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Serological test for *West Nile* virus in horses of Los Ríos, Ecuador

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Abstract

The aim of this study was to determine the serological presence of *West Nile* virus (WNV) in horses of wetland "Abras de Mantequilla", Los Ríos, Ecuador, during the rainy season of 2012. The samples received were selected according to the presence or absence of disease which belonged to horses in the study area. During the study, a total of 118 horses were examined by ELISA detection of IgG and IgM antibodies. The incidence was 36.44% and the prevalence was 33.05%. The highest incidence occurred at the Mapancillo (28%) locality and the lowest was in La Luz (13%). Age distribution was: 1-5 years (32.55%); 6-10 years (27.91%); 11-15 years (23.26%); 16-20 years (16.28%). The 53.48% of the infected animals were males and 46.52% were female. All animals were criollos mixed race. The presence of WNV was demonstrated through the detection of IgG and IgM antibodies in blood serum of horses at the wetlands "Abras de Mantequilla" Los Ríos, Ecuador.

Keywords: *West Nile* virus; ELISA IgG and IgM: equines.

Prueba serológica para el virus del *Nilo Occidental* en los caballos de Los Ríos, Ecuador

Resumen

El objetivo de este estudio fue determinar la presencia serológica del virus del *Nilo Occidenta*l (VNO) en los caballos de los humedales "Abras de Mantequilla", Los Ríos, Ecuador, durante la temporada de lluvias del 2012. Las muestras analizadas fueron registradas de acuerdo a la presencia o ausencia de enfermedad y que pertenecía a los caballos en el área de estudio. Durante el estudio, un total de 118 caballos se examinaron por ELISA para la detección de anticuerpos IgG e IgM. La incidencia fue del 36,44% y la prevalencia fue del 33,05%. La incidencia más alta se produjo en la localidad Mapancillo (28%) y la más baja fue en La Luz (13%). La distribución por edad fue: 1-5 años (32,55%); 6-10 años (27,91%); 11-15 años (23,26%); 16-20 años (16,28%). El 53,48% de los animales infectados eran machos y 46,52% hembras. Todos los animales fueron de raza mixta-criolla. La presencia indirecta del virus, se demostró a través de la detección de anticuerpos IgG e IgM en el suero sanguíneo de los caballos en los humedales "Abras de Mantequilla" Los Ríos, Ecuador.

Palabras Clave: virus del *Nilo Occidental*; ELISA IgG e IgM; equinos.

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I. INTRODUCCIÓN

West Nile fever (FON) is caused by the West Nile virus (WNV) genus *Flavivirus*, family *Flaviviridae*. FON is an emerging infectious zoonoses. The birds are amplifying hosts and biological vector is *Culex* mosquito. However, other sorts and even mosquito, ticks transmit it. Susceptible hosts are human and equine. Mammals such as cats, dogs, goats, sheep, pigs, cows, rabbits, squirrels, bats, lemurs and rodents can also become infected (Valles and Sánchez 2000 and Burgueño et al. 2013).

WNV was detected in the Western Hemisphere in August 1999, when an epidemic occurred in the city of New York. WNV is enzootic in eastern and southeastern United States, today. Because of the migratory patterns of birds in America, this problem has spread to several countries in a short time (CDC 199 and CDC 2000). FON involves American countries: Canada, Cayman Islands, USA, Guadeloupe, Dominican Republic, Bahamas, Puerto Rico, Cuba, Jamaica, Haiti, French West Indies, Brazil, Colombia, Trinidad and Tobago, El Salvador, Costa Rica, Guatemala, Mexico, Venezuela and Argentina (Soler and Vera 2007 and Díaz et al. 2011). Coello et al. (2011) serological evidence was found against WNV from 2007 to 2009 in horses of Los Rios, Ecuador. This area is a migration route of birds from North America.

The aim of this research was to demonstrate the presence of WNV in Los Rios, Ecuador, through a serological study of antibodies in horses.

II. DESARROLLO

1. Methodology

Materials and methods

Location: wetlands "Abras de Mantequilla" is located in the municipalities of Vinces, Pueblo Viejo and Baba, in the province of Los Ríos, Ecuador. Sampling period: February and June of 2012. Type of research: panel and longitudinal. Population and sample: the population was 506 horses and the sample was118 animals (36.87%). The locations studied in the wetland "Abras de Mantequilla" were: La Piedad, La Luz, Los Playones, Mapancillo and Jobo.

Sampling methodology: blood sampling was performed with the consent of the owners, in the early hours of the morning. Biosafety standards (cleaning and bath investigator, appropriate clothing, insect repellent and cold boxes) were met. Samples were centrifuged at 3000 rpm for 5 minutes to separate blood serum. The serum was stored at -20 °C for further analysis in the area of Virology, in the National Institute of Hygiene and Tropical Medicine "Leopoldo Perez Izquieta". ELISA method was used to detect specific antibodies (IgM and IgG) against WNV, according to the protocols of the World Health Organization (WHO) and Pan American Health Organization (PAHO) and the Center for Control and Prevention (CDC) (PAHO-WHO-CDC 2003) and World Organization for Animal Health (OIE 2013). Reactive serum it was considered positive by the ELISA test and used the conjugate HRP6B6C1-CDC. (OMS/OPS/CDC, 2013). Incidence was calculated as the quotient of positive animals in February, among the total sample, multiplied by one hundred percent. Prevalence was calculated as the quotient of positive animals in June, among the total sample, multiplied by one hundred percent. The animals were classified by age ranges 5 years, sex, and race.

