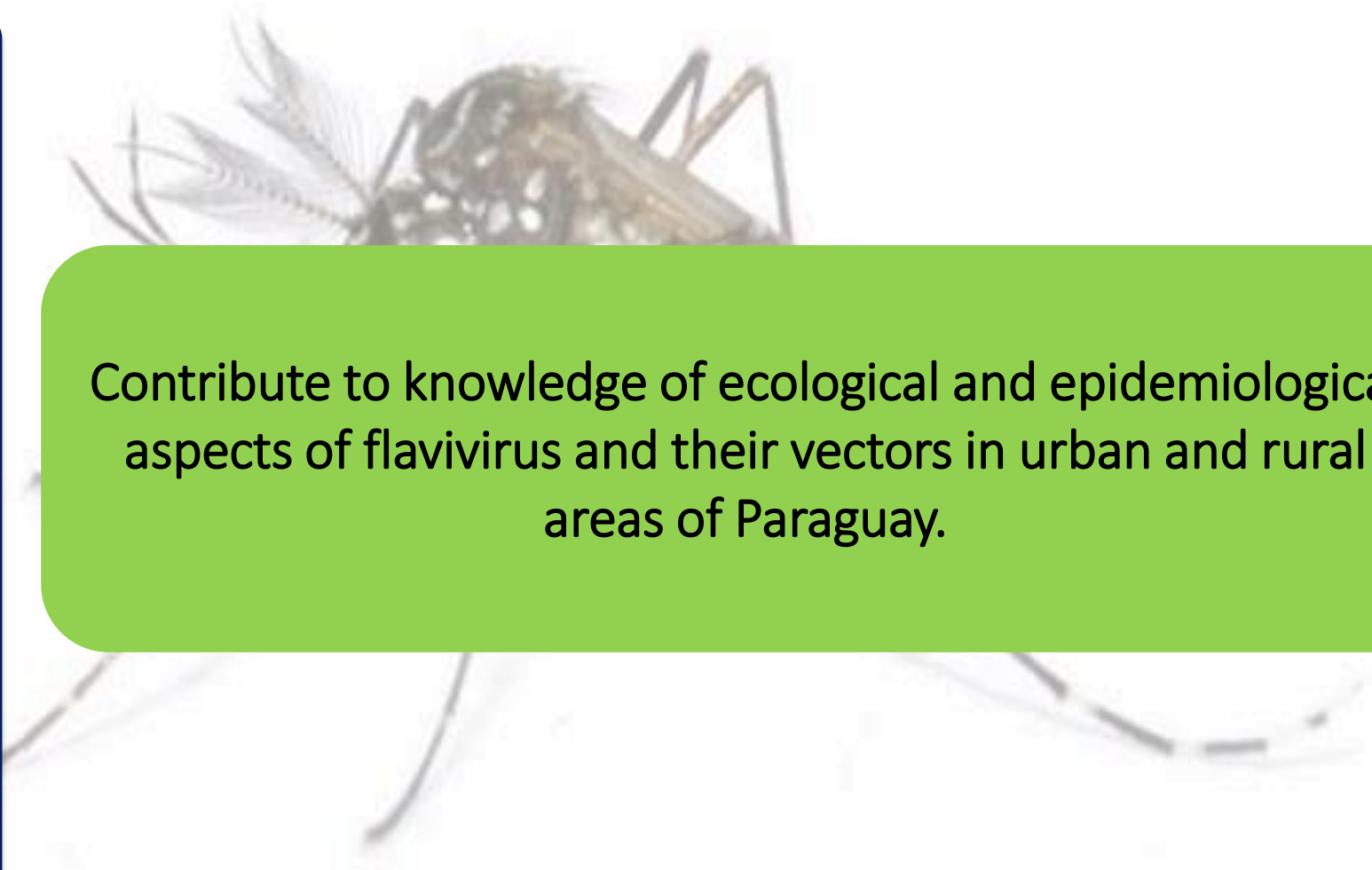
### RESERVOIR

**220 species of resident and migratory birds**

(Castillo y Clay, 2006)

## OBJECTIVE OF THE PROJECT

Contribute to knowledge of ecological and epidemiological aspects of flavivirus and their vectors in urban and rural areas of Paraguay.



