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SHORT COMMUNICATION

First report of molecular detection of *Carnivore bocaparvovirus 1* (former CnMV: Canine Minute Virus) in Chilean domestic dogs

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ABSTRACT. *Carnivore bocaparvovirus 1* (CnMV) infects dogs worldwide and can be responsible for different clinical signs in neonates and bitches during gestation. There is a limited number of CnMV sequences available in the NCBI database, so far. The present preliminary study aimed to obtain evidence of CnMV infection status among domestic dogs in Chile and evaluate the genetic relatedness of the sequences obtained with any type of bocaparvovirus already reported. Two partial nucleotide sequences of the VP2 coding gene were obtained from forty blood samples and compared with 23 available sequences of *Carnivore bocaparvovirus* from 1 to 6. Chilean sequences grouped within *Carnivore bocaparvovirus* 1 group, being sequence 5434 (Accession number, MH544475) more similar to the Japanese strain HM-6 and sequence 5423 (Accession number, MH544476) to the Chinese strain SH1.

Key words: *Bocaparvovirus*, canine parvovirus type 1, minute virus of canines, *Parvoviridae,* canine.

INTRODUCTION

Carnivore bocaparvovirus 1 (formerly canine minute virus (CnMV); minute virus of canines (MVC), and also known as canine parvovirus type 1) is a viral species belonging to *Bocaparvovirus* genus, within the *Parvovirinae* sub-family and family *Parvoviridae* family (Adams *et al* 2014). Currently, this genus includes 21 species. The vast majority of these viruses were identified using molecular discovery approaches, and they have to be isolated yet (ICTV). CnMV is genetically related to *Bocaparvovirus* of ungulates and primates, in addition to 5 more species that infect carnivores (*Carnivore bocaparvovirus* 2 to 6) and is genetically distinguishable from other genera within the *Parvovirinae* sub-family that infect canines such as *Protoparvovirus* (Formerly *Parvovirus* genus) (ICTV)[1].

The genome of CnMV consists of single-stranded (ss) DNA, 5,097 to 5,390 bp in length (Ohshima *et al* 2004, Shan *et al* 2010, Schwarts *et al* 2014) encoding three capsid proteins, VP1, VP2, and VP3 (Schwarts *et al* 2012). All parvoviruses encode a nonstructural protein NS1 that is essential for viral DNA replication (Li *et al* 1990) and packaging of viral DNA into the capsid (Cotmore and Tattersall 2005). Sequencing of both NS1 and VP2 genes has been used for phylogenetic analyses and epidemiological investigations in CnMV (Guo *et al* 2016).

Infection with CnMV has been associated with enteric and respiratory symptoms in neonates, reproductive failures with embryonic or fetal death after early transplacental infection and with late abortion, but most infections occur sub-clinically (Kapoor *et al* 2012, Bodewes *et al* 2014, Choi *et al* 2015). The clinical outcome caused by CnMV is most commonly seen in animals between the ages of 1 to 5 weeks (Harrison *et al* 1992, Carmichael *et al* 1994, Järplid *et al* 1996). Serological evidence indicates that CnMV infection is widespread in the dog population, with a 5 to 70% seroprevalence worldwide (Carmichael *et al* 1994, Mochisuki *et al* 2002).

Although there is information about the presence of CnMV in canines from several countries of different continents including North America, Europe, and Asia, its presence in our country has not been registered. The present study aimed to obtain evidence of the CnMV infection status among domestic dogs in Chile[1].

MATERIAL AND METHODS

Forty blood samples from diseased patients were referred to the Bioingentech Laboratory between November 2017 and June 2018 for molecular detection of *Carnivore protoparvovirus 1*(also known as *Canine parvovirus type* 2). The samples were analysed through conventional PCR using two commercial kits with in-house primers developed by Bioingentech for *Canine Parvovirus type 2* and also for *Canine minute virus*. The PCR protocols amplified partial sequences of 435 bp for *Carnivore protoparvovirus 1* and 286 bp of the gene coding for VP2 of CnMV. The PCR conditions consisted of initial denaturation for 3 min at 94 ºC, followed by 30 amplifications cycles of denaturation for 30 s at 95 ºC, primer annealing at 56 ºC for 30 s, and extension at 72 ºC for 30s. The final extension step was performed for 5 min at 72ºC. After amplification, 5 µL of each reaction mixture was subjected to electrophoresis

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[1] ICTV. *The online 10th report of the International Committee on Taxonomy of Viruses. Genus: Bocaparvovirus*. https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/parvoviridae/1041/genus-bocaparvovirus , accessed 24 february 2020.

in 1% agarose gel, and the amplified gene products were visualized under UV light after GelRed™staining.

