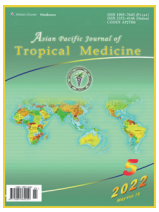

Letter to Editor

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.345947

5-Year Impact Factor: 2.285

Evidence of SARS-CoV-2 infection in pets, captive non-human primates and farm animals in Central Africa

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It has been reported that the host of SARS-CoV-2 can be extremely wide due to the conservation of angiotensin converting enzyme 2 in mammals[1]. Several studies have reported natural or experimental infection with SARS-CoV-2 in pets, farm and captive wild animals[2-4]. However, to the best of our knowledge, there was no published study assessing virus circulation among domestic or captive animals in Central, West and East Africa. In this study, we assessed the presence and circulation of SARS-CoV-2 or other betacoronaviruses in pets, livestock and animals kept in captivity in conservation centers in Haut-Ogooué province, southeastern Gabon.

Samples were collected retrospectively and prospectively from August 2020 to July 2021 in Haut-Ogooué province and in Ogooué-Ivindo province. The ID screen® SARS-CoV-2 Double Antigen ELISA Multi-Species (Innovative Diagnostics, Grabels, France) was used for the detection of IgG and IgM antibodies against the nucleocapsid (N protein) of SARS-CoV-2 in dogs, cats, pigs and non-human primates sera. The ELISA was performed according to the manufacturer's instructions. Then, from the RNA extracted from oropharyngeal and rectal swabs, using the QIAamp viral RNA minikit (Qiagen, Hilden, Germany), two separate real-time RT-PCRs were used to detect SARS-CoV-2 in all samples, one targeting the RNA-dependent RNA polymerase gene and the other the *E* gene, as previously described[5], using the Superscript III One-step RT-PCR kit (Invitrogen, Germany). To investigate the presence of other coronaviruses in the animals studied, all the samples initially analyzed by both real-time RT-PCRs were submitted to a nested reverse transcription-polymerase chain reaction[6]. This study was approved by the national review board approval under No. AR021/20/MESRSTTENCFC/CENAREST/CG/CST/CSAR.

In total, 347 animals were recruited, including 139 dogs, 11 cats, 9 wiskered *Cercopithecus*, 36 common chimpanzees, 1 *Macaques*, 1 gorilla, 71 mandrills, 1 white-nosed *Cercopithecus*, 48 pigs, 29 sun-tailed *Cercopithecus*, and 1 white-collared Mangabey (Table 1). Additionally, of the sampled dogs and cats, 17 (12.2%) and 7 (63.6%), respectively, were from households whose owners had confirmed SARS-CoV-2 infection or suspected COVID-19 symptoms. The overall seroprevalence of SARS-CoV-2 infection was 1.1% (4/346). Among the 346 animals tested by ELISA, 1 (0.3%) was classified as indeterminant, which was a Large White adult pig, sampled in May 2021 from another farm in Franceville. Of the 300 individuals studied during the pandemic, antibodies against SARS-CoV-2 were found in 4 (1.3%) of them, all from the city of Franceville. None of the samples collected in the pre-pandemic period were found to be positive. The seroprevalence of SARS-CoV-2 infection was 0.7% (1/139), 2.1% (1/48) and 6.9% (2/29), respectively in dogs, pigs and

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How to cite this article: Maganga GD, Ngoubangoye B, Koumba JP, Lekana-Douki S, Kinga Moussadji IC, Tsoumbou TA, et al. Evidence of SARS-CoV-2 infection in pets, captive non-human primates and farm animals in Central Africa. Asian Pac J Trop Med 2022; 15(5): 232-235.

Article history: Received 4 April 2022 Revision 23 May 2022
Accepted 24 May 2022 Available online 31 May 2022

Table 1. Overview of the animals in the study.

