SARS-CoV-2 Spillback to Wild Coatis in Sylvatic–Urban Hotspot, Brazil

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We tested coatis (*Nasua nasua*) living in an urban park near a densely populated area of Brazil and found natural SARS-CoV-2 Zeta variant infections by using quantitative reverse transcription PCR, genomic sequencing, and serologic surveillance. We recommend a One Health strategy to improve surveillance of and response to COVID-19.

By November 2022, the COVID-19 pandemic had resulted in >630 million cases of disease worldwide (*1*). During the outbreak, natural occurrence of SARS-CoV-2 infections in animals was a hallmark; infections have been reported mainly in companion, domestic, captive, and farmed animals but also in wildlife (*2*,*3*). As of September 2022, the World Organisation for Animal Health had recorded 26 animal species infected with SARS-CoV-2 in 36 countries (*2*), indicating that the virus is able to cross the species barrier, thereby increasing risk of new transmission cycles and animal reservoirs (*2*,*3*).

Coatis (*Nasua nasua*) from South America are small diurnal mammals (family Procyonidae) that are omnivorous, terrestrial, synanthropic, and opportunistic. Coatis interact easily with humans and are often seen foraging for human food, especially from trash (*4*,*5*). We investigated the transmission of SARS-CoV-2 to a coati population living in an urban park near a large anthropized area of Brazil.

We collected serum samples and anal and oral swab samples during February–August 2021 from 40 free-living coatis inhabiting Mangabeiras Municipal Park in Belo Horizonte, Brazil (Appendix Table, Figure 1, https://wwwnc.cdc.gov/EID/article/29/3/22-1339-App1.pdf). Trained professionals captured coatis during 4 periods (February, June, July, and August), using appropriate personal protective equipment (laboratory coats, gloves, N95 face masks, and face shields) in accordance with all biosafety guidelines. Ethics approval was obtained for this study (Appendix).

Coatis were captured in Tomahawk Live Traps (https://www.livetrap.com/index.php) (70 cm × 35 cm × 40 cm) baited with banana pieces. Animals were anesthetized with Zoletil 100 (Virbac, https://vet-uk.virbac.com) by intramuscular injection (7–10 mg/kg body weight), clinically evaluated, identified, and marked with polypropylene earrings and microchips. After anesthesia recovery, each coati was released at their capture site.

We stored anal and oral swab specimens by using RNAlater (ThermoFisher Scientific, https://www.thermofisher.com) and extracted RNA by using QIAmp Viral RNA Mini Kits (QIAGEN, https://www/qiagen.com). We performed quantitative reverse transcription PCR targeting the nucleocapsid N1 and N2 regions (*6*) and sequenced PCR positive samples by using nanopore technology. We performed phylogenetic analysis by using IQ–TREE2 (*7*) and maximum-likelihood reconstruction.

We detected SARS-CoV-2 RNA in 2 (5%) female coatis that had no clinical signs of infection (Table). We obtained a complete genomic sequence from the anal swab specimen from coati 535 (99% average coverage). The genomic sequence of SARS-CoV-2 obtained from the anal swab specimen from coati 535 indicated this variant belonged to the Zeta lineage (B.1.1.28.2, P.2) (Figure). The P.2 variant was initially detected in the state of Rio de Janeiro, Brazil, in July 2020 and was considered a variant of interest (*9*).

We performed plaque reduction neutralization tests (PRNT) on serum samples from all captured coatis to detect SARS-CoV-2 neutralizing antibodies (*8*). We serially diluted serum samples to obtain 1:20,

Table. Specimens from 2 SARS-CoV-2 RNA–positive coatis in study of SARS-CoV-2 spillback to wild coatis in sylvatic–urban hotspot, Brazil*

Coati ID	Collection date	Sex	Sample	SARS-CoV-2†	N1 Count‡	N2 Count‡
C341	2021 Feb 17	F	Oral swab	Positive	33	37
			Anal swab	Negative	NA	NA
			Serum	Negative	NA	NA
C535	2021 Feb 18	F	Oral swab	Positive	20	24
			Anal swab	Positive	30	33
			Serum	Negative	NA	NA

*Oral and anal swab and serum samples were collected from 40 wild coatis inhabiting Mangabeiras Municipal Park in Belo Horizonte, Brazil. We performed quantitative reverse transcription PCR targeting the nucleocapsid N1 and N2 regions of SARS-CoV-2 RNA for each sample. ID, identification, NA, not applicable.
†Specimens positive or negative for SARS-CoV-2 RNA by PCR.
‡PCR cycle threshold count.