# METHODOLOGY

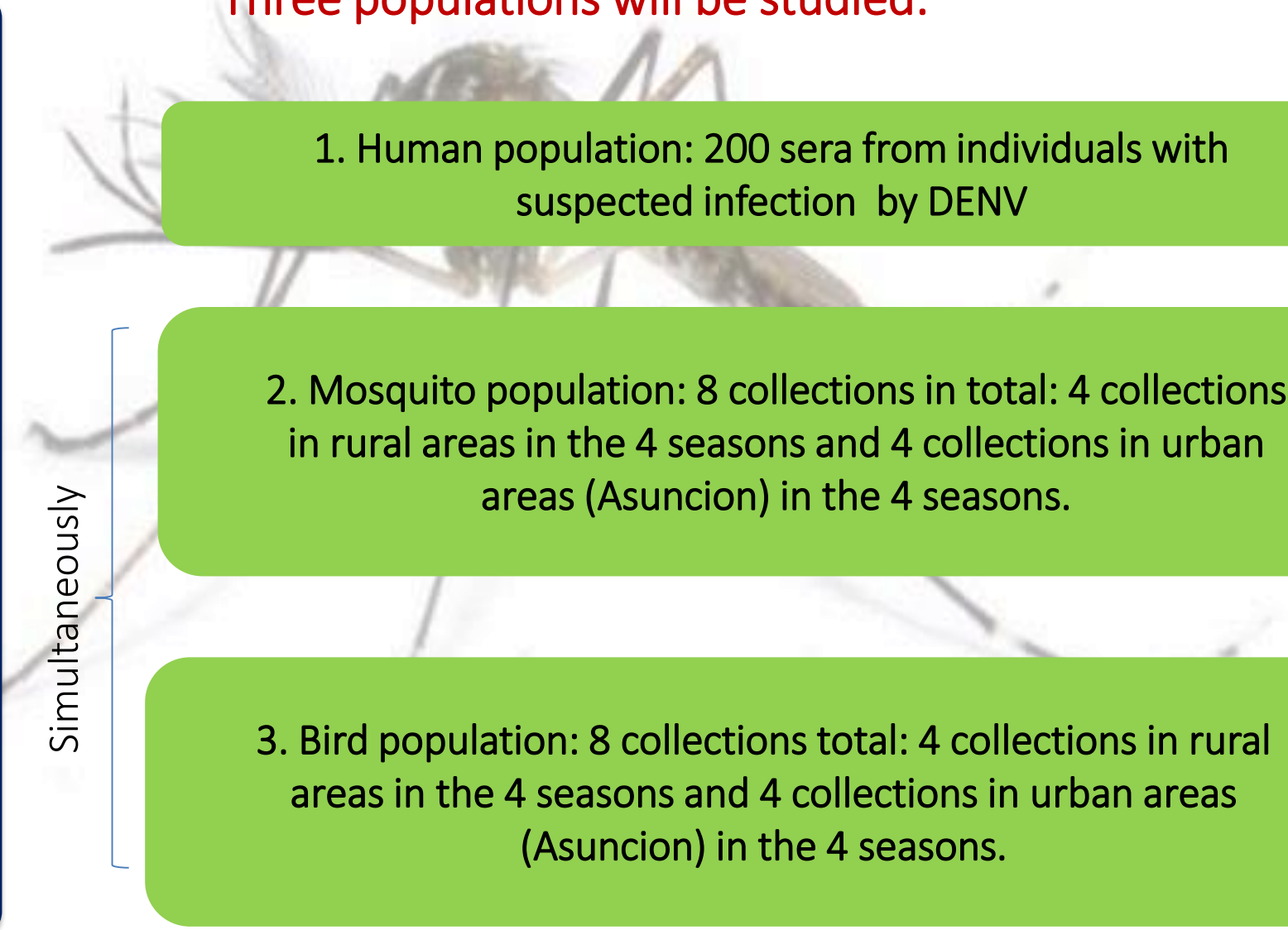
## Three populations will be studied:

