

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

BATISTA, H. B. C. R.<sup>1</sup>; CARNIELI-JR, P.<sup>1</sup>; OLIVEIRA, R. N.<sup>1</sup>; CASTILHO, J.G.<sup>1</sup>

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 (LD<sub>50</sub>) 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 LD<sub>50</sub> died 6 to 9 days post infection and the mice infected with 1 and 0,1 LD<sub>50</sub> 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.

<sup>1</sup> Pasteur Institute of São Paulo

### 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.

FERNANDES, M. E. S.; ALMEIDA, E.; CRUZ, P. S.; PEREIRA, P. M. C.; CHAVES, L. B.; SCHEFFER, K. C.; CAPORALE, G. M. M.; SILVA, S. R.; FERNANDES, E. R.; LUIZ, F. G.; KATZ, I. S. S.; SILVA, A. C. R.

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)

SCHEFFER, K. C.<sup>1</sup>; DI GREGORIO, M. C.<sup>1</sup>; BATISTA, A. M.<sup>2</sup>; ROSATO, P. N.<sup>3</sup>; CAMPOS FILHO, E.<sup>2</sup>; SILVA, A. C. R.<sup>1</sup>; CAPORALE, G. M. M.<sup>1</sup>; CRUZ, P. S.<sup>1</sup>; CHAVES, L. B.<sup>1</sup>

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.

<sup>1</sup> Instituto Pasteur, São Paulo, Brazil

<sup>2</sup> Biólogo Autônomo

<sup>3</sup> Laboratório de Análises Clínicas Anclivet, Praia Grande, São Paulo, Brazil

### Evaluation of neutralizing antibodies in cats prime vaccinated for rabies

SILVA, V. A.; GAMOM, T. H. M.; SILVA, A. C. R.; CAPORALE, G. M. M.; CHAVES, L. B.; CENTOAMORE, N. H. F.; SOUZA, T. C. P.; SCHEFFER, K. C.

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