

**TITLE:** A MASSACHUSETTS INFECTIOUS BRONCHITIS VIRUS STRAIN CAUSING KIDNEY TROPISM AND LESION IN A NATURALLY INFECTED BROILER FLOCK.

**AUTHORS:** KASMANAS, T. C.; HASS, T.; HIPOLITO, A. M. S.; SILVA, R. W.; SILVA, F. A.; EVANGELISTA, T. C.; MONTASSIER, H. J.; FERNANDO, F. S.

**INSTITUTION:** UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”, UNESP JABOTICABAL (VIA DE ACESSO PROF. PAULO DONATO CASTELLANE S/N JABOTICABAL-SP, BRAZIL); LABORATÓRIO DE SANIDADE ANIMAL (RODOVIA WALDIR CANEVARI KM 06 NUPORANGA-SP, BRAZIL)

**ABSTRACT:**

The infectious bronchitis virus (IBV) caused a highly contagious disease resulting in great economic losses. IBV is a coronavirus that belongs to *Gammacoronavirus* genus and cause upper respiratory disease, although some strains can affect kidneys. Brazilian genotype, BR strains, is frequently associated with renal lesions, but Mass-like nephropathogenic strains have not been reported in Brazil until 2016. Infectious bronchitis was diagnosed in a 25-day-old commercial broiler flock in September of 2016 in north of São Paulo state, Brazil. The first signs were depression and respiratory distresses. Postmortem examination revealed increased tracheal mucus and moderate renal congestion. Trachea and kidney samples were collected. Part of the samples was cooled in BHI with antibiotics and part in 10% buffered formalin. The samples were submitted to RNA extraction using RNeasy mini Kit (Qiagen) and then analyzed by RT-qPCR using primer set described by Callison and others (2006) in parallel with a standard curve of viral load quantification. Positive samples were processed and then inoculated in chicken embryonated eggs for virus isolation. The allantoic fluids (AL) from 3 inoculated embryos were collected for RT-qPCR amplification. Total RNA from AL was obtained using Trizol LS Reagent (Life Technologies) and RT-PCR was performed as previously described by Marandino and others (2015) using One-Step RT-PCR (Qiagen). The amplified DNA products were purified and submitted to bi-directional DNA sequencing. The sequences obtained were aligned with Bioedit v. 7.1.9 and tree was generated with Mega 6.0. Naturally infected birds showed moderate lymphocytic infiltration in interstitium, interstitial edema, degeneration and mild necrosis of renal epithelial cells. In the tracheas, moderate to severe scaling with lymphocyte and heterophilic infiltrate, degeneration and necrosis of the ciliated epithelial cells and deciliation were found. The viral load ranged from 5.23 to 5.86  $\log_{10}$  among tracheal samples and from 3.24 to 4.19  $\log_{10}$  in renal samples. The isolated was called IBV/Brazil/NUP/0316 and the nucleotide sequences of full S1 gene were submitted to GenBank (Sequence ID KY465747). The isolated is closely related to Mass genotypes with 98% identity with Mass-M41 reference strain (GenBank AY561711). These results suggest that Mass strains with nephropathogenic potential are circulating in Brazil and escaping from conventional vaccine-induced immunity of Mass genotype.

**Keywords:** broiler, infectious bronchitis virus, kidney, mass-like strain

**Development Agency:** Laboratório de Sanidade Animal Nuporanga