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Short communication

Betacoronavirus 1 in alpacas (*Vicugna pacos*) in the High Peruvian Andes

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ABSTRACT

Genetic sequences highly related to Bovine coronavirus (BCoV) were detected in fecal samples from Peruvian 1–3 week old alpaca crias located on six farms in Puno department, some of which shared pastures with cattle. A total of 60 samples were screened for coronavirus using a nested PCR amplification of a fragment of the RNA-dependent RNA polymerase (RdRp) gene. Sequences from 11 positive samples were highly similar to the Kakegawa, Quebec and Mebus BCoV strains (99.5–100.0%) and 99.2% identical to an alpaca Coronavirus (CoV) previously detected in the USA. The detection of genetic sequences related to BCoV from Peruvian alpaca crias suggests possible role of this virus on enteric disorders etiology in the High Andes.

1. Introduction

Coronaviruses (CoV) have long been recognized as causative agents of diseases that negatively impact production in livestock, poultry and companion animals (Holmes, 2003). Bovine coronavirus (BCoV) is classified in the Order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, genus *Betacoronavirus*, and species Betacoronavirus 1. It is prevalent worldwide and antibodies can be detected in the majority of cattle (Hasoksuz et al., 2008). Host-shifting events involving in cross species transmission in BCoV have been described in the past involving captive wild ruminants (Alekseev et al., 2008), dogs (Kaneshima et al., 2006) and humans (Holmes, 2003). Enteric infections associated with BCoV have been recognized in alpaca crias (Cebra et al., 2003) and has been detected in intestinal tissue from adult alpacas suffering hemorrhagic enteritis (Genova et al., 2008). This article reports the occurrence of a betacoronavirus similar to BCoV in fecal samples from 1 to 3 week old alpaca crias in the High Peruvian Andes, based on partial nucleotide sequencing of RNA-dependent RNA polymerase (RdRp) gene.

2. Materials and methods

2.1. Samples and localization

Between February and March 2009, 60 fecal samples with normally consistency from 1 to 3 week old asymptomatic crias, of six farms of the *La Raya* experimental research station in Melgar Province, Puno Department, Peru, which is located at 4200 m above sea level, were used in this study. Three of these farms, alpacas and cattle shared the same pastures. The method of sampling from animals complied with the principles of Ethical Committee in the use of animals of the School of Veterinary Medicine and Animal Science of University of São Paulo.

2.2. RNA extraction and nested PCR reaction

Total RNA of fecal samples was extracted using TRIzol Reagent™ (Invitrogen®), and cDNA synthetized using 50 ng of random primers (Invitrogen®) and 200 U of Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Invitrogen®) following the manufacturer's recommended protocol. Detection of CoV was performed using a pancoronavirus nested PCR reaction with specific primers targeting the RdRp gene (Chu et al., 2011). Positive and negative control reaction were Kakegawa strain of BCoV and Dietylpirocarbonate (DEPC) treated water respectively. Platinum

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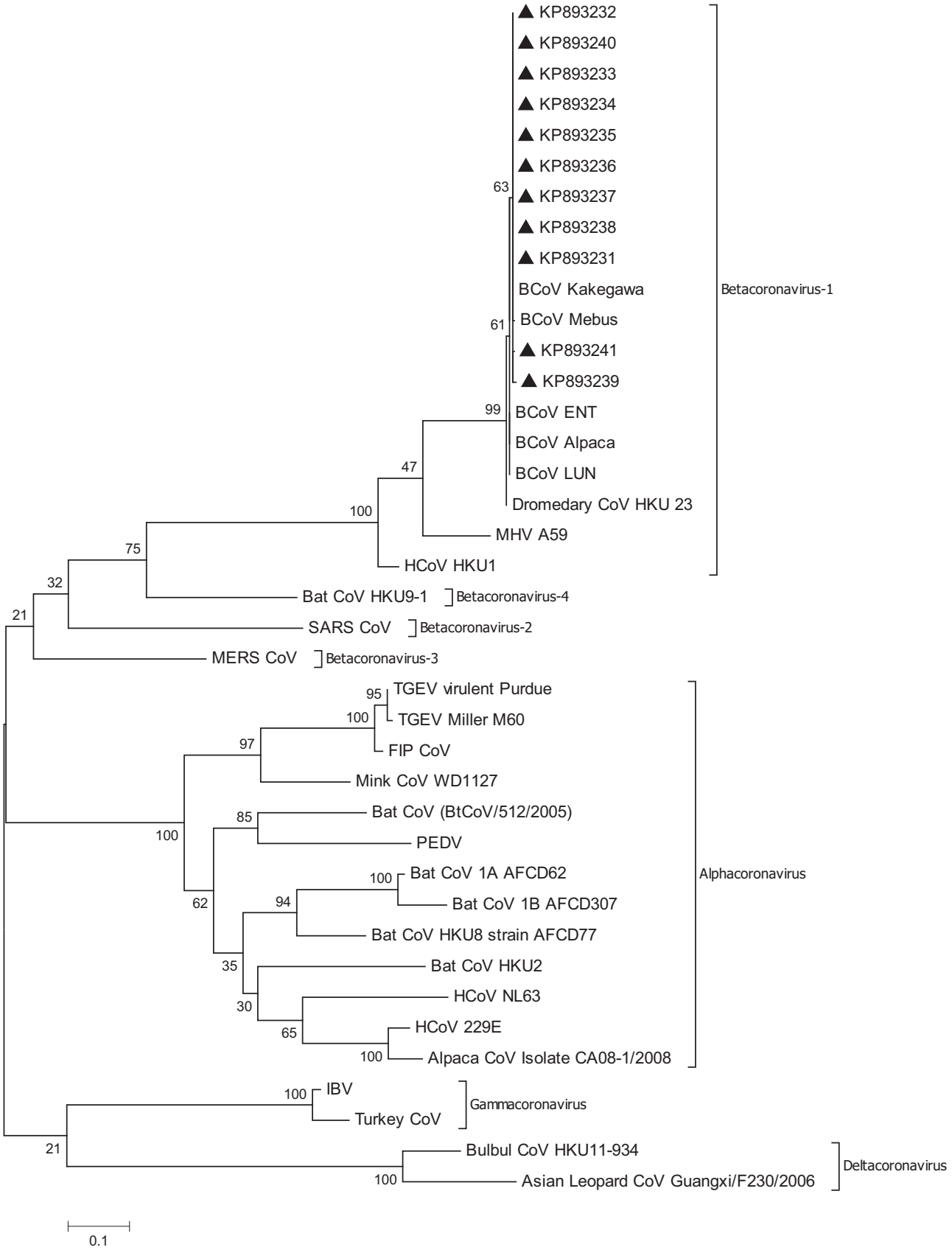

