Rocio virus: an overview
El Virus Rocio: Una visión general

Joel Henrique Ellwanger, Valéria de Lima Kaminski, José Artur Bogo Chies
Laboratório de Imunogenética, Programa de Pós-Graduação em Genética e Biologia Molecular,

* Corresponding authors: Joel Henrique Ellwanger / José Artur Bogo Chies. Laboratório de Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS). Av. Bento Gonçalves, 9500, Campus do Vale, Porto Alegre - RS, Brasil. Phone: +5551 33086737.
E-mails: joel.ellwanger@gmail.com, jabchies@terra.com.br

© 2017 Todos los derechos reservados

Key words: emerging diseases, Flavivirus, outbreak, Rocio virus, viral encephalitis, viral ecology.

Introducción

Natural environments are rich in biodiversity and potentially harbor a large number of pathogens, including unknown viruses. Some of these microbes have the capacity to infect humans, although generally use only wild animals as hosts. Unbalances and changes in the natural environment that approximate hosts or pathogen vectors to the human population act as facilitators to the emergence of new infectious diseases in humans (1-5). In addition, the simple fact of humans penetrate into wild natural environments where pathogens are naturally found facilitates infections from wild animals to humans. For example, a study (6) performed in the Netherlands showed that active forestry workers are at high risk to develop Hemorrhagic fever with renal syndrome and Lymphocytic choriomeningitis, both viral diseases. We can also assume that people became more susceptible to infections traveling to regions where an endemic disease occurs or visiting natural environments where vectors of pathogens are found.

Abstract

Rocio virus (ROCV) was identified as responsible for an outbreak of human encephalitis viral in Brazil between 1975 and 1980. Currently, ROCV is considered an emerging virus and represents a constant threat to the Brazilian population, which at any moment may suffer from a new outbreak of encephalitis by ROCV. Despite the importance of this pathogen, little is known about its biological aspects. Aiming to draw attention to this virus, in this review we described epidemiological, pathological, morphogenetic, and ecological aspects about ROCV. Some historical facts regarding the virus are also presented in this article.

Key words: emerging diseases, Flavivirus, outbreak, Rocio virus, viral encephalitis, viral ecology.
Epidemiology and historical facts

ROCV belongs to the Flavivirus genus, as well as other well-known viruses, like Dengue, Saint Louis encephalitis and Yellow Fever (12). It is considered an emerging zoonosis in Latin America (13). Except the data accessed on experimental conditions, all information about ROCV were obtained through the analysis of an outbreak of human acute encephalitis that occurred in the Vale do Ribeira and Baixada Santista regions (São Paulo State, southeastern Brazil). Although the outbreak only have been detected in 1975, it is likely to have already started in 1973. The end of the outbreak occurred in 1980 (12, 14). Five cases of infections by ROCV were registered outside the São Paulo State during the outbreak, all of them in Paraná, a state bordering São Paulo (15). The outbreak of enROCV affected 1,021 people, caused approximately 100 deaths, and left sequelae in more than 200 individuals affected by the disease (12). The mortality rate of the disease was estimated at 13% (16). Adult males (aged 15-30 years) were the most severely affected (16, 17). Soon after the end of the outbreak, it was suggested that out-of-door exposure during routine work could be one of the causes for this group have been the most affected by enROCV (17).

ROCV was isolated and identified for the first time in 1975 from samples of the central nervous system (CNS) tissue of a 39-year-old male who died from the disease. The name "Rocio" was given because it was the name of the village where the patient lived, located in the city of Iguape (São Paulo State, Brazil). Two sentinel mice exposed to the environment of the outbreak were infected by ROCV. Moreover, the virus was also detected in a Rufous-collared sparrow collected in the same region (18). This data showed the circulation of the ROCV and its capacity to infect different animal species in the ecological system where the outbreak occurred.

