

Detection of Coronavirus in Capybaras (*Hydrochoeris hydrochaeris*) by Transmission Electron Microscopy in São Paulo, Brazil

Detección de Coronavirus in Capybaras (*Hydrochoeris hydrochaeris*)
por Microscopía Electrónica de Transmisión en São Paulo, Brasil

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SUMMARY: Coronaviruses are enveloped, positive-stranded RNA, belongs to the *Nidovirales* order, *Coronaviridae* family and are classified into three groups. Groups 3a and 3c comprising the avian coronaviruses. They are worldwide in distribution and can cause respiratory, enteric and in some cases hepatitis and neurological diseases in a wide variety of animals, resulting in economic losses to breeders and breeding. In this study, we describe an outbreak of diarrhea in a herd of capybaras found in the city of Piracicaba, São Paulo, Brazil. The animals had sudden symptoms of anorexia, profuse and watery diarrhea, yellowish or greenish, dehydration, weight loss, sharp and twisting of limbs leading to death in less than a week, mostly weaned and with an age of three months. A total of 59 stool samples and 10 fragments of small intestine were processed for negative staining technique (rapid preparation). By transmission electron microscope a large number of particles with morphology similar to coronavirus, pleomorphic, rounded or elongated features with radial projections form a corona and measuring 80-140 nm in diameter, was visualized in all samples analyzed. The technique was extremely useful for rapid viral diagnosis in the affected animals.

KEYWORDS: Coronavirus; Capybaras; Transmission electron microscopy.

INTRODUCTION

Coronaviruses are worldwide in distribution, highly infectious, and extremely difficult to control. They can cause respiratory, enteric and in some cases hepatic and neurological diseases in a wide variety of animals (Gorbalenya *et al.*, 2004), resulting in economic losses to breeders and breeding and dairy cattle industry (Boileau & Kamil, 2010).

In cases of diarrhea, it has been detected in diverse species as cattle (Dea & Garzon, 1991; Tsunemitsu *et al.* 1995; Catroxo *et al.*, 1999; 2007; Brandão *et al.*, 2002), equine (Cappellaro *et al.*, 1988), swine (Cappellaro *et al.*, 1998), dog (Hagiwara *et al.*, 1989; Catroxo *et al.*, 1998), monkey (Catroxo *et al.*, 1997) and bird (Catroxo *et al.*, 2000).

The enteric coronaviruses is characterized by anorexia, prostration, dehydration, dyspnea and death of animals of all ages, mainly young and those submitted to a confinement system (Fenner *et al.*, 1992).

The virus replicates itself in the villi of small intestinal absorptive cells and differentiated cells in the crypts of the colon, resulting in replacement by immature cuboidal cells, desquamation and villus shortening, decreasing the absorption surface of the intestine, causing an imbalance and mal-absorptive diarrhea that progresses to a state of dehydration, which leads in most cases, the death of animals (Dea *et al.*, 1981, Heckert *et al.*, 1990; Cappellaro *et al.*, 1998).

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The incubation period ranges from 2 to 8 days and the morbidity reaches 100% by the fourth day (Guard & Fecteau, 2009).

Infection is primarily via fecal-oral and to a lesser extent, respiratory or aerosol routes (Thomas *et al.*, 2006).

Coronaviruses are enveloped, positive-strand RNA, belongs to the Nidovirales order, Coronaviridae family and are classified into three groups. Groups 1 and 2 comprising the mammalian coronaviruses and group 3a and 3c, the avian coronaviruses (Woo *et al.*, 2009). They have the high frequency of recombination and high mutation rates that may allow them to adapt to new hosts and ecological niches (Herrewegh *et al.* 1998; Woo *et al.*, 2006).

The capybara (*Hydrochoerus hydrochaeris*) is a large herbivore rodent mammal, semi-aquatic habit, found throughout the national territory. Its captive breeding has been increasing in Brazil due to the marketing of beef in the national and international market, whose protein content is high and fat low. Reproduction is more efficient in these animals than in the bovine species and diet of low-cost are factors that economically incentive breeding. Moreover, the leather is appreciated in the international market due to the impermeability, lightness and resistance, being used in the manufacture of shoes, gloves and clothing. The oil, extracted from subcutaneous fat can be used for medicinal purposes, and fur in the manufacture of brushes and upholstery (Silva, 1986; Arouca *et al.*, 2000; Oda, 2002).

In spite of several factors encouraging breeding, little has been discussed about the diseases that affect this species and the importance of these animals as reservoirs of infectious agents (Sarkis, 2002).

