

OCCURRENCE OF RABIES VIRUS IN WILD POPULATION OF MARMOSETS *CALLITHRIX GEOFFROYI* (HUMBOLDT, 1812) IN SOUTHEASTERN BRAZIL

LA APARICIÓN DE VIRUS DE LA RABIA EN LA POBLACIÓN SILVESTRE TITÍES DE MONO TITÍ DE CABEZA BLANCA (HUMBOLDT, 1812) EN EL SURESTE DE BRASIL

OCORRÊNCIA DO VÍRUS DA RAIVA NA POPULAÇÃO SELVAGEM DE SAGUIS *CALLITHRIX GEOFFROYI* (HUMBOLDT, 1812) NO SUDESTE DO BRASIL

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ABSTRACT: Background: Zoonosis, which causes acute and lethal encephalitis, is transmitted through the inoculation of the virus present in the saliva of mammals of several species. In Brazil, wild rabies is a challenge for epidemiological surveillance and a significant increase has already been observed in human cases. Some of the main wild reservoirs are: wild dog (*Cerdocyon thous*), marmosets (*Callithrix* sp) and hematophagous bats (*Desmodus rotundus*). Considering cases of positivity in marmosets in the Northeast of Brazil, together with the increasing number of cases in wild animals, this work aimed to survey the occurrence of the virus in a wild population. The animals were necropsied from the tramway ES-060, all of the *Callithrix geoffroyi*. The material collected for analysis of the rabies virus consisted of brain, which was conditioned and identified for later analysis at the Institute of Agricultural and Forest Defense of Espírito Santo (IDAF). The 44 samples gave negative results. The study suggests that the occurrence of the virus should be investigated in other localities, especially in regions closer to sites that have already been reported cases of rabies, and that the use of trampled animals is feasible for a better understanding of wildlife health.

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Keywords: Zoonosis. Nonhuman primates. Wild animals. Wild rabies.

RESUMEN: La zoonosis causante de una encefalitis aguda y letal, la rabia es transmitida por medio de la inoculación del virus presente en la saliva de mamíferos de diversas especies. En Brasil, la rabia silvestre es un desafío para la vigilancia epidemiológica y ya se ha constatado un aumento significativo en casos humanos. Algunos de los principales depósitos silvestres son: perro de matorral (*Cerdocyon thous*), saguis (*Callithrix* sp) y murciélagos hematófagos (*Desmodus rotundus*). En el caso de los animales silvestres, teniendo en cuenta casos de positividad en saguis en el Nordeste de Brasil, junto con el creciente aumento de casos en animales silvestres, este trabajo tuvo como objetivo hacer un levantamiento de la ocurrencia del virus en una población silvestre. Se han necropsiado 44 animales, procedentes de atropellamiento en la carretera ES-060, todos de la especie *Callithrix geoffroyi*. El material recolectado para análisis del virus rábico consistía en encéfalo, que fue acondicionado e identificado para posterior análisis en el Instituto de Defensa Agropecuaria y Forestal del Espírito Santo (IDAF). Las 44 muestras dieron resultados negativos. El estudio sugiere que la ocurrencia del virus debe ser investigada en otras localidades, principalmente en regiones más cercanas a los locales que ya se han notificado casos de rabia, y que el uso de animales atropellados es viable para la mejor comprensión de la salud de la vida silvestre.

Palabras-clave: Zoonosis. Primates-no humanos. Fauna. Rabia silvestre.

RESUMO: Zoonose causadora de uma encefalite aguda e letal, a raiva é transmitida por meio da inoculação do vírus presente na saliva de mamíferos de diversas espécies. No Brasil, a raiva silvestre é um desafio para a vigilância epidemiológica e já foi constatado um aumento significativo em casos humanos. Alguns dos principais reservatórios silvestres são: cachorro-do-mato (*Cerdocyon thous*), saguis (*Callithrix* sp) e morcegos hematófagos (*Desmodus rotundus*). Levando em consideração casos de positividade em saguis no Nordeste do Brasil, juntamente com o crescente aumento de casos em animais silvestres, este trabalho objetivou fazer um levantamento da ocorrência do vírus em uma população silvestre. Foram necropsiados 44 animais, provenientes de atropelamento na rodovia ES-060, todos da espécie *Callithrix geoffroyi*. O material coletado para análise do vírus rábico consistia em encéfalo, que foi acondicionado e identificado para posterior análise no Instituto de Defesa Agropecuária e Florestal do Espírito Santo (IDAF). As 44 amostras deram resultados negativos. O estudo sugere que a ocorrência do vírus deve ser investigada em outras localidades, principalmente em regiões mais próximas aos locais que já foram notificados casos de raiva, e que o uso de animais atropelados é viável para a melhor compreensão da saúde da vida silvestre.

Palavras-chave: Zoonose. Primatas-não-humanos. Animais selvagens. Raiva silvestre.

INTRODUÇION

In the last five decades 60% of the human pathogens studied and 75% of the emerging diseases that affected humans are derived from zoonoses (SILVA, 2009). Although rabies has been known for a long time, it is one of the zoonoses that still causes serious public health problems in many developing countries. It is an infectocontagiosa disease caused by a neurotropic virus that affects all the mammals, leading to an acute encephalomyelitis with high mortality (KOUZNETZOFF *et al*, 1998; KOTAIT *et al*, 2009).

The transmission of the virus can be by the saliva of infected animals, resulting in an acute, progressive encephalomyelitis with a high level of mortality (KOTAIT *et al*, 2009). The virus belongs to the Mononegavirales order of the family Rhabdoviridae and genus Lyssavirus, with 7 genotypes currently identified, and in Brazil only genotype 1 (Rabies 1.2 virus - RABV) has been described (BADRANE *et al*, 2001). This genotype is the most widely distributed and is of major epidemiological importance because of its association with a greater number of cases of Lyssavirus encephalitis in humans compared to other genotypes (OLIVEIRA, 2009).

It has features like RNA of simple enveloped tape and morphology that resembles a revolver projectile, with size of 75nm of diameter and of 100 to 300nm of length. Its wrapping has a double layer of phospholipid membrane with glycoprotein spicules with a size of approximately 9nm. (KOTAITET *et al*, 2007, ITO *et al*, 2001, MAPA, 2005, FAVORETTO *et al*, 2002).

For the post mortem diagnosis there are different techniques, one of them is the one of direct immunofluorescence, is realized by the examination of the encephalon of the suspect animal, using fluorescent antibodies against the rabies virus. The biological test for viral isolation is the confirmatory examination recommended by the Ministry of Health and is carried out by means of inoculation in mice or cell culture (LEVINSON, 2010). The main wild reservoirs of the rabies virus in Brazil are *Cerdocyon thous*, the marmoset (*Callithrix* sp) and the hematophagous bat (*Desmodus rotundus*) (KOTAIT *et al*, 2007; SODRÉ *et al.*, 2010; AGUIAR *et al*, 2011).

According to the World Health Organization (WHO) every 15 minutes a person dies infected with rabies virus. According to the Secretariat of Health Surveillance of the 428 cases of human rabies registered in Brazil during the years of 1999 and 2012, 8% were caused by aggressions from wild animals, including: bats, marmosets, cubs of the bush and racoon (BRASIL, 2013). Wild rabies has become more important because of the synanthropic habits of wild animals, which over time have reached urban areas (KOTAIT *et al*, 2007). Laboratory diagnosis is one of the fundamental surveillance tools (BRASIL, 2012). Between 1990 and 2010, 35 cases of rabies were reported in the state of Ceará (FAVORETTO *et al*, 2006; State Secretariat of Health of Ceará, 2010), where these animals represent a major problem in relation to disease control (MORAES *et al*, 2010), mainly due to their possession as pets (AGUIAR *et al*, 2011).

From 2000 to 2009, 163 cases of rabies were recorded in humans in Brazil, and 5 of these had as their source of infection primates (WADA *et al*, 2011). Rabies transmission can also occur among individuals of different species that are not considered natural reservoirs of the virus and is a phenomenon known as "spillover" and results from interspecies contact that may lead to the determination of new virus variants (CORONA, 2016).

Even though all mammals are susceptible to infection, only a few species are of greater importance as a reservoir of disease (CDC, 2015). It is known that non-human primates are reservoirs of several infectious agents with implications for public health (SOUZA, 2012). Due to the great diversity of species in Brazil, it is necessary that researches with the various species be carried out more frequently, aiming at a better knowledge of pathogens and related diseases, which will serve as a basis for species conservation and prevention of zoonoses (CATÃO-DIAS, 2003).