The PCR products that amplified CnMV from 2 samples were purified using the Kit Wizard® SV gel and PCR clean-up system (Promega). Finally, the purified PCR products were sequenced to verify that the bands corresponded to the correct genes. Sequencing was performed on an ABI PRISM® 3130 Applied Biosystems instrument at the Pontifical Catholic University of Chile. Once obtained, the two sequences were submitted to GenBank (strain 5423: accession number, MH544476 and 5434: accession number, MH544475) and were further evaluated by multiple pairwise comparisons with another 23 international sequences of *Carnivore bocaparvovirus* species from 1 to 6 that are reported infecting carnivores (canine, feline, mink) and the species type of the genus *Bocaparvovirus* (*Ungulate bocaparvovirus* 1) available in GenBank using the Clustal Omega program (Larkin *et al* 2007). Subsequently, the evolutionary history was inferred with the Maximum Likelihood method and Jukes-Cantor model (Jukes and Cantor 1969), and the test of phylogeny was the bootstrap method with 500 replications. The analysis involved 26 nucleotide sequences and a total of 260 positions in the final dataset. The evolutionary studies were conducted in MEGA7 (Kumar *et al* 2006).

RESULTS AND DISCUSSION

From 40 blood samples analysed, there were 3 samples positive to CnMV (7.5%), 72.5% (29/40) to *Carnivore protoparvovirus 1* and 7.5% (3/40) presented coinfection with both viruses. The samples positive to CnMV were obtained from puppies between two and three months of age, and only two samples were sent for sequencing.

