on-line
28º CONGRESSO DA SOCIEDADE BRASILEIRA DE MICROSCOPIA E MICROANÁLISE
12 a 15 JUL 2021
50 anos SBMM

Bovine coronavirus. Detection by transmission electron microscopy techniques.

Catroxo, M.H.B.[1*], Martins, A.M.C.R.P.F.[1], and Santos, E.M.[1]

[1*] Electron Microscopy Laboratory, Biological Institute, São Paulo, SP, Brazil, e -mail: marcia.braga@sp.gov.br

Coronaviruses infect humans and a wide diversity of animal species causing respiratory, enteric, neurologic and hepatic disorders. They constitute a zoonotic risk to global public health because their ability to adapt to new species and establish sppilover events [1]. Several humans CoVs associated with acute gastroenteritis were of suspected zoonotic origin including spillover from cattle [2]. Bovine Coronavirus (BCoV) belongs to the genus *Betacoronavirus* and *Coronaviridae* family. It is an enveloped, RNA virus with a genome size of 32 kb, encoding five main structural proteins, the nucleocapsid, the hemagglutinin esterase, the membrane, the spike (S), and the envelope proteins [3]. BCoV has been implicated in severe diarrhea in neonatal calves from 1 day to 3 months of age, winter dysentery in adult cattle and respiratory infections in calves and feedlot cattle. Animals become infected through the faecal-oral route or inhalation of aerosols. Diseases caused by BCoV leads to significant economic losses both in beef and dairy industry worldwide due to mortality, reduced growth and drastic reduction in milk yield [4]. During the period from 2011 to 2021, approximately 430 samples of bovine feces or small intestine fragments from clinical cases were sent to the Electron Microscopy Laboratory of the Biological Institute of São Paulo, SP, Brazil, for viral diagnostic. The samples were processed for transmission electron microscopy utilizing, negative staining (rapid preparation), immunoelectron microscopy and immunocytochemistry techniques. For the negative staining, the clinical samples were suspended in phosphate buffer 0.1 M and pH 7.0 and placed in contact with metallic grids. Next the grids were blotted with filter paper and negatively stained at 2% ammonium molybdate, pH 5.0 [5]. For the immunoelectron microscopy technique, the screens were incubated with a virus-specific antibody and with viral suspension drops. Upon the screens were contrasted at 2% ammonium molybdate [6]. For the immunocytochemical detection [6], the screens were placed in contact with viral suspension and with primary polyclonal antibody drops. The grids were subsequently incubated in protein A drops in association with 10 nm gold particles (secondary antibody). Grids were then contrasted at 2% ammonium molybdate [7]. Using a Philips EM 208 transmission electron microscope all the samples were analyzed by negative staining technique and a great number of coronavirus particles (Fig.1), pleomorphic, rounded or elongated shaped, with characteristic radial projections forming a corona and measuring 80-140 nm in diameter (Fig. 1, arrow) were observed in 314 (73%) samples. The presence of aggregates formed by antigen-antibody interaction, characterized the positive result obtained, at the immunoelectron microscopy technique for coronavirus (Fig. 2). In the immunocytochemistry technique, the antigen-antibody reaction was strongly enhanced by the dense colloidal gold particles (Fig. 3, arrow). The techniques used are greatly effective for a rapid diagnosis of bovine coronaviruses and can be used in routine procedures to identify the viral agent of this important disease.