Some antigenic and genetic studies with rabies virus isolates in this species have shown that the variant found is the most divergent already isolated in Brazil (FAVORETTO *et al*, 2002). *Callithrix* is a species of seagrass found mainly in Brazil (ACCIOLY, 2000), and the *Callithrix geoffroyi* (HUMBOLDT, 1812) is popularly known as the endemic white-fronted marmoset of the Atlantic Forest. It is a

neotropical primate that occurs in southern Bahia, eastern region of Minas Gerais and, practically, in every state of Espírito Santo (PASSAMANI AND RYLANDS, 2000). That species invests much of the time foraging in the hottest hours of the day. Their feeding consists of fruits during the morning, vegetable sap, in the early hours of the day and in the late afternoon small reptiles and insects, which constitute a source of protein (PASSAMANI AND RYLANDS, 2000).

MATERIAL E METHODS

Samples of brains were collected in 44 *C. geoffroyi* specimens from the runway, being 44 animals, 27 were males and 17 females. Although they were runners, they all had their skull intact. The material collected during the necropsy was packaged and identified according to the biosafety standards (Technical Commission of Biosafety, 2005) and transported under refrigeration from 2 to 8°C. The samples were stored at -20 ° C in a freezer and subsequently analyzed by the direct immunofluorescence technique and biological test by intracerebral inoculation in mice.

The procedures that were used in the preparation and reading of the slides followed the guidelines recommended by Dean *et al.*, (1996) with the changes in the incubation period of the conjugate proposed by Roehe *et al.*, (2002). Reaction reading was performed on the Zeiss® brand binocular immunofluorescence microscope, equipped with a HBO 50 mercury vapor lamp, VGI excitation filter and Zeiss® 43 barrier filter, at 400X magnification in the dark field. Each impression was independently examined and classified by two observers under double-blind system, considering the mode of control of measurement pathways (Rouquayrol and Almeida 2003).

RESULTS AND DISCUSSION

Studies have shown that temperature variation has little interference in rabies tests, since carcasses of animals that were at high temperatures and advanced stage of putrefaction presented results positive when they were tested (MCELHINNEY *et al.*, 2014; BELTRAN *et al.*, 2014), thus discarding the possibility that the results of this

study are false negatives. All 44 samples were negative and despite the result, it is important to note that in Brazil the disease is endemic in several parts of the country, and in the wild cycle a larger number of canines and non-human primates are observed (ALBAS *et al*,2011) .

In the state of Ceará, wild animals that have emergent importance are the hand-peeled (*Procyon cancrivorous*) and marmosets (*Callithrix sp*) (ARAUJO *et al*, 2008), where it is common to breed *C. jacchus* as a pet and have already been registered cases of aggressions with reports of cases of human rabies. In 2005 alone, a total of 44 deaths were recorded in the North and Northeast regions of Brazil, including cases of marmoset attacks on humans kept as companion animals (WADA *et al*, 2011), and due to the proximity to the regions where they are registered positive cases, it is necessary to investigate and control the viral agent in the adjacent populations.

With a broad and complex mammals, each region of Brazil has its kind of interest involving cases positive for rabies (SILVA *et al*, 2012). The close contact between humans and marmosets is a risk factor (AGUIAR *et al*, 2011), mainly because these animals adapt well to urban environments and arouse the interest of approach in parks and areas where they occur. Determining the incidence and distribution of infectious pathogens in wild populations is an urgent need (OTTO, 1998), mainly because the risk of the occurrence of a particular disease and its impact on biodiversity depends on the epidemiological knowledge of the agents and the relationships with the possible hosts (BALLOU *et al*, 2000). Rabies virus is of great importance in public health, but it must also be taken into account as a threat to the conservation of various species of mammals (GASCOYNE *et al*, 1993).

The monitoring of viral circulation in wild animals is still very scarce, and in 20 years less than 150 samples were analyzed in Espírito Santo until 2013 (MARINE, 2015), in this study, 44 animals were analyzed in less than one year , suggesting that the use of carcasses for scientific purposes is of great value to help in monitoring the health of these species without requiring that these animals be captured in their habitat, taking advantage of materials that would be discarded.

FINAL CONSIDERATIONS

The circulation of rabies virus in wild animals has been reported in marmosets in Brazil, however, the circulation of this virus and the actual characteristics should be better investigated. The use of biological material from trampled animals facilitates access to information and research to understand, monitor and know the infectious agents of populations, without the need to collect animals in free life, using environmental loss for scientific gain

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