Journal of Zoonotic Diseases
https://jzd.tabrizu.ac.ir/
Published by the University of Tabriz
Online ISSN: 2717-2910

Coronavirus prevalence in bats from Panama

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Article type:
Original article

Keywords:
RT-PCR
Reservoir
Mitigation
Pandemic
Coronavirus

Article history:
Received:
September 28, 2024
Revised:
October 23, 2024
Accepted:
October 30, 2024
Available online:
December 8, 2024

Abstract

Bats are natural reservoirs of various types of viruses, especially coronaviruses, constituting approximately 31% of all viruses in this group. Bats can become a source of direct or indirect transmission, infecting humans and generating epidemic and even pandemic outbreaks. Panama has more than 118 species of bats recorded, of which some are likely hosts of various types of coronaviruses. However, studies have yet to be conducted in our country to determine the existence and prevalence of coronavirus in bats since they might be a risk for zoonotic transmissions. To determine the prevalence of coronavirus in bats from Panama, 217 specimens of 48 species of bats were captured in different sites of the country. Fecal samples were collected from the captured specimens to determine the presence of coronavirus by amplifying a 440 bp region of the RNA-dependent RNA polymerase (RdRp) gene using conventional RT-PCR and real-time PCR. No positive results for the presence of coronavirus were obtained in any of the 217 samples tested with the two PCR techniques used in this work. Positive and negative controls for all step procedures yielded the expected results with both techniques. The negative results obtained in our study in detecting coronavirus do not necessarily imply that bats in Panama are not reservoirs of coronavirus. Our results suggest that the prevalence of coronavirus in the bat species collected in Panama is considerably low, which did not allow us to detect positives. Further studies analyzing more samples per species and more species will confirm these findings.

Introduction

Coronaviruses are positive, non-segmented, single-stranded RNA viruses with an envelope featuring several surface proteins, including several tens of molecules of a trimeric protein called spike (S) that gives the virus a corona-like appearance (1). Coronaviruses belong to the family Coronaviridae of the order Nidovirales and infect a wide range of vertebrates, including humans, birds, snakes, mice, and other wildlife (2,3).

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https://doi.org/10.22034/jzd.2024.63733.1307
https://jzd.tabrizu.ac.ir/article_18905.html
Cite this article: Fuentes W., Castro-Pérez E., Samudio R., de Chial M., and C.W.R. Delgado. Coronavirus prevalence in bats from Panama. Journal of Zoonotic Diseases, 2024X

Seven types of coronaviruses have been identified from the mid-1960s to date that have infected humans (4,5). Human coronaviruses (HCoVs) 229E, OC43, NL63, and HKU1 exhibit relatively low virulence (6) and have been associated with 15% of common colds (7). However, the remaining three types, MERS-CoV, SARS-CoV, and SARS-CoV-2, exhibit different pathogenicity and a high mortality rate (8).

MERS-CoV, responsible for the Middle East respiratory syndrome coronavirus isolated in 2012 in Saudi Arabia (9), generated 2494 cases, 858 deaths, and a case fatality rate of 34.4 % (10). SARS-CoV, responsible for severe acute respiratory syndrome due to coronavirus, was first identified in China (11,12), produced a total of 8422 919 deaths and a case fatality rate of 11 % in the period from November 2012 to August 2013 (13). SARS-CoV-2, responsible for severe acute respiratory syndrome due to coronavirus 2, has produced to date (July 2024) 775 million cases and a total of 7 million deaths, representing a 0.9% fatality (14). In Panama, on the same date and according to statistics from the World Health Organization (WHO), there have been 1,0444,665 cases and 7,899 deaths, a fatality of 0.756%. (14). These statistics place Panama at Central America's highest rate of infections.

Bats are natural reservoirs for several types of viruses. These natural reservoirs can be the source of virus transmission to humans, usually through an intermediary. For instance, OC43 hCoV emerged as a strain derived from bovine coronavirus (15). In the case of SARS-CoV, MERS, and SARS-CoV-2, the natural reservoir appears to be the bat (16). The genome of SARS-CoV-2 shares 96 % identity with BatCoV RaTG13, a bat virus of Rhinolophus affinis (17). SARS-CoV-2, like all other animal-derived coronaviruses, undergo evolution and genetic recombination either within its natural reservoir or when passing from one species to another. When transmitted to humans, these changes can generate variants with high pathogenic potential (18-21). Studies on the evolution of SARS-CoV-2 suggest that transmission from bats to humans occurred after the virus underwent mutations in the S and N proteins (22).