After the end of the outbreak in 1980, little evidence indicated the circulation of the ROCV among Brazilian population. Tavares-Neto et al. (19) identified in 1984 the presence of antibodies against ROCV in a 12-year-old girl from the village of Corte de Pedra (Bahia State, Brazil). Iversson et al. (20) described serological findings (IgM antibodies) against ROCV in two children from the rural area of the Vale do Ribeira. Sera used for the analyzes were collected in 1987. Subsequently, Straatmann et al. (15) reported eight cases of infections by ROCV in the Brazil. The authors drew attention that these serological findings should be interpreted with caution. This prudence is needed due to the possibility of antigenic cross-reactivity with other flaviviruses. Recently, de Figueiredo and Figueiredo (21) cited two cases of ROCV infection (detected in 2010) in individuals from the city of Manaus (Amazon State, Brazil). Manaus is located at a distance greater than 2,000 km from the original enROCV outbreak region. Although scarce, these data indicate the circulation of the ROCV in Brazil, as well as the potential to infect different animal species in the ecological system where the outbreak occurred.

Vectors and transmission

Mosquitoes are the vectors of the ROCV. Different authors considered Aedes scapularis and Psorophora ferox as potential vector species of the virus (24-27). Laporta et al. (27) showed that people are highly exposed to bites by Aedes scapularis and Psorophora ferox in the region where the enROCV outbreak occurred. However, these species are present and can be considered potential vectors of the ROCV in other regions where there are no recorded cases of the disease, as the states of Goiás (28) and Rio Grande do Sul (29). Culex mosquitoes may also be considered as potential vectors of the ROCV (17).
There is no evidence of other natural routes of ROCV transmission besides mosquito bites. Lopes et al. (16) drew attention to the fact that when assessing families living where the enROCV outbreak occurred, in 75% of cases only one family member got sick. The same authors described that there are no reported cases of infections among the medical staff who took care of the patients during the outbreak. Iversson et al. (30) also found no higher anti-ROCV antibodies prevalence among persons cohabiting with patients affected by enROCV as compared to other individuals. Such evidence indicates that there is no direct ROCV transmission from human to human.

The inability of ROCV to be transmitted between humans without the interference of specific vectors suggests that this pathogen is not able to sustain long epidemics. To cause a long epidemic, in addition to transmission from non-human animals to humans, many cycles of transmission between humans would also be necessary (31). Although the natural ROCV route of transmission appears to be restricted to mosquito bites, other possible routes of transmission should not be neglected. For example, early in the Zika virus epidemic in Brazil, it was believed that the virus was transmitted only by the bite of mosquitoes. However, currently it is known that other forms of transmission, such as sexual transmission, are also possible (32). Laboratory infections by ROCV were also reported (33). It is believed that transmissions in the laboratory environment may have been caused by aerosol, during the manipulation of samples with large viral loads in inappropriate biosafety conditions (23).

### Structural and molecular characteristics

ROCV is morphologic and morphogenetic similar to other flaviviruses. Viral particles are spherical (34) and their size range from 34 to 43 nm (34, 35). In agreement with Harisson et al. (36), mature virus particles have a mean diameter of 39 nm. Using an animal model, Tanaka (35) showed that in the brain infected by ROCV, viral particles are found in the light of the reticular system of the cytoplasm and in the cisterns of the Golgi complex. Infected cells showed no mitochondrial changes. Moreover, the same author has found no evidence of the participation of the nucleolus in ROCV replication. A complete list of ROCV proteins and their corresponding lengths can be found in the studies of Junglen et al. (37) and Medeiros et al. (38).

Figueiredo et al. (39) described a Reverse Transcription – Polymerase Chain Reaction (RT-PCR) method to identify Brazilian flavivirus-ese, including ROCV. Posteriorly, Medeiros et al. (38) sequenced and characterized the entire ROCV genome. ROCV is a single-stranded and positive-sense RNA virus. The viral genome is composed of 10,794 nucleotides including an open reading frame of 10,275 nucleotides. This open reading frame is flanked by a 5' non-coding region of 92 nucleotides and a 3' non-coding region of 427 nucleotides. Interestingly, this was the first study to sequence and characterize the complete genome of a Brazilian Flavivirus. Baleotti et al. (40) performed a phylogenetic study of 15 strains of 10 Brazilian flaviviruses based on nucleotide and amino acid sequences of the NS5 gene. In this study, the authors grouped the viruses into three main branches: (I) dengue, (II) Japanese encephalitis virus (JEV) complex, and (III) yellow fever branches. ROCV belongs to the JEV branch according to neighbor-joining and parsimony phylogenetic trees. Medeiros et al. (38) carried out multiple protein and phylogenetic analyses and reinforced the close relationship between ROCV and Ilheus virus (ILHV), as previously described by other authors (40, 41). However, despite such close relationship, data published by Medeiros et al. (38) confirmed that ROCV is a distinct pathogen from ILHV.

