Detection of Coronavirus-like Particles in Wild, Exotic and Captive Animals by Transmission Electron Microscopy Catroxo, M.H.B.^{1*}; Martins, A.M.C.R.P.F.²; Miranda, L.B.³; Milanelo, L.⁴;

Santos, E.M.⁵

1,2,5 Electron Microscopy Laboratory, Research and Development Center in Animal Health, Biological Institute, São Paulo, SP, Brazil

³Federal University of Goiás, Goiânia, GO, Brazil ⁴Tietê Ecological Park, São Paulo, SP, Brazil *Corresponding Author: Marcia H.B. Catroxo

Biological Institute, Avenida Conselheiro Rodrigues Alves, 1252, Vila Mariana, São Paulo, SP, Brazil *Corresponding Author

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Abstract— Coronaviruses infect humans and a wide diversity of mammalian and bird species causing respiratory, enteric, neurologic and hepatic disorders. Due to their facility of adapting to new species and establishing sppilover events, coronaviruses pose a risk to global public health. Considering the zoonotic risk of coronaviruses, their role in wild species that host wild animals, as well as their ability to adapt to new species, seems to be the fundamental key to understanding their pathophysiology. The objective of this work was to report the presence of coronavirus-like particles in wild, exotic and captive animal species, in fecal or small intestine samples, using negative staining technique for transmission electron microscopy. 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. This report is the first worldwide occurrence of coronaviruses in Falco peregrinus, Tayassu tajacu and Tayassu pecari and the first occurrence in Brazil in Sus scrofa, Nosua nosua, Puma concolor and in Rhea Americana.

Keywords— Coronavirus, wild, exotic and captive animals, Transmission electron microscopy.

I. INTRODUCTION

The transmission of pathogens from wild animals to humans is called zoonotic spillover, which indicates that this phenomenon plays an important role in the emergence of new infectious diseases, representing a global burden on public health. Although associated with several outbreaks, it is still a poorly understood phenomenon. Coronaviruses circulate in nature in several animal species. There is a consensus that the possible origin of the virus comes from a wild animal, considering that most of the new viruses that crossed the evolutionary barrier and infected humans throughout the epidemiological history of viral diseases, were of animal origin, such as SARS and MARS that caused worldwide epidemics [1]. While still preliminary, current data suggest bats are the most likely initial source of the current Covid -19 outbreak, which began in December 2019 in Wuhan, China [2; 3].

Activities and processes that increase human interaction with the different animal species and pathogens they host, which include handling, poaching and indiscriminate consumption of wild animal meat and by-products without sanitary measures, are associated with an increased risk of spillover events[4]. These factors associated with habitat degradation and fragmentation, climate change and infectious diseases contribute to the extinction of many species of wild animals [5].

Coronaviruses have crossed these species barriers and during the last two decades, three zoonotic coronaviruses have been identified as the cause of large-scale disease outbreaks, Severe Acute Respiratory Syndrome (SARS), Middle Eastern Respiratory Syndrome (MERS) and Syndrome of Acute Swine Diarrhea (SADS) [6]. Coronavirus infection results in respiratory, enteric, neurological, and liver disorders [7].

All pathogenic human coronaviruses have their origin in animals [8], and studies have been carried out in an attempt to obtain clarification on the capacity of domestic and wild animals to be potential reservoirs [9].

Considering the zoonotic risk of coronaviruses, their role in wild species that host wild animals, as well as their ability to adapt to new species, seems to be the fundamental key to understanding their pathophysiology [8].

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) [10].

Coronaviruses infect humans and a wide diversity of mammalian and bird species causing respiratory, enteric, neurologic and hepatic disorders [7]. Most coronaviruses replicate in epithelial cells of the respiratory tract producing respiratory symptoms while others infect epithelial cells of the enteric tract, causing diarrhea severely, sometime fatal, in young farm animals, causing serious damage to livestock [11; 12].

The objective of this work was to report the presence of coronavirus particles in wild, captive and companion animal species, in fecal or small intestine samples sent to the Electron Microscopy Laboratory of the Biological Institute for identification of the viral agent.

II. MATERIAL AND METHODS

Description of cases

Wild boar (*Sus scrofa*) – In 2001, around 8 wild boars, from farms located in Paraguaçu Paulista, Serra Azul and Ribeirão Preto, of São Paulo State, Brazil, suddenly presented yellowish and watery diarrhea, loss of appetite, dehydration and death. One of the animals was found dead.

Collared peccary (*Tayassu tajacu*) e White-lipped peccary (*Tayassu pecari*) – In 1995, feces were collected from 2 collared peccaries and 2 white-lipped peccaries from CIZBAS (ESLQ/USP Animal Science and Wildlife Research Committee ESALQ/USP), located in Piracicaba, SP, Brazil, where the animals were kept in the picket sector for experimental studies to determine levels of energy and protein for growth and weight gain. The animals had diarrhea with pasty and yellowish stools, loss of appetite and dehydration. Animals after supportive treatment recovered. In this place, a group of rheas (*Rhea americana*) was also kept for experiments related to the assessment of the relationship between nutrition and bone deformities of the legs during the growth period. Two of these animals suddenly developed profuse, yellowish diarrhea, followed by anorexia, prostration, dehydration and death.

Brocket deer (*Mazama gouazoubira*) – In 1995, a young female brocket deer was rescued and sent to the Serra do Itapety Municipal Park, Mogi das Cruzes, SP. After identification, marking and routine examinations, it was observed that the animal presented watery, yellowish diarrhea, anorexia, apathy and dehydration.

Coati (*Nosua nosua*) – In 2003, a free-living male coati, coming from the Tietê Ecological Park, SP, was found seriously prostrate and with dyspnea in the surroundings of the Park. The animal was attended at the CRAS (Wild Animal Recovery Center), where it was medicated, but after 3 days it died. During necropsy it was possible to observe the presence of severe pyothorax associated with pneumonia, pericarditis and intestines containing yellowish stools.

Peregrine falcon (*Falco peregrinus*) – In 2004, during a survey of viral agents among the birds in the Tietê Ecological Park, feces samples were collected from a rescued peregrine falcon victim of an illegal bird trade. The animal presented watery and greenish stools.

Montain lion (*Puma concolor*) During a survey of viral agents carried out among animals kept in captivity in a breeding site, located in the municipality of Carapicuíba, SP, in 2004, fecal samples from 2 puppies of Montain lion (*Felis concolor*) were collected, among others. The animals, with approximately 10 months of age, were kept in a cage together with their parents, from birth. From three months, the puppies were suddenly separated from their parents, being weaned with the purpose of adapting them to human coexistence. Soon after, they presented watery, yellowish diarrhea and signs of dehydration. Samples of feces from adult animals were also collected.

Golden-faced lion tamarin (*Leontopithecus chrysomelas*) – During a study carried out among primates from an Ecological Park located in Americana, SP, in 2004, fecal samples were collected from 6 animals (3 males and 3 females), adults, natives of the south of Bahia, belonging to a group that were kept in enclosures, for research related to captive reproduction. Pasty stools had a yellowish color.

Ferret (*Mustela putorius furo*) - Stool samples from 86 ferrets (offspring aged 20 days to 6 months and adults aged 2 to 7 years) were referred from clinical cases for investigation of the viral agent, between 2006 and 2018. The main clinical symptoms and signs presented by the animals were, coryza, hyporexia, weight loss, periodic colitis, emesis, dehydration, abdominal distension, and yellowish watery to pasty diarrhea, sometimes with mucus or blood, lasting from 2 days to 1 month. One animal died.

Chinchilla (*Chinchilla lanígera*) – A domestic chinchilla suddenly showed clinical signs of anorexia, diarrhea, prostration and sudden death, in 2010. During necropsy, it was observed that the intestines were dilated, containing watery and yellowish stools.

Buffaloes (*Bubalus bubalis*) – Fecal samples from two 60-day-old male buffaloes (*Bubalus bubalis*) from a property in Pilar do Sul, São Paulo State, Brazil, were sent to search for viral agents in 2009. The animals had acute enteritis for 20 days, with yellowish stools.