Bats constitute approximately 20% of all mammals (23); they are distributed in all continents except Antarctica and occupy diverse ecological niches in various habitats. In Panama, 118 of the 145 bat species reported for Central America have been recorded (24,25). Only one regional study from 2013 involving several countries has been conducted to detect the presence of coronavirus in bats, and a portion of the samples were from Panama and included some of the species recorded in the country (26). Panama is a country of high international traffic because it is a hub of tourism, marine, flights, regional business and trade zones, and other activities that might contribute to the dispersion of diseases. Due to the various outbreaks, epidemics, and recent pandemic events associated with coronaviruses, the prevalence of coronavirus in bats may have increased or changed by dispersion due to contamination or other sources. Therefore, it is essential to determine the prevalence of this type of virus in their natural reservoirs to predict the risks of possible zoonotic transmission and spread to humans. In this study, 217 fecal samples from 48 different bat species, thus increasing the number of species in 2013 report, were collected from 12 sites throughout Panama to determine the coronavirus's presence by amplifying a region of the RNA-dependent DNA polymerase (RdRp) gene.

Materials and methods

Study area

The study was conducted at 12 sites in the Republic of Panama (Figure 1) in the provinces of Panama, Coclé, Darién, Comarca Ngäbe-Buglé, Chiriquí, and Veraguas. Captures were made between July 2021 to October 2022. The bats were captured using mist nets 3 meters above ground level. The captured specimens were released from the net and placed in cotton bags for a few minutes to tranquilize them. We identified the species and determined their sex,

forearm length, weight, and maturity stage (juvenile or adult) for each specimen.

