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Artículo de revisión

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

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Resumen

El virus Rocío (ROCV) causó un brote epidémico de encefalitis viral en Brasil entre 1975 y 1980. En la actualidad este virus es considerado un agente emergente y representa una constante amenaza a la población brasilera, la cual en algún momento podría sufrir otro brote de infección por este virus. A pesar de la importancia de este patógeno, poco se sabe sobre sus aspectos biológicos. Así, con el objetivo de despertar el interés de la comunidad científica sobre este vírus, en la presente revisión describimos sus aspectos: epidemiológico, patológico, morfogenético y ecológico. Además algunos hechos históricos son relatados en este artículo.

Palabras clave: Enfermedades emergentes, Flavivírus, brote epidémico, virus Rocio, encefalitis viral, ecologia viral.

Abstract

Rocio virus (ROCV) was identified as responsible for an outbreak of human encephalitis 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.

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.

Many viruses that until recently were absolutely unknown, are currently identified, classified, and with their ability to infect humans well recognized. This is the case of the Human Immunodeficiency Virus (HIV), discovered in the early 1980s and currently hosted by nearly 37 million people worldwide (7). Some viruses cause infections in a seasonal pattern, such as Influenza viruses (8). Other pathogens arise, cause few/isolated infections and then disappear, as is the case of the Sabiá virus (SABV). To date, only two natural infections by SABV have been reported, both in Brazil and with fatal outcomes (9-11). Little is known about the biological aspects of this pathogen. Similarly to the pathogens that cause isolated human infections, some viruses arise, cause outbreaks or epidemics, and then virtually no additional cases of new infections are recorded over a long time. Rocio virus (ROCV) is an example of pathogen that showed this infection behavior.
ROCV was identified and described in Brazil during an outbreak of human encephalitis (encephalitis by ROCV - enROCV). This outbreak began in the middle of the 1970s and ended at the beginning of the 1980s. ROCV can cause a serious pathological condition and is a potential causative agent of new outbreaks/epidemics in the Brazilian population. However, similarly to the SABV, little is known about ROCV. In addition, this pathogen can be considered a neglected virus by both scientific and public health authorities. Aiming to draw attention to this pathogen, in this article, we reviewed the biological aspects of the ROCV and synthetically present some historical facts about this virus.

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). Despite 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 humans with anti-ROCV antibodies from four cities of the Bahia State (Brazil). Although these data indicated the circulation of the ROCV in 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 different regions of Brazil after the end of the outbreak in 1980.
Few authors have focused on the historical aspects of the ROCV outbreak. Thus, data on these aspects are also scarce. However, Villela and Natal (22) performed a relevant work addressing the coverage of the media on the ROCV outbreak. The authors note that in face of the reports released by the print media about enROCV, tourists stopped going to the affected area. Consequently, a crisis occurred in the local market, causing revolt in the traders. Recently, Azevedo (23) made an important report for the Brazilian newspaper O Globo recalling historical facts about the outbreak. Interestingly, the journalist described reports from people who had a prominent role in the study and combat of the ROCV during the outbreak.

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 mosquitos 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 nucleon in ROCV replication. A complete list of ROCV proteins and their corres-pondent 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 flaviviru-ses, including ROCV. Posteriorly, Medeiros et al. (38) sequenced and characterized the entire ROCV genome. ROCV is a single-stran-ded 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 1. Signs, symptoms, and sequelae of a ROCV infection*.

General signs and symptoms	Encephalitis signs / neurologic symptoms (generally appear later)	Sequelae
abdominal distention, aerophobia, 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	blindness, consciousness alterations, convulsions, deafness, dysarthria, dyslalia, meningeal irritation, motor abnormalities (especially gait and impaired equilibrium), reflex disturbances,	disturbances in visual, auditory and olfactory acuity, dysarthria, dysphagia, memory disturbances, motor abnormalities (especially gait and impaired equilibrium), motor incoordination, paresthesia, sphincter incontinence, strabismus

*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).

Rosemberg (45) studied eight human cases of enROCV. In the brain, the more damaged structures were thalamus, dentate nucleus, substantia inominata, brain stem, spinal cord, and basal nuclei. Gray matter was the most injured region. In the thalamus, a definitive loss of nerve cells was observed. Interstitial mononuclear infiltration, microglial proliferation, thalamic inflammatory necrosis, and perivascular lymphocytic cuffing were described amongst the pathological findings. It is important to highlight that, to the best of our knowledge, this was the first author to demonstrate the neuropathological findings of the infection by ROCV in humans, having already published preliminary findings in 1977 (46). Moreover, Harrison et al. (36) demonstrated that ROCV could cause changes beyond the CNS. In their study, using hamsters as an animal model, heart and pancreas were the organs most affected by the infection.

Infection and pathogenesis of the ROCV were addressed by few authors until now, although relevant results were obtained from these studies. For example, Barros et al. (43) evaluated the contribution of cytokines and nitric oxide (NO) to the outcome of infections by Brazilian flaviviruses and to the aspects of the replication of these pathogens, including ROCV, and their results suggested an absence of NO involvement in ROCV infectivity. Posteriorly, Dias de Barros et al. (44) using the Balb/C mouse strain found that ROCV induces neuronal degeneration and apoptosis in the CNS, and that this phenomenon was associated with an inflammatory process. In relation to neuronal death caused by ROCV, it is still debated whether neuronal death is caused directly by the viral replication or if it is the result of the inflammatory process induced by the viral infection (47). In a hamster model to study the persistence of the ROCV infection/pathogenesis, Henriques et al. (48) showed that ROCV can be found in viscera, brain, blood, serum, and urine. The virus was also detected by quantitative RT-PCR in the brain, liver, and blood (as long as three months after infection). In addition, the virus caused histopathological changes in the liver, kidney, lung, and brain. Viral antigens were detected in these organs up to four months after infection. Moreover, ROCV induced a strong immune response in the animals. These results indicate that ROCV affects different organs than the brain in a persistent way. However, these data should be interpreted with caution, since they came from an animal model (using intraperitoneal infection). It is not known whether these results would be similar in humans.

Chávez et al. (47) demonstrated in mice that the CC-chemokine receptor 5 (CCR5) and macrophage inflammatory protein (MIP-1 α) are important in the outcome of the ROCV infection. Shortly, infecting CCR5 and MIP-1 α knockout and wild-type mice with ROCV, knockout mice survived longer and had reduced brain inflammation as compared to the wild-type animals. Based on these data, the authors suggested that CCR5/MIP-1 α axis contributes to the migration of infected cells to the brain and affects ROCV pathogenesis.

Recently, Franca et al. (49) studied the immune response induced by ROCV using an experimental mice 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.

Ecological aspects and surveillance

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 Sete 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 individuals who were not exposed to high-risk areas of infection by ROCV, 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.

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.

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