### Infection and pathogenesis

As well as other flaviviruses, ROCV can cross the blood-brain barrier and cause encephalitis (42-44). The virus incubation period is 7–14 days. Young men are the individuals most affected by the disease (12). Signs, symptoms, and sequelae of the ROCV infection are described in Table 1.

<table>
<thead>
<tr>
<th>General signs and symptoms</th>
<th>Encephalitis signs / neurologic symptoms (generally appear later)</th>
<th>Sequelae</th>
</tr>
</thead>
<tbody>
<tr>
<td>abdominal distention, aerophonia, anorexia, coma with respiratory complications, falling, fever, headache, hyperemia of the oropharynx and conjunctivae, lacrimation, lassitude, lethargy, malaise, mastication, myalgia, nausea, photophobia, stupor, urinary retention, vomiting, weakness</td>
<td>blindness, consciousness alterations, convulsions, deafness, dysarthria, dyslalia, meningeval irritation, motor abnormalities (especially gait and impaired equilibrium), reflex disturbances</td>
<td>disturbances in visual, auditory and olfactory acuity, dysarthria, dysphagia, memory disturbances, motor abnormalities (especially gait and impaired equilibrium), motor incoordination, paresthesia, sphincter incontinence, strabismus</td>
</tr>
</tbody>
</table>

*This table lists many signs or symptoms known or possible to happen during ROCV infection. However, signs/symptoms/sequelae do not necessarily occur together and/or in all patients (14, 16, 42, 44).*

---

**Table 1. Signs, symptoms, and sequelae of a ROCV infection**
The amount of knowledge regarding ecological aspects about ROCV is scarce. For example, the reasons for the appearance and disappearance of the ROCV in the Vale do Ribeira are still a mystery (44). Since the end of the enROCV outbreak in the 1980s, some authors believed that besides mosquitoes acting as vectors, birds were also involved in the transmission cycle of the ROCV as natural hosts (25). This is quite likely because ROCV was found in a rufous collared sparrow (Zonotrichia capensis) in the country of Serté Barras (São Paulo State, Brazil) (18). Currently, it is still believed that wild birds are responsible for keeping the virus in the form of a naturally occurring zoonosis (44, 51).

Ferreira et al. (52) called attention to the fact that there are records of the enROCV in people who do not have left the area around their homes. The authors also presented data suggesting the circulation of the ROCV among wild birds in the Atlantic Forest region of the São Paulo State. According to these authors, the pathogen could move from São Paulo State to other Brazilian regions through migratory birds. The circulation of the virus through birds would explain the cases of the enROCV in humans, as highlighted by Figueiredo (51). This possibility makes the reemergence of the ROCV a permanent threat to the Brazilian population.

Importantly, other animals, besides mosquitoes and birds, can host and be involved in the transmission cycle of the ROCV. Two strains of the virus were isolated from sentinel mice exposed in the city of Cananéia (São Paulo State, Brazil) (18). Casseb (53) found a prevalence of 5.61% for ROCV antibodies in domestic herbivores in the Pará State. ROCV also circulates among water buffaloes (Bubalus bubalis) in Brazilian Amazon (54). In addition, horses seem to be important hosts of the ROCV. One equine seropositive for ROCV was found by Pauvolid-Corrêa et al. (55) in the Brazilian Pantanal region. Silva et al. (56) reported serological data suggesting that ROCV previously circulated among horses in different regions of Brazil (northeast, west-central, and southeast). Considering the presence of antibodies anti-ROCV in horses from different parts of Brazil, these authors stand out that other outbreaks of ROCV may be occurring without being detected. 