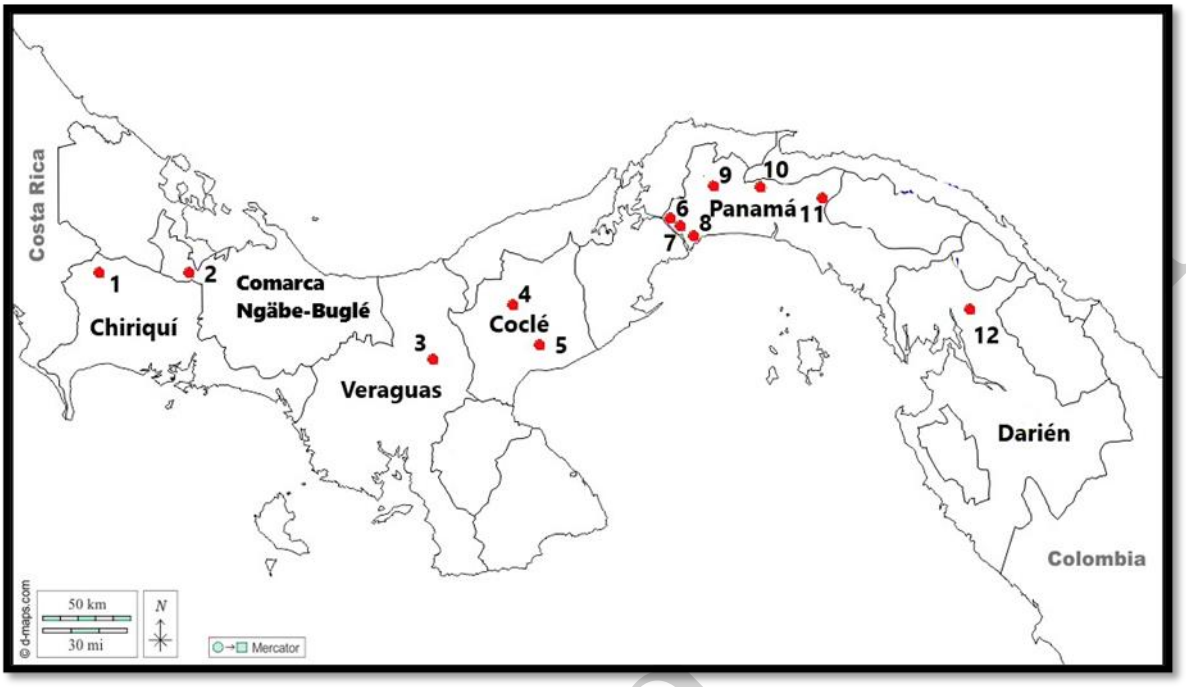

Fig. 1. Map depicting the capture sites. Numbered red circles indicate the specimens' capture sites. The capture sites were the following: 1- Mount Totumas Cloud Forest- Province of Chiriquí (MTCF) 2- Fortuna Cabins-Comarca Ngäbe-Buglé (FCCNB), 3- Calobre-Province of Veraguas (CPV), 4- La Saldaña- Province of Coclé (LSC), 5- Centro Regional Universitario de Coclé- Province of Coclé (CRUC), 6- Parque Nacional Soberanía-Province of Panamá (PNS), 7- Summit rainforest & golf resort-Province of Panama (SRF), 8- Metropolitan Natural Park- Province of Panama (MNP), 9- Cerro Azul- Province of Panama (CAP), 10- Mamoní Valley- Province of Panama (MVP), 11- Bunorgándi Private Reserve- Province of Panama (BPR) and 12- Darién Regional University Center- Province of Darién (CRUD)

Sampling

Generally, the captured bats excreted fecal material collected from the bags with sterile swabs. We performed rectal swabbing for sampling when specimens did not excrete fecal material. A total of 217 stool samples were collected and placed in PrimeStore MTM® Transport Medium for Nucleic Acid Testing (Longhorn Vaccines and Diagnostic, LLCMD, USA). This transport medium inactivates, stabilizes, and preserves the RNA in the collected samples. Once the samples were obtained, the specimens were marked with a collar according to their size and age to avoid obtaining samples from the same specimen twice by any chance. The samples were transported to the laboratory for further analysis.

RNA extraction and cDNA synthesis

The tubes with transport medium containing the samples were briefly shaken with a vortex to obtain a homogeneous suspension. The swab was removed from the medium, and 280 µl of suspension was used for RNA extraction. The extraction was performed with the commercial QIAamp Viral RNA mini kit (QIAGEN, Valencia, California, USA) (Cat. Num. 52906) according to the procedure described by the manufacturer.

Because coronavirus-infected bat feces samples were unavailable, SARS-CoV2 RNA and citrus tristeza virus (CTV)-infected citrus leaves were used as positive controls for RNA extraction viability and PCR amplification capacity. In particular, SARS-CoV2 RNA was used as a

positive control in cDNA synthesis, conventional PCR, and real-time PCR. Citrus leaves were used as a control for RNA extraction, cDNA, and conventional PCR. CTV, like coronavirus, is a large, positive-sense, single-stranded RNA virus.

The cDNA synthesis was performed from 10 µL of RNA. The High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Cat. Num. 4374966, Applied Biosystems) was used according to the procedure described by the manufacturer. The temperature conditions used in the T100™ Thermal Cycler (BIO-RAD) for cDNA synthesis were 65°C for 5 min, 25°C for 5 min, 42°C for 60 min, and 65°C for 20 min (27). The final reaction volume was 20 µL. The cDNA was stored at -80°C for subsequent use in amplification reactions.

Conventional PCR

The presence of the coronavirus RdRp gene was assessed by amplification of a 440 bp region. (28), using specific primers IN6 forward 5'-GGTTGGGACTATCCTAAGTGTGA-3' and IN7 reverse 5'-CCATCATCA GATAGAATCATCATCATA-3'. We used 5 µL of cDNA from each sample and positive controls as templates for amplification. The same volume of nuclease-free water was used as a negative control. One µL of each primer, 12.5 µL of GoTaq® Hot Start Green Master Mix (Promega, Madison, USA) (Cat. Num. M5122), and 5.5 µL of nuclease-free H_2O were used in a final volume of 25 µL. Amplification conditions used in the T100™ Thermal Cycler (BIO-RAD) settings included an initial step of 95°C for 2 min, followed by 45 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min, concluding with a final extension at 72°C for 10 min.