1. Human population: 200 sera from individuals with suspected infection by DENV

2. Mosquito population: 8 collections in total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

3. Bird population: 8 collections total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

Simultaneously



# METHODOLOGY

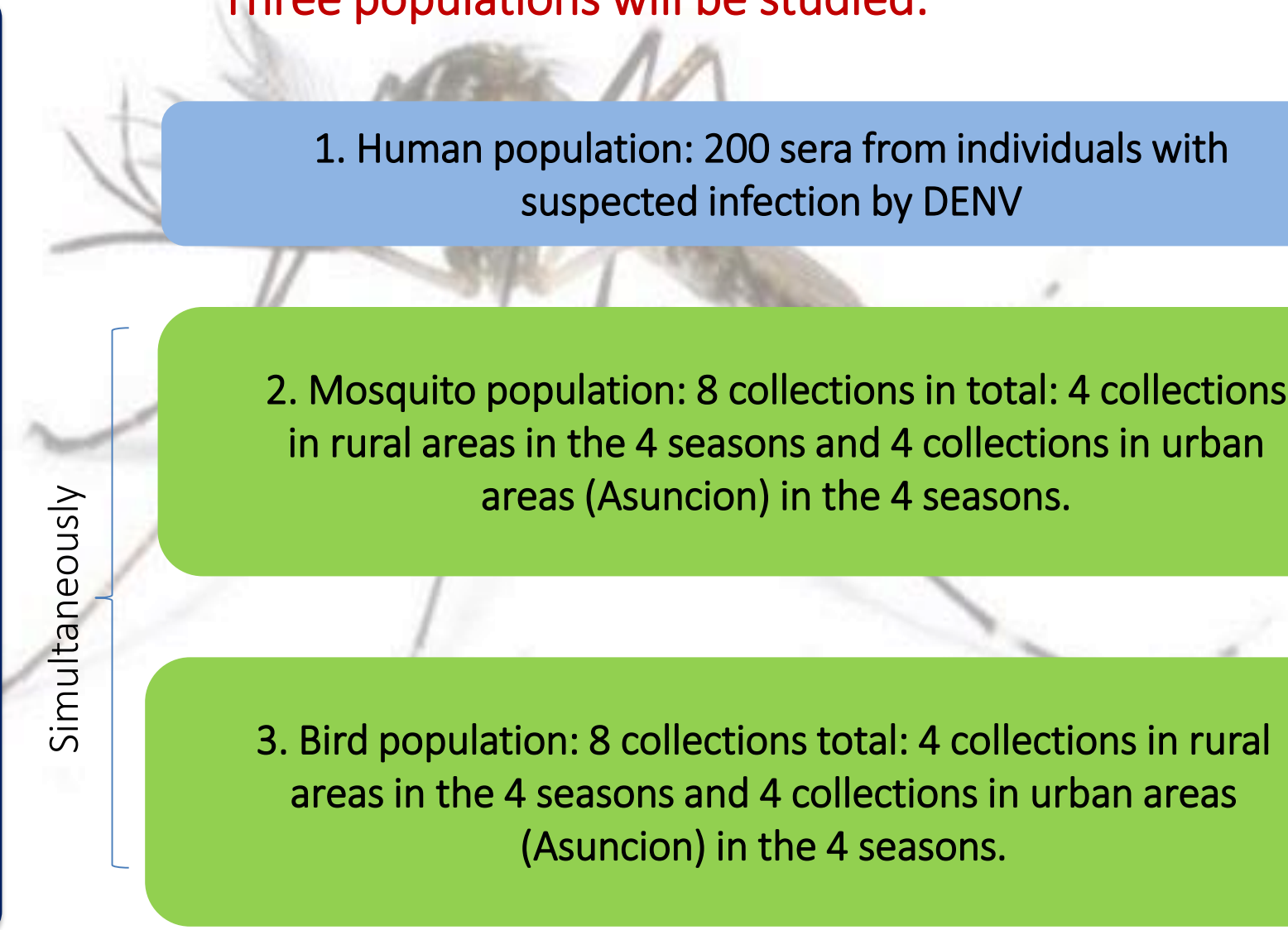
## Three populations will be studied:

1. Human population: 200 sera from individuals with suspected infection by DENV

2. Mosquito population: 8 collections in total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

3. Bird population: 8 collections total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

Simultaneously



# METHODOLOGY

Three populations will be studied:

1. Human population: 200 sera from individuals with suspected infection by DENV

2. Mosquito population: 8 collections in total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

3. Bird population: 8 collections total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

Simultaneously





# METHODOLOGY

## Three populations will be studied:

1. Human population: 200 sera from individuals with suspected infection by DENV

2. Mosquito population: 8 collections in total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

3. Bird population: 8 collections total: 4 collections in rural areas in the 4 seasons and 4 collections in urban areas (Asuncion) in the 4 seasons.

Simultaneously

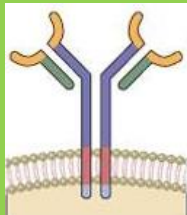
Two different techniques will be applied:

METHODOLOGY

INDIRECT TECHNIQUES:  
Seroprevalence antibody  
detection



Plaque reduction  
neutralization test  
(PRNT)

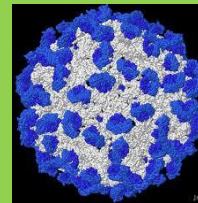


Neutralizing  
antibodies  
(WNV, SLEV)  
BIRDS SERA

DIRECT TECHNIQUES:  
Prevalence of viral genome  
detection



RT-*nested* PCR



Flavivirus genome,  
SLEV, WNV, etc.

