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Eastern Equine Encephalitis virus, a re-emerging wild arbovirus in wild hosts, posing a threat to animal and human health

Virus de Encefalitis Equina del Este, un arbovirus silvestre reemergente en hospedadores silvestres, que representa una amenaza para la salud animal y humana

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ABSTRACT

This is a bibliographic review, which explains about the generalities, transmission, characteristics, genome, replication, epidemiology, infections in various species, diagnosis, treatment, control and prevention of the Eastern Equine Encephalitis virus (EEEV); causing Eastern Equine Encephalitis (EEE), which is a zoonotic disease with worldwide epidemiological importance; It occurs with wild cycles among birds through the Culex

mosquito preferably, and the latter can accidentally transmit the virus to horses and humans, where they can cause meningoencephalitis in both. The EEEV could contribute to the appearance of epidemic or epizootic outbreaks of emerging or re-emerging infections in susceptible populations; Likewise, the EEEV is of importance in wildlife (by the interaction in wild animals), animal health (causes death in horses) and public health (causes death in humans).

Key words: *Eastern Equine Encephalitis Virus*, Vector, Eastern Equine Encephalomyelitis, zoonosis

RESUMEN

El presente es un revisión bibliográfica, que explica sobre las generalidades, transmisión, características, genoma, replicación, epidemiología, infecciones en varias especies, diagnóstico, tratamiento, control y prevención del virus de Encefalitis Equina del Este (VEEE), causante de la Encefalitis Equina del Este (EEE), que es una enfermedad zoonótica con importancia epidemiológica mundial; se presenta con ciclos silvestres entre aves a través del mosquito Culex preferentemente, y este último puede transmitirse el virus accidentalmente a equinos y humanos, donde pueden producir en ambos meningoencefalitis. El VEEE podría contribuir a la aparición de brotes epidémicos o epizoóticos de infecciones emergentes o reemergentes en poblaciones susceptibles; así mismo, el VEEE es de trascendencia en vida silvestre (por la interacción en animales silvestres), salud animal (produce muerte en equinos) y salud pública (produce muerte en humanos).

Palabras clave: Virus de la encefalitis equina del este, vector, encefalomielitis equina del este, zoonosis

INTRODUCTION

The Eastern Equine Encephalitis Virus (VEEE), currently called the Madariaga virus, is an arbovirus (viruses transmitted by arthropod vectors such as: mosquitoes, ticks, mites, fleas) (Go, et al. 2014) and is an important veterinary pathogen and human that belongs to the Togaviridae family, is found within the seven antigenic complexes of the Alphavirus genus (Arrigo. et al. 2010). This virus causes Eastern Equine Encephalitis (EEE), which has an incubation period between 5 to 14 days and is characterized by causing from a feverish disease, up to causing fatal meningoencephalitis in equines and humans, with percentages of mortality in humans between 30 to 70% (OIE, 2017; Rocheleau et al. 2017) and in equines between 70 to 90% (CFSPH, 2017; Bingham, et al. 2014), that is why it is considered the most deadly arbovirus (OIE, 2017; Morens, 2019), and it is a zoonosis of animal origin, a mandatory report for the World Organization for Animal Health (OIE), in addition It is important to mention that there are no safe and efficient human alphavirus vaccines (Torres R. et al. 2017).

TRANSMISSIÓN

The VEEE transmission cycle in North America is maintained among passerine birds as reservoirs and amplifying hosts; Other reservoirs may be reptiles and amphibians. Recently, it has been suggested that snakes play a role in the enzootic transmission cycle. The main

enzootic vector in swamp habitats is the ornithophilic mosquito, Culsette melanura, but mosquito species such as C. peccator, C. erraticus and Uranotaenia sapphirina, can also transmit the virus and cause serious disease in humans, horses, pigs, dogs and some species of birds. (Go, et al. 2014)

In South American ecosystems, the VEEE appears when infected migratory birds (amplifying hosts) from North America reach the humid or wild areas of great ecological impact and transmit the virus to native birds, through the mosquitoes of the Culex genus, however, the infected vector bites mammals, but the susceptible hosts for the disease are equines and humans (PAHO / WHO, 2017; Torres, et al. 2017). Go et al. describes rodents and reptiles as reservoirs in South American ecosystems. (Fig. 1).