III. METHODS

Negative staining technique (rapid preparation). Samples of feces and small intestine fragments from the animals 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 [13].

IV. RESULTS

Using a Philips EM 208 transmission electron microscope all the samples were analyzed analyzed by negative staining technique and a great number of coronavirus-like particles, pleomorphic, rounded or elongated shaped, with characteristic radial projections forming a corona and measuring 80 -140nm in diameter, were observed in all samples (figures 1-12)

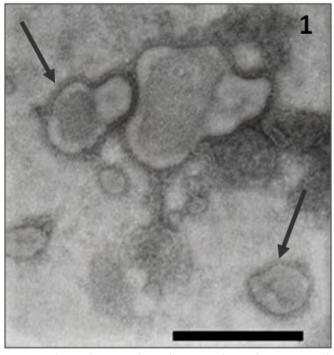


FIGURE 1: Pleomorphic coronavirus-like particles in small intestine suspension of the wild boar (Sus scrofa), showing radial projections of the envelope (arrow). Bar: 160 nm.

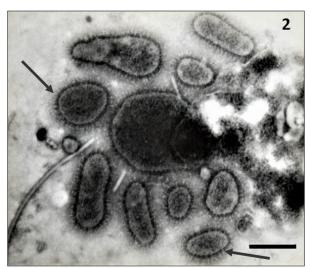


FIGURE 2: Grouping of elongated coronavirus-like particles in feces of collared peccaries (*Tayassu tajacu*), showing characteristic radial projections forming a solar corona (arrow). Bar: 70 nm

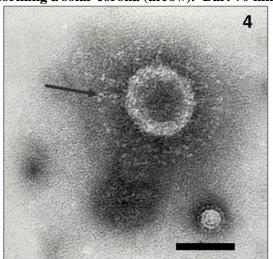


FIGURE 4: Coronavirus-like particle showing a characteristic envelope in the form of a solar corona or goblet (arrows), in feces of *Rhea americana*. Bar: 64 nm.

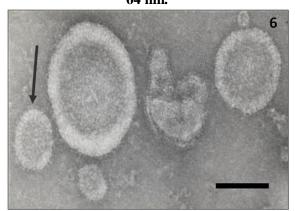


FIGURE 6: Oval coronavirus-like particles showing a characteristic electron dense central corion in the small intestine of *Nosua nosua* (arrow). Bar: 120 nm

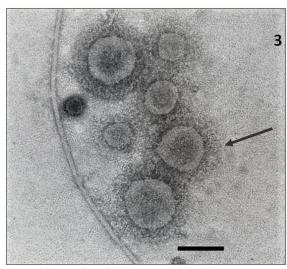


FIGURE 3: White-lipped peccary (*Tayassu pecari*) stool suspension with compact distinctive projections that extend in a corona over the particle surface (arrow).Bar: 90 nm



FIGURE 5: Coronavirus-like particles showing spaced and petal-shaped spikes (arrows) in feces of *Mazama gouazoubira*. Bar: 120 nm

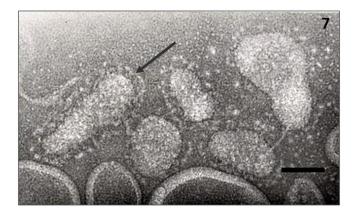


FIGURE 7: coronavirus-like particles exhibiting thin, wispy, and widely spaced spikes in feces of Falcon peregrine (arrow). Bar: 54 nm.

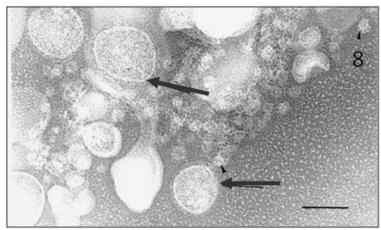


FIGURE 8: Coronavirus-like particles showing a fringe of shorter spikes (arrow) and astrovirus (arrow head) in feces of *Felis concolor*. Bar: 70 nm.

