One Health 18 (2024) 100709

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

SARS-CoV and SARS-CoV -2 cross-reactive antibodies in domestic animals and wildlife in Nigeria suggest circulation of sarbecoviruses

Ebere R. Agusi [a,b], Jacob Schön [c], Valerie Allendorf [c,*], Emmanuel A. Eze [b], Olayinka Asala [a], Ismaila Shittu [a], Anne Balkema-Buschmann [c], Kerstin Wernike [c], Ishaya Tekki [a], Mark Ofua [d], Omowunmi Adefegha [e], Oluwatoyin Olubade [a], Oluyemi Ogunmolawa [a], Klaas Dietze [c], Anja Globig [c,*], Donata Hoffmann [c], Clement A. Meseko [a]

[a] National Veterinary Research Institute, Vom, Nigeria
[b] University of Nigeria, Nsukka, Nigeria
[c] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald, Isle of Riems, Germany
[d] SaintMarks-Lagos Urban Forest Sanctuary Initiative (LUFASI), Lagos, Nigeria
[e] Federal College of Agriculture, Akure, Nigeria

ARTICLE INFO

Keywords:
One Health
SARS-CoV-2
Zoonosis
Sarbecovirus
Serology
Nigeria

ABSTRACT

Anthropogenic exposure of domestic animals, as well as wildlife, can result in zoonotic transmission events with known and unknown pathogens including sarbecoviruses. During the COVID-19 pandemic, SARS-CoV-2 infections in animals, most likely resulting from spill-over from humans, have been documented worldwide. However, only limited information is available for Africa. The anthropozoonotic transmission from humans to animals, followed by further inter- and intraspecies propagation may contribute to viral evolution, and thereby subsequently alter the epidemiological patterns of transmission. To shed light on the possible role of domestic animals and wildlife in the ecology and epidemiology of sarbecoviruses in Nigeria, and to analyze the possible circulation of other, undiscovered, but potentially zoonotic sarbecoviruses in animals, we tested 504 serum samples from dogs, rabbits, bats, and pangolins collected between December 2020 and April 2022. The samples were analyzed using an indirect multi-species enzyme-linked immunosorbent assay (ELISA) based on the receptor binding domain (RBD) of SARS-CoV and SARS-CoV -2, respectively. ELISA reactive sera were further analyzed by highly specific virus neutralization test and indirect immunofluorescence assay for confirmation of the presence of antibodies. In this study, we found SARS-CoV reactive antibodies in 16 (11.5%) dogs, 7 (2.97%) rabbits, 2 (7.7%) pangolins and SARS-CoV-2 reactive antibodies in 20 (13.4%) dogs, 6 (2.5%) rabbits and 2 (7.7%) pangolins, respectively. Interestingly, 2 (2.3%) bat samples were positive only for SARS-CoV RBD reactive antibodies. These serological findings of SARS-CoV and/or SARS-CoV-2 infections in both domestic animals and wildlife indicates exposure to sarbecoviruses and requires further One Health-oriented research on the potential reservoir role that different species might play in the ecology and epidemiology of coronaviruses at the human-animal interface.

1. Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulted in the deaths of more than six million people worldwide [1]. In Nigeria, the first confirmed human case of COVID-19 was announced on February 27, 2020, when a foreign national arriving via Lagos Airport in Nigeria tested positive for the virus [2]. The second case was a Nigerian citizen transiting from Milan to Lagos who had contact with the index case. Thereafter, the number of cases increased exponentially in successive waves [3]. Despite an increase in the incidence figures in the second wave, the case fatality rate during the second wave declined, and most confirