Detection of Coronavirus in Giant Anteater (*Myrmecophaga tridactyla*) by Transmission Electron Microscopy in São Paulo, SP, Brazil.

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Abstract— *Coronaviruses belong to the order Nidovirales, family Coronaviridae and have four genera, Alphacoronavirus, Betaconavirus, Gammacoronavirus and Deltacoronavirus. They infect humans and several animal species, causing various diseases. Coronavirus constitute zoonotic risk to global public health because of their ability to adapt to new species and establish sppilover events. In this study, we evaluated the presence of coronavirus particles in the feces of giant anteaters (Myrmecophaga tridactyla). Under the transmission electron microscope, particles with coronavirus-like morphology, pleomorphic, rounded or elongated with radial projections forming a corona and measuring 80-140 nm in diameter, were visualized in all examined samples. The technique used was extremely useful for rapid viral diagnosis in affected animals. This report is the first occurrence of coronavirus in Giant anteater (Myrmecophaga tridactyla).*

Keywords— Coronaviruses, Giant Anteater (Myrmecophaga tridactyla), Transmission electron microscopy.

I. INTRODUCTION

Coronaviruses infect humans and a wide diversity of animal and bird species causing respiratory, enteric, neurologic and hepatic disorders [1].

They constitute a zoonotic risk to global public health because their ability to adapt to new species and establish sppilover events [2].

Due to their zoonotic potential, they have a strong tendency to cause catastrophic impacts, such as the recent human viral pandemics, originating from bat, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Covid 19 [3].

In animals, the coronavirus has also caused devastating diseases, such as porcine epidemic diarrhea (PEDV) that eliminated 10% of the US pig population in less than a year [4, 5, 6] and the infectious bronchitis virus that decimated flocks of chicken and turkey in different parts of the world, causing economic losses to the poultry industry [7].

Coronaviruses are positive-stranded RNA viruses, belong to the order *Nidovirales*, family *Coronaviridae* and have four genera, *Alphacoronavirus* (human coronavírus NL63 (HCoV-NL63), porcine transmissible gastroenteritis coronavírus (TGEV), PEDV, and porcine respiratory coronavírus - PRCV), *Betacoronavirus* (SARS-CoV, MERS-Cov, bat coronavírus HKU4, mouse hepatites coronavírus (MHV), bovine coronavírus (BCoV), and human coronavírus OC43, *Gammacoronavirus* (avian (infectious bronchitis coronavírus - IBV) e *Deltacoronavirus* (porcine deltacoronavirus (PdCV) [8].

Companion animals, such as cats, dogs, ferrets, horses and alpacas can also be infected [9].

The electron micrographs of coronavirus revealed a diverging spherical outline with some degree of pleomorphism, virion diameters varying from 60 to 140 nm, and distinct spikes of 9 to 12 nm, giving the virus the appearance of a solar corona [10].

Coronavirus genome measures 27 to 32 kb and encodes three tipos of proteins, structural, accessory, and non-structural proteins. The structural proteins comprise the nucleocapsid (N), spike (S), membrane (M), and envelope (E) proteins [11].

The S protein mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry.
The M protein is the most abundant protein and defines the shape of the viral envelope and the pleomorphic variability in different species. The E protein is the smallest of the major structural proteins and participates in viral assembly and budding. The N protein is the only one that binds to the RNA genome and is also involved in viral assembly and budding [12]. The roles of most of the accessory proteins remain poorly understood, but, the SARS coronavirus encodes accessory proteins that antagonize the development of type I interferon (IFN) responses [13]. The nonstructural proteins reassemble to form a viral replicase-transcriptase complex, consisting of the RNA-dependent RNA polymerase (RdRp, nsp12), helicase (nsp13), nsps with accessory functions [14].

Viral replication occurs quickly and mainly in the villous epithelial cells of the small intestine, resulting in marked villous atrophy due to necrosis [15, 16].

Animals become infected through the faecal-oral route, respiratory or inhalation of aerossol [17, 18].

The incubation period is 2–8 days [19] and the morbidity is very high, up to 100% [20].