Locations	Sampling period	Species	Serum ELISA positive/ Serum tested (%)	qPCR positive buccal swabs/ Swabs tested (%)	qPCR positive rectal swabs/ Swabs tested (%)
Franceville	Pre-pandemic	Common chimpanzee	0/3 (0)	0/3 (0)	0/3 (0)
		White-nosed *Cercopithecus*	0/1 (0)	0/1 (0)	0/1 (0)
		Wiskered *Cercopithecus*	0/2 (0)	0/2 (0)	0/2 (0)
	During the pandemic	Dogs	1/53 (1.9)	0/53 (0)	0/53 (0)
		Cats	0/10 (0)	0/10 (0)	0/10 (0)
		Pigs	1/48 (2.1)	0/48 (0)	0/48 (0)
		Common chimpanzee	0/23 (0)	0/23 (0)	0/23 (0)
		Wiskered *Cercopithecus*	0/6 (0)	0/6 (0)	0/6 (0)
		Mandrills	0/57 (0)	0/57 (0)	0/57 (0)
		Macaques	0/1 (0)	0/1 (0)	0/1 (0)
		Sun-tailed *Cercopithecus*	2/29 (6.9)	0/29 (0)	0/29 (0)
		White-collared Mangabey	0/1 (0)	0/1 (0)	0/1 (0)
Moanda	During the pandemic	Cat	0/1 (0)	0/1 (0)	0/1 (0)
		Dogs	0/47 (0)	0/47 (0)	1/47 (2.1)
Bakoumba (Lekedi Park)	Pre-pandemic	Common chimpanzee	0/2 (0)	-	-
	During the pandemic	Common chimpanzee	0/8 (0)	0/8 (0)	0/8 (0)
		Gorilla	-	-	0/1 (0)
		Mandrills	0/14 (0)	0/14 (0)	0/14 (0)
		Wiskered *Cercopithecus*	0/1 (0)	0/1 (0)	0/1 (0)
Bongoville	During the pandemic	Dog	0/1 (0)	-	-
Zadie Department	Pre-pandemic	Dog	0/38(0)	-	-
			4/346 (1.1)	0/308 (0)	1/309 (0.3)

Note: A total of 347 animals were recruited, but only 346 were tested for serology except for a gorilla sampled at Lekedi Park in Bakoumba whose rectal swab was collected. Buccal and rectal swabs could not be collected for all 347 animals, but rather for animals collected in the pre-pandemic period (n=308 and n=309).

sun-tailed *Cercopithecus* (Table 1). For the seropositive dog (a young male of the Borboel breed), COVID-19 status of the household was unknown. The infected pig (a Large White young female) came from a farm in Franceville for which the status of the personnel with respect to COVID-19 was unknown. As for the 2 seropositive sun-tailed *Cercopithecus*, owners are females, one child and one adult, originated from CIRMF primatology center. These monkeys did have close contact with an human individual who tested positive for COVID-19 and was subsequently hospitalized.

SARS-CoV-2 RNA was detected in rectal swab of 1 out of 309 (0.3%) animals tested and particularly in 1 out of 139 (0.7%) dogs in the study, only by real-time RT-PCR targeting the *E* gene (Table 1). The cycle threshold value obtained was 39.1. The dog was a Basenji breed from the city of Moanda, lived with a COVID-19 infected symptomatic human with a positive PCR SARS-CoV-2 result. The nested pancoronavirus PCR were negative on all samples (buccal and rectal swabs) tested.

In Africa, except in southern Africa, no studies have been conducted to date to investigate the infection of domestic, captive or wild animals with SARS-CoV-2. Only one study had reported the infection of animals (lions and puma) in captivity in South Africa[3]. Our study revealed a relatively low overall seroprevalence, and a seroprevalence of 0.7% in dogs. The seroprevalence is lower compared to those found in other studies, some used the same ELISA test as in our study[2,7–9]. The differences observed between our study and previous studies may suggest a low circulation of SARS-CoV-2 in Gabon compared to other countries. The dog found to be seropositive in our study came from a household in which the owners had not tested positive for COVID-19. This would suggest that the infection in the owners could have been asymptomatic. Furthermore, in the studies cited above, cats were found to be infected and the seroprevalences of SARS-CoV-2 obtained in these pets appeared to be higher than those found in dogs. However, in this study, no cats were found infected with SARS-CoV-2. This could be explained by the small number of cats sampled, as cats are not very popular as pets in our region. The results by city showed that the virus circulated exclusively in the city of Franceville. This finding is not very surprising because approximately 67.4% (234/347) of the samples were taken in this city and also, it is the city in the country with the second highest COVID-19 prevalence. In addition to pets, animals living in captivity (in zoos) and farm animals have been shown to be susceptible to and infected with SARS-CoV-2. Thus, although there is no evidence in the literature of SARS-CoV-2 infection of pigs, this study also focused on these farm animals. Serology performed on the pigs found one seropositive pig out of 48 tested (2.1%). The result obtained would suggest an exposure of pigs to SARS-CoV-2 and a receptivity of this species to the virus. However, a study carried out on wild boars in Croatia showed anti-SARS-CoV-2 antibodies in wild boars[10]. Our results showed the presence of anti-SARS-CoV-2 antibodies in 2 sun-