**Ecological aspects and surveillance**

Ferreira et al. (52) called attention to the fact that there are records of the enROCV in people who do not have left the area around their homes. The authors also presented data suggesting the circulation of the ROCV among wild birds in the Atlantic Forest region of the São Paulo State. According to these authors, the pathogen could move from São Paulo State to other Brazilian regions through migratory birds. The circulation of the virus through birds would explain the cases of the enROCV in humans, as highlighted by Figueiredo (51). This possibility makes the reemergence of the ROCV a permanent threat to the Brazilian population.

Recently, Franca et al. (49) studied the immune response induced by ROCV using an experimental mouse model. In this study, interleukin 33 (IL-33) signaling was essential to attenuate the development of the enROCV by downregulating the expression of nitric oxide synthase in the CNS.

Results obtained with the development of a vaccine against ROCV were published in 1980. The vaccine was tested in humans. However, the immunogenicity of the vaccine was not satisfactory (50). To date, there is no effective vaccine against ROCV. Figueiredo (51) pointed out that the development of a broad-reactive JEV complex vaccine offering protection to the ROCV and other viruses of the same complex is required.
Recently, Neves and Machado (57) warned about the re-emergence risk of the ROCV in Brazil and highlighted the importance of epidemiological surveillance of the ROCV circulation. We add that this surveillance must be carried out both in animals (wild or domestic) and humans. Khan et al. (58) developed a DNA microarray platform (SMAvirusChip) for screening a large set of viruses transmitted by small mammals and arthropods, including ROCV. Methods like this can be very useful for the early detection of the ROCV circulation in the population.

ROCV is classified as an emerging virus (51). The emergence or re-emergence of a pathogen among the human population is a complex event. For example, it is believed that the emergence of the HIV/AIDS in Africa has been caused by a series of ecological and social changes (59). Similarly, according to Pedroso and Rocha (5), the contributing factors to the emergence of the ROCV (among other diseases) were: ecological changes, economic development, and manipulation of land (classified as major factors).

As specific factors, the authors quote agriculture, dams, deforestation and reforestation, changes in water ecosystems, floods and droughts, famine, and climate change.

Zoonoses are infectious diseases transmitted naturally between humans and non-human animals (wild or domestic) (60). Taking this definition into consideration, ROCV can be considered a zoonosis. In agreement with Slingenbergh et al. (60), the emergence of zoonoses and the spread of diseases are usually caused by human activity, being humans also responsible for prevention of such situations. For this, the fight against emerging diseases requires the effort of professionals from different areas (61). This is due to the complex and different socioecological factors that influence the dissemination of a pathogen among the human population. In other words, the emergence of diseases can be considered the result of disturbances in human, animal, and environmental health (62). In our point of view, ROCV is within this context. However, in order to prevent the possible re-emergence and spread of the ROCV among humans, the study and the better understanding of the basic ecological aspects of this virus are necessary (63).

**Conclusion**

The diversity of the Brazilian nature hides several pathogens. Most of them will probably never cause infections in humans. However, sometimes, due to socioenvironmental disturbances, some new infections, originally derived from wild or even domesticated animals, may emerge amongst human populations. This was the case of the ROCV, which emerged in Brazil in 1975 and caused an outbreak of human encephalitis. Due to the lack of knowledge about this pathogen, it is not possible to known with certainty which factors contributed to its emergence and to its subsequent “disappearance”.

Morphogenetically, ROCV resembles other flaviviruses. Data obtained from experimental studies and through the analysis of samples from people who died of enROCV indicate that ROCV causes a very complex and serious pathological state, and this must be taken as an important alert to our health services. Measures for monitoring virus circulation between human and non-human animals are required. The development of a vaccine against ROCV is also essential to prevent a new outbreak of encephalitis among Brazilian population.

From a scientific perspective, the history of the ROCV is quite interesting. We believe that this overview will help to gather relevant information on the historical, pathological, epidemiological, morphogenetic, and ecological aspects about ROCV. Moreover, we hope that this study will help to raise the scientific community’s interest about ROCV and to alert public health authorities regarding the importance of surveillance of this pathogen.

**Referencias bibliográficas**

Referências bibliográficas