Polymorphism of rabies virus nucleoprotein from samples isolated in the Rio de Janeiro state, Brazil*

Polimorfismo da nucleoproteína do vírus da raiva de amostras isoladas no Estado do Rio de Janeiro

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Rabies virus belongs to genus Lyssavirus of the family Rhabdoviridae. The viral nucleoprotein is responsible for several functions in viral particle such as, packing and protects the RNA genome, and participates of transcription and replication of genome. Rabies cause large loss in livestock industry from Rio de Janeiro State, Brazil. All regions of the State are affected by rabies virus transmitted by vampire bat. This work aims to evaluate the changes between the sequences of the nucleoprotein of rabies virus isolated in Rio de JaneiroState. The nucleoprotein gene of 32 samples was sequenced. The sequences length were 1,301 nucleotide, that encoding 433 amino acids. These sequences were aligned with reference sequence Pasteur virus fixed strain (PV GenBank accession number M13215), using ClustalW method. The nucleotide and amino acid polymorphism were analyzed manually using the BioEdit software. Among the samples of herbivores were showed 95 (7.30%) positions with mutation of nucleotides. When comparing the 32 samples of herbivores with PV (GenBank M13215), we observed that there were 257 (19.75%) positions with mutation. Replacements of the transition type were more frequent than the transversion type. Mutations in third nucleotide of the codon were more frequent than in first and second nucleotide. Among the sequences of amino acid from herbivores were showed 13 positions of amino acid substitution. It was observed an increase of 21 replacement of amino acid when the herbivores sequences were compared with PV, totalizing 34 amino acid mutations. The samples of rabies virus show high polymorphism of its nucleotides, but at the level of amino acid the polymorphism is low and even when there are changes in many cases amino acid replacement occurs by the same biochemical amino acid group. Thus the mutations found among the samples have little difference in functionality of the protein, but the nucleotide mutations can be used in rabies epidemiological studies.

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Shelters of vampire bats: identifying and mapping in rural areas of Rio de Janeiro and Espírito Santo states, Brazil*

Abrigos de morcegos hematófagos: identificação e mapeamento em áreas rurais dos Estados de São Paulo e Espírito Santo

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In Brazil, there are 150 species of bats, all of them from suborder Microchiroptera. Three of them correspond to vampire bats: *Desmodus rotundus, Diphylla ecaudata* and *Diaemus youngi.* The common vampire bat *D. rotundus* has been the main reservoir of rabies virus in rural areas. Shelters of bats can be classified as natural or artificial. The study was conducted between 2008 and 2011. Twenty-one shelters were mapped with a GPS unit. *D. rotundus* were captured in nine field trips, using mesh nets set up in front of shelters. Ten shelters were mapped in Campos dos Goytacazes; six were artificial and four were natural; they were located at S22°00'26,4"–W041°40'00,3"/S21032'14,7"–W041020'31,3"/S21048'31,2"–W041038'20,7"/S21048'29,4"–W041038'22,0"/S22000'40,6"–W041039'98,0"/S21057'53,2"–W041027'59,0"/S21058'18,3"–W041027'03,2"/S21047'09,6"–W041014'00,8"/S21046'02,6"–W041035'44,0"/S21047'54,2"–W041036'28,2". Six shelters were found in Cardoso Moreira; half of them were artificial, and the other half were natural; they were located at S21032'07,5"–W041035'46,9"/S21032'29,0"–W041036'31,8"/S21027'29,4"–W041034'36,2"/S21026'43,7"–W041033'43,1"/S21030'28,7"–W041027'20,5"/S21032'29,9"–W041036'31,8". Two shelters were mapped in Miracema; one was natural, and the other was artificial; their location was S21°23'39,3"–W042°04'32,4"/S21019'29,1"–W042007'49,1". One shelter was found in Quissamã; it was artificial and located at S22°05'15,7"–W041°41'20,2". Another shelter was found in Bom Jesus do Norte; it was artificial and located at S21°06'45,8"–W041°40'55,6". The last shelter was mapped in Italva; it was natural and located at S21°27'06,4"–W041°43'34,2". In a field trip to Campos dos Goytacazes (S22°00'26,4"–W041°40'00,3"), four male bats were captured. In Miracema (S21°23'39,3"–W042°04'32,4"), 28 males and 23 females were captured in three field trips. In Quissamã, 117 bats (59 males and 58 females) were captured in three field trips too. In a field trip to Bom Jesus do Norte, 26 males and 46 females were captured. In the last field trip, to Italva, one male and 13 females were captured. All mapped shelters were located in rural areas (distant from urban centers), and most of them were far from human habitations. Most shelters were artificial, showing the direct influence of humans on the spread of bats. All field trips were made during the day, except the one to Bom Jesus do Norte, made at night. In Italva, one vampire bat *D. ecaudata* was found. A total of 263 bats were captured. Vampire bats play an important role in rabies transmission, then the identification and mapping of their shelters are essential to control the rural cycle of the disease.