1:40, and 1:80 dilutions and measured 50% and 90% neutralizing activity against SARS-CoV-2. Twenty (50%) coatis had SARS-CoV-2 neutralizing antibodies in ≥1 dilution at the 50% level; at the 90% level, 13 (32.5%) coatis had detectable neutralizing antibodies in ≥1 dilutions and 7 (17.5%) coatis had SARS-CoV-2 neutralizing antibodies in all 3 dilutions. We observed neutralizing antibodies in all 3 serum dilutions for coati 535 (Appendix Figure 2).

We were unable to confirm the mode of SARS-CoV-2 transmission to the coati population. However, we found evidence for human-to-animal transmission; the P.2 genomic sequence from coati 535 was the same variant circulating in humans within the area during the study period. Furthermore, 50% of the coati population had antibodies against SARS-CoV-2, suggesting a cluster of natural exposure and infections within this population. Our results support indirect

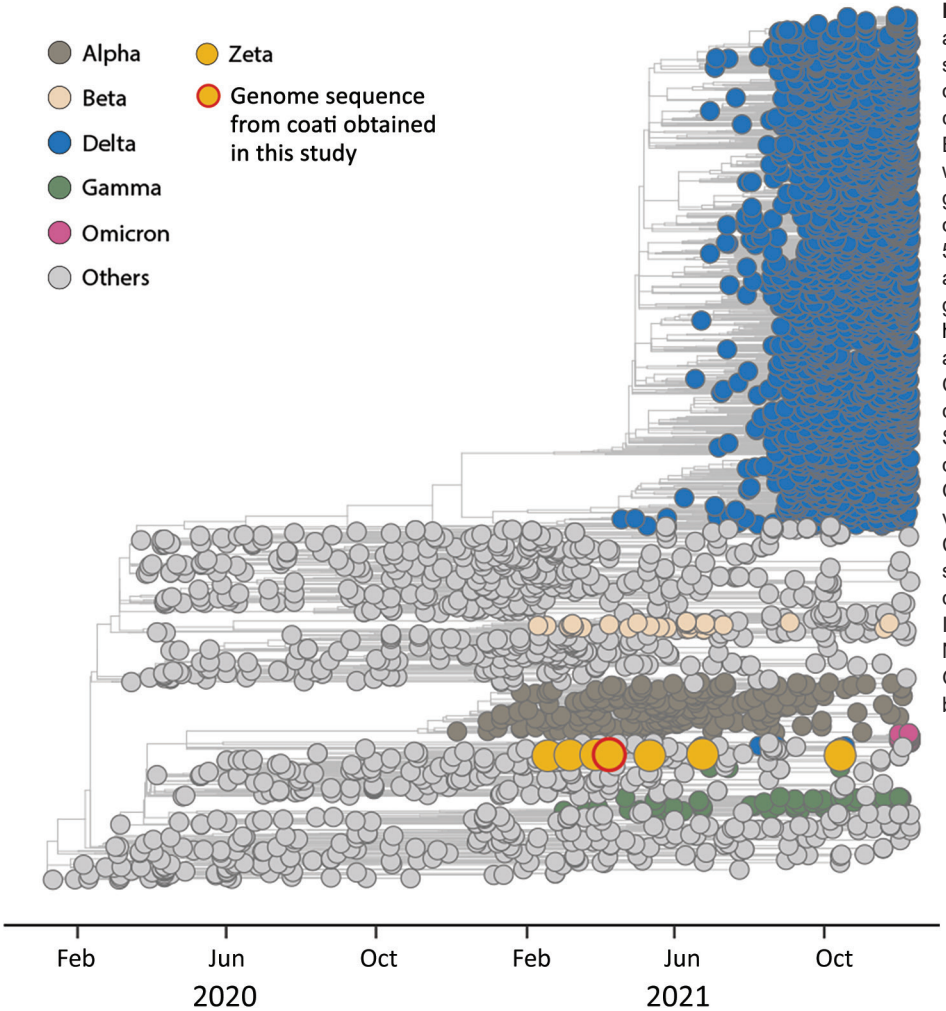

Figure. Time-scaled phylogenetic analysis of SARS-CoV-2 sequences, by variant type, in study of SARS-CoV-2 spillback to wild coatis in sylvatic–urban hotspot, Brazil. Maximum-likelihood method was used to compare the complete genomic sequence of SARS-CoV-2 obtained from coati (*Nasua nasua*) 535 (red-outlined yellow circle) and 3,441 SARS-CoV-2 reference genomic sequences (GISAID, https://www.gisaid.org) from around the world collected through October 2021. Colors represent clades corresponding to different SARS-CoV-2 variants of concern described by the World Health Organization; yellow indicates Zeta variant sequences. The SARS-CoV-2 sequence generated in this study was deposited in the GISAID database (accession no. EPI_ISL_8800460) and SisGen (Sistema Nacional de Gestão do Patrimônio Genético, https://www.sisgen.gov.br; no. A627307).

contact of coatis with contaminated human trash and food scraps in dumpsters and in the bordering urban areas of the park or potential direct close contact with infected human visitors (Appendix Figure 1).