The primers 5'-TTATATGGACGACGACGAGACAAAGA-3' and 5'-CCAAGCTGCCTGACATTAGT-3' were used to amplify a 655 bp fragment of the CTV coat protein gene. In contrast, the primers 5'-ACAGGTACGTTAATAGTTAATAGCGT-3' and 5'-ATATTGCAGCAGCAGTACGCACACACA-3' primers were used to amplify an approximately 112 bp fragment of the SARS-CoV2 E protein gene and the primers 5'-TAA TCA GAC AAG GAA CTG ATT A-3' and 5'-CGA AGG TGT GAC TTC CAT G-3' were used to amplify an approximately 109 bp fragment of the SARS-CoV2 N protein gene. Amplification conditions in the 2720 Thermal Cycler (Applied Biosystems) for CTV were 35 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 1 min, and 72°C for 5 min, while for both SARS-CoV2 genes were: 95°C for 2 min, 45 cycles at 95°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 min.

Nested PCR

To rule out the possibility of negative results due to sequence variations among samples in the primer nesting region of the RdRp gene, a nested PCR with degenerate primers was performed according to the procedure described by Quan et al. (29). The initial (first) PCR used 1 µL of each of the primers 5'-CGTTGGIACWAAYBTVCCWYTICARBTRGG -3' and 5'-GGTCATKATATAGCRTCAVMASWGCNACN ACNACATG-3', 15 µL of GoTaq® Hot Start Green Master Mix (Promega, Madison, USA) (Cat. Num. M5122), 5 µL of cDNA; and 8 µl of nuclease-free water in a final volume of 30 µL. Amplification conditions used in the T100™ Thermal Cycler (BIO-RAD) for the first PCR were one cycle at 95°C for 15 min; 15 cycles at 95°C for 30 s, and a touchdown starting at 65°C (-1°C/cycle) for 30 s and 72°C for 45 s; 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s; and one cycle at 72°C for 5 min.

In the second PCR, 4 µL were used as a template from the first PCR, 1 µL of each of the primers 5'-GGCWCCWCCWCCHGG NGARCAATT-3' and GGWAWCCCCAYTGYTGWAYRTC, 12.5 µL of GoTaq® Hot Start Green Master Mix (Promega, Madison, USA) (Cat. Num. M5122) and 6.5 µL of nuclease-free water were used in a final volume of 25 µL. The conditions for the second (Nested) PCR were 35 cycles at 94°C for 30 s, 50°C for 30 s and 72°C for 45 s, and one cycle at 72°C for 5 min.

Real-time PCR

To rule out the possibility of negative results due to the lower limit of detection (LOD) of the conventional PCR technique, we also performed real-time PCR using Sybr Green, which is more sensitive than conventional PCR. We performed real-time PCR using MicroAmp® Fast Optical 96-Well Reaction Plates with Barcode (0.1 mL) (Cat. Num. 4346906, Applied Biosystems) with 0.5 µL of each degenerate primer for RdRp gene 5'-CGTTGGIACWAAYBTVCCWYTICARBTRGG-3' and 5'-GGTCATKATAGCRTCAVMASWGCNACNACNACATG-3' (the same primers used in nested PCR), 6.25 µL of PowerTrack™ SYBR™ Green Master Mix (Cat. Num. A46109, Applied Biosystems) and 5.25 µL of cDNA were used for a final volume of 12.5 µL. Amplification conditions used in the Thermal Cycler QuantStudio™ 5 were 95°C for 10 min, 40 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 45 s.

Results

A total of 217 feces samples from 48 different bat species were analyzed, representing 40% of the total 118 species of bats reported in the country. The species with the highest number of samples analyzed were *Artibeus jamaicensis* (56/217), *Carollia perspicillata* (30/217), *Uroderma convexum* (11/217), *Carollia castanea* (9/217) and *Artibeus intermedius* (8/217). One hundred and fourteen samples (114) out of 217 (52%) were sampled from these six species. Meanwhile, between 1 and 6 samples were obtained from the remaining 42 species.

No positive results were obtained from any of the 217 samples tested with the different PCR techniques and strategies used, while the positive controls generated the expected amplification products: 655 bp for CTV and 109 bp and 112 bp for SARS-CoV2, N, and E gene, respectively; in the conventional PCR (Fig. 2). In real-time PCR, the Ct values for the positive control (SARS-CoV2) were 4.2 and 9.4 for N and E genes, respectively. In contrast, no Ct values were recorded for the negative controls. The amplification of some bat samples and positive control are shown in Fig. 3. The PCR results and collection sites by species are summarized in Table 1.