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Diagnóstico molecular por PCR de ectima contagioso em caprinos e ovinos

Molecular diagnosis by PCR of contagious ecthyma in goat and sheep

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O ectima contagioso (EC) é uma virose aguda que acomete caprinos e ovinos, amplamente disseminada em todo o mundo. No Brasil, onde a ovinocaprinocultura é amplamente praticada para produção de pele, carne e leite, EC tem sido relatado como uma das principais enfermidades infecciosas de caprinos e ovinos. Em certas situações, EC pode ser clinicamente confundido com enfermidades vesiculares, como a febre aftosa, sendo assim necessário um teste laboratorial direto para definição do diagnóstico diferencial, preferencialmente de rápida execução, como a reação em cadeia da polimerase (PCR), que ainda não tem sido amplamente testada com amostras brasileiras do vírus EC. O presente trabalho foi desenvolvido com o objetivo de avaliar uma PCR para diagnóstico de EC. O DNA foi extraído de crostas de animais clinicamente afetados por EC e de sobrenadantes de células de córnea fetal caprina (FCC 40) após passagem dessas amostras. A PCR foi realizada empregando-se os oligonucleotídeos iniciadores PPP3 (5'-tac gtg gga agc gcc tcg ct-3') e PPP4 (5'-gcg agt ccg aga aga ata cg-3') que amplificam um produto de 235 pb do gene B2L, da cepa NZ2 do vírus EC. As condições de ciclagem consistiram em incubação inicial a 94º C por três minutos, seguida de 30 ciclos: desnaturação a 94º C por 30 segundos, anelamento a 65º C por 30 segundos, extensão a 72º C por um minuto, 72º C por dez minutos e uma etapa final a 4º C. Foram processadas oito amostras de caprinos e 25 de ovinos, originários dos Estados da Paraíba, Pernambuco, Sergipe e Bahia. De todas as amostras, foi amplificado um fragmento do tamanho esperado. Para confirmação do diagnóstico, doze produtos de amplificação da PCR foram sequenciados. A análise das sequências através do método de *neighbor-joining*, usando os parâmetros do modelo Tamura 3 de substituição de nucleotídeos com mil replicatas, mostrou que entre o grupo de sequências analisadas há 99% de similaridade e 67%, quando comparado com outras amostras brasileiras e asiáticas. A significativa divergência com outras amostras indica que é necessária a avaliação de um maior número de amostras de diferentes regiões do País para validar a PCR como teste de diagnóstico. Espera-se que a PCR definitivamente validada possa ser usada para diferenciação de EC da febre aftosa, sobretudo como suporte às ações do Programa Nacional de Prevenção e Erradicação da Febre Aftosa, no qual os caprinos e ovinos são considerados animais sentinelas.

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Natural co-infection with infectious hypodermal and hematopoietic necrosis virus (ihhnv) and infectious myonecrosis virus (imnv) in *litopenaeus vannamei* in northeast Brazil*

Coinfecção natural com o vírus da necrose hipodérmica e hematopoiética infecciosa (VNHHI) e o vírus da mionecrose infecciosa (VMI) em Litopenaeus vannamei no Nordeste do Brasil

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Cultivation of whiteleg shrimp (*Litopenaeus vannamei*) constitutes a growing aquaculture industry in Northeast Brazil. Similar to other animals that are intensively farmed, shrimp experience disease outbreaks, which are a constant threat and cause eventually significant economical losses. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) and infectious myonecrosis virus (IMNV) are prevalent epizootic viral agents in Brazil. In a routine monitoring program for the diagnosis of IHHNV and IMNV, using molecular techniques like conventional PCR, reverse transcription coupled with PCR (RT-PCR) and absolute quantitative real time PCR (qPCR), we found that most positive samples of shrimp were simultaneously co-infected with both viruses. This survey is the first to show the occurrence of a natural co-infection that is caused by IHHNV and IMNV in penaeid shrimp attacked by viral disease that were cultivated in Northeast Brazil. Taken together, RT-PCR can be readily employed in the routine diagnostic screening program for shrimp viruses in the aquaculture industry. Moreover, in combination with qPCR, diagnosis of the viral load and co-infection can be absolutely assessed and the data used to assist differential management plans for epizootic control.

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Differential diagnosis of active hypodermal and hematopoietic necrosis virus based on gene choice and reverse transcription coupled with PCR*

Diagnóstico diferencial do vírus da necrose hipodérmica e hematopoiética na forma ativa com base na escolha do gene e transcrição reversa por meio de PCR

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The Pacific whiteleg shrimp *Litopenaeus vannamei* (Penaeidae) is one of the most important cultivated species in world aquaculture. In Brazil, the Northeastern states are home to the main shrimp producers. As shrimp aquaculture has expanded and intensified, diseases have progressively become one of the most serious threats to this industry. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is an enzootic viral agent in Brazilian shrimp farms. These viruses are usually diagnosed by histological methods. However, to detect sub-clinical or acute IHHNV infection, more refined methods based on molecular techniques have been utilized. We found that by using "universal" primers and a single-step PCR diagnostic test, it was difficult to distinguish between non-infective forms of the virus and active IHHNV. Detection of IHHNV was more accurate when we used two alternative molecular strategies, namely 1) single-step PCR amplification based on gene choice and 2) reverse transcription coupled with PCR. This communication presents the results of