Direct electron microscopy technique is the most perfect method to detect the presence of enteric viruses in stool samples (Arcangeletti *et al.*, 2005). It is generally used for the diagnosis of enteric coronaviral infections (Dea & Garzon; Tsunemitsu *et al.*, 1995; Cappellaro *et al.*, 1998; Catroxo *et al.*, 2007).

Considering the efficiency and speed of this technique, this study aimed to detect viral agents in fecal and intestine samples during an outbreak of diarrhea occurred in a herd of capybaras, in the city of Piracicaba, Brazil.

MATERIAL AND METHOD

Description of the outbreak. In the period from June to

December 1995, an outbreak of diarrhea occurred in a herd of capybaras (*Hydrochoerus hydrochaeris*), located in Piracicaba, SP, Brazil, where the animals were kept in individual sectors (maternity, nursery, picket and reproduction) for experimental studies to determine levels of energy and protein for growth and weight gain. For verification of weight gain the capybaras were captured every two weeks, and submitted to conditions of intense stress. Suddenly, animals showed symptoms of anorexia, profuse and watery diarrhea yellowish or greenish, dehydration, severe weight loss and twisting of anterior limbs, leading many animals to death in less than a week, mostly weaned and at three months of age. Stool samples from 59 capybaras (22 maternity sector, 10 pickets, 9 nursery, 17 of reproduction and one free-living capybara) and fragments of small intestines of 10 capybaras of the maternity sector were collected, kept under refrigeration and sent to the Electron Microscopy Laboratory of the Biology Institute of São Paulo to investigate viral agents. Feces and small intestine fragments were processed for transmission electron microscopy using negative staining (rapid preparation) technique.

Negative staining technique (rapid preparation). In the negative staining process, feces and small intestine fragments were suspended in phosphate buffer 0.1 M, pH 7.0. Drops of the obtained suspension were placed in contact with metallic copper grids with carbon stabilized supporting film of 0.5% collodium in amyl acetate. Next, the grids were drained with filter paper and negatively stained at 2% ammonium molybdate, pH 5.0 (Brenner & Horne, 1959; Hayat & Miller, 1990; Madeley, 1997). Observations were made in a Philips EM 208 electron microscope, at 80 kV.

RESULTS

Necropsia. Gross lesions confined to the gastro-intestinal tract and a stomach distended and congested were observed during the necropsy in 10 (100%) capybaras. The small intestine was also distended with yellow foamy fluid. The intestinal wall was thin and translucent due to severe atrophy of intestinal villi.

Negative staining technique (rapid preparation). Under the transmission electron microscopy in 49 (83%) of 59 feces samples and in the 10 (100%) samples of intestinal suspension, coronavirus particles were visualized in a great number when negatively contrasted with ammonium molybdate 2% and pH 5.0 (Figs. 1, 2). The viral particles showed pleomorphism marked, rounded or elongated shaped (Fig. 2), with characteristic radial projections forming a corona (Figs. 3, 4), and mostly presenting a central electron dense corion (Fig. 3) and measuring 80-140 nm in diameter. The club-shaped surface projections measured approximately 20 nm long.

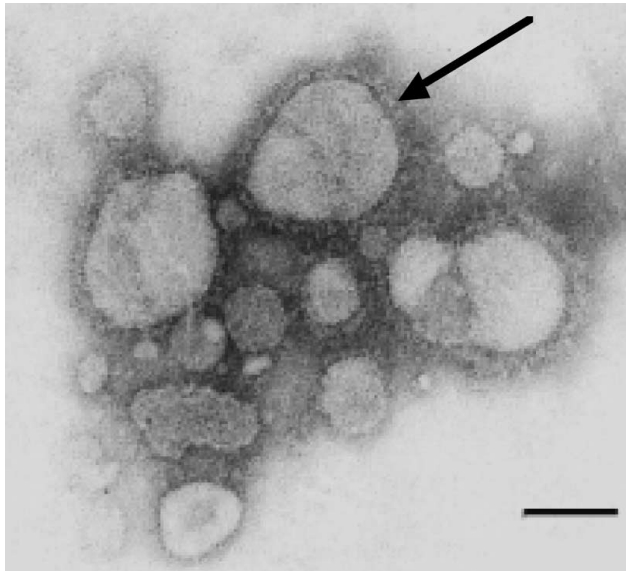


Fig. 1. Negatively stained showing coronaviruses particles grouping and external envelope (arrow). Bar: 80 nm.