The giant anteater (*Myrmecophaga tridactyla*) is the largest known species of anteater and the only species in Myrmecopahaga [21], listed as "vulnerable" by IUCN. It has become extinct in some parts of its geographic distribution, such as Uruguay. In Brazil it is considered extinct in the states of Vitoria, ES and Rio de Janeiro, RJ, and in condition of vulnerability in other states at great risk of disappearance in Central America [22].

The main threats to the survival of the species are hunting and habitat destruction, being an animal susceptible to being fatally hit by fires and traffic-accidents [21].

The incidence of studies on the presence of coronavirus in animals with potential reservoir has been little discussed, especially in animals at risk of vulnerability [23].

Electron microscopy techniques (rapid preparation) allow an accurate detection of viral particles, especially those of the coronavirus, enabling a rapid diagnosis in samples of different specimens [24].

To the detriment of the efficiency of detection by means of electron microscopy, this study sought to evaluate the presence of coronavirus particles in fecal samples of giant anteaters from the Ecological Park of the State of São Paulo, SP, Brazil.

II. MATERIAL AND METHODS

Description of the case: In 2016, there were cases of diarrhea among giant anteaters (*Myrmecophaga tridactyla*), from Parque Ecológico of State São Paulo, SP, Brazil. Fecal samples from the five individuals were sent to the Electron Microscopy Laboratory of the Biological Institute, São Paulo, SP, Brazil, for research on viral agents. The feces were watery to pasty and yellowish to greenish in color.

Negative staining technique (rapid preparation): The 5 fecal samples received were processed using the negative staining technique (rapid preparation) and subjected to examination using a Philips EM 208 transmission electron microscope.

In the negative staining technique, stool samples were suspended in phosphate buffer 0.1 M, pH 7.0. Drops of the obtained suspensions were placed in contact with metallic copper grids with carbono stabilized supporting film of 0.5% collodion in amyl acetate. Next, the grids were drained with filter paper and negatively stained at 2% ammonium molybdate, pH 5.0 [25].

III. RESULTS

Through the negative staining technique (rapid preparation) and under the transmission electron microscope Philips EM 208, it was possible to identify coronavírus like-particles, rounded or elongated, enveloped, with an average diameter of 140 nm, containing radial projections typical in the form of a solar corona, measuring 10 nm. (Fig.1 and Fig. 2).

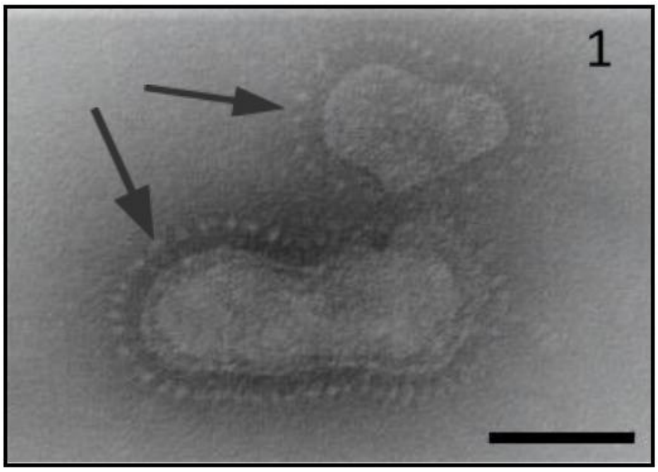

FIGURE 1: Negative staining of coronavirus particles in a suspension of giant anteater feces, rounded or elongated, pleomorphic, containing a characteristic envelope in the shape of a solar corona. Observe thin, wispy, and widely spaced spikes forming the envelope. Bar: 120 nm.

