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Molecular characterization of canine kobuvirus in wild carnivores and the domestic dog in Africa

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ABSTRACT

Knowledge of *Kobuvirus* (Family *Picornaviridae*) infection in carnivores is limited and has not been described in domestic or wild carnivores in Africa. To fill this gap in knowledge we used RT-PCR to screen fresh feces from several African carnivores. We detected kobuvirus RNA in samples from domestic dog, golden jackal, side-striped jackal and spotted hyena. Using next generation sequencing we obtained one complete *Kobuvirus* genome sequence from each of these species. Our phylogenetic analyses revealed canine kobuvirus (CaKV) infection in all four species and placed CaKVs from Africa together and separately from CaKVs from elsewhere. Wild carnivore strains were more closely related to each other than to those from domestic dogs. We found that the secondary structure model of the IRES was similar to the *Aichivirus*-like IRES subclass and was conserved among African strains. We describe the first CaKVs from Africa and extend the known host range of CaKV.

Introduction

Kobuvirus is a recently described and emerging genus in the family *Picornaviridae* (Knowles et al., 2012). Currently this genus is divided into three species, *Aichivirus A* (formerly *Aichi virus*), first described from gastroenteritis in humans in the Aichi Prefecture, Japan, in 1989 (Yamashita et al., 1991), *Aichivirus B* (formerly *Bovine kobuvirus*) and *Aichivirus C* (www.picornaviridae.com). Kobuviruses are small, non-enveloped viruses with a single stranded, polyadenylated, positive-sense RNA genome that encodes a single polyprotein (Reuter et al., 2011). The genome contains a long 5′ untranslated region (UTR) which contains the internal ribosome entry site (IRES), a single open reading frame (ORF) that encodes a polyprotein, and a 3′ UTR. Within the picornavirus family, there are four classes of IRES elements (Type I–IV) and a new IRES subclass was recently described in *Aichivirus* (Yu et al., 2011). The polyprotein is proteolytically cleaved into a leader protein (L), and the structural (P1) and non-structural (P2 and P3) regions (Fig. 1a, Reuter et al., 2011). The role of the 3′-UTR is currently unclear, although there is evidence of its involvement in replication (Brown et al., 2004). Furthermore, conserved barbell-like secondary structures in the 3′-UTR have been recently described in distantly related picornaviruses (Boros et al., 2012). Recombination has been widely reported in the *Picornaviridae* (Lukashev, 2010, Sweeney et al., 2012) and is considered to play a significant role in the diversification of this family (Oberste et al., 2004). Recombination is expected to occur in kobuviruses but assessment of its potential importance is hampered by the relatively small number of published complete *kobuvirus* genomes available (Lukashev, 2010).

Current knowledge of *Kobuvirus* infections in carnivores is extremely limited. *Kobuvirus* infection has been reported in three domesticated carnivores (the ferret, *Mustela putorius furo*, the domestic cat, *Felis catus* and the domestic dog, *Canis lupus familiaris*). The novel *Kobuvirus* described from ferrets in Europe belongs to the *Aichivirus B* species (Smits et al., 2013). Feline kobuvirus (FeKV) and canine kobuvirus (CaKV) belong to the *Aichivirus A* species. FeKV infection has only been reported in domestic cats in Asia (Chung et al., 2013). CaKV infection has been