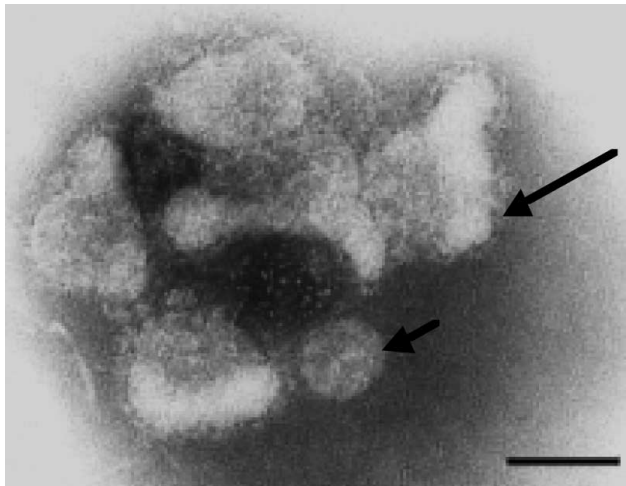


Fig. 2. Negatively stained coronaviruses particles showing pleomorphism marked, rounded (minor arrow) or elongated shaped (big arrow). Bar: 90 nm.

DISCUSSION

In this study, we observed by transmission electron microscopy, the presence of coronavirus in feces of capybaras, during an outbreak of diarrhea in a herd.

Clinical signs observed in the affected animals were anorexia, prostration, dehydration, dyspnea and death, accompanied by yellowish or greenish diarrhea with liquid to pasty consistency, recurrent type, represented by intermittency of symptoms with periods of improvement followed by diarrhea episodes.

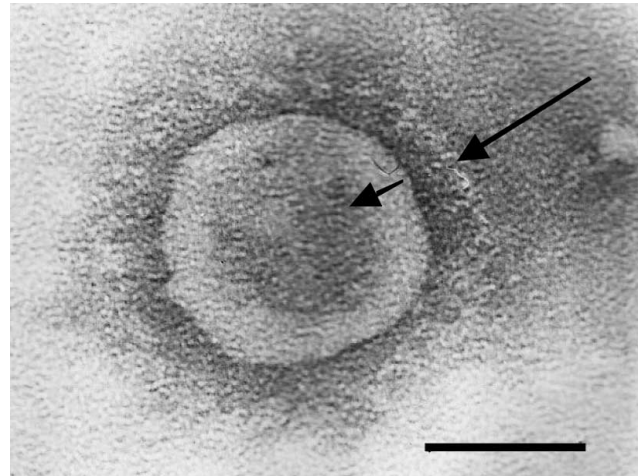


Fig. 3. Negatively stained coronavirus particle showing a central electron dense corion (minor arrow) and characteristic radial projections forming a corona (big arrow).

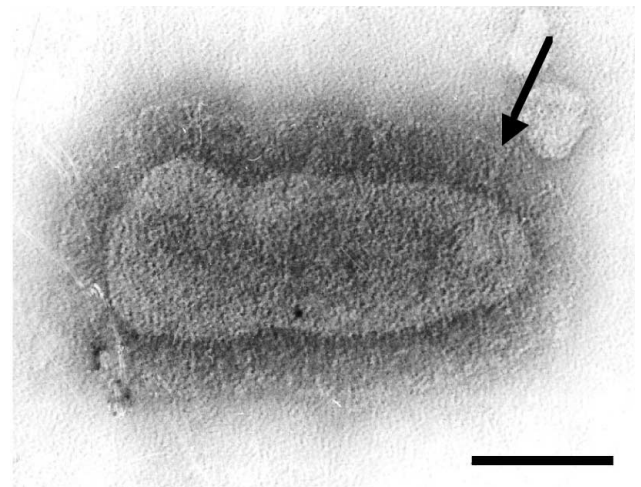


Fig. 4. Negatively stained coronavirus particle exhibiting thin, wispy, and widely spaced spikes. Bar: 100nm.

Similar outbreaks have been reported in cattle (Brandão *et al.*, 2007; Barry *et al.*, 2009), swine (Cappellaro, 1998), equine (Cappellaro *et al.*, 1988), dog (Hagiwara *et al.*, 1989; Dezengrini *et al.*, 2007; Decaro *et al.*, 2010), sheep (Pass *et al.*, 1982), feline (Pedersen *et al.*, 2008) and avian (Circella *et al.*, 2007) and among wild animals of zootechnical breeding, such as collared peccary (Catroxo *et al.*, 1995), rhea (Catroxo *et al.*, 1996 a); white lipped peccary, (Catroxo *et al.*, 1996 b), ostrich (Els & Josling, 1998) and wild boar (Bersano *et al.*, 2001).