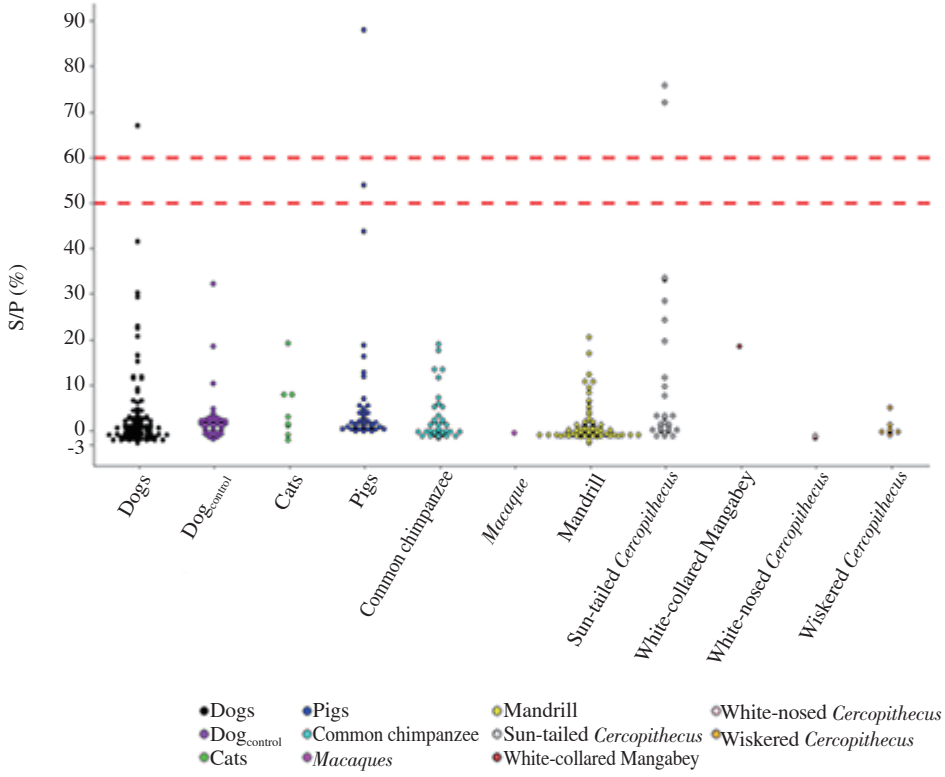

Figure 1. Representation of the results of the SARS-CoV-2 ELISA assay from the animals sera. S/P (%): Sample/positive or sample to positive ratio. Samples tested by ELISA were considered positive if the S/P (%) was greater than or equal to 60% (cutoff OD_{450} value represented by upper red dash line), indeterminant when the S/P (%) ranged between 50% and 60%, and negative if the S/P (%) was less or equal to 50% (represented by lower red dash line).

tailed *Cercopithecus*. The infected sun-tailed *Cercopithecus* were found to have contact with a worker at the Primatology Center who subsequently tested positive for COVID-19 and was symptomatic. These results support the susceptibility of non-human primates to SARS-CoV-2. In contrast to the serological results, almost all animals tested negative by PCR, except for one dog in which SARS-CoV-2 RNA was amplified by real-time PCR targeting the *E* gene. The confirmatory test with the RNA-dependent RNA polymerase gene was negative, so it is difficult to confirm the infection with SARS-CoV-2 as this result could be considered as a non-specific result because dogs can also be infected by different coronaviruses able to react with the *E* gene amplification. Techniques such as viral isolation and high-throughput sequencing could be performed for a better detection and characterization of the virus. All swabs tested were negative for Pancoronavirus PCR.

To the best of our knowledge, this study reports for the first time in Central Africa the circulation of SARS-CoV-2 in pets, captive and farm animals. Although these results need to be confirmed by further analysis, such as serum neutralisation and indirect immunofluorescence, they nevertheless highlight the need to monitor pets, farmed and captive animals in order to assess the role these animals play in the epidemiology of the disease and the risk to the conservation of nonhuman primates.

Conflict of interest statement

All authors disclose no conflict of interest. No financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

Acknowledgments

We acknowledge all animal owners and the managers of the Lekedi Park for their cooperation. We also acknowledge Karen Saylors and Elsa Assengone for the English revision and Mr Mfoumbi Ibinda Guy Arnault Rogue for his help in editing citations. The CIRMF is supported by the Government of Gabon, Total-Fina-Elf Gabon, and the Ministère de la Coopération Française.

Funding

It was supported by the Centre Interdisciplinaire de Recherches Médicales de Franceville.

Authors' contributions

GDM and BN conceptualized and designed the study; GDM, BN, JPK and JBLK defined the intellectual content; GDM, BN, JPK and TGNM searched for literature; GDM, BN, JPK, ICMK, TAT carried out experimental studies and data acquisition; GDM, JPK, SLD and AMMB performed data analysis; GDM and JPK drafted the manuscript. All authors have read and approved the final version of the manuscript.

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