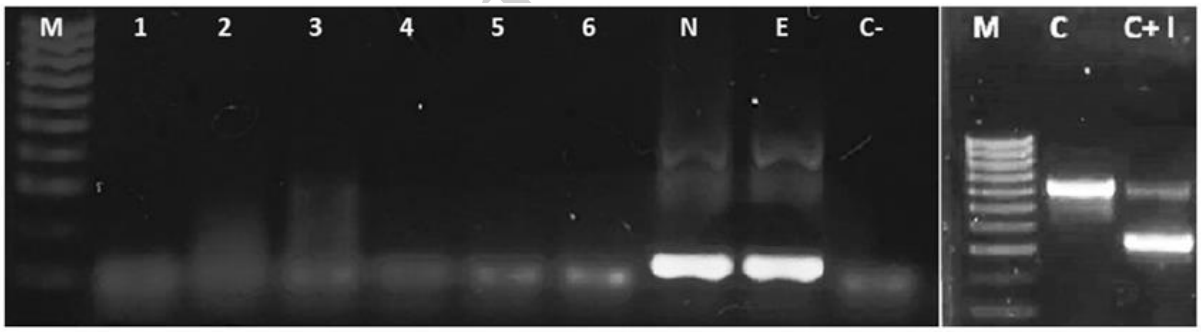

Fig. 2. Gel electrophoresis of conventional PCR for positive controls and bats feces samples. Left gel. M, 100 bp molecular marker; lanes 1-6, bats negative samples for coronavirus; N positive amplification of SARS-CoV2 N gene; E, positive amplification of SARS-CoV2 E gene; C, negative control. Right gel. M, 100 bp molecular marker; C, positive amplification for CTV coat protein gene (600 bp); C+1, positive amplification of CTV coat protein gene and internal control Nad5 gene (300 bp).

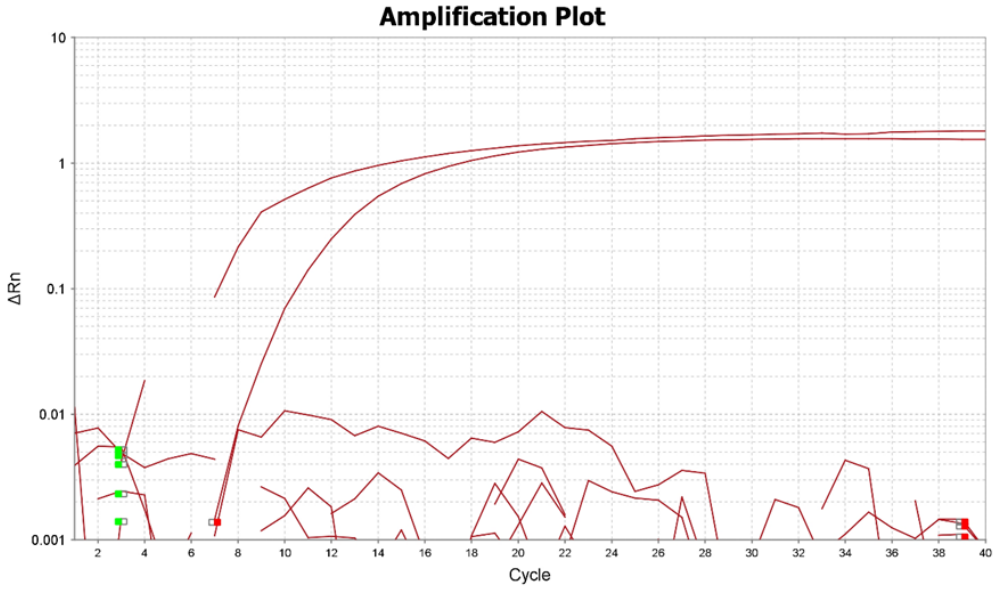

Figure 3. Real-time PCR of positive controls and bats feces samples. The amplification plot was observed for positive controls SARS-CoV2, N and E genes. In contrast, no amplification was observed in feces bat samples.

