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ESTABLISHMENT OF THE BASELINE FOR EBOLA VIRUS IN POTENTIAL HOSTS FROM TROPICAL AMERICA

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ABSTRACT

Introduction: In recent years has been an increase in emerging and re-emerging viral infectious diseases. Those are a great challenge for public health; one of them is Ebola, a viral hemorrhagic fever. It is natural hosts and carriers are several species of mammals of the order Chiroptera, Primates, Artiodactyla, and Rodentia, and in the southeast of Mexico, there is an ecologically similar environment.

Objective: This study aimed to identify the possible presence of the Ebola virus in several mammalian species (bats and rodents) with distribution in tropical Mexico with similar ecological conditions to the recorded host in tropical Africa.

Study design: This work was developed in 75 liver samples of 12 different species of rodents and bats native to Mexico to which RNA was extracted to determine the presence of EBOV-RNA by RT-qPCR.

Results: The samples analyzed, was a negative result for the presence of EBOV-RNA, indicating no previous infection of Ebola virus.

Conclusion: In Mexico, there are several viral diseases with significant impacts on the human population. New emerging viral zoonotic diseases may occur in Mexico, representing a significant threat to Public Health among them Ebola disease.

Keywords: Chiroptera, Ebolavirus, Mammals, Real-Time PCR, Rodentia, Tropical America.

INTRODUCTION

In recent years there has been an increase in new infectious diseases (emerging) and others that were already considered controlled (re-emerging diseases), in which the pathogenic cause is a virus (Gire et al., 2012). Viral diseases are a great challenge for public health. The appearance of novel viruses and variations in epidemiological patterns, in addition to the absence of vaccines, growing of antiviral resistance and bioterrorism, are reasons that make up the situation for the emergence and re-emergence of diseases with widespread and pandemic potential Gire et al., 2012, Genne, 2007).

Ebola is a viral hemorrhagic fever caused by the Ebola Virus (EBOV), which is a single-stranded RNA virus and a member of the Filoviridae family. It is native to tropical areas of central and western Africa, in particular of the Democratic Republic of Congo, Uganda, Gabon, and Ivoire Coast. Natural hosts and carriers have been registered to several species of mammals of the order Chiroptera, Primates, Artiodactyla and Rodentia (Leroy et al.,2005, Leroy et al.,2004, Morikawa et al., 2007, Olival et al., 2014, Swanepoel et al., 1996). The natural hosts of the Ebola virus are frugivorous bats of the family Pteropodidae (Organization WH, 2017). However, experimental studies have shown that the eve also replicates in rodents and shrews (Morvan et al., 1999).

On the other hand, in the southeast of Mexico, mainly in the states of Campeche, Chiapas, Oaxaca, Quintana Roo, Tabasco and Yucatan, there is an ecologically similar environment and with species of mammals of the same taxonomic orders (Gual-Díaz,2017, Solari and Martínez-Arias, 2014), that could be guests or carriers in case of being exposed accidentally. Also, this region presents great diversity, richness and distribution of mammals, especially frugivorous bats (121 species, of which 17 species are frugivorous specialists of the Phyllostomidae family as *Artibeus, Dermanura, Carollia, Chiroderma, Enchistenes, Sturnira, Uroderma,* and *Vampyressa* and rodents (63 species), mainly of the genus *Baiomys, Oryzomys, Peromyscus, Reithrodontomys, Sigmodon* and *Oligoryzomys* of the family *Cricetidae* (Álvarez-Castañedaet al., 2017) that can act as reservoirs of zoonoses (Lorenzo et al., 2017).

Chiroptera has been designated as the most likely natural hosts of EBOV (Barrette et al.,2009, Daszak, 2010) for their unique immune system (Bosio et.,2003, Lubaki et al., 2016). Due to their diversity and wide geographic distribution around the world (Feldhamer et al., 2015), bats are considered

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an essential reservoir for new emerging viral diseases, not only for Ebola but also for others such as SARS-Coronavirus, Marburg fever and encephalitis due to the virus Nipah. Closer contact can transmit rabies and other infectious non-viral diseases (Brauburger et al.,2012). Rodents also represent a serious concern as reservoirs of emerging viruses (Luis et al., 2013); they are apter in the short term to have relatively short gestation periods and many offspring that begin their early reproduction (Han et al., 2015).