Our findings agree with results from a zoo in Illinois, USA, that also confirmed SARS-CoV-2 in a coati by using molecular methods (*2*,*10*). Those results reinforce the susceptibility of coatis to SARS-CoV-2 infection and suggest possible virus shedding and transmission capacity of coatis. Viral RNA detection in both oral and anal swab specimens from coati 535 (Table) and presence of neutralizing antibodies indicate that viral replication occurred in this host. Therefore, our findings highlight possible SARS-CoV-2 enzootic maintenance in nature, including in fragmented green areas close to urban settings. Because of the potential for SARS-CoV-2 interspecies transmission, we recommend establishing a One Health strategy to improve surveillance and ability to respond to COVID-19 emergency health events.

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Ms. Stoffella-Dutra is a PhD student in microbiology at the Federal University of Minas Gerais. Her primary research interests focus on virology, viral zoonoses, epidemiology, and wildlife disease ecology.

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Babesia microti Causing Intravascular Hemolysis in Immunocompetent Child, China

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We report a case of *Babesia microti* infection in an immunocompetent child <5 years of age that caused fever and severe intravascular hemolysis. Physicians in China should be aware of babesiosis, especially in the differential diagnosis of immune hemolytic anemia with negative results for antiglobulin tests.

Babesiosis, caused by tickborne zoonotic protozoa of the genus *Babesia*, is an emerging health risk to humans. Among the known *Babesia* species infecting humans, *B. microti* is the most common cause of human babesiosis (*1*). In China, *B. microti* has caused >100 human cases of babesiosis (*2*), but nearly all have been reported in adults, particularly the elderly.

B. microti babesiosis has rarely been reported in immunocompetent children in China. We reported a case of severe intravascular hemolysis caused by *B. microti* infection in an immunocompetent preschooler from Shandong Province, China.

The patient, a girl 4 years and 9 months of age, had fever develop (highest temperature 39°C) on September 30, 2021. Antimicrobial drug treatment was not effective. Four days later, her urine became dark, and she had abdominal pain. On October 9, 2021, she was admitted to the hospital because of severe anemia and abnormal laboratory test results (Table). She had shock after a discharge of dark brown urine.

To stabilize her vital signs, we began repeated blood transfusion for supportive treatment. Azithromycin and immune regulatory treatment (high-dose methylprednisolone, 10 mg/kg/d for 3 days, and intravenous immunoglobulin, 1 g/kg/d for 2 d) were not effective. Her symptoms worsened, and her hemoglobin level remained at <60 g/L (Appendix Figure, panel A, https://wwwnc.cdc.gov/EID/article/29/3/22-0888-App1.pdf). On the basis of those findings, we excluded congenital hemolytic anemia and autoimmune hemolytic anemia.

We examined her blood smear and observed parasites in the erythrocytes (Appendix Figure, panel B). We used a genus-specific 18S rRNA PCR described previously (*3*) to confirm *Babesia* infection by amplification of a 515-bp fragment (Appendix Figure, panel C). Test results for malaria infection was negative. Subsequent sequencing of the fragment and BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the nucleotide sequence showed 100% similarity with *B. microti* RI strain. Those results confirmed the diagnosis as a *B. microti* infection causing severe intravascular hemolytic anemia.

The girl's parents recalled that the child had been in a wild chestnut forest in a suburb of Zaozhuang City, Shandong Province, on September 14, 2021. They found ≈20 red papules and an itching sensation on the trunk and limbs. The papules subsided within 3 days. There were no other complications of babesiosis, such as splenic infarction, acute respiratory distress syndrome, or disseminated intravascular coagulation.

The child was given atovaquone and azithromycin (*4*) for 21 days, and the urine color became clear within 24 hours. The frequency of erythrocyte transfusion was reduced gradually, and the hemolysis was controlled (Appendix Figure, panel A). On the 10th day after the treatment began, molecular detection

[1]These authors contributed equally to this article.