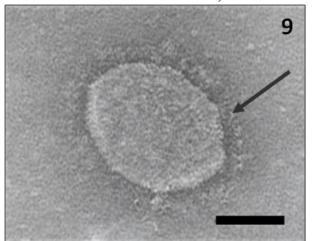


FIGURE 9: Coronavirus-like particles showing delicate spikes that make up the envelope in *Leontopithecus chrysomelas* (arrow). Bar: 60 nm.

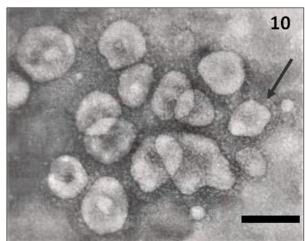


FIGURE 10: Large numbers of rounded coronavíruslike particles showing a characteristic envelope and an electron-dense central corion in feces of *Mustela* putorius furo (arrow). Bar: 130 nm.

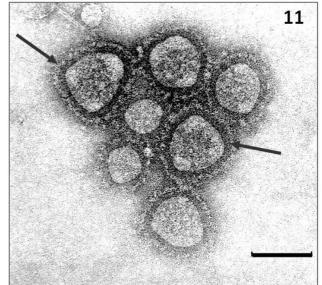


FIGURE 11: Coronavirus-like particles displaying petal or globet-shaped spikes on the surface of the virion in small intestine suspension of *Chinchilla lanigera* (arrow). Bar: 160 nm.

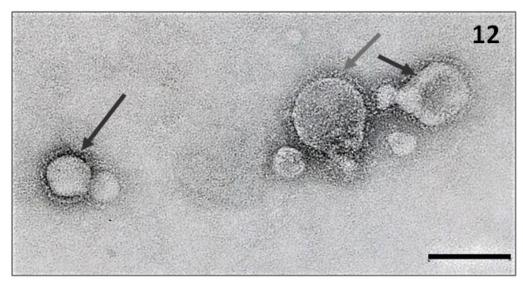


FIGURE 12: Rounded (big arrow) and pleomorphic (minor arrow) coronavirus-like particles with typical envelope (gray arrow), in feces of *Bubalus bubalis*. Bar: 140 nm.

V. DISCUSSION

Anthropogenic and natural actions are closely linked to ecological changes that can influence the epidemiology of zoonoses, affecting wild animals that can serve as reservoirs [14].

The knowledge accumulated so far shows that their occurrence may not result from the direct presence of animals, and even less from high levels of biodiversity, but from human action and the way we interact with the natural environment. In fact, the reduction in the number of species and their habitats appears to be a much more direct cause for the spread of wild-borne diseases [15; 16].

In our study we found coronavirus particles in several species of wild animals. The particles were pleomorphic, rounded or elongated, containing radial projections or spikes that emerge from the envelope forming a corona and measuring 80-140 nm in diameter. The morphological characteristics of these particles correspond to those described by other authors in other species of wild animals such as capybaras [17], Giant Anteater [18]; ferrets [19]; doves [20], owls [21], buffaloes [22]; [23], frogs [24], and, minks [25].

The presence of watery to pasty diarrhea with yellowish coloration was the main clinical sign observed in all animals. Other signs, such as, anorexia, apathy, prostration, dehydration and death, were also found in the animals in our research, also reported in other wild animal species [17, 18, 19, 20, 21].

Coronaviruses have been associated with important enteric processes in several animal species, which can cause high mortality in breeding, causing damage to livestock [11].

The Moontain lion puppies of the our study were suddenly separated from their parents, a procedure recommended for adapting to human life, and were suddenly subjected to a stress factor. Since the parents maintained a position of healthy carriers, favoring the contagion of the offspring, probably after weaning, when there is a decrease in immunity. According to Pedersen et al. (2004) [26] factors such as confinement, mating, feeding and handling practices constitute important procedures that cooperate in favor of the release of viral processes, especially in carriers animals. Animals were co-infected with astroviruses. These viruses, however, have been associated with mild diarrhea in cattle [27], sheep [28], swine [29], dogs [30] and birds [31]. In cats, however, they are responsible for severe diarrhea (Aydin &Timurkan, 2018) [32]. In most cases, astroviruses have been isolated in association with other viruses, also present in the feces of diseased animals [11].