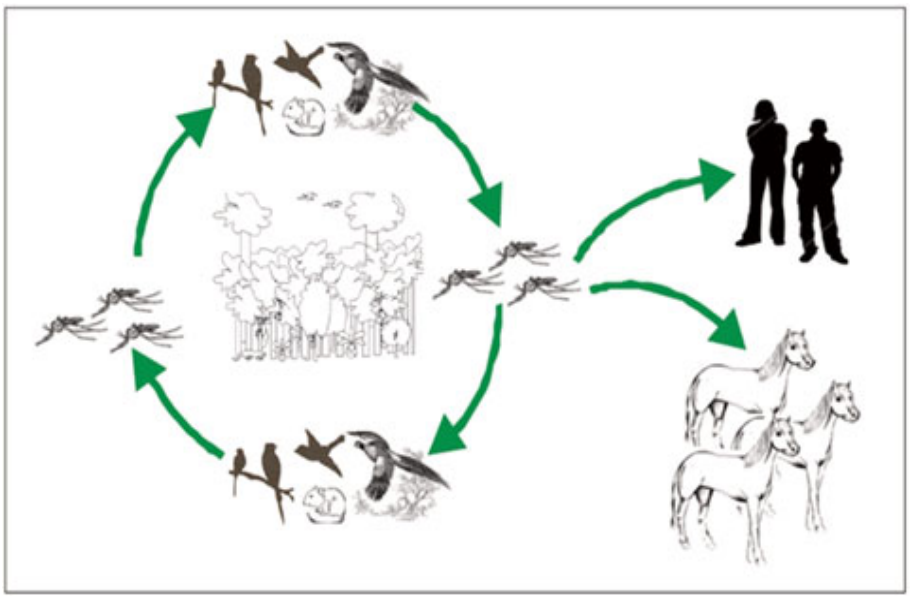

Fig. 1. Biological cycle of VEEE.
Available in:
https://www.paho.org/hq/index.php?option=com_content&view=article&id=8301:2013-encefalitis-equina-este&Itemid=39850&lang=es

Furthermore, it is important to highlight that VEEE isolates in Culex Melanoconion and Culex pedroi in South America and Culex taeniopus in Central America, are suggested to be the main enzootic and potentially epizootic vectors. (Arrigo, et al. 2010)

Also, the transmission of the VEEE involves various birds of the order Passeriformes and Columbiformes. (Molaei, et al. 2013), 26 species of mosquitoes, lice and chicken mites (CFSPH, 2017), amphibians, reptiles (Bingham, et al. 2014) and mammals such as wild rodents, marsupials, opossums (Mesa et al, 2005), bats (Blohm, et al. 2018; Benvenuto, et al. 2019), monkeys, dogs, goats, and small mammals. (Benvenuto, et al. 2019) establishing a **Transmission Network** between various reservoirs, amplifying hosts, vectors and susceptible hosts. Although the virus mainly causes diseases in horses and humans, occasional cases of encephalitis have also been reported in sheep, cows, deer, South American camelids (llamas and alpacas) and pigs (Go, et al. 2014). (Fig. 2)

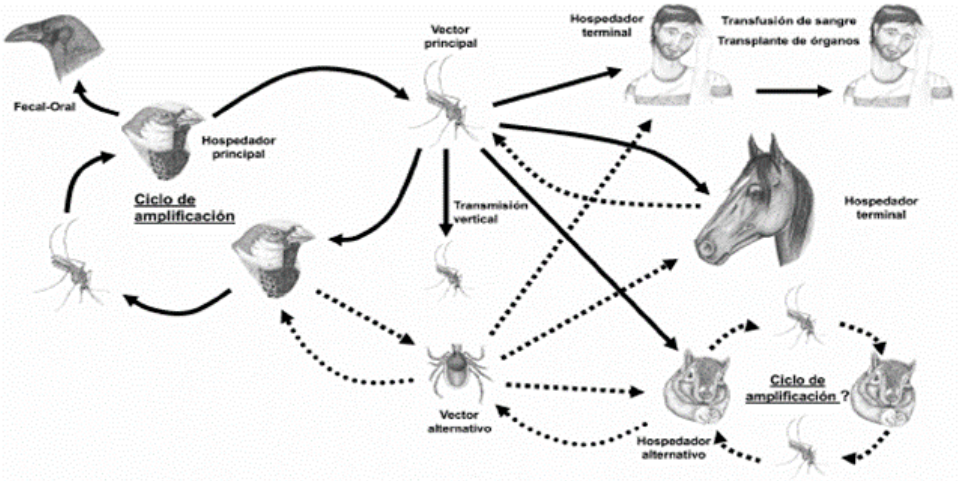

Fig. 2. Transmission network of VEEE.
Available in: https://www.researchgate.net/publication/262590189.

FEATURES AND VIRAL GENOME

The causative agent of EEE is an enveloped, spherical and icosahedral virus, 65 to 70 nm in diameter, with a linear, non-segmented RNA genome, and positive polarity, with a size between 9.7 and 11.8 kb; the genome at the 5 'end is protected by methylation and at the 3' end it is polyadenylated (Fig. 3).

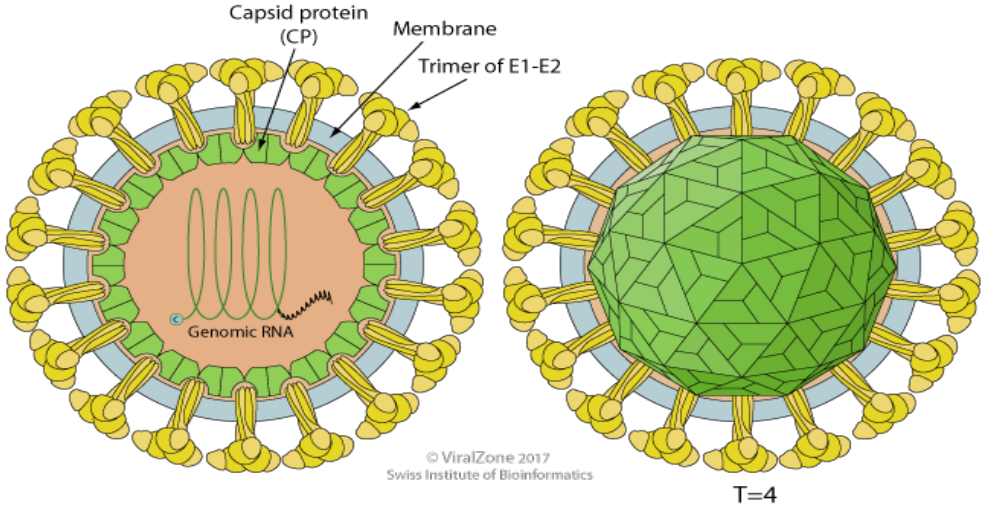

Fig. 3. Eastern Equine Encephalitis Virus.
Available in: https://viralzone.expasy.org/625?outline=all_by_species

The first two thirds of the genome in its 5 'portion encode for four non-structural proteins (nsP1 to nsP4) that make up a complex of enzymes required for viral replication. The rest of the genome codes for structural proteins: the capsid protein C, and the envelope glycoproteins E1 and E2, which are immunogenic; Of these, E2 presents the highest antigenic variability (Fig. 1). Furthermore, being a enveloped virus, they are susceptible to common disinfectants such as 1% sodium hypochlorite, 70% ethanol, glutaraldehyde and 2% formaldehyde, and do not survive outside the host (Kuhn, 2007), (Fig. 4).

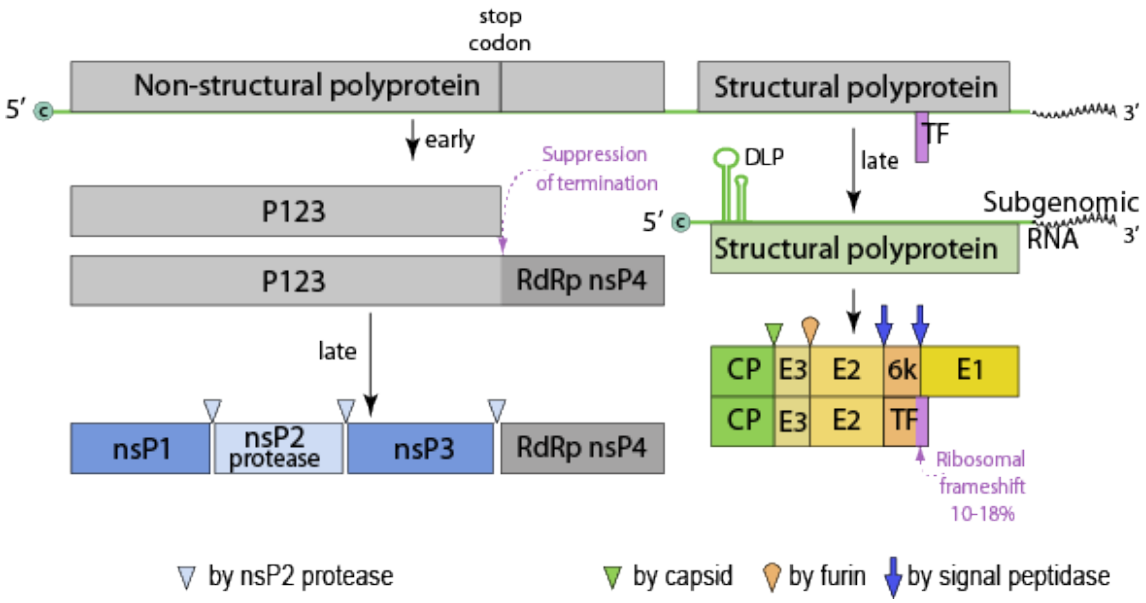

Fig. 4. Viral Genome.
Available in: https://viralzone.expasy.org/625?outline=all_by_species

In the VEEE, 4 viral types (I, II, II and IV) with high virulence and genetic variability are distinguished (Mesa et al, 2005; Arrigo, et al. 2010). The subtype / lineage I corresponds to the circulating strains in North America and the Caribbean and are the most virulent for horses and humans (Go, et al. 2014), and the other strains prevail in Central and South America, the latter being the more divergent, which leads to differences in their ecology and adaptation to different vertebrate hosts and mosquitoes, facilitating the appearance of epidemics or epizootics (Arrigo, et al. 2010).

VIRAL REPLICATION

Viral replication begins when the viral E glycoprotein binds to the host's cellular receptor, then enters the cell through catrin-mediated endocytosis; the viral membrane is then fused with the host endosomal membrane; the viral genome is then released into the cytoplasm. The positive-sense viral strand is then translated into a polyprotein, which is then cleaved into non-structural proteins necessary for RNA synthesis (replication and transcription). (Kuhn, 2007)

Subsequently, replication occurs in cytoplasmic viral factories on the surface of endosomes, but the strand of a positive polarity strand synthesizes a double-stranded genome (dsRNA). The dsRNA genome is then transcribed and then replicated, thus providing viral mRNAs and novel one-strand (+) genomes (ssRNA). Then, the expression of the subgenomic RNA (gRNA) that gives rise to the structural proteins is promoted. Subsequently, the assembly of the capsid in the cytoplasm occurs; finally, the capsid is enveloped by sprouting in the plasma membrane where the virion leaves the host cell (Kuhn, 2007).

MATERIALS AND METHODS

HISTORY AND EPIDEMIOLOGY:

The VEEE has a wide geographical distribution in the United States, Canada, the Caribbean, Central and South America, therefore, it is prevalent in the Americas from Canada to Argentina (PAHO / WHO, 2017).

The virus was first isolated in 1933 from New Jersey horses in the United States (USA); then in 1955, in this country it was isolated in mosquitoes, rodents and humans, and in 1960 in birds according to Casals (1964) and Armstrong (2013), then in 1982 it was isolated in dogs (Weaver, et al. 1994). From February 2012 to March 2013, winter transmission of VEEE in mosquitoes from 3 Florida wildlife parks and host preference in cattle, dogs, rabbits, humans, raccoon, possums, deer, wild boar, crocodiles, frogs, and turtles were determined. (Bingham, et al. 2014). The United States is believed to be at the highest risk of infection and between 2003 and 2016 VEEE was detected in 33 states by at least one species, with 20 states with human disease; a total of 121 human cases were reported during this time (a median of eight cases per year), with the majority of cases originating from Florida, Massachusetts, and New Hampshire. (Oliver, et al. 2016; Gill, et al. 2019). On the other hand, between January 1 and September 30, 2013, from a total of 997 serum collected from wild birds, a seroprevalence of 2% was determined (Pedersen, et al. 2016); And in the summer and fall of 2019, nine US states reported 36 human cases (14 of them fatal) (Morens, 2019).

In Canada, from a total of 196 equine serum samples, taken between March 7 to July 4, 2012, 18 positive samples were determined, registering a seroprevalence of 9.18% (Rocheleau, et al. 2013). In this country, viral activity has been recorded in mosquitoes and horses, in the latter, clinical cases were presented annually from 2008 to 2010 (total = 43) in the southern part of the province of Quebec, suggesting that the virus may have become endemic in this area. In 2012, a serological study against VEEE in horses, carried out in the same region, revealed that more than 6% of the horses had been infected, suggesting that there are ecological niches (that is, appropriate habitats, vectors and viruses) to maintain the transmission of VEEE, including possible spread to humans. (Rocheleau et al, 2017) utbreaks in horses are common and are often accompanied by high case fatality rates. Eighty to 90% of infected horses develop acute and lethal disease, and about 66% of survivors develop severe neurological sequelae. (Go, et al. 2014).

As is evident, in North America, there are frequent cases of EEE in humans, equines, wild and migratory birds, therefore, it is worth investigating in various ecosystems, since infected migratory birds could carry the VEEE, from North America to Central American ecosystems and South Americans; and outbreaks or epidemics / epizootics could occur in a very short period of time, since migratory birds reach South American ecosystems from North America in 3 to 6 months (Piter, 2014).

Likewise, viral activity has been determined in the Dominican Republic in 1949, where it was isolated in monkeys and rodents. In Jamaica, in 1962 it was identified in horses and rodents (Casals, 1964).

In 1958, in **Panama** it was determined in horses and rodents (Casals, 1964), and between 1962 and 1986 it was determined in horses and birds. (Weaver, et al. 1994); likewise, in 2010

it was isolated in the Darien province in an epidemic / epizootic in humans and equines (Carrera, et al. 2014), a seroprevalence in humans of 19.4% and in equines of 26.3% was also reported (Carrera, et al. 2018); In addition, there is serological evidence in the population between 2 to 5% (Lednicky, et al. 2019).

Haiti, in April 2015, reported viral isolation in an 8-year-old boy with feverish symptoms of 39 ° C, cough, headache, and myalgia. (Lednicky, et al. 2019).
The presence of the virus in South American ecosystems has been reported in: Trinidad, Guyana, Brazil, Venezuela, Colombia, Ecuador, Peru, Brazil and Argentina.
However, in **Trinidad** in 1959, it was isolated from mosquitoes and rodents. (Casals, 1964).
As in **Guyana** in 1950 it was determined in horses (Weaver, et al. 1994).

