

# Diagnosis to Detect Porcine Transmissible Gastroenteritis Virus (TGEV) by Optical and Transmission Electron Microscopy Techniques

Diagnóstico para Detectar el Virus de la Gastroenteritis Porcina Transmisible por Técnicas de Microscopía Óptica y Electrónica de Transmisión

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**SUMMARY:** The porcine transmissible gastroenteritis is a highly severe contagious disease, caused by virus of the Coronaviridae family, genus Coronavirus. Its epizootic shape can reach a rate of up to 100% mortality in piglets under two weeks of age as a result of severe dehydration. In this study fragments of small intestine and stool samples were collected from 75 autopsied pigs from properties. The samples of the fragments were frozen and sent to the Laboratory of Electron Microscopy, Instituto Biológico, SP, Brazil, for histological and transmission electron microscopic analyses. According to histological H&E technique, atrophy, villous necrosis and destruction of the enterocytes were observed in 35 (46.6%) out of the 75 fragments of the small intestine samples. On the immunohistochemistry technique 19 (25.3%) fragments were positively stained with DAB in the Ag-Ac reaction (MabTGEV). In 19 (25.3%) positive samples analyzed by *in situ* hybridization, a brown stain of enterocytes was observed, mainly in the epithelial cells of the villi. By the negative staining technique, we visualized enveloped, pleomorphic coronavirus particles, with typical radial projections resembling solar corona, with 140 nm diameter in 21 samples (28%) of the small intestine fragments and in 16 (21.3%) stool samples. In the ultrathin sections of 21 (28%) samples of small intestine, complete coronavirus particles with 80 nm diameter were seen among the microvilli and in the cytoplasm of epithelial cells. Immature particles with 60 nm diameter, budding from cell membrane and from a rough endoplasmic reticulum and also inside the vacuoles were visualized. In 19 (25.3%) out of 75 analyzed samples of small intestine, the antigen-antibody interaction was characterized by aggregation of viral particles in the immunoelectron microscopy technique. In the immunocytochemistry technique, the antigen-antibody interaction was strongly enhanced by the dense colloidal gold particles over the coronavirus in 19 (25.3%) out of the 75 samples of fragments of small intestine examined.

**KEYWORDS:** Porcine Transmissible Gastroenteritis; Coronavirus; Histopathology; Transmission Electron Microscopy.

## INTRODUCTION

The porcine transmissible gastroenteritis is described as a highly contagious disease caused by coronavirus. It belongs to the Nidovirales order, Coronaviridae family, *Alphacoronavirus* genus, group Ia (Woo *et al.*, 2009). They are single-stranded, positive sense RNA viruses. Morphologically they are pleomorphic, with radial projections with a form like-club giving an aspect of solar corona. They have a diameter of 75-160 nm (Cappellaro *et al.*, 1998; Cleri *et al.*, 2010). These viruses are prone to genetic evolution by accumulation of point mutations in genes coding for structural and non-structural proteins and by analogous recombination between members of the same antigenic group (Decaro *et al.*, 2010).

The viral envelope is composed of five proteins (M, sM, HE, S and I) (Schwegmann-Webels *et al.*, 2002). The binding cell receptor to the viral spike glycoprotein (S) via aminopeptidase initiates infection. A second mechanism mediates binding cell receptor to the sialic acid seems to play a role in different strains of enteropathogenicity (Schwegmann-Webels *et al.*, 2002). Additionally, the 5'-terminal sequence differentiates porcine transmissible gastroenteritis virus (TGEV) from other existing coronavirus (Barrera-Vale, 2005).

This disorder was first described in 1946 by Doyle & Hutchings. It affects pigs of all ages and presents as characteristic symptoms, severe watery diarrhea