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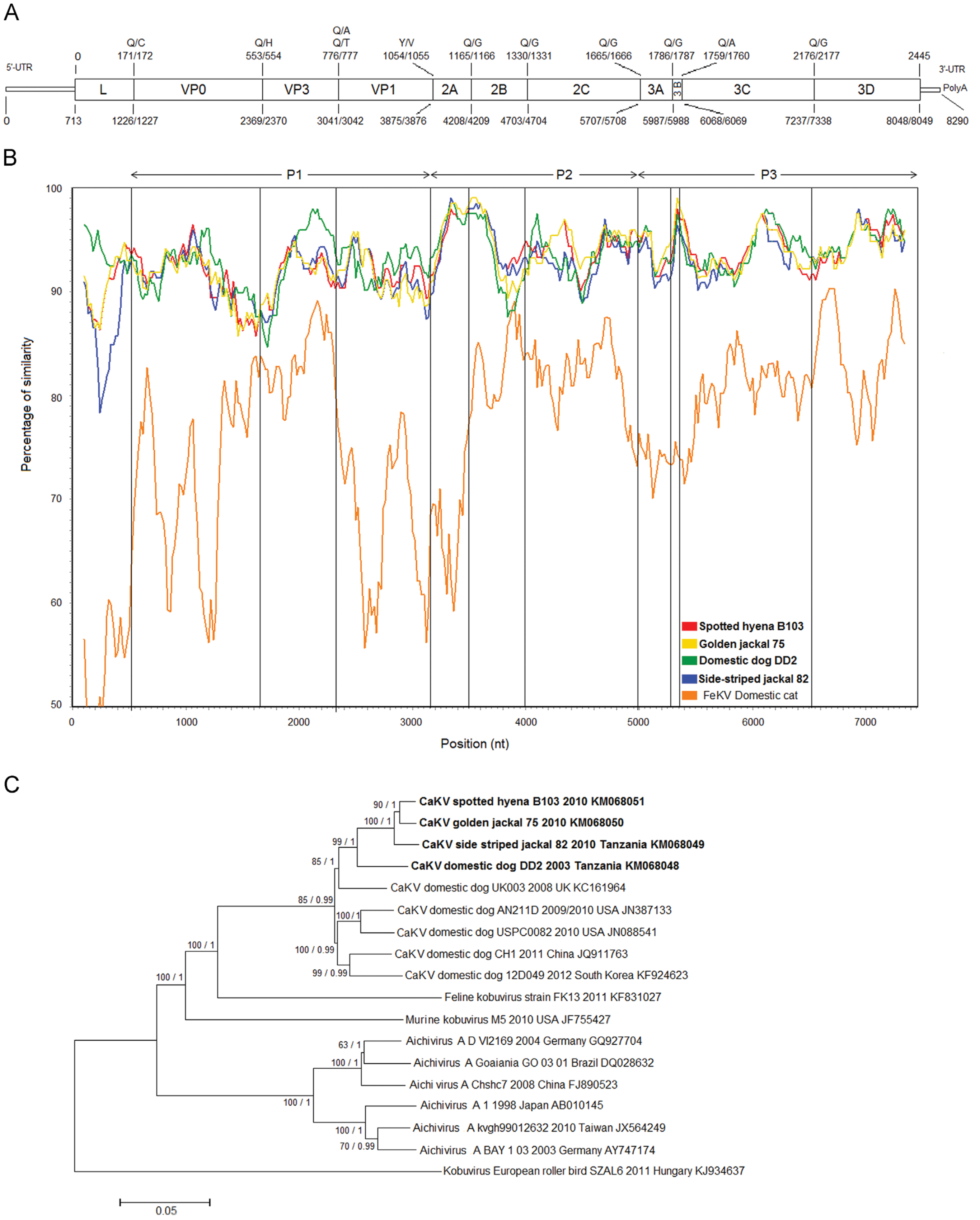

Fig. 1. Comparison of the complete single ORF of Tanzanian strains with known kobuvirus *Aichivirus A* strains. (a) Graphical depiction of the complete genome of canine kobuvirus (CaKV). The predicted positions of cleavage sites, amino-acid (above bar) and nucleotide (below bar) are shown. (b) Similarity plot analysis: The percentage similarity of complete ORF sequences from Tanzanian strains (plotted in different colors) compared against the most closely related strain UK003 (KC161964). CaKV ORF from Tanzanian host species: domestic dog (green); side-striped jackal (blue) golden jackal (yellow) spotted hyena (red). FeKV ORF, domestic cat from Asia (orange). (c) Phylogenetic relationships between kobuviruses from Tanzania, East Africa with strains within the *Aichivirus A* group based on complete ORF nucleotide sequences. Numbers at the nodes indicate bootstrap percentage values from 1000 replicates followed by Bayesian posterior probabilities. Strains obtained in this study are depicted in bold. GeneBank accession numbers for each complete kobuvirus ORF used in this analysis are quoted on their respective branches.