Studies have shown that cats can be naturally infected with SARS-COV-2. During a survey conducted among felines in Spain, a lion tested positive for SARS-CoV-2 RNA and a Zoo puma located in South Africa had a confirmed diagnosis for the viral RNA after coming into contact with an infected handler. Another investigation showed that a snow leopard was positive for viral RNA at a Zoo in Kentucky, United States [33].

The brocket deer (*Mazama gouazoubira*) and the golden-faced lion tamarin (*Leontopithecus crysomelas*) in our work were also subjected to stressful conditions. The first one was rescued and sent to a Wildlife Recovery Center, a foreign place to the

animal and the monkeys were kept in enclosures for research related to captive reproduction, due to its status as an endangered species, according to the International Union for the Nature Conservation (UICN) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio) [34]; [35]. In addition, their habitat is increasingly fragmented, which raises concerns about the species' survival [36, 37]. The phylogenetic proximity between non-human primates and humans favors the cross-transmission of infectious agents and poses a threat. It must be considered, therefore, that unlike most mammal species, non-human primates are sociable species which exponentially expands the spread of shared intraspecific diseases [38], highlighting the yellow fever that caused an outbreak in 2016 and 2019 [39] and Zika cases [40]. According to Guimarães et al. (2020) [41] although the natural cross-transmission of SARS-CoV-2 between humans and non-human primates has not been demonstrated, the global spread of the virus represents a potential threat. Thus, establishing preventive surveillance, and control measures for viruses of the family *Coronaviridae* in Neotropical non-human primates populations is crucial for their conservation. White-tailed deer and some monkey species are susceptible to SARS-CoV-2 infection [42].

The Long-tailed Chinchilla (Chinchilla lanigera), included in our work, is a species currently threatened with extinction, as the animal is widely used as a pet and in fur production in the early 2000s. Because the chinchila is an exotic species in Brazil, there are few reports of viral infections in this species [43]. It is likely that chinchillas are carriers of coronaviruses. According to the CDC (Center Disease Control) [44] about 75% of infectious diseases that affect humans originate in other animals. Similar to the chinchilla, the mink, a mustelid species also threatened, has been bred for years focused on the production of skins [45]. Mink, chinchillas, raccoons, foxes and rabitts are bred for fur production in several countries [46]. According to a report by PETA - People for the Ethical Treatment of Animals (2021) [47], these animals are constantly exposed to various conditions of exhaustion, such as extreme confinement in breeding sites with dubious sanitary conditions, fear, poor nutrition, diseases, physical and psychological disorders and death inhuman. These conjunctions can trigger several diseases whose viral agents are housed in many of these species. The World Health Organization has reported the emergence of a mink-associated SARS-CoV-2 variant [48]. People in Denmark and the Netherlands are already infected with a mutant SARS-CoV-2 (Y453F) originated from minks [49]. The susceptibility of mink to SARS-CoV-2 was not a surprise, as they are closely related to ferrets that have already been found to be susceptible in experimental inoculation [50,51, 52]. During an outbreak of epizootic mink catarrhal gastroenteritis, coronavirus particles were detected by transmission electron microscopy [25].