MOSQUITO POOLS  
FEBRILE HUMAN SERA

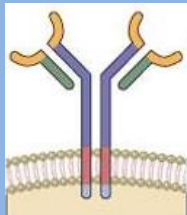
## Two different techniques will be applied:

# METHODOLOGY

INDIRECT TECHNIQUES:  
Seroprevalence antibody  
detection



Plaque reduction  
neutralization test  
(PRNT)

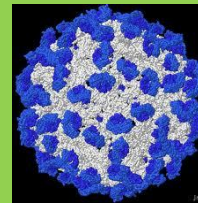


Neutralizing  
antibodies  
(WNV, SLEV)  
BIRDS SERA

DIRECT TECHNIQUES:  
Prevalence of viral genome  
detection



RT-*nested* PCR



Flavivirus genome,  
SLEV, WNV, etc.

MOSQUITO POOLS  
FEBRILE HUMAN SERA



Universidad  
Nacional  
de Córdoba

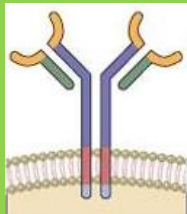
## Two different techniques will be applied:

# METHODOLOGY

INDIRECT TECHNIQUES:  
Seroprevalence antibody  
detection



Plaque reduction  
neutralization test  
(PRNT)

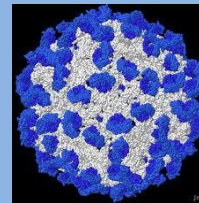


Neutralizing  
antibodies  
(WNV, SLEV)  
BIRDS SERA

DIRECT TECHNIQUES:  
Prevalence of viral genome  
detection



RT-nested PCR



Flavivirus genome,  
SLEV, WNV, etc.

MOSQUITO POOLS  
FEBRILE HUMAN SERA



# METHODOLOGY

## Collection of mosquitoes



### CDC light traps

- supplemented with dry ice
- 4 days (from 18:00 until 09:00)



### Transported alive in refrigerated containers

- Liquid nitrogen



# METHODOLOGY

## Mosquito pools



## Identification of mosquitoes

By species, sex, collection date and site and non-engorged and engorged status

## Homogenized Mosquito pools

1–30 mosquitoes of the same specie will be homogenized in MEM and macerated.

## Homogenates were centrifuged

11,400 g during 30 min at 4°C

Supernatants will be stored in tubes at -70°C until utilization.

# METHODOLOGY

## Mosquito pools

### Identification of mosquitoes

By species, sex, collection date and site and non-engorged and engorged status

### Homogenized Mosquito pools

1–30 mosquitoes of the same specie will be homogenized in MEM and macerated

### Homogenates were centrifuged

11,400 g during 30 min at 4°C

Supernatants will be stored in tubes at -70°C until utilization.

# METHODOLOGY

## Mosquitoe pools

### Identification of mosquitoes

By species, sex, collection date and site and non-engorged and engorged status

### Homogenized Mosquitoe pools

1–30 mosquitoes of the same specie will be homogenized in MEM and macerated

### Homogenates were centrifuged

11,400 g during 30 min at 4°C

Supernatants will be stored in tubes at -70°C until utilization.

## What we hope to accomplish with this project??

HUMAN POPULATION WITH SUSPECTED INFECTION BY DENV



Identified the cases of infection by other flavivirus than Dengue

Identified variants of Flavivirus circulating and compared to those detected in the region.

## What we hope to accomplish with this project??

MOSQUITOES POPULATION



Identify the species of mosquitoes infected by flavivirus and also identify the viral species.

Recognize variants of Flavivirus circulating in our country and compared to those detected in the region.

Identify seasonal variation of mosquitos species.

## What we hope to accomplish with this project??

BIRDS POPULATION



Identify the bird species that could act as potential hosts of the flavivirus analyzed (St. Louis encephalitis virus, the West Nile virus).

Recognize seasonal variation of birds species.



# PERSPECTIVE

Standardisation of RT-nested PCR generic for Alphavirus (in progress)

EXPANSION PROJECT: Perform detection of Alphavirus in samples collected under the project.

EXPANSION PROJECT: Perform collections of rodents that could act as hosts of arbovirus

MOSTICAW WORSKHOP

5 al 7 de Octubre 2016

**THANK YOU**