In **Brazil** (Belen) in 1955 it was isolated in sentinel monkeys and rodents. (Casals, 1964) and in 1976 it was isolated in mosquitoes (Weaver, et al. 1994), in 2007 in 135 equines of the South Pantanal 47.7% of seroprevalence was determined (Pauvolid, et al. 2010), between May From 2008 to August 2009, in the Brazilian states of Pernambuco, Ceará and Paraíba, out of a total of 229 equines, a case fatality rate of 72.92% was presented (Silva, et al. 2015), in addition, between 2005 to 2013 it was isolated in equines and hamsters (Oliveira et al, 2014) and between 2015 and 2016 a human case of VEEE with lineage III was determined in Mato Grosso. (De Sousa, et al. 2019)

Between 1973 and 1974 in **Venezuela** the virus was isolated in sentinel hamsters (Walder et al, 1976), then between 1976 to 1981 it was isolated from horses, rodents and mosquitoes (Weaver, et al. 1994). In 1984 it was isolated in mosquitoes from the La Guajira region (Walder et al, 1984). In 2018, a case was reported in a 12-year-old girl with undifferentiated acute febrile disease, and was also identified as a strain of lineage III. (Blohm, Et al. 2018)
In **Colombia**, between July and September 1969, along the margins of a swampy freshwater area 50 km from the interior of the port of Tumaco, near the Ecuadorian border, the VEEE was isolated in 2 sentinel hamsters (Sanmartín et al , 1971).
It is important to highlight that the first isolation of the VEEE in South America occurred in **Argentina**, in 1936, from a horse (Sabattini, et al. 1985), then again in 1959 (Buenos Aires) it was detected in equines (Casals, 1964) . In 1980, a new enzootic subtype of VEEE (AG80-663) was isolated in mosquitoes from Chaco, in addition, neutralizing antibodies were determined in horses and rodents (Sabattini, et al. 1985), then, in 1981, an EEE epizootic occurred in horses from four sectors of the Santiago del Estero province, with an incidence of 17%, and a fatality rate of 61% (Sabattini, et al. 1991). Later, in 2019, viral detection in Culex spp mosquitoes was reported. and phylogenetic analysis showed amplified fragments of VEEE belonging to the lineage / subtype III of the South American VEEE complex (Stechina, et al 2019).
Importantly, in temperate regions of South America (eg Argentina), VEEE infections often occur during the summer (Go, et al. 2014)
In **Uruguay**, a typical North American epizootic lineage was detected in Culex pipiens mosquitoes and an equine seroprevalence against Madariaga Virus was reported between 3-4% (Burgueño, et al. 2018).

Peru, during the year of 1970, the VEEE was isolated in rodents (Weaver, et al. 1994); on the other hand, in a study carried out on mosquitoes between April 1996 to August 1998, the presence of VEEE was determined; in 2005 the virus was also detected in the Loreto mosquito (Turrel, et al. 2005) and in 2007 it was established between 2 to 3% of seroprevalence in humans (Aguilar, et al. 2007). In 2011, a human serological prevalence of 1.5% was established in an Indigenous community called Nueva Esperanza in the Peruvian Amazon. (Pérez, et al. 2019)

Although Arrigo (2010) describe that the South American strains of VEEE are associated only with equine disease and that they are not clearly associated with human disease; however, some authors evidence important human cases in recent years, in some South American countries such as: Peru in 2011 (Pérez et al, 2019), Brazil 2015-2016 (De Souza, et al. 2019) and Venezuela in 2016 (Benvenuto, et al. 2019). However, Benvenuto (2019) describe that the EEE virus has the potential to become a global pathogen due to its transmission mechanisms mediated by urban mosquitoes such as Aedes aegypti and Culex theileri.

On the other hand, **in Ecuador**, VEEE activity has been reported in 1974, when it was isolated from mosquitoes in the Manglaralto area, Santa Elena province (Weaver, et al. 1994; Calisher, et al. 1983) and in May 2013, 2 serological cases were reported in equines in the Chongón sector (Guayas Province), in the vicinity of a water dam (OIE, 2013; Bingham, et al. 2014).