Fig. 1. Maximun likelihood phylogenetic tree of 397-nt fragment of the RNA dependent RNA polymerase (RdRp) gene. The sequences from this study are shown with a black triangle.

Taq DNA polymerase (Invitrogen®) was used for PCR and nested according to the manufacturer's protocol.

2.3. Sequencing and phylogenetic analysis

Amplicons of CoV were purified using ExoSAP-IT PCR product cleanup (Affymetrix®), and sequenced with an ABI prism 3500 genetic DNA analyzer (Applied Biosystems®). The Maximum likelihood tree using GTR as substitution model was constructed using MEGA v. 6.06 software (Tamura et al., 2013).

3. Results

Eleven of the 60 fecal samples were positive for coronavirus. These positive samples came from crias on two different farms, located approximately 100 m from each other, both with mixed alpaca-cattle pastures. Phylogenetic analysis of the generated sequences (393 nt) all segregated within the species Betacoronavirus 1 (Fig. 1).

The majority of the sequences obtained in this study (GenBank KP893231, KP893232, KP893233, KP893234, KP893235, KP893236, KP893238, KP893240, all from farm 1, and KP893237, from the farm 2) were identical to AB354579 (Kakegawa strain BCoV) and AF220295 (Quebec strain BCoV) in the area under analysis, while KP893239 and KP893241 (both from farm 1) had a similarity of 99.5% and 99.7%, respectively.

4. Discussion

When compared to the previously reported RdRp sequence of an alpaca betacoronavirus from the USA (DQ915164) the nucleotide identities of the Peruvian partial sequences were 98.4% (KP893239), 98.9% (KP893241) and 99.2% (KP893231, KP893232, KP893233, KP893234, KP893235, KP893236, KP893237, KP893238, and KP893240).

The nested-PCR detected CoV in 11/60 fecal samples, was further characterized as Betacoronavirus 1 (Fig 1). Betacoronavirus 1 includes strains of CoVs, which are very similar to BCoV found in domestic and wild ruminants, as well as to strains found in canines, porcines and humans, that very probably originated from a common ancestor similar to BCoV (Perlman and Netland, 2009).

The Peruvian sequences were more closely related to the Kakegawa, Mebus and Quebec strains of BCoV (100% of identity), but less related to the USA isolate of alpaca CoV (99.2%). This could be due to the geographical isolation of these farms maintaining a strain of virus in circulation in the population, unlike the North American alpaca Cov strain (Jin et al., 2007).

New World Camelids co-located with cattle for over 500 years in South America and, although the identification of coronavirus infection and diarrhea in alpacas is recent, it is possible that the virus crossed between species during earlier interspecies contact (Primo, 1992; Cebra et al., 2003). The genetic analysis of the S gene would help to the better understand the nature of the interspecies transmission event that could led to the origin of the alpaca coronaviruses (Perlman and Netland, 2009; Bidokhti et al., 2013).

To our knowledge this is the first detection of a BCoV-like coronavirus in fecal samples from Peruvian alpaca crias, despite the clinical significance of the BcoV-like coronavirus identified in this study remains to be determined due to this study design.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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