Statistical analysis: data were analyzed by SAS software (Statistical Analysis System), version 9.3 (2013) to evaluate descriptive statistics (mean and standard deviation) and multiple range test of Tukey was used for comparison of averages, with the determining standard error (SE) and the probability value (p), in the analysis of variance (ANOVA).

2. Results and discussion

The incidence of WNV had a decreasing trend between the blood serum samples at the beginning and end of the rainy season (Table 1) in 118 horses. IgG antibodies were reduced by 6.78% and IgM antibodies were reduced by 3.39% in animals that were sampled in the wetland "Abras de Mantequilla", Los Rios, Ecuador. The total positive animals, including two samples of blood serum was 33.05%. The incidence was of 36.44%. The prevalence was 33.05%.

Table 1. Serology incidence of WNV in beginning and ending rainy season.

Antibodies		IgG	IgM	Positive total	I	II
February, beginning	Absolute value	43	22	43	22	22
	Relative value (%)	36.44	18.64	36.44	18.64	18.64
June, ending	Absolute value	35	18	39	15	14
	Relative value (%)	29.66	15.25	33.05	12.71	11.86
Diference	Absolute value	8	4	4	7	8
	Relative value (%)	6.78	3.39	3.39	5.93	6.78

I: how many of the animals that were positive for IgG antibodies were also positive for IgM
II: how many of the animals that were positive for IgM antibodies were also positive for IgG

The results match those of CDC (1999) which determined that the presence of IgG and IgM is an effective method to detect the presence of WNV serological. This technique has been used in USA and Canada since 1999. The serological evaluation has been an effective indirect evidence, to detect the movement of the pathogen, since WNV has spread to other countries in the Americas (Mattar et al. 2005). Mattar et al. (2005), Diaz et al. (2011) and Lindsey (2011) also reported serological evidence of infection in horses and other species in different countries of the Americas.

Human infections by WNV have been reported in the USA, Cuba, and Argentina, in relation to infected horses (Soler and Vera, 2007 and CDC 2013). This disease has a high rate of incidence and prevalence in the USA, where up to November 2013, there had been 39,557 positive cases of encephalitis (CDC 2013) and 1668 people had died from WNV (CDC 2013). In this investigation, it was also found a high incidence and prevalence of WNV.

In this research, only two horses show clinical symptoms of the disease, but it was failed to isolate the virus. However, in some South American countries like Argentina, WNV has been isolated in equine brain (Morales 2006), with symptoms of the disease. The relative distribution of positive cases in the first sampling localities in the first rains in February showed that more animals were found in Mapancillo (26%); followed by Los Playones (23%), Jobo (21%), La Piedad (16%) and La Luz (14%).

In the sampling at the end of the rainy season in June, the largest number of positive cases were again presented in Mapancillo (28%); followed by Los Playones and Jobo, both with 23%; and La Piedad and La Luz, in the two locations with 13%. The areas studied are routes of migratory birds from North America to Ecuador. This is a risk factor for the transmission of various types of microorganisms in the wetland "Abras de Mantequilla" including WNV. Soler and Vera (2007), Diaz (2008) and Vasquez (2010) demonstrated that birds are multiplier hosts of WNV amplifiers. Migratory birds that come with WNV may influence the development of the transmission to other susceptible species including horses and humans as in wetlands like this, where they are so abundant.

Seropositive samples for IgG and IgM antibodies had the following age distribution: 1-5 years (32.55%); 6-10 years (27.91%); 11-15 years (23.26%); 16-20 years (16.28%). The 53.48% of the infected animals were males and 46.52% were female. All animals were criollos mixed race. IgG antibodies indicate past infections. The serological examination showed that the infection is recent. This is because the presence of IgM antibodies can be detected only up to 500 days after infection. This could indicate that the entry of the virus into the country is activated by the migratory birds. However, studies are needed in other wetlands in the country, to confirm the finding. This exploratory study allows to demonstrate the serological evidence of WNV circulation in Ecuador. The results of this work could enable health authorities to strengthen epidemiological surveillance systems in Ecuador.

III. CONCLUSIONS

The presence of WNV was demonstrated through the detection of IgG and IgM antibodies in blood serum of horses, in the wetlands "Abras de Mantequilla" Los Rios, Ecuador. The incidence was of 36.44%. The prevalence was 33.05%. The highest incidence occurs in Mapancillo (28%) locality and the lowest was in La Luz (13%). Age distribution were: 1-5 years (32.55%); 6-10 years (27.91%); 11-15 years (23.26%); 16-20 years (16.28%). The

53.48% of the infected animals were males and 46.52% were females.

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