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Coronavirus outbreak in cheetahs: Lessons for SARS

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In November 2002, a flu-like outbreak caused by a coronavirus now known as SARS-CoV occurred in Guangdong Province in China. In the space of 9 months the disease traveled to 29 countries, infected 8098 people and caused 774 deaths [1]. The SARS epidemic spread with alarming speed among health care workers attending SARS patients (e.g., 112 health care workers at Prince of Wales hospital in Hong Kong became infected contemporaneously), but also indirectly among residents of an apartment complex, many who did not have physical contact with a clinically ill patient. Although the epidemic subsided by May 2003, there is still no clear understanding of the precise mode of transmission, no firm laboratory based diagnostic test, no vaccine, and no efficient treatment for SARS [2].

Recently published reports show that a virus closely related to the SARS-CoV was discovered in samples collected in Chinese food markets from Himalayan palm civets, raccoon dogs, snakes, bats and monkeys [3,4], and that SARS-CoV can infect domestic cats and ferrets [5]. Yet unconfirmed results [3,4] imply that Himalayan palm civets are one animal reservoir for SARS-CoV, and suggest that the deadly SARS-CoV recently emerged from an animal species.

A fatal epizootic of a related coronavirus in captive African cheetahs at Winston Safari park in the early 1980s may offer comparative insight into the prospects for a coronavirus-based epidemic [6]. The affected animals died of feline infectious peritonitis (FIP), caused by a feline coronavirus (FCoV, also called FIPV). The presence of a cheetah coronavirus (Aju-CoV, for *Acinonyx jubatus* coronavirus) was inferred based upon the presence of FIPV antibodies and the observation of coronavirus-like particles [6,7]. In domestic cats FCoV occurs in two varieties: virulent FIPV which causes severe FIPV antibody mediated fatal disease in about 5–10% of infected cats, and a subclinical enteric feline coronavirus (FECV) infection. Within months of arrival of the two infected cheetahs to Winston Safari, other cheetahs in the park fell ill. Retrospective serum samples tested for antibodies demonstrated that prior to 1982 all cheetah serum were negative, but within six months of the Sacramento cheetahs' arrival, 100% of the cheetahs had seroconverted, most with titers >1600 (Supplemental Figure S1). Ninety percent of the 60 cheetahs in the park developed disease symptoms including jaundice, diarrhea, weight loss, gingivitis, hepatic and renal pathology. With a mortality of 60% within 2–3 years, this was the most extreme outbreak of coronavirus in any species recorded.

To characterize the genomic disposition of the cheetahs' Aju-CoV strain, PCR primers based on alignment of seven coronavirus gene segments (*pol1a, pol1b, S, M, N, 7a/7b*, and *3'UTR*), were used to amplify cDNA from archived cheetah liver and kidney tissues collected during the Winston outbreak. Tissues from five different cheetahs were successfully used for amplification of 439 bp of the *pol 1b* gene, 405 bp of *pol 1a*, 316 bp of *N-7a*, and 187 bp in the *3'UTR* region. Phylogenetic analyses of aligned virus genome sequences confirm the monophyly of three previously discovered antigenic groups of coronavirus, plus the divergent SARS-CoV genome (Figure 1 and Supplemental Data). The cheetah isolates were nested within a group of domestic cat viruses using *pol 1a*, *N-7a* and also in a polyphyletic intermix with the *3'UTR* gene segment. The phylogenetic analyses indicate a close similarity of the Aju-CoV and the FCoV strains, suggesting the cheetah virus is closely related to, if not indistinguishable from, domestic cat isolates. The most likely scenario to explain these results is that FCoV jumped from the domestic cat into the cheetah. Interestingly, the two cheetahs exported from Sacramento to Winston had visited the U.C. Davis veterinary hospital, where many domestic cat FCoV isolates were originally isolated, suggesting an opportunity for cross species transmission.

Comparisons of SARS-CoV, FCoV and Aju-CoV reveal important epidemiological lessons. First, the virus in cheetahs and in humans emerged abruptly from distinct species reservoirs [3,4,6]. Second, in cats, cheetahs and humans, the viruses are highly contagious, spreading rapidly through close quarters in weeks, if not days. Third, despite these similarities, there is a clear difference in age sensitivity. For SARS, there were virtually no childhood cases, and the mortality reaches over 50% in people over 65 or with pre-existing medical complications [8]. In cheetahs and domestic cats, mortality is the highest in neonates, infants or sub-adults; 85% of cheetah cubs succumbed to the Winston outbreak [6]. Fourth, while mortality among humans with SARS symptoms and house cats with FCoV is low, around 5–10%, cheetahs with Aju-CoV exhibited the opposite extreme, showing 90% morbidity and over 60% mortality.

