Is the "*in vivo*" transfection of antibodies against rabies virus a potential mechanism for rabies treatment?

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Rabies is a worldwide zoonotic disease caused by rabies virus (RABV). According to the World Health Organization, more than 55,000 people still die of rabies every year. The domestic dogs are the major responsible for transmission of the virus for people.The human rabies transmitted by dog is considered a neglected disease. Rabies can be controlled by correct prophylaxis, but since the first case of the rabies cure, different studies have been made to search potential mechanisms of rabies treatment. This work was carried out in order to examine the toxicity and the efficiency of "*in vivo*" transfection of antibodies against rabies. In this study mice were infected by intracerebral route with 100, 10, 1 and 0,1 lethal doses (LD50) of RABV isolated from dog. The negative control group was inoculated with minimum essential medium (MEM). All mice were submitted to "*in vivo*" transfection of antibodies against rabies after 24 hours. For the transfection, was used a cationic reagent, the lipofectamine and a polyclonal antibodies against rabies. Food and water were offer "at libidum" for the inoculated mice that were observed daily and the dates of death were recorded. The mice infected with 100 and 10 LD50 died 6 to 9 days post infection and the mice infected with 1 and 0,1 LD50 survived of the infection. None mice of the negative control died after the transfection. Our results show that the "*in vivo*" transfection of antibodies against rabies is effectiveness when low infectious doses of RABV is inoculated in mice, in addition the lipofectamine and the antibodies against rabies are not toxic for use by intra-cerebral route. In conclusion the "*in vivo*" transfection is a potential treatment mechanism of the human rabies transmitted by dogs, but more experiments could be realized to confirm these results.

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Neutralizing antibodies in serum and CSF in *antemortem* diagnosis of suspected cases of rabies received in the laboratory diagnosis of rabies at the Pasteur Institute from january 2011 to april 2013.

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Different techniques can be used for *antemortem* diagnosis in suspected cases of human rabies. The techniques for detecting virus in saliva, cerebrospinal fluid (CSF), hair follicle, corneal impressions include direct immunofluorescence (DIF), RT-PCR and gene sequencing. However, the neutralizing antibody (VNA) titers in serum and CSF are important to ascertain how the individual is responding immunologically against rabies virus infection. The aim of this study was to analyze the techniques SFIMT and RFFIT, the presence of VNA in serum and CSF of patients suspected of rabies that had no history of the serovaccination. From January 2011 to April 2013 there were received serum and/or CSF of 19 suspected cases of rabies sent to the serology laboratory for the presence of VNA in serum and CSF by Rapid Fluorescent Inhibition Test (RFFIT) and Simplified Fluorescence Inhibition Microtest (SFIMT). Five cases were confirmed by the joint evaluation of virological and serological techniques. In three cases were confirmed the disease progress by monitored sampling on successive days. The highest titer of VNA was 3.59 IU/mL in serum sample of a case with a single sample, and 0.25 IU/mL in CSF samples from another case, and they were collected near the date of death. Some samples showed a low titre of VNA, which may be related to the collection period in relation to stage of disease, because in most cases the production of antibodies occurs late in cases of infection by rabies virus. These results show that the presence of VNA in samples of unvaccinated patients may be indicative of virus infection, and emphasize the importance of early diagnosis in suspected cases of rabies.

Hemolysis in serum samples as a factor of pre analytical variability in the evaluation of rabies virus neutralizing antibodies by the rapid fluorescent focus inhibition test (RFFIT)

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Among the factors that establish the pre-analytical variability, some studies report that hemolysis is the main interferential in several tests. However, no studies were found on immunofluorescence tests or that using cell cultures. The aim of this study was to evaluate the interference of different degrees of hemolysis serum samples in the evaluation of virus neutralizing antibodies (VNA) by rapid fluorescent focus inhibition test (RFFIT). We obtained 27 blood samples from dogs vaccinated for rabies. Hemolysis was caused by mechanical induction and freezing. Hemolysated sera were classified visually and by measurement of hemoglobin made by spectrophotometry. The evaluation of VNA in the samples was performed by RFFIT. The results were statistically analyzed by the Mann-Whitney test. Four samples and their hemolyzed aliquots were selected, added to BHK-21 cell and maintained at 37°C for 20 hours to evaluate the interference caused by hemolysis. Sera were classified as without hemolysis (up 0.5), 1 + (0.6 to 1.0), 2 + (1.1 to 1.5), 3 + (1.6 to 2.6), 4 + (2.7 g / dL or more). The results of VNA titers showed differences when we compared the serum without hemolysis with 2 +, 3 + and 4 + however, this difference was not statistically significant (P>0.05). The reading of the results was difficult in the first dilution of sera with 2 + hemolysis by irregular cell growth, and in sera with 3 + and 4 + for the formation of clusters and cell death. It is concluded that hemolysis can interfere with antigen-antibody or virus-cell binding and has a high degree of interference in sera with 3 + and 4 + by lysing the cell or changing its growth pattern.

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Evaluation of neutralizing antibodies in cats prime vaccinated for rabies

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In some countries, the number of pet cats is increasing and in some places already exceeds the number of pet dogs. The behavioral aspects of these animals as varied degree of dependence on humans, a greater number of individuals in the colonies and their predatory instinct increase the risk of infection by rabies virus. The aim of this study was to analyze the immune response of cats who would travel to the European Community and that received only one dose of cell culture rabies vaccine, in the triennium

2009-2011. We analyzed the request forms of virus neutralizing antibodies (VNA) titration. Were selected samples from animals that had received only one dose of vaccine until the date of blood collection. Information on age, race and period of vaccination and blood collection were evaluated. Serum samples were tested by Rapid Fluorescent Focus Inhibition Test (RFFIT) for determination of VNA. The animals with less than one year were considered young and aged greater than or equal to one year were considered adults. Titers of VNA = 0.50 IU/mL were considered as protectors. Of the total 120 samples, 90.8% (109) had protective titers of VNA, regardless of race, age or vaccination period. Approximately 9.2% (11) of the animals had titers of VNA lower than protective levels, independent of age and the period of vaccination and the collection of material. As for race, 88% (8) of the samples that were not protective bonds were mixed breed cats. It was concluded that there was satisfactory immune response in the animals analyzed. Studies are needed to evaluate immunity against other factors of the population, mainly socioeconomic, since most of cats are semi domiciled or feral, increasing the risk contact with the rabies virus.