INFECTIONS IN VARIOUS SPECIES

Birds: They function as amplifying hosts for the virus, although they are generally asymptomatic; however, diseases with high titer viremia and high mortality rate have been reported in Chukar partridges, pheasants, egrets, brilliant Ibis (Plegadis falcinellus), rock pigeons, sparrows, psittacine birds, ratites, ostriches, chickens, pigeons, Pekin ducks and cranes (Go, et al. 2014). In addition, EEE produces in birds: depression, drowsiness, a decrease in egg production and an increase in mortality (OIE, 2017).

Other species: The EEE virus has been described to cause the disease in cows, sheep, pigs, white-tailed deer and dogs (OIE, 2017).

Equine: In equines they function as sentinel animals of the disease, but tend to be the first to develop clinical signs and often serve as an indicator of onset of an outbreak or epidemic; therefore, rapid detection of VEEE in equine specimens is critical for controlling disease outbreaks in humans, horses, and other animal species (Go, et al. 2014). Equine disease occurs with: High fever, malaise, dullness, depression, poor appetite, aimless walking, often circling, head pressure at corners, blindness, stepwise and uncoordinated gait, recline, seizures, and death (in more than 80% of cases) (Mackay, 2017).

Humans: EEE in humans presents with fever, myalgia, headache, irritability, stiff neck, confusion, drowsiness or stupor, focal weakness, focal neurological deficiency, disorientation, tremors, paralysis of the cranial nerves, seizures, or altered mental status, Cerebral edema and / or death may occur.

Although some patients go into a coma, also, eyelid edema, abdominal pain, vomiting and diarrhea can be observed (CFSPH, 2017; Gill, et al. 2019). Also, VEEE produces multi-organ failure of the lung, liver, heart, brain and kidneys (Reddy, et al. 2008); Furthermore, hemorrhagic enteritis has been described (OIE, 2017).

Compared to infected humans, children under 15 years of age or over 50 years of age are more likely to develop EEE (Gill, et al. 2019).

RESULTS
DIAGNOSIS

An accurate and efficient diagnostic system for VEEE is important for clinical management, epidemiological surveillance systems, pathogenesis studies as well as for differentiating VEEE encephalitis from other arboviral diseases such as West Nile Virus, St. Louis Encephalitis, among other. (CDC, 2020).

There are different diagnostic systems that can be used to diagnose VEEE infection, which are: Direct and indirect detection methods.

Among the direct methods are**:** Viral Isolation, Electron microscopy, Molecular detection and sequencing. For research purposes, an attempt at viral isolation can be made, carrying out: Capture of mosquitoes and sentinel animals.

On the other hand, among the indirect methods of detection of antibodies against VEEE there are: Hemagglutination Inhibition, ELISA, Seroconversion: and the NTRP test to confirm the antibodies. (OIE, 2017; CDC, 2020).

TREATMENT

There is no specific treatment for EEE in humans, the treatment of severe conditions is symptomatic and supportive and includes hospitalization to provide intensive supportive care, such as intravenous fluids and nutrition, respiratory support and the prevention of secondary infections. (CDC. 2020)

CONTROL AND PREVENTION

The strategies are aimed at controlling the vector by eliminating mosquito breeding sites, using barrier methods such as metal grids, repellents, and using appropriate clothing to decrease exposure to the vector. Likewise, the establishment of an active epidemiological surveillance system in birds for the detection of possible deaths among wild populations, vector surveillance, identification of other potential vectors and surveillance in farm animals (horses, cows, dogs) and beings Humans are vital to prevent the spread of the disease; on the other hand, the OIE recommends vaccination in equines (Go et al. 2014; OIE, 2017)

CONCLUSIONS

It is important to carry out studies of detection of VEEE, since it is a fatal pathogen, with epidemiological importance, and of notable relevance in wildlife due to the affections in birds, in animal health because it causes death in equines and in public health due to fatal encephalitis in humans. Likewise, this article aims to publicize how VEEE is an important reemerging arbovirus in tropical and subtropical ecosystems, which makes its study important, since it is currently in epidemiological silence.

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