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Appendix

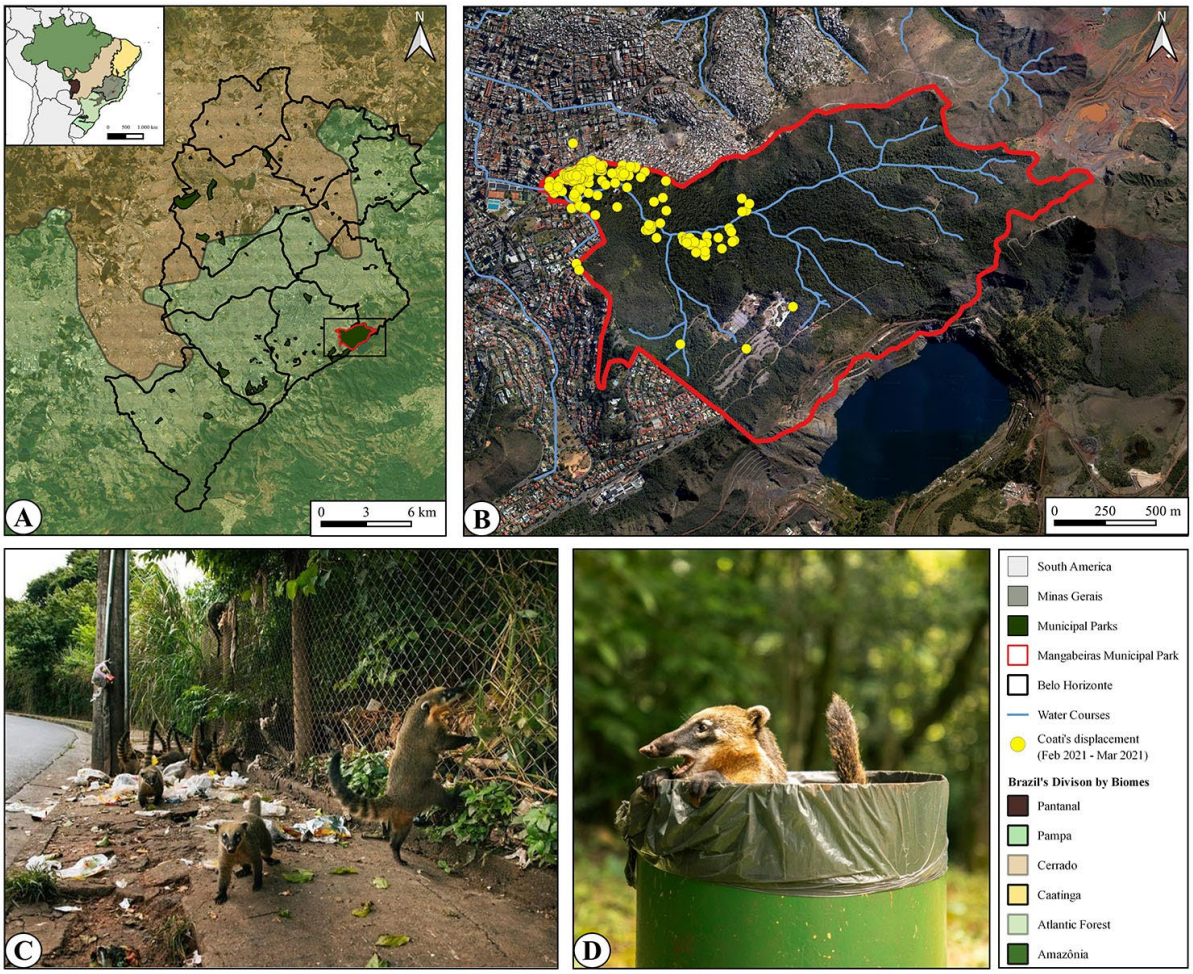
Ethics Approval

The study was approved by SISBIO (Sistema de Autorização e informação em Biodiversidade, license no. 75831), Ethical Committee on Animal Use (CEUA) of Universidade Federal de Minas Gerais (protocol nos. 158/2020 and 100/2021), SisGen (Sistema Nacional de Gestao do Patrimonio Genetico, no. A627307), and Fundação de Parques Municipais e Zoobotânica de Belo Horizonte (protocol no. FU 004–2020).