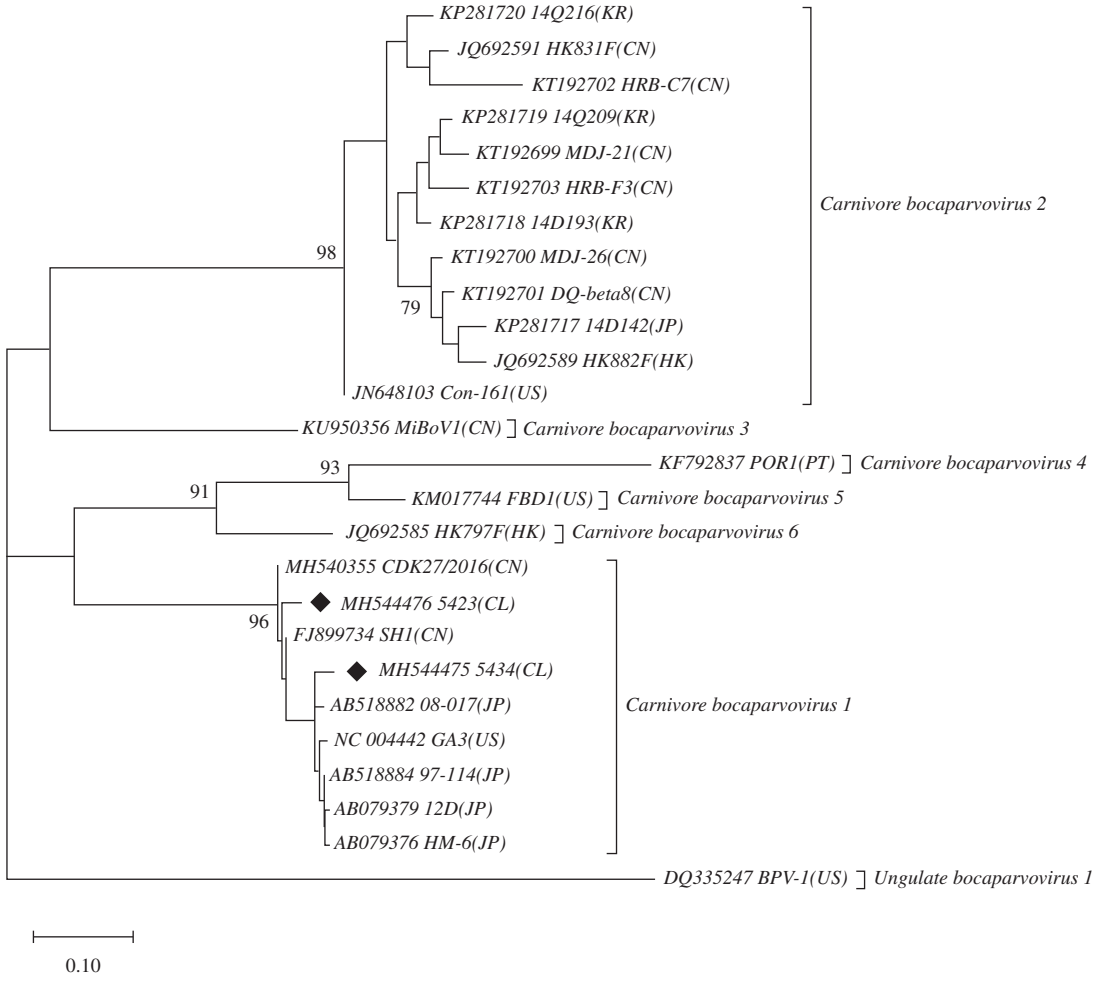

Figure 1. Phylogenetic analysis based on the nucleotide sequence of partial VP2 gene of *Carnivore bocaparvovirus*1. Phylogenetic tree constructed using the Maximum Likelihood method and Jukes Cantor model method with 500 bootstrap replicates. Only bootstrap values >70% are displayed over the tree branches. GenBank accession numbers, strain names, and countries of isolation are shown. Black rhombus indicate Chilean sequences obtained in this study. There was a total of 260 positions in the final dataset. Location codes: Japan (JP), Chile (CL), United States (US), China (CN), Hong Kong (HK), Portugal (PT), Korea (KR). Bovine parvovirus 1 (BPV, *Bocaparvovirus*).

The two partial Chilean sequences of CnMV are epidemiologically unrelated, but they shared 94.6% identity between them and 94.6-97.6% with partial sequences from China, Japan, and the United States genotyped as *Carnivore bocaparvovirus* 1 (data not shown). The highest identity percentages were observed between sequence 5434 and the Japanese HM-6 (97.6%) and between sequence 5423 and the Chinese SH1(97.37%).

In this first phylogenetic approach, the two Chilean partial VP2 sequences grouped within CnMV species, together with sequences from the United States, China, and Japan including the U.S. reference strain GA3 (GenBank accession no. AF495467). The remaining sequences were located in the *Carnivore bocaparvovirus* species 2 to 6 (figure 1).

The first evidence of CnMV in Chile reflects what happens around the world, with a wide distribution of these biological agents in enteric cases or subclinical infections. For instance, clinical specimens from 346 dogs were examined by PCR for CnMV presence in Japan. Specific gene fragments from samples of four diseased puppies were detected (1.2%). In addition, the same study shows that 5.0% of dogs possessed antibodies against CnMV (Mochisuki *et al* 2002). Likewise, Guo *et al* (2016) indicated that 15 out of 201 (7.5%) faecal samples from dogs affected with diarrhea were positive for CnMV in veterinary hospitals in China.

CnMV should be considered when young pups die suddenly with signs of respiratory or enteric disease or in cases of sudden death with heart failure (Pratelli *et al* 1999). Epidemiological investigation in China revealed a high co-infection rate of CnMV with other enteric viruses (*Carnivore protoparvovirus 1,* canine coronavirus, canine kobuvirus) suggesting CnMV as a potential enteric pathogen that could be associated with viral diarrhea in dogs (Guo *et al* 2016).

Similarly, the CnMV detected in Chile occurred in the context of enteric disease of young dogs' concomitant with infections with *Carnivore protoparvovirus 1*. Unfortunately, we have no information if the coinfected puppies also presented respiratory or cardiac problems. The epidemiology of CnMV infections must be investigated in our country to corroborate the link of these infections with respiratory, cardiac or exclusively enteric outcome. Further studies are needed to determine the prevalence of CnMV in the domestic dog population in Chile and to clarify the genetic relationships among Chilean and international CnMVs from the whole genome or the combination of complete VP2 and NS1 gene analyses. As it is a global concern, more experimental evidence should be generated regarding the role of CnMV in puppy mortality and dog reproductive disorders.

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