

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 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.

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 (RFFIT). 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 ≥ 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.¹; CHAVES, L. B.²; SCHEFFER, K. C.²; KANTEK, D. L. Z.¹; CRAWSHAW-JÚNIOR, P.; MORATO, R. G.¹; MAY-JÚNIOR, J. A.; AGUIAR, D. M.³

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

investigations, as this feline specie is protected through an epidemiological peculiarity, the viral strain that occurs in jaguars must be better evaluated and epidemiological characteristics as the way of infection must be determined. Genotypic and phylogenetic analysis of rabies virus that occurs in jaguars could be interesting to clarify issue of rabies in the Brazilian pantanal.

¹ ICMBio/MT/Brazil

² Instituto Pasteur, São Paulo/Brazil

³ Universidade Federal de Mato Grosso, MT/Brazil

Positivity and classification of bats submitted for rabies diagnosis at Pasteur Institute over the period from 2007 to 2012

IKEDA, M. A. K.; BARROS, R. F.; LIMA, J. Y. O.; FERREIRA, J. S.; PEREIRA, P. M. C.; FAHL, W. O.; IAMAMOTO, K.; MORI, E.; FERREIRA, E.; ACHKAR, S. M.; SCHEFFER, K. C.

The bats are a major reservoir of rabies virus and have a relevant importance in the disease transmission. The aim of this study was to evaluate the bat population submitted for rabies diagnosis. Data were analyzed from 18.805 bats received by the diagnostic section of the Pasteur Institute of Sao Paulo, originated from several counties of the state of Sao Paulo over the period from 2007 to 2012. These specimens were morphologically classified according to their family, at the time of execution of the rabies diagnosis technique. The central nervous system from these animals was submitted to direct immunofluorescence test and, when viable, to viral isolation. From the total of bats received, 76.92% bats belonged to the Molossidae family, 12.14% to the Phyllostomidae family, 9.52% to the Vespertilionidae family, 0.02% to the Noctilionidae family and 1.37% to a group of bats whose identification was not possible to establish. Regarding positivity, 261 (1.44%) bats were diagnosed positive, 94 (36.01%) were from Vespertilionidae, 87 (33.33%) were from Phyllostomidae, 79 (30.27%) were from Molossidae and 01 (0.38%) was unable to classify. We also observed a total of 197 (1.26%) bats that were not submitted to the diagnosis due to poor preservation of the samples. These results showed that Molossidae was the main family received for rabies diagnosis; however, the positivity was higher in the Vespertilionidae family. The dynamic population investigation of the species is necessary in order to promote a better understanding of rabies seasonality in bats. These data reinforce the importance of an active search for suspect animals in order to establish new control strategies of these animals considering the epidemiologic surveillance of rabies and other zoonosis.

Instituto Pasteur, SP, Brazil

Indissociabilidade da presença de corpúsculos de Negri e da inflamação parenquimatosa em amostras de sistema nervoso central de herbívoros acometidos pela raiva

ACHKAR, S. M.1; FERNANDES, E. R.1; GUEDES, F.1; MORI, E.1, DEL FAVA, C.2; DUARTE, M. I. S.3

Introdução: A raiva é uma meningoencefalomielite que acomete diferentes espécies. Estudos da literatura mencionam que possa haver uma dissociação entre a presença de infecção viral e inflamação. **Objetivo:** caracterizar as alterações histopatológicas no sistema nervoso central (SNC) de equinos e bovinos infectados pela raiva e comparar a presença de corpúsculos de Negri pela

coloração de hematoxilina-eosina (HE) e por técnica de imunohistoquímica, correlacionando a presença do antígeno viral e a inflamação. **Métodos:** 05 amostras de SNC de bovinos e 05 de equinos foram analisadas pela coloração de HE. O antígeno da raiva foi pesquisado pela técnica de imunohistoquímica (IHQ), utilizando-se anticorpo policlonal antivírus da raiva e método Estreptavidina-biotina peroxidase. Diferentes regiões do SNC foram avaliadas: córtex, hipocampo, cerebelo e tronco encefálico. A caracterização histopatológica e imunohistoquímica foram realizadas semiquantitativamente de acordo com a intensidade de achados observados. **Resultados:** as alterações histopatológicas encontradas nas amostras de SNC dos herbívoros estudados, independentemente das espécies, foram: meningite, congestão vascular, necrose neuronal, edema perivascular, perivasculite e vasculite, cromatólise e nódulos microgliais, sendo em grau discreto a moderado na maioria dos casos. Corpúsculos de Negri nas diferentes regiões cerebrais foram evidenciados em 90% das amostras, sendo o cerebelo a região mais acometida. A avaliação do antígeno viral por imunohistoquímica apresentou positividade em praticamente todas as amostras, com exceção de uma amostra de equino. O cerebelo também foi a região que apresentou maior positividade para o antígeno viral por imunohistoquímica. **Discussão e conclusões:** O processo inflamatório deu-se concomitantemente com a presença de corpúsculos de Negri e de material antigênico nas diferentes regiões do SNC de ambas as espécies, havendo correlação positiva entre inflamação e imunopositividade para o antígeno viral.

¹ Instituto Pasteur de São Paulo

² Instituto Biológico

³ Faculdade de Medicina da Universidade de São Paulo

Molecular techniques for rabies virus detection in organs of frugivorous bats

SCHEFFER, K. C.1; FAHL, W. O.1; LAMAMOTO, K.1; CARNIELI JUNIOR, P.1; CARRIERI, M. L.1; MORI, E.1; OLIVEIRA, R. N.1; ITO, F. H.2

The aim of this study was to detect the rabies virus (RABV) presence in different organs of frugivorous bats using molecular techniques such as RT-PCR, hnRT-PCR, and the Real Time RT-PCR. Thirty bats of the genus *Artibeus* were selected and resulted as positive by the DFA test and N2A-cells inoculation test using brain tissue in both tests. Samples of salivary gland tissue, urinary bladder tissue, kidney tissue, lung tissue, stool, and skull lavage were collected for molecular assays. The organs and the stool were diluted at 1:10 (w/v) and the urinary bladder was diluted at 1:20 (w/v). The RT-PCR and the hnRT-PCR were performed using specific nucleoprotein gene-target primers. The product obtained by reverse transcription technique was submitted to the Real Time RT-PCR technique, using primers and probe specific for the RABV antigenic variant 3. For the 180 samples evaluated, the sensitivity results detected by the RT-PCR, hnRT-PCR and Real Time RT-PCR techniques were: 56.25%, 82.57%, and 82.19%, respectively. The results obtained by RT-PCR showed lower sensitivity of this technique compared with the hnRT-PCR and Real Time RT-PCR techniques, excepted for skull lavage samples. A comparison of hnRT-PCR and Real Time RT-PCR techniques performed by Fisher's exact test showed that the proportion of positives was non-significant ($P > 0.05$) among skull lavage, organs and stool. Thus, the results suggest that hnRT-PCR and Real Time RT-PCR techniques can be used as complementary methods for the rabies diagnosis and are sensitive to be used in detecting RABV in different organs and extra neural tissues of bats.

¹ Instituto Pasteur, SP, Brazil

² Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, SP, Brazil