Table 1. PCR results and collection sites by species

Species	Sites	N	CP	NP	RP
Anoura geoffroyi	MTCF	5	-	-	-
Artibeus intermedius	MVP, CRUC	8	-	-	-
Artibeus jamaicensis	FCCNB, CPV, LSC, CRUC, PNS, SRF, MNP, CAP, MVP, BPR, CRUD	56	-	-	-
Artibeus lituratus	CPV, BPR	2	-	-	-
Artibeus phaeotis	MVP	2	-	-	-
Artibeus sp.	MTCF	1	-	-	-
Artibeus toltecus	FCCNB	4	-	-	-
Artibeus watsoni	FCCNB, MVP	3	-	-	-
Carollia perspicillata	FCCNB, CPV, LSC, PNS, SRF, MNP, MVP, BPR, CRUD	30	-	-	-
Carollia brevicauda	MTCF, MVP	2	-	-	-
Carollia castanea	CAP, MVP	9	-	-	-
Carollia sp.	FCCNB	1	-	-	-
Chiroderma trinitatum	MVP	1	-	-	-

Desmodus rotundus	CRUC, MVP	3	-	-	-
Dermanura Sp.	FCCNB	1	-	-	-
Enchisthenes hartii	FCCNB	1	-	-	-
Gardnerycteris keenani	MVP	1	-	-	-
Glossophaga soricina	CRUC	1	-	-	-
Glossophaginae	FCCNB	1	-	-	-
Glossophaga commissarisi	CPV	1	-	-	-
Glossophaga sp.	BPR	1	-	-	-
Hsunycteris thomasi	FCCNB	1	-	-	-
Lichonycteris obscura	MVP	1	-	-	-
Lonchophylla concava	MVP	1	-	-	-
Lasiurus ega	MVP	1	-	-	-
Lophostoma silvicolum	CAP	1	-	-	-
Lophostoma brasiliense	BPR	1	-	-	-
Myotis sp.	MVP	2	-	-	-
Micronycteris hirsuta	PNS	1	-	-	-
Micronycteris microtis	FCCNB	1	-	-	-
Myotis riparius	MTCF	1	-	-	-
Noctilio leporinus	PNS	6	-	-	-
Pteronotus mesoamericanus	SRF	1	-	-	-
Platyrrhinus helleri	MVP	3	-	-	-
Phyllostomus discolor	LSC	2	-	-	-
Pteronotus personatus	BPR	1	-	-	-
Saccopteryx bilineata	MVP	1	-	-	-
Sturnira mordax	MTCF	5	-	-	-
Sturnira hondurensis	MTCF	2	-	-	-

Sturnira burtonlimi	MTCF	5	-	-	-
Sturnira lilium	MTCF	1	-	-	-
Sturnira sp.	MTCF, FCCNB, LSC	6	-	-	-
Uroderma convexum	CPV, SRF, MNP, MVP, BPR	11	-	-	-
Uroderma magnirostrum	MVP	1	-	-	-
Vampyrodes Caraccioli	MVP	1	-	-	-
Vampyressa thyone	PNS, MVP, BPR	4	-	-	-
Vampyriscus nymphaea	CAP, MVP	5	-	-	-
Vampyrodes major	SRF, BPR	2	-	-	-
SARS-CoV2	NA	NA	+	NA	+
CTV	NA	NA	+	NA	NA
Water	NA	NA	-	-	-

Positive amplification (+). No amplification was observed (-). Not applicable (NA). N = number of individuals captured; CP = conventional PCR; NP = nested PCR; RP = real-time PCR. Sites' abbreviations are shown on the map's legend (Fig. 1).

Discussion

In a previous study from 2013, 1394 bat feces samples from Costa Rica, Ecuador, Brazil, and Panama were analyzed, of which 714 were collected in Panama and belonged to 30 bat species (26). In the present study, we covered more species (48) than in the previous report, which is significant considering that reports indicate differences in coronavirus prevalence and virus load among species (32), even though the number of samples was smaller. Sixteen of the species sampled were the same in both studies. In the samples collected in Panama by Corman et al., 2013, coronaviruses were detected only in *Artibeus jamaicensis* (3 samples), *Artibeus lituratus* (2 samples), and *Phylostomus discolor* (2 samples). The percentage of positivity observed was 0.98 %. However, by species, the percentage of positivity was 1.02 % (3/295), 4.76% (2/42), and 20% (2/10) for *A. jamicensis*, *A. lituratus*, and *P. discolor*, respectively.