All ferrets in our survey presented yellowish diarrhea. Two types of coronaviruses affect ferrets, enteric coronavirus (FREVC) and systemic coronavirus (FRSCV), an emerging disease of ferrets. FREVC is known to cause gastroenteric disorders (epizootic catarrhal enteritis) and FRSCV for its pyogranulomatous similarity to Feline Infectious Peritonitis, in cats. In epizootic catarrhal enteritis the stools are greenish, but in the hypersection phase there may be a period of poor digestion or malabsorption and the stools become yellowish containing a granular material [53, 54], where coronavirus particles can be easily identified by electron microscopy, during the acute phase of the disease process [53]. Two animals had bloody diarrhea and one of them died. The severity of clinical signs varies and the presence of blood in the stool can occur in the presence of secondary infections, causing ulcerations of the intestinal wall [53]. The presence of coronavirus in feces of ferrets with diarrhea has been reported in Brazil [19]. The color of the feces, together with clinical signs and symptoms, suggests that the animals were affected by epizootic catarrhal enteritis. In Europe, Asia and the United States, Mustela putorius furo was used for commercial fur farming and is currently considered a pet [55]. The results of experimental inoculation of SARS-CoV-2 indicate that cats and ferrets are highly susceptible [52]. Currently in Brazil, the ferret is considered a pet, and its commercialization is monitored by IBAMA in order to control pathogens and avoid ecological instability [55, 56]. Natural infections of SARS-CoV-2 have been reported in various animal species, including ferrets [57]. Both mink and ferrets belong to the Family Mustelidae. Therefore, the pattern of infection suggests that other members of the family Mustelidae may be susceptible to SARS-CoV-2 and therefore require further investigation [42].

The collared peccaries (*Tayassu tajacu*) and white-lipped peccaries (*Tayassu pecari*) in our survey are species currently raised in captivity for meat consumption. The breeding of these species in captivity has been widespread in several countries in recent decades, potentially because the meat has quality standards close to those required in the consumer market and due to the demand for exotic meats, mainly in specialized restaurants. Captive breeding of collared peccaries and white-lipped peccaries can minimize the effects of predatory hunting, habitat fragmentation, trafficking and species extinction, which could result in ecological instability. The free life or captivity Tayassuids are susceptible to the main infectious agents that also affect domestic pigs, including the swine transmissible gastroenteritis (TGEV) and pathogens with zoonotic potential [58, 59].

Wild boar meat (*Sus scrofa*) is highly appreciated and sought after all over the world due to peculiar flavor and nutritional content, however, its consumption is not recommended due to health risks [60]. Wild boar meat is consumed, mainly in the form of barbecue, in the South and Southeast regions (https://revistapesquisa.fapesp.br/carne-de-javaporco-pode-transmitir-doencas/) [61]. Several coronaviruses (PEDV, PDCV, SADS-CoV and TGEV), which originated from interspecies, infect the pig (*Sus scrofa*) and cause acute gastroenteritis in neonatal piglets and death of animals, leading to economically relevant problems to the pig industry [12, 62]. Frequent contact between humans and swine could lead to a higher risk of cross-species transmission or virus recombination [63]. Zhou et al. (2018) [62] reported that SARS-CoV-2 could use ACE2 from four animal species including the porcine ACE2 as the receptor to enter the cell in vitro, showing that pigs might be potentially susceptible to SARS-CoV-2 infection and could be a potential intermediate host. Crossbred pigs (*Sus scrofa domesticus*) with 8-week-old were found to be susceptible to SARS-CoV-2 infection following oronasal inoculation. Although SARS-CoV-2 RNA was detected in the oral fluids and nasal wash collected from two pigs, live virus was isolated only from one animal [64]. Based on the phylogenetic tree and recombination detection program, Hu et al. (2020) [63] stated that swine and mice could be probable reservoirs for the Sars-CoV-2.

The buffaloes in our work came from beef breeding. In Brazil, the exploitation of buffaloes is primarily intended for meat production, however, from the 1980s onwards, there was a growing interest in their dairy or dual purpose (beef and milk) exploitation [65]. The presence of coronaviruses has been reported in feces of young buffaloes with diarrhea on properties in the State of São Paulo, Brazil [66] and in Italy [67]. The productivity of bovine and buffaloe herds is closely related to success in raising calves. Among the main causes of failure in buffaloe and bovine breeding, neonatal diarrhea stands out, responsible for the highest rates of morbidity and mortality in these breeding [68]. Bovine coronavirus (BCoV) is the etiological agent responsible for acting and/or aggravating infections associated with diarrhea and respiratory diseases in cattle of all ages, being an extremely contagious virus that affects the health of the herd and the world economic sector due to its high morbidity and significant mortality rate among calves [69]. Bovine CoVs have been reported to infect children and thus possess zoonotic potential. Spillover is managed by the interaction of viral-specific proteins like S protein and host ACE2 receptor [70].

The coati (*Nosua nosua*) included in our research presented severe pyothorax associated with pneumonia and pericarditis. Coronaviruses cause enteric and respiratory problems in cattle (Franzo et al., 2020) [71] and bovine-like CoVs have been identified in wild species such as elks [72], minks [73], raccoons [74] and in alpaca [75, 76]. In montains lions vascular necrosis and necrotizing myocardial changes have also been observed [77]. SARS-CoV-2 was recently detected in a coati mundi at an Illinois Zoo, in the United States. Samples of several zoo species, including coatimundi, were collected and tested after a zoo tiger showed signs of virus infection [78]. The coati (*Nosua nosua*) is host to several etiological agents that can be harmful to its populations and others with zoonotic potential. In urban areas, coatis can be found in close association with humans and domestic animals [79].

The rheas and the peregrine falcon from our work suddenly developed profuse diarrhea. In birds, the disease caused by the coronavirus is infectious bronchitis, whose main clinical signs are respiratory, including sneezing, nasal discharge, tracheobronchiolar rales, conjunctivitis and sinusitis. Strains of IBV that affect the kidney, but can result in diarrhea, severe dehydration and mortality due to kidney failure [80]. The chickens and pheasants are natural hosts of IBV [81], although IBV isolates have also been reported in peafowl, turkey, teal, geeze, quill, duck, parrot, quail, penguin and guinea fowl [82]. Coronavirus particles have already been found in free-living animals such as doves, owls and in passerines such as the Lined Seedeater (Sporophila lineola) [20, 21, 83]. Probably the rheas acquired the disease through contact with infected chickens present in the creation, or through the feces of wild birds that carry the virus. A similar occurrence was reported in a breeding of rheas in the United States, where the bird showed weakness, ataxia, enteritis and death, after introducing new birds into the breeding. Coronavirus particles were detected in small intestine fragments by transmission electron microscopy [84]. The peregrine falcon could also be acting as an IBV host and suddenly develop diarrhea after being removed from the wild, considering that the bird was a victim of illegal trade. The presence of the IBV in wild and exotic birds can be assigned by the interaction between species or by its close proximity to commercial poultry farms [85]. The variation in host range, tissue tropism, and pathogenesis of CoVs is mainly due to variations in the glycoprotein and lead to different antigenic forms, serotypes, or variant strains [86, 87]. Although the presence of the coronavirus in wild birds might suggest the possibility that they are reservoirs of COVID-19 with transmission to humans, infection of COVID-19 in poultry would be unlikely, mainly due to the incompatibility of cell receptors that would not recognize the same type of virus [88].

People with COVID-19 can spread the virus to animals during close contact. It is important for people with suspected or confirmed COVID-19 to avoid contact with pets and other animals to protect them from possible infection [78].

It is likely that the new coronavirus is circulating in wild reservoir animals and that these have the potential to emerge very quickly and cause a new epidemic due to human consumption habits and their interrelationship with nature [88].

Crucially, efforts to reduce poaching and the indiscriminate consumption of wild animals must be conducted to change the normative values of consumers through education, raising awareness not only for public health, but also for protection and welfare animal living and global biodiversity [89].

VI. CONCLUSION

Studies aimed at determining the presence of the coronavirus in wild animals should be continued to better characterize the interspecies barrier transmission between these viruses and different animal species in order to avoid possible losses, mainly due to the fact that many species are included in the list endangered species official. Attention should be given to public health and veterinary surveillance programs, including monitoring of wild animals in order to contribute information to assist prevent future emergencies, outbreaks and pandemics [90].

This report is the first worldwide occurrence of coronaviruses in *Falco peregrinus, Tayassu tajacu* and *Tayassu pecari* and the first occurrence in Brazil in *Sus scrofa, Nosua nosua, Puma concolor* and in *Rhea Americana*.

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