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Rabies virus neutralization assays: comparison of three fluorescent inhibition tests in cell cultures

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The Rapid Fluorescent Focus Inhibition Test (RFFIT) and the Fluorescent Antibody Virus Neutralization Test (FAVN) are the rabies virus neutralization assays recognized by World Health Organization and the World Organization for Animal Health, to quantify rabies virus neutralizing antibodies (VNA). In the Pasteur Institute of Sao Paulo/Brazil, the Simplified Fluorescence Inhibition Microtest (SFIMT) is the method used for titration of VNA in serum samples of vaccinated individuals. The aim of this study is the comparison of VNA titration methods: FAVN, RFFIT and SFIMT. One hundred ninety-three sera samples from dogs and cats were analyzed by the three methods. The statistical tests used to compare the techniques were the McNemar test and Kappa coefficient (CI=95%) to qualitative analyses (<0.5 IU/mL and = 0.5 IU/mL) and Student's t-test for quantitative evaluation of mean of the VNA titers. The VNA titers ranged between 0.09 IU/mL to 7.79 IU/mL for FAVN test, 0.05 IU/mL to 9.55 IU/mL for RFFIT and 0.12 IU/mL to 3.70 IU/mL for SFIMT. The dilution factor values in LogD50 ranged from 0.48 to 2.38 (GM=1.57) for FAVN, 0.42 to 2.60 (GM = 1.79) for RFFIT and 1.17 to 2.68 (GM=2.08) for SFIMT. Qualitative analysis of the results showed considerable agreement between the tests (p-value=1.0; Kappa=0.73). In the quantitative analysis of VNA titers means, for FAVN (GM=1.68 IU/mL) the mean was numerically lower than RIFFT (GM=2.1 IU/mL), and between FAVN and SFIMT (GM =1.36 IU/mL) it was numerically higher. The determinations of diagnostic sensitivity and specificity between FAVN and RIFFT were 94.9% and 80.6% and between FAVN and SFIMT were 94.2% and 74.1% respectively. The FAVN, RFFIT and SFIMT showed good agreement, because statistics do not differ in their percentages in the evaluation of VNA.

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Deficiency in the humoral immune response to vaccine rabies virus in domestic dogs prime vaccinated

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The World Health Organization (WHO) and the Office International des Epizooties (OIE) consider as being the reference *status* of protection against rabies titers of virus neutralizing antibodies (VNA) = 0.5 IU/mL to maintain a protective immune response in animals. The aim of this study was to evaluate, according to age, race and period of vaccination and blood collection, the immune response in dogs prime vaccinated with rabies cell culture vaccine. Based on request forms, 432 samples of animals received at the Pasteur Institute of Sao Paulo at the period of 2009 to 2011, those receiving a single dose of vaccine by the time of blood collection were analyzed. We evaluated the information on age, race and period of vaccination until to blood collection. The evaluation of VNA to rabies virus was performed by Rapid Fluorescent Inhibition Test (RFFITT). In this study, we considered animals with less than 12 months as puppies and with over 12 months as adults. Of total samples analyzed, 21.76% (94) had titers lt;0.5 IU/mL and among these, 63 (67.02%) samples were puppies. When considering the interval between administration of the vaccine and blood collection, 74 (60.63%) samples did not achieve protective titers in the first six months interval between vaccination and test showing a window period especially important in puppies. With regard to race, there was no significant variation. It was concluded that the puppies are more susceptible to infection by rabies virus than adults, proving the need for a second dose of vaccine in the primary vaccination, which would increase the possibility of a rapid, efficient and lasting immune response.

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Detection of rabies virus antibodies in free-living jaguars (Panthera onca) in the pantanal of Mato Grosso, Brazil

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The proximity to domestic animals has been considered an important cause of disease of wildlife, and has led to recent epidemics in endangered species around the world. In this study, exposure to rabies virus in eleven free-living jaguars (Panthera onca) captured from July 2010 to November 2012 in two protected areas in the Pantanal/MT/Brazil, was screened by Simplified Fluorescent Inhibition Microtest (SFIMT) and Rapid Fluorescent Focus Inhibition Test (RFFIT). Serum sample from each jaguar was analyzed twice in different days. Considering the presence of virus neutralizing antibodies (VNA) in samples with titers = 0,10, three jaguars had low positive titers for each test performed, for a frequency of 27.3%, but only a jaguar showed rabies-neutralizing antibodies on both SFIMT and RFFIT (0,19/0,12 and 0,14, respectively). Low titers of VNA have been detected in other species of wild carnivores, including apparently healthy free-living jaguars, suggesting a non-lethal infection. In our study, we could not correlate or presumed the cause of death of a jaguar that showed the highest rabies-neutralizing antibodies and reacted on both tests. Therefore, it was not possible to infer about the possible effects of the virus in this animal health. Although some species of wild animals are known to serve as rabies reservoirs, nothing is known about wild felids as reservoirs, precluding any conclusion about the role of wild cats in the circulation of the rabies virus. Prevalence in free-living jaguars require further