There are two plausible explanations for the cheetah's extreme sensitivity to Aju-CoV. One is that slight mutational differences transform a relatively benign coronavirus strain to a virulent 'hot' strain. Among coronaviruses this scenario has been proposed, but not proven, for the transition of FECV to FIPV in cats [9]. This hypothesis is less likely for the cheetahs, as in the outbreak mentioned above lions became infected concurrently with
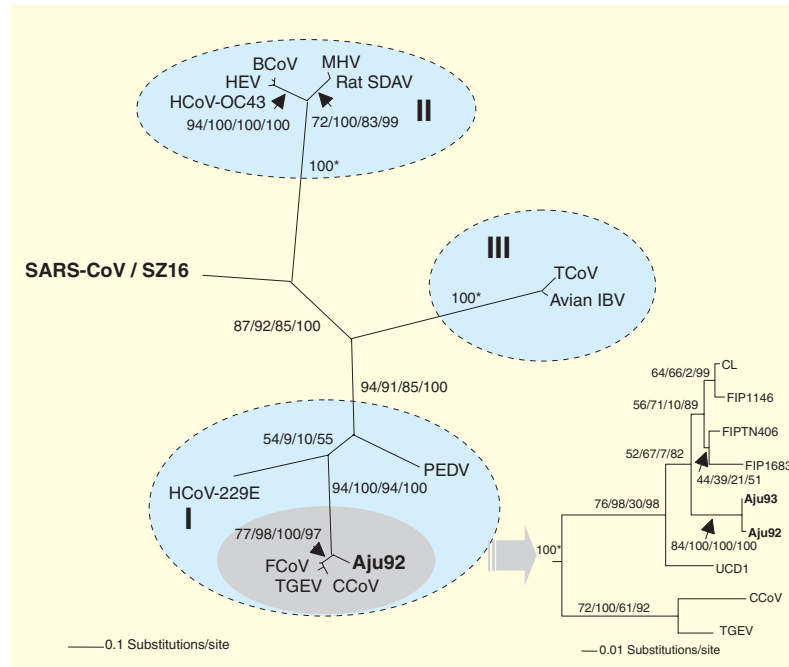
Figure 1.
Phylogenetic analysis of Aju-CoV *pol 1b* (439bp) sequences from archived liver and kidney tissues from cheetahs during the coronavirus outbreak, unrooted maximum likelihood tree under the GTR + Γ + I model of sequence evolution (-ln likelihood = 3347.49). Numbers plotted along the branches indicate bootstrap values and Bayesian posterior probabilities shown as percentages in the following order: Maximum Likelihood/ Maximum Parsimony/ Minimum Evolution/ Bayesian. The three major coronavirus antigenic groups are supported by the genomic data (blue shading and roman numerals). Abbreviations: human coronavirus 229E (HCoV-229E), canine coronavirus (CCoV), feline coronavirus (FCoV), porcine transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), human coronavirus OC43 (HCoV-OC43), bovine coronavirus (BCoV), porcine hemagglutinating encephalomyelitis virus (HEV), rat sialodacryoadenitis (SDAV), mouse hepatitis virus (MHV), turkey coronavirus (TCoV), avian infectious bronchitis virus (avian IBV), SARS coronavirus from human (SARS-CoV) and from palm civet (SZ16). The inset depicts portions of the unrooted maximum likelihood tree for *pol 1a* (405bp) under the GTR + Γ + I model of sequence evolution (-ln likelihood = 3368.60) CL, FIP1146, FIPTN406, FIP1683 and UCD1 are FCoV strains from domestic cats. Cheetah isolates from Winston, Oregon outbreak are indicated as Aju.

the cheetahs, but developed no overt symptoms [6]. Also, experimental transmission of the Aju-CoV to three domestic kittens failed to cause disease [6]. Perhaps also relevant is that when the human SARS-CoV was induced in domestic cats, they developed no symptoms [4].

Cheetahs are known as the world's fastest land animal but also for their extreme genetic uniformity, a consequence of their escape from extinction some 12,000 years ago. Remarkably, unrelated cheetahs accept skin grafts from non-relatives, a characteristic of highly inbred laboratory strains of mice or rats [8]. The most likely explanation for the high mortality in cheetahs is their genetic uniformity, particularly at immune

genes like the MHC. This may have rendered the species susceptible to an emerging virulent strain that had evolved to circumvent the defenses of the first victim. If this hypothesis is correct, the greater genetic diversity of domestic cats and humans may reduce the severity of the epidemic, and also contribute to the occurrence of rare genetically determined SARS-CoV super-spreaders who can infect with high virulence. This explanation emphasizes the critical role of intrinsic genomic diversity among immune defense genes in any fatal epidemic. As such, the search for explicating genetic susceptibility polymorphisms that may inform prognosis, spread therapy, and prevention of emerging

pathogens seems warranted, particularly to anticipate future episodes of the deadly SARS coronavirus.

Supplemental Data
Supplemental Data are available at http://www.current-biology.com/supplemental

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