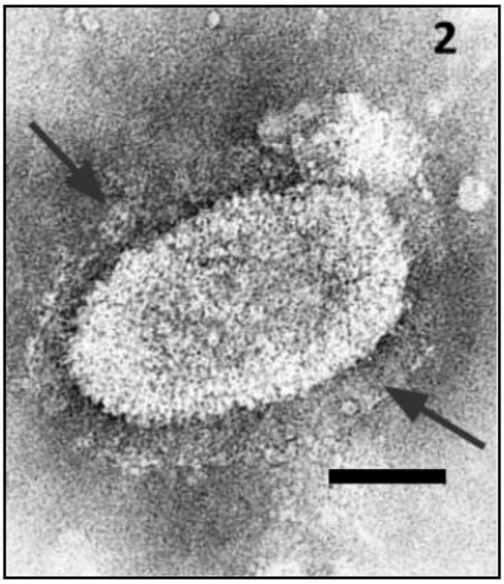

FIGURE 2: Coronavirus particle showing a characteristic envelope in the form of a solar corona or goblet (arrows), negatively contrasted with 2% ammonium molybdate. Bar: 50 nm.

IV. DISCUSSION

Observation under the transmission electron microscope, using the negative staining technique, clearly showed typical particles of coronavirus in the 5 fecal samples, confirming a small outbreak of infection among specimens of giant anteaters from Parque Ecológico of State São Paulo SP, Brazil.

The particles were pleomorphic, rounded to elongated, containing a characteristic envelope with typical radial projections in the shape of a solar corona and measuring an average of 140 nm in diameter.

Similar cases of outbreaks in wild animals with the presence of coronavirus particles have already been reported in Collared peccary [26]; Brocket deer [27], Greater rhea[28], White-lipped peccary[29], Golden-faced lion tamarin [30], Coatis [31], Montain lion [32], capybaras [33], pigeons [34], wild boar [35], peregrine falcon[36], ferrets [37], buffaloes [38].

The coronavirus has also been reported in other species of wild animals, such as dromedaries [39], rodents ([40]; bats [41], gorillas [42] and wild birds [43].

We observed that the spikes of the coronavirus particles in our study measured 10 nm long, as also described by [10] in human coronaviruses.

The negative staining technique is commonly used to detect coronaviruses in other animal species, such as swines[44], dogs, [45] bovines[46, 47] and equines[48], and in humans [10, 49].

The presence of yellowish or greenish diarrhea with watery to pasty consistency in the animals in our study was also reported in other coronavirus research in Brocket deer, Collared peccary, White-lipped peccary, Greater rhea, Golden-faced lion tamarin, dogs, bovines, wild boar, coati, Montain lion, capybaras, equines, ferrets, pigeon, buffaloes, swines, dogs, bovines and equines [26, 27, 28,29, 30, 45, 46,31,32, 47, 33, 34, 35, 36, 48, 37, 38,44].

New studies on the animal ability to be a reservoir for the coronavirus must be conducted to develop a more accurate viral origin [50].

Ecological assessments regarding the human-nature interrelationships should be studied, also addressing the interactions within breeding centers and efforts that allow a direct and indirect management with animals, since new infectious diseases can develop and lead to new worldwide damages, taking as an example the new coronavirus pandemic started in 2019 [51].

Integrative management together with surveillance planning programs for giant anteaters would reduce the occurrence of new outbreaks, avoiding economic and faunal losses [52].

Giant anteaters, like other mammals, have a sociable behavior with humans [21], therefore, it is extremely important for public health to better understand the aspects involved in possible contagions.

Considering that spillovers are a consequence of environmental degradation by human activity, studies must be instituted in order to understand how they arise and avoid new occurrences. Considering that bats and other wild animals act as host of several viral types, genetic and computational research must be developed for the previous knowledge of the viruses they carry, as well as the affinity of such viruses for the proteins of human cells, constituting an alert to avoid contagion and the prior establishment of prevention and treatment measures [53].

A better understanding of the animal's coronaviruses, their capacity for cross-species transmission, and the sharing of genetic information may facilitate improved prevention and control strategies for future emerging zoonotic coronaviruses [3].

The correct application of transmission electron microscopy techniques allows a quick clinical diagnosis, and, therefore, the elaboration of prophylactic and disease control measures, avoiding the rampant dissemination of viruses [54].

The use of the negative staining technique (rapid preparation), allowed to capture photographic images with greater focus, allowing to accurately and smoothly visualize the club-shaped surface projections, and can be widely used in the research of coronavirus particles in samples from different specimens.

Coronavirus has already been reported in different animals of the mammal class; however, this is the first occurrence in giant anteater (*Myrmecophaga tridactyla*).

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