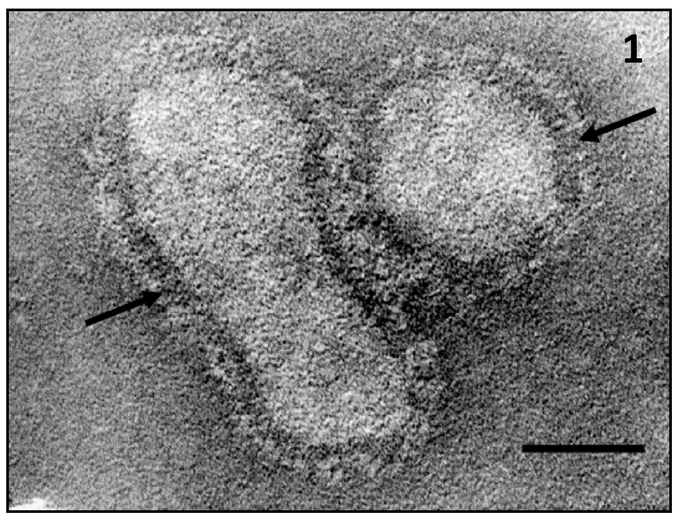

Fig. 1 - Negative staining of coronavirus particles, rounded and elongated, containing characteristic envelope in the shape of a solar corona (arrows). Bar: 54 nm.

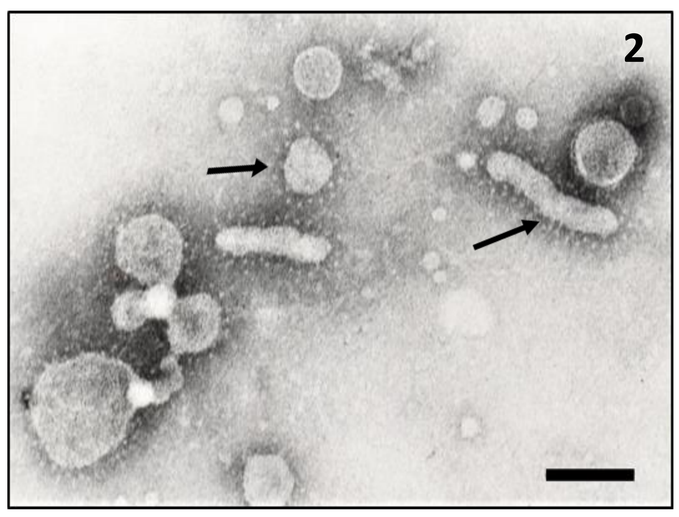

Fig. 2 - In the immunoelectron microscopy technique the coronavirus particles were aggregated by antigen-antibody interaction. Observe thin, wispy, and widely spaced spikes forming the envelope (arrows). Bar: 130 nm.

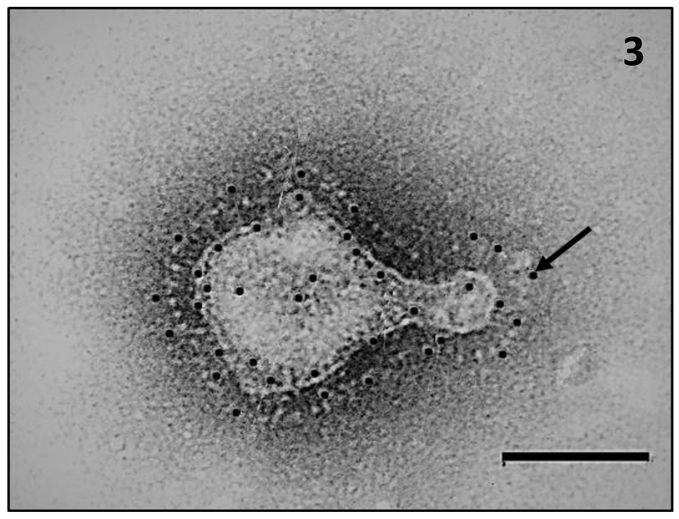

Fig. 3 - Antigen-antibody interaction strongly enhanced by the dense gold particles over the coronaviruses (arrow). Bar: 100 nm.

References

[1] K. Dhama, et al., Clin.Microbiol. Rev., 33 (2020) 1-48
[2] S. Su et al., Trends Microbiol, 24 (2016) 490-502
[3] T. Suzuki et al., Viruses, 12 (2020) 183
[4] M.J. Boileau, S. Kapil, Food Anim. Practice, 26 (2010) 123-146
[5] S. Brenner, R.W. Horne, Biochem. Biophys. Acta, 34 (1959) 103.
[6] M.A. Hayat, S.E. Miller. Negative Staining. Mc. Graw-Hill Publ. Company. New York, 1990.
[7] S. Knutton, Methods Enzymol., 253 (1995) 145-58.