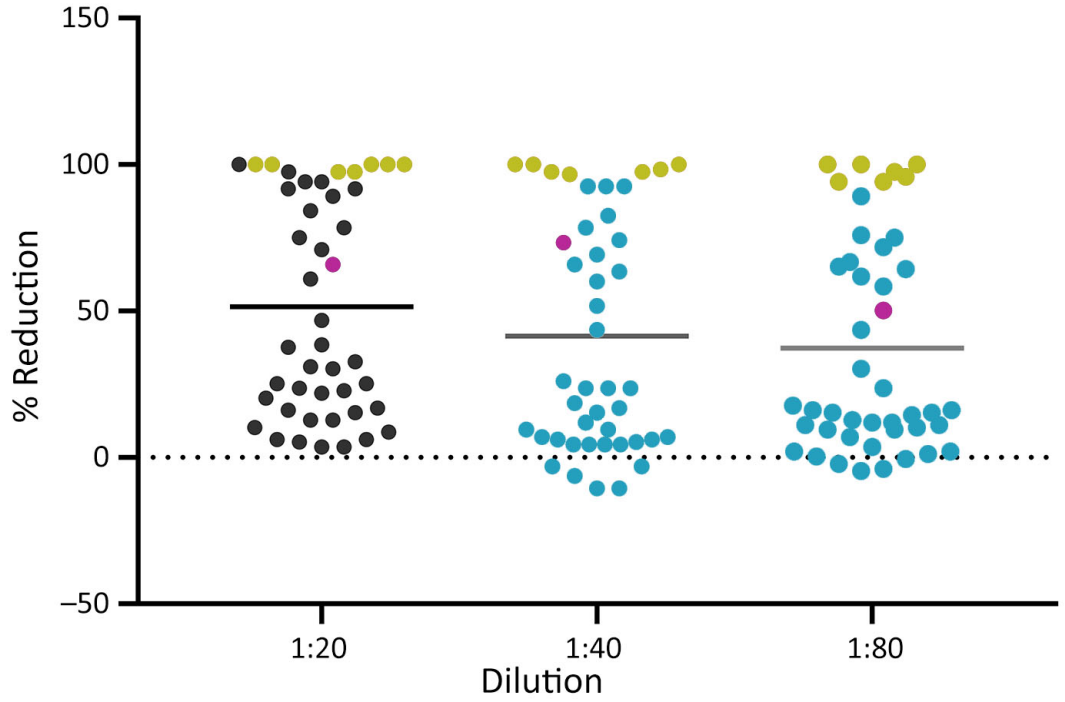
Appendix Table. Numbers of captured coatis categorized by collection date, sex, and age in study of SARS-CoV-2 spillback to wild coatis in sylvatic–urban hotspot, Brazil*

	Sex		Age†			
Capture month	M	F	Young	Immature adult	Adult	Total
February	3	5	3	3	2	8
June	4	6	3	3	4	10
July	6	9	3	1	11	15
August	4	7	1	2	8	11
Total no. animals	17	27	10	9	25	44

*Collection comprised 40 coatis (*Nasua nasua*) (62.5% female and 37.5% male) and 4 additional recaptures. Live traps (n = 25) were placed at 5 strategic points of passage for coatis (5 traps set per point) in Mangabeiras Municipal Park in Belo Horizonte, Brazil. The 5 capture points were: Caraça (n = 19 captured coatis), Casa de bonecas (n = 6), Mirante (n = 3), Praça das águas (n = 6), and Trilha 12 (n = 10).
†Biometrics analyses were performed to estimate age.



Appendix Figure 1. Overview of Mangabeiras Park in southeastern Brazil in study of SARS-CoV-2 spillback to wild coatis in sylvatic–urban hotspot, Brazil. A) Map of Brazil subdivided by its biomes and neighboring countries of South America, and municipal parks (dark green). Mangabeiras Municipal Park (19°56′ S and 43°54′ W) is surrounded by a red line. B) A satellite view of Mangabeiras Municipal Park (encircled by red line) in the city of Belo Horizonte highlighting the water courses (blue lines) and 341 coati displacements by georeferenced spots (global positioning system) (yellow circles) during February and March of 2021. Animal monitoring showed displacement throughout the park border areas that had residences and urban settings. C, D) Coatis in Mangabeiras Park in contact with human trash and food remnants. Photographer: Augusto Gomes. Maps were created with QGIS 3.24.3 software (https://www.qgis.org/pt_BR/site), using the coordinate reference system DATUM SIRGAS 2000 (UTM 23S).



Appendix Figure 2. Scatter plot of plaque reduction neutralization tests of serum samples from coatis. SARS-CoV-2 neutralizing antibodies were measured in serum samples by using the plaque reduction neutralization test. Samples were serially diluted to 1:20, 1:40, and 1:80. Solid lines represent the average reduction percentage of all tested samples and dotted line is 0% reduction. Solid circles represent all samples tested at each dilution (n = 44) (4 additional samples were from recaptures; 3 of those showed seroconversion when compared with the previous collection date). Magenta circles represent coati 535, which was positive for SARS-CoV-2 RNA by PCR. Green circles represent 7 coatis that had 90% plaque reduction (neutralizing activity) in all 3 dilutions.