Some studies report that the prevalence rate of coronavirus in bats varies between 3 to 10 % (30). However, a global study that analyzed 12,000 samples reported a rate of 8.6 % (31). Studies conducted in eleven countries in the Americas report positivity varying by country from 0.3 to 29.3 % (32). The variation in the prevalence rate of coronavirus in bats might related to several factors that may influence the detection and percentage of coronavirus positivity, which are consistent with the present study. For instance, factors like sample size and type (sources like feces, saliva, etc.), developmental stage of specimens, temporal patterns (season), food availability, species type, habitat, reproductive cycle, age, and colony density (33,34).

Based on the percentage of positivity per species reported by Corman et al. (2013), the detection of a positive sample in the species that tested positive required a sample size of 98 specimens from *A.*

jamaicensis, 21 of *A. lituratus* and 5 of *P. discolor*. Four hundred twenty-one stool samples and rectal swabs performed in Costa Rica reported a rate of only 1% (35). In that study, the positive species were *A. jamaicensis* (1/76), *Carollia perspicilata* (1/49), *Carollia castanea* (1/16), and *Glossophaga soricina* (1/21). The number of samples collected of those species in the present investigation was lower than that collected in both previous studies (Table 1). Likely, the lower number of specimens collected per species in our study reduced the probability of positive detection. A study conducted in Malaysia to test for coronavirus in bats also reported negative results (36). However, the sample size per species might not necessarily be a limiting factor to detecting coronavirus since positives have been detected with five or fewer samples in species such as *Lichonycteris obscura*, *Lonchorhina aurita*, *Cynomops planirostris*, and *Myotis riparius*; however, in *Saccopteryx bilineata*, *Sturnira hondurensis*, and *Phyllostomus hastatus* more than one hundred individuals were analyzed, and no positives were detected. These differences in positivity rates suggest that some bat species are more susceptible to coronavirus infection than others, but the reasons for this susceptibility are not well-known (32). Based on these variation rates reported, they suggest that between 150 to 400 fecal samples per species are required to maximize the probability of positive detection (31).

Sample type is critical in detecting coronavirus in bats as the probability of detection is related to the concentration of the virus in the sample. Coronaviruses exhibit tropism towards the intestinal tract (37,38,39). Therefore, there is a higher concentration of viral particles in feces than in other sample sources. All samples used in our study were rectal swabs or stools. Therefore, the type of sample used in our case was a manageable factor that impacted the observed results.

The stage of development is a factor associated with the percentage of positivity. Juveniles and adults show significant differences with 51.09% and 26.22%, respectively (34). Higher positivity in juveniles was also observed in other studies (31,40,41,42,43,44). The decrease in maternal antibodies passively received from the mother has been suggested as one of the causes of increased viral load in juveniles and, thus, the higher possibility of coronavirus detection (45). In our study, 6.91 % (15/217) of all samples analyzed were juveniles. The distribution of juvenile samples by species was *Artibeus jamaicensis* (6), *Vampyriscus nymphaea* (3), *Myotis sp*. (2), *Uroderma convexum* (1), *Artibeus toltecus* (1), *Sturnina sp* (1) and *Carollia perspicillata* (1).

Also, evidence indicates that the different seasons during the twelve months of the year show differences in variation in the prevalence of bat coronaviruses. For instance, in a study in Hong Kong, the prevalence of SARSr-Rh-BatCov and Rh-BatCov HKU2 coronaviruses was higher in spring (March-May) when the weather is warm and humid, and the first summer rainfall begins (June) than in the other seasons of the year (37). In Thailand, adults and juveniles positive for coronavirus were detected from May to October, while only adults and only juveniles were positive in January and April, respectively (44). These seasonal variations in virus concentrations may impact the probability of positive detection. In Yunan province, China, a study reported that the probability of detection of SL-Cov was higher between late summer and autumn and that the variations may be related to the life habits of the species (46). In African and Asian countries, studies reported more coronavirus positives in the dry season than in the rainy season (31).

The samples in our study were collected from July 2021 to October 2022; however, most samples (76%) were collected in the rainy season (July to December). This seasonal variation in prevalence observed in other countries likely occurs in Panama, which could explain the negative results.