Bats and rodents are closer to the habitat of human beings due to ecological changes such as deforestation, so the transmission of new infectious agents could cause epidemics (Gire et al., 2012). An example was presented in 1967 in San Leandro ranch about 35 miles south-west of Palenque, Chiapas, México, where an outbreak of hemorrhagic fever epidemic occurred due to the RNA of a virus (arenavirus) located in Mexican mice of the species *Peromyscus mexicanus*, causing a febrile illness in human disease similar to dengue hemorrhagic fever. This outbreak was preceded three years earlier by the destruction of forested areas in the epidemic zone and an increase in disturbing proportions in the abundance of rodents (*P. mexicanus*) in and around the houses in the area of the epidemic (Goldsmith et al., 1971). Also, in Ocozocoautla de Espinoza, Chiapas antibodies against arenavirus have been found in *P. mexicanus* (Cajimat et al., 2012, Milazzo et al., 2010).

The objective of this study is to identify the possible presence of the Ebola virus in several mammalian species (bats and rodents) with distribution in tropical Mexico with similar ecological conditions to the recorded host in tropical Africa, considered as possible reservoirs of viruses that produce viral hemorrhagic fevers in humans in the south-southeast region of Mexico.

MATERIALS AND METHODS

This work was developed in different species of rodents and bats native to Mexico, some of which, due to their migratory characteristics, can inhabit North, Central, and South America.

Ethics statement

The animals were collected under the scientific collection permit number FAUT-0143 of CL (official letter No. SGPA/DGVS/002779/18) and following the protocols established by for capture and handling, methods followed the animal care and use guidelines of the American Society of Mammalogists (Sikes et al., 2016).

Study area and dates

1. In February, April, July, September of 2017 and September of 2018 rodents and bats were collected in different sampling sites in urban and peri-urban areas of three states of Mexico:

1) Chiapas (n=10): 5 localities in the municipality Berriozábal, 3 in the municipality Salto de Agua, and 2 in the municipality Ocozocoautla de Espinoza.

2) Quintana Roo (n=3): 1 in the municipality Othón P. Blanco, and 2 in the municipality Puerto Morelos.

3) Campeche (n=3): 3 in the municipality of Calakmul. The geographical address and ecological characteristics of the sampling sites are available in Figure 1.

Mammal Sampling

The rodents and bats were collected by 150 Sherman traps and four mist nets, following Álvarez-Castañeda protocols (Álvarez-Castañedaet al., 2017). The location was placed one and 5 km away from the human settlements since the vectors and reservoirs of hemorrhagic fever viruses have peri-urban habits and can move up to 5 km approximately. The rodents and bats collected were taxonomically described (Álvarez-Castañedaet al., 2017) and euthanized by dislocation of cervical vertebrae (Sikes et al., 2016).

The collected specimens were handled complying with biosecurity standards, such as the use of disposable laboratory lab coats, eye protection lenses, latex gloves, and unique masks to avoid inhalation of possible pathogens and other contaminating particles. From each specimen, external measurements, weight, sex, reproductive condition, habitat type, and geographical position were obtained. Tissue samples (liver) were taken from all specimens with the help of sterile tweezers and scissors and placed in sterile 2 ml tubes with RNA later and preserve at -20 °C. All specimens and tissues are deposited and cataloged in the Mammal Collection of El Colegio de la Frontera Sur, San Cristobal de Las Casas, Chiapas.

RNA extraction

RNA was extracted from liver samples to determine the presence of EBOV RNA. The tissues were homogenized in 140 ul of sterile water by Virsonic 100 (VirTis Company, USA), RNA extraction was carried out with the QIAamp Viral RNA Mini extraction kit (Qiagen, USA). According to manufacturer instructions. The obtained RNA was stored at -80°C.

RT-qPCR

Molecular detection of the viral RNA was performed by RT-qPCR, the reaction mixture was made according to the manufacturer, using the EBOV kit (Techne, UK) and the One-step 2x Master Mix kit (Roti Molecular, VEN), with the following conditions: 10 ul Buffer 2X, 1ul Mix primers, and probe, 4 ul water, and 5ul of the sample. A specific sequence of the virus was used as a positive control, reading the results for the probe in the FAM channel and the internal control provided in the reaction, in the VIC channel. The real-time amplification was carried out in a 7500 fast thermal cycler (Applied Biosystem, USA), amplification conditions were 15 min at 45°C, 2 min at 95°C, 45 cycles at 95°C for 10 sec and 60°C for 60 sec, the data collection was in the last step.

