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Diagnosis of rabies and eastern and western equine viral encephalitides in equids by multiplex Hemi-Nested RT-PCR technique

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esta sirva como obstáculo aos morcegos hematófagos. A maioria dos abrigos localizavam-se próxima a corpos d'água. Foram identificados 10 abrigos de *Desmodus*, todos do tipo artificial. O número médio de morcegos foi de 11 por abrigo. Os *buffers* dos abrigos 3, 5 e 6 concentram a maior número de óbitos registrados. Os resultados mostraram que 25 (37,9%) óbitos estavam contidos em áreas de influência de 3 km de três abrigos próximos (abrigos 3 e/ou 5 e/ou 6). Não foram considerados os abrigos de *Desmodus* localizados nos municípios vizinhos. Verifica-se o caráter rural da doença, porém há vários bairros residenciais circundados pela área rural, o que evidencia o risco de infecção humana. Este trabalho reforça que a incorporação das ferramentas e técnicas de geoprocessamento auxiliam na compreensão da composição da paisagem e padrões ambientais, da ocupação do solo sendo de grande utilidade na vigilância epidemiológica da raiva como de outras zoonoses. Verifica-se a escassez de trabalhos utilizando ferramentas do geoprocessamento e de análise espacial na ocorrência da raiva.

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DIAGNOSIS OF RABIES AND EASTERN AND WESTERN EQUINE VIRAL ENCEPHALITIDES IN EQUIDS BY MULTIPLEX HEMI-NESTED RT-PCR TECHNIQUE

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Several viral zoonoses affect the equids causing neurological diseases, including rabies and Eastern and Western equine encephalitides (EEE and WEE). Clinical diagnosis is often not conclusive, in a way that laboratory diagnosis is essential. Data from the Laboratory of Rabies Diagnosis at the Pasteur Institute of São Paulo, between 2000 and 2010, demonstrate that approximately 75% of submitted equid samples, wich animals presented neurological symptoms, were negative for rabies, emphasizing the importance of achieving a differential diagnosis for equine encephalitis caused by alphaviruses. The aim of this study was to test the suitability of using multiplex hemi-nested RT-PCR for the diagnosis of rabies, EEE and WEE in equids central nervous system samples. We used the primers 21G, 304 and 504 directed to the N gene of rabies virus, and the primers cM3W, M2W, nEEE and nWEE directed the NSP1 gene of WEE and EEE viruses. A preliminary study of the primers was carried out, as well as their use in a hemi-nested RT-PCR, evaluating the optimal annealing temperature, the analytical sensitivity and specificity and the reproducibility of the technique in positive field samples for rabies and EEE. Based on the established protocol for the hemi-nested RTPCR, variations in reagents concentrations for the multiplex hemi-nested RT-PCR protocol were perfomed. After establishing the protocol for this reaction, the tests to verify the analytical sensitivity and specificity and reproducibility were performed and the results were compared to those obtained by hemi-nested RT-PCR. In the detection threshold test, the analytical sensitivity was similar for both techniques, resulting in 10- 1.7 for the three standard virus CVS, EEEV and WEEV. In the detection threshold test using a sample with the three viruses, a high specificity of the primers was verified and the multiplex hemi-nested RT-PCR was able to detect the three viruses simultaneously. There was no difference in the proportions of samples detected as positive for rabies obtained by both techniques, according to the Fisher exact test (P = 1.0000). However, for EEE positive field samples, the proportion of samples detected as positive by the hemi-nested RT-PCR a difference in the proportion obtained by multiplex hemi-nested RT-PCR (P <0.0001) was observed. Although it was not possible to use WEE positive field samples in this study, the results suggest that its detection would be possible by multiplex hemi-nested RT-PCR. Thus, data suggest that the multiplex hemi-nested RTPCR technique could be applied to detect rabies and WEE, but with some limitations for the EEE detection, in a way that new studies will be carried out. Financial support: CNPq and Pasteur Institute of São Paulo

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IMPLEMENTATION OF THE FLUORESCENT ANTIBODY TECHNIQUE NEUTRALIZATION VIRUS TEST IN RABIES LABORATORY DIAGNOSIS OF PASTEUR INSTITUTE OF SÃO PAULO / BRAZIL

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The Fluorescent Antibody Virus Neutralization Test (FAVN) is used routinely in many laboratories for the reference measurement of rabies virus neutralizing antibodies (VNA) in serum of animals to confirm the efficacy of vaccination against rabies, which is required to authorize the transit of these animals in countries free of rabies. The World Organization for Animal Health (OIE) recommends a VNA title ≥ 0,5 IU/mL to ensure that the animal has immunity against the rabies virus. The rabies laboratory diagnosis of Pasteur Institute of São Paulo/Brazil (IP/SP) is a national reference of the Ministry of Health for rabies diagnosis and performs the measurement of VNA in humans and animals serum samples for proof of immunization against rabies. The objective of this study was to implement the FAVN in the laboratory routine of rabies diagnosis at IP/SP using as reference the Rapid Fluorescent Focus Inhibition Test (RFFIT). Initially, the Challenge Virus Standard (CVS) was titrated by the FAVN and RFFIT methods, performing serial dilutions from 10-1 to 10-12 and determining the dilution of 100 TCID50 (50% infectious dose in tissue culture) or 100 FFD50 (50% of the dose forming focus) for FAVN and RFFIT, respectively. A total of 97 serum samples from animals vaccinated against rabies with different ranges of VNA previously titrated by RFFIT, and 15 samples from unvaccinated animals were selected. Statistical analysis of agreement was performed considering the results in a qualitative analysis (< 0,5 and ³ 0,5) using the Kappa test. The CVS title was 10-6 in TCID50 for FAVN and 10-5 in FFD50 for RFFIT. The FAVN showed high specificity with titers <0,09 IU/mL and LogD50 <0,83 in samples from unvaccinated animals. Qualitative analysis of the results showed a substantial agreement between the two methods (kappa = 0,66, p <0,001). The titles of the sera from vaccinated animals were 0,12 IU/ml to 5,92 IU/ml (GM = 0.92 IU/mL) for FAVN and 0,13 IU/mL to 9,55 IU/mL (GM = 1,34 IU/mL) for RFFIT. The dilution factor in LogD50 values varied from 0,74 to 2,27 (GM = 1,55) for FAVN and 1,08 to 2,48 (GM = 1,84) for the RFFIT. The obtained results showed concordance within the parameters of specificity and sensitivity between FAVN and RFFIT methods. In this context, the implementation of FAVN in the rabies diagnosis laboratory IP/SP has a great importance for rabies epidemiological investigation by the VNA evaluation in animal serum samples and may complement the techniques already in use in the IP/SP.