

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.

Instituto Pasteur, São Paulo, Brazil

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

FERREIRA, J. S.; TROTI, A. C. P.; SCHEFFER, K. C.; CAPORALE, G. M. M.; SILVA, A. C. R.; CHAVES, L. B.

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 LogD<sub>50</sub> 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.

Instituto Pasteur, São Paulo, Brazil

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

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

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  $\geq 0.5$  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.

Instituto Pasteur, São Paulo, Brazil

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

ONUMA, S. S. M.<sup>1</sup>; CHAVES, L. B.<sup>2</sup>; SCHEFFER, K. C.<sup>2</sup>; KANTEK, D. L. Z.<sup>1</sup>; CRAWSHAW-JÚNIOR, P.; MORATO, R. G.<sup>1</sup>; MAY-JÚNIOR, J. A.; AGUIAR, D. M.<sup>3</sup>

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