RESULTS

A total of 40 rodent specimens (Family Cricetidae) of the species *Peromyscus mexicanus, P. aztecus, P. leucopus, Sigmodon toltecus, Reithrodontomys gracilis, Oryzomys couesi, Mus musculus, Rattus rattus*, and 35 bats specimens (Family Phyllostomidae) of the species *Artibeus jamaicencis, A. lituratus, A. toltecus*, and *A. phaeotis* were collected. All species were collected in different types of vegetation: cloud forest, high and low perennial forest, low perennial forest with patches of cloud forest, low deciduous forest, acahual, acahual with medium forest, urban acahual with scattered trees, secondary vegetation, pasture with patches of low forest, corn crops, rural households with fruit trees and wooded urban areas (Figure 1).

The most abundant bats species was *A. jamaicensis*, with 20 individuals, and for the rodents *P. mexicanus*, with 15 individuals. The less abundant bat species was *A. phaeotis*, and for the rodents were *Oryzomys couesi, M. musculus*, and *R. rattus*, in all cases with only one individual each.

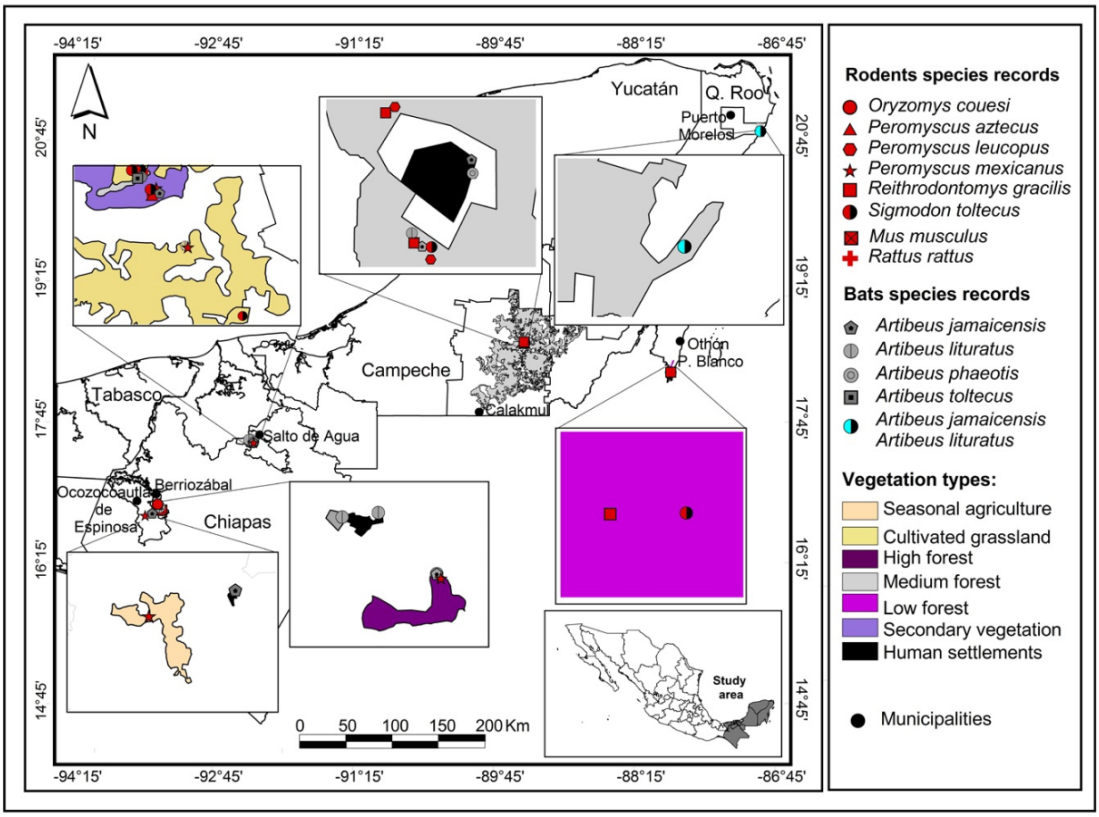
The site and date of sampling and frequency for each specimen are presented in Table S1. The necropsies after the euthanized did not show morphological changes that indicated a pathological condition.

The 75 samples analyzed by RT-qPCR, was a negative result for the presence of EBOV RNA, amplified the internal control, with an average CT value of 22 (Figure 2A), the negative control (Figure 2B) and positive control (Figure 2C), indicating no previous infection of EBOV.

