Title: DEVELOPMENT AND ANTIGENIC ANALYSIS OF A RECOMBINANT ORF-2 PROTEIN OF HEPATITIS E VIRUS GENOTYPE 3.

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Abstract:

Hepatitis E (HE) is an infectious disease caused by a non-enveloped, single-stranded, positive sense RNA virus from the Hepeviridae family. HE virus (HEV) genome contains three open reading frames (ORF1, ORF2 e ORF3). ORF 1 encodes for a non- structural polyprotein involved in viral replication; ORF 2 encodes for the viral capside protein and ORF 3 encodes for a protin related to viral pathogenesis. Based on nucleotide sequence analysis, HEV is classified in four major genotypes (HEV-1 to HEV-4). Domestic swine and other mammals are reservoirs for genotypes 3 and 4 that are ubiquitously distributed and associated with HE. In Brazil there are few epidemiological data available related to human or animal HE. In this study we expressed HEV-3 ORF-2 and evaluated its immunogenic and antigenic properties. HEV-3 RNA was isolated from pigs naturally infected (Londrina – Paraná, Brasil) and cDNA was synthesized by retrotranscription. A fragment of the carboxyl region of the ORF-2 (amino acids 394 to 661) was cloned into a T7 expression vector containing an N-terminal maltose binding protein containing a polyhistidine tag and a TEV cleavage site. After expression and cell disruption, the protein was purified by Ni-NTA chromatography. Soluble rORF-2 was obtained after expression in pET20-modified vector and 10 mg of purified protein per liter of culture medium were obtained. Following SDS-PAGE, the rORF-2 fusion protein was detected as a band of 78 kDa and, after TEV cleavage, a band was observed at 33 kDa, as expected. Wistar rats (n=2) received 50 µg of protein antigen with Freund's complete adjuvant by subcutaneous injection. Booster immunization was performed after 21 days using incomplete Freund's adjuvant. The immunization process resulted in high levels of IgG antibodies detected by ELISA. Five piglets were infected by intravenous injection with a HEV-3 preparation. Blood and stools were collected during the first 21 days after infection. Using rat polyclonal antibodies anti-rORF-2 in a dot-blot assay we were able to detect HEV-3 present in stools from commercial pigs and infected piglets (both RT-PCR positive). Antibodies against HEV-3 developed during pig infection were able to recognize rORF-2 by western blotting. In conclusion, we presented a useful system to develop a rORF-2 of HEV-3 with conserved antigenic properties. This antigen will be used to develop a serological assay to detect HEV-3 in both human and animals.

Key words: Hepatitis E, ORF2 protein, Diagnosis, Zoonosis