Food shortage can cause stress and impact the immune response in bats, thus making them more susceptible to infection. In *Carollia perspiscillata*, food deprivation delays the metabolic response of

the immune system to exposure to bacterial lipopolysaccharides (47). On Barro Colorado Island, in Panama, a study reported that the primary food source during most of the year for *A. jamaicenis* and *A. lituratus* are fig fruits, which in the latter part of the wet season and at the beginning of the dry season are scarce (48). Even though the samples of *A. jamaicensis* and *A. lituratus* were not collected in Barro Colorado, the availability of this fruit is the same at the national level.

Diet type influences the risk of infection by parasites or pathogens in bats in natural environments (49,50). Carnivorous and hematophagous bat species have a higher risk of acquiring infectious diseases, including parasites and pathogens, due to contact between the bat and its prey, further facilitating transmission (51). Of the three species in Panama that have been reported positive (26), two are primarily frugivorous (*A. jamaicensis* and *A. lituratus*), and only *P. discolor* is omnivorous as it feeds mainly on insects, meat and also fruits, and nectar.

Events related to the reproductive cycle, such as pregnancy and lactation, can induce physiological and energetic stress and influence antiviral activity (52). Mormopterus francoismoutoui, a tropical insectivorous bat, showed a substantial increase in coronavirus detection rate after birth (53). We did not observe gravid or lactating females in our study. Size, colony density, and shelter composition can also affect prevalence by changing the transmission rate (50). A higher prevalence of SARS-CoVs was reported in shelters where the most abundant species was *Rhinolophus sinicus*. A lower prevalence was detected in the same shelter where the most abundant species was *Aselliscus stoli* (54). Shelter size and type appear to affect the likelihood of virus transmission between species due to close physical contact in caves with a high density of individuals (55). Because the samples analyzed in the present study were collected from individuals captured using nets placed in flight paths, information on refuge type or colony density is unknown.

The negative results obtained in our study on coronavirus detection do not necessarily imply that bats in Panama are not reservoirs of coronavirus. Our results suggest that the prevalence of coronavirus in the species of bats collected in Panama is considerably low, which did not allow us to detect positive specimens due to the number of samples analyzed by species. In addition, we do not rule out the possibility that the virus concentration (viral load) was below the detection limits of the PCR techniques and strategies used.

several species with high colony densities have been previously identified. Since we validated all procedures with controls for every step, we ruled out the possibility that the data obtained resulted from errors or mistakes in the methods used for RNA extraction, cDNA synthesis, and PCR amplification. These controls included the results of amplifying the CTV coat protein gene from citrus leaves infected with this virus and the SARS-CoV2 N and E protein genes. Finally, we recommend implementing a permanent national surveillance program to detect coronaviruses in natural bat populations and other potential reservoir species. This work may provide the basis for such a program for bat coronavirus and other zoonotic diseases in Panama. The study may also serve as a reference for

Conclusions

Molecular analyses by both PCR strategies did not detect coronavirus-positive fecal samples in any of the 217 specimens collected from the 48 species of bats from Panama. The results obtained in this study do not necessarily imply that bats in our country are not natural reservoirs of coronavirus. Instead, the data means that the prevalence and/or viral load, if any, might be very low. More samples per species and even more sensitive methods, like pre-concentrating the samples or using TaqMan probes, would confirm these results and conclusions. Extending the sampling period to cover all months of the rainy and dry seasons is also necessary. It is also recommended that collections be carried out in bat shelters such as caves and other places where

other countries in the area since Panama contains 81.3% of the 145 bat species reported in the Central American region that could potentially be carriers of coronavirus and vectors for the zoonotic disease.

Acknowledgments

We thank Sociedad Mastozoológica de Panamá (SOMASPA) for providing the logistics and equipment required for the fieldwork and all the students and staff accompanying us on the collecting tours. To Parque Natural Metropolitano, Parque Nacional Soberanía, and Summit Rainforest & Golf Resorts for permission to conduct the captures in their facilities and Gorgas Memorial Institute for Health Studies for providing the transport and stabilization media for samples.

Ethical approval

This research was conducted with the authorization of Panama's Ministry of Environment with the approval ID ARG-016-2023.

Conflict of interest statement

The authors declare no conflicts of interest.

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