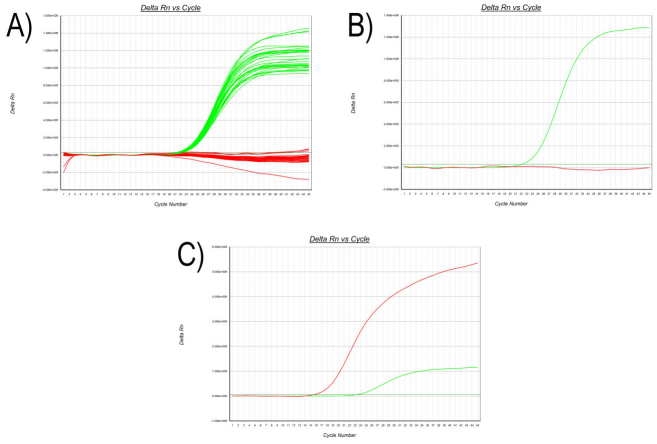
DISCUSSION

In tropical regions of Mexico, there are several viral diseases with significant impacts on the human population, including hemorrhagic fevers such as dengue, zika, and chikungunya (Rojas et al., 2018). The latter is a disease that originated in Africa, Asia, and the Indian subcontinent and appeared in Mexico as a significant outbreak in 2015 (Organization WH, 2017). Therefore, new emerging viral zoonotic diseases may occur in Mexico, representing a significant threat to Public Health among them Ebola virus disease, which produces a severe and infectious viral hemorrhagic fever that affects humans.

Mexico is not the exception to that social and environmental transformation with outbreaks of emerging and re-emerging diseases in recent decades. With environmental changes, the reproduction niches of A. aegypti have increased with the increase in the number of cases of dengue fever, especially with the introduction of the DEN3 genotype. Such as the dengue virus, leptospirosis has become the most differential diagnosis for dengue. Some other virus and bacteria have been identified and reported by Instituto de Diagnóstico y Referencia Epidemiológicos (INDRE), Secretaría de Salud (Flisser et al., 2002). Another viral hemorrhagic fever re-emerging in tropical America is yellow fever; it is originated in Africa and was brought to the western hemisphere during the slave trade era, with the first epidemic reported in 1648 in the Yucatan (Staples and Monath, 2008). Since 1980, yellow fever has re-emerged across Africa and in South America. A total of 18 735 yellow fever cases and 4522 deaths were



Figures 1. Geographical address and ecological characteristics of the sampling sites and species records.



Figures 2. Real-time PCR graphics. (A) show internal control in samples and non-amplification of EBOV, (B) show the negative control, and (C) show the positive control.

reported just in four years from 1987 to 1991 (Robertson et al., 1996).

The presence of emerging and re-emerging diseases has been accelerated as a result of different factors that have allowed the rapid dissemination of etiologic agents. The origins of emerging infectious diseases are significantly correlated with socioeconomic, environmental and ecological factors, and provide a basis for identifying the regions in which new emerging zoonotic areas are most likely to originate (Olival et al., 2017).

The south-southeast region of Mexico for its tropical ambience is of great importance for the study of emerging zoonotic diseases, including those that produce viral hemorrhagic fevers, since this region have:

1) great diversity, richness, and distribution of mammals (rodents and bats),

2) ecologically similar environments and species of mammals of the same taxonomic orders that could be carriers of the Ebola virus and other hemorrhagic fevers,

3) various viral diseases with significant impacts on the human population, including hemorrhagic fevers such as dengue, zika and chikungunya,

4) a high human population density (69 inhabitants / km2),

5) high levels of marginalization among its inhabitants (Instituto Nacional de Estadística,2011, Urbano Sddaty, 2014),

6) increase in environmental deterioration due to the uncontrolled development of activities such as mining and deforestation for extensive livestock and agriculture (Urbano Sddaty, 2014), which results in alterations in the patterns of wildlife, contributing to the development of viral zoonotic hemorrhagic diseases.

CONCLUSION

In order to contain and respond quickly to possible current outbreaks of hemorrhagic dengue fever and other viral hemorrhagic fevers in the tropical region of Mexico, it is necessary to continue with the study on the distribution of potential reservoirs of zoonotic viral diseases in southeastern Mexico, identify localities where there have been outbreaks of viral hemorrhagic fevers and include other families of bats (Molossidae, Vespertilionidae) and other taxonomic orders of mammals, such as Eulipotyphla (shrews), Artiodactyla (deer) and Primates (monkeys).

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