142 - DETECTION AND CHARACTERIZATION OF FIBROPAPILLOMA-ASSOCIATED TURTLE HERPES-VIRUS IN MARINE TURTLES FROM BRAZIL

Rodenbusch, C.R.[1]; Almeida, L.L.[1], Marks, F.S.[1], Baptistotte, C.[2]; Pires, T.T.[3]; Werneck, M.R.[3]; Damasceno, T.[3]; Allieve, M.[4]; Canal, C.W.[1]
1 Laboratório de Virologia, Faculdade de Veterinária, UFRGS; 2 Centro Tamar-ICMBio, 3 Fundação Pró-Tamar, 4 CECLIMAR, UFRGS, Rio Grande do Sul, Brasil.
Email: carlarodenbusch@yahoo.com.br

Fibropapillomatosis (FP) is a neoplasic disease of marine turtles characterized by the presence of epithelial fibropapillomas and internal fibromas. Although the tumors are considered bening, the disease can be life-threatening; large cutaneous tumors can interfere with the turtles' locomotion, vision, swallowing, and breathing, and visceral tumors can be locally invasive and affect organ function. The prevalence of FP has increased over the past four decades. The first report was on the green turtle (*Chelonia mydas*), but confirmed cases have now also arisen in the loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) species. FP was described in Brazil for the first time in 1986. Differences in the prevalence of FP have been observed between different geographic locations in Brazil, but the nation-wide tumor prevalence average in Chelonia mydas was 15,41% between 2000 to 2005. The etiologic agent of FP is most likely a herpesvirus (chelonid herpesvirus 5–ChHV 5); however, environmental cofactors may be involved. Herpesvirus polymerase gene sequences have been detected by PCR in DNA from 100% of the tested fibropapillomas and fibromas. The aim of this work was to identify by PCR and characterize by DNA sequencing the fibropapilloma-associated turtle herpesvirus in marine turtles from Brazil. FP samples will be collected during June/2009 to May/2010 in the Ceará, Bahia, São Paulo, Espírito Santo and Rio Grande do Sul States. Samples of skin without the tumor will be used as negative controls. The PCR amplifies a 483-bp fragment of the turtle herpesvirus DNA Polymerase gene. The obtained sequences will be aligned with sequences already described in the literature. Preliminary results show that one Brazilian sample is similar to the AF299107 Australian sample because it presents no deduced amino acid change. With respect to the samples AF299108 (Australia), AF035004 (Florida), AF299110 (Barbados) and AF299109 (Mexico), the Brazilian sample displays 1, 2, 4 and 5 amino acids changes, respectively. It can be concluded that the ChHV 5 Brazilian sample is more closely related to the Australian samples.

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143 - HYPERIMMUNE SERUM PRODUCTION USING A CAPSID PORCINE CIRCOVIRUS 2 RECOMBINANT PROTEIN EXPRESSED IN *E. COLI* AND ITS USE IN IMUNOFLUORESCENCE ASSAYS

Machado, J.P.[1,2]; Braga, D.A.M.[1]; **Pinheiro, A.L.B.C.**[1]; Salgado, R.L.[1]; Vargas, M.I.[2]; Fietto, J.L.R.[1], Silva Júnior, A.[1]; Almeida, M.R.[1]
[1]Universidade Federal de Viçosa, Laboratório de Infectologia Molecular Animal, BIOAGRO, Viçosa, MG, Brasil. Laboratório de Histopatologia veterinária, Departamento de Veterinária, Universidade Federal de Viçosa.
E-mail: marcia@ufv.br

Porcine circovirus 2 (PCV2) is a member of *Circoviridae* family. It consists of a non-enveloped virus single-stranded circular DNA about 1.76 kb. PCV2 is the major etiological agent of porcine circoviruses diseases and it is responsible for serious economical losses in the swine industry. In this assay was produced a hyperimmune serum to PCV2 using a purified recombinant capsid protein expressed in E. coli, after that this serum was used in Imunofluorescence assays. The hyperimmune serum was obtained from habits, inoculating three shots in intervals of fifteen days, subcutanelly, containing each shot about 100µg of recombinant protein previously purified by affinity chromatography. The purified protein was emulsified using a saponine adjuvant at 1 mg/mL proportion of inoculated protein. Between every inoculating interval the serum conversion was evaluated by means of ELISA technique. To verify the PCV2 antibody affinity, a *Western blotting* assay was conduced using as antigen a viral inoculums developed in PK15 cells infected with PCV1/PCV2, PCV1 and PCV2 infected cells. The imunofluorescence technique used PK15 infected cells with PCV2 affixed at sheets and treated with serum at dilutions (1:50, 1:100, 1:200, 1:400 e 1:800). Subsequently, the cells were treated with the secondary antibody marked by fluorescein at a prefixed 1:800 dilution. In ELISA assays were verified an increase in antibody levels between the inoculation intervals. In *Western blotting* assays were verified the specificity of the hyperimmune serum to the PCV2 capsid protein, without cross-reaction to the PCV1 capsid protein. Imunofluorescence assays have shown better results at 1:200 dilutions, observing specific fluorescence and low background score. Therefore was possible producing a specific serum to PCV2 used as an important tool to identify the virus at isolated samples and for other biologic assays.

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144 - THE INFLUENCE OF MAGNESIUM CHLORIDE IN THE REPLICATION OF BOVINE HERPESVIRUS TYPE 1

Yamamoto, K.A.[1]; Rincão, V.P.[1]; Linhares, R.E.C.[1]; Nozawa, C.[1]
[1] Departamento de Microbiologia, Centro de Ciências