Studies indicate that among animals there is a large reservoir for coronavirus not yet been recognized, such as for influenza virus (Holmes, 2009). The coronavirus has a high frequency of recombination and high mutation rate (Lai & Cavanagh, 1997) generating a diversity of strains and genotypes of new species becoming able to adapt to new hosts and ecological niches which might cause zoonotic outbreaks with disastrous consequences (Woo *et al.*).

By transmission electron microscope it could be visualized by negative staining, in 49 fecal and in 10 intestinal samples, a great number of particles with typical coronavirus morphology, measuring on average 140 nm in diameter. The morphological characteristics of these particles correspond to those described by other authors, who also used this technique in avian (Dea & Tissen, 1988; Catroxo *et al.*, 1996a; Circella *et al.*, 2007), dog (Catroxo *et al.*, 1998); Hagiwara *et al.*), cattle (Benfield & Saif, 1990; Dea & Garzon, 1991; Tsunemitsu *et al.*; Catroxo *et al.*, 1999; Barrera Valle *et al.*, 2005; Jeong *et al.*, 2005; Catroxo *et al.*, 2007), monkey (Catroxo *et al.*, 1997), swine (Capellaro *et al.*, 1998).

According to Fenner *et al.*, the virus more severely affects young animals, spreading very quickly and producing severe economic losses even before the establishment of control measures. In our study, the most affected animals were the weaned at three months.

During the experimental study in the herd, the capybaras were constantly submitted to intense stress situation, since they were captured every two weeks for check weighing.

In cattle, other risk factors such as, closed confinement, improper ventilation, inadequate handling of food, the constant presence of visitors or the introduction of a new animal on the property, may lead to the onset of the outbreak (Boileau & Kapil).

The application of good practices in the management of a herd of capybaras, reduce sanitary order problems preventing economic losses (Arouca *et al.*; Nogueira & Cruz, 2007).

Capybaras may play an important role in public health because they act as a viral reservoir, both in the wild and rural areas. Besides, the fact that they are social animals in relation to other animals and humans, it makes possible the viral transmission and the appearance of zoonoses (Gonzalez, 1995; Roman, 1999).

In suspected viral infection, the use of the method of transmission electron microscopy for detection of viral particles in stool samples is required when there is a need of a rapid epidemiological clinical diagnosis and in cases of unsatisfactory diagnoses (Arcangeletti *et al.*) and particularly valuable for following outbreaks of naturally acquired or viral gastroenteritis (Brandt *et al.*, 1981).

In this study, the use of negative contrast technique for transmission electron microscopy, which is essential to detect coronavirus in capybaras, allowed a rapid prophylactic and control measures in the herd of capybaras.

Although the enteric coronaviruses is often reported in several animal species, this is the first occurrence in capybaras (*Hydrochoeris hydrochaeris*).

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RESUMEN: Coronavirus están envueltos en una cadena positiva de ARN, (orden *Nidovirales*, familia *Coronaviridae*) y se clasifican en tres grupos. Grupos que integran la tercera 3c y los coronavirus aviario. Se distribuyen por todo el mundo y pueden causar enfermedades respiratorias, entéricas y, en algunos casos, hepatitis y enfermedades neurológicas en una amplia variedad de animales, resultando en pérdidas económicas a la ganadería y los criadores. En este trabajo se describe un brote de diarrea en un rebaño de capybaras en la ciudad de Piracicaba, São Paulo, Brasil. Los animales presentaron súbitamente, los síntomas de anorexia, diarrea, profusa y acuosa, de color amarillento o verdoso, deshidratación, pérdida de peso, agudo, torsión de los miembros y la muerte en menos de una semana, en su mayoría con tres meses de edad. Un total de 59 muestras de heces y 10 trozos de intestino delgado se procesaron por la técnica de contraste negativo (preparación rápida). En todas las muestras analizadas, al microscopio electrónico de transmisión se visualizó un gran número de partículas con morfología similar a los coronavirus, pleomórficas, con características redondeadas o alargadas con proyecciones radiales en forma de corona midiendo de 80-140 nm de diámetro. La técnica es sumamente útil para el diagnóstico rápido de virus en animales afectados.

PALABRAS CLAVE: Coronavirus; Capybara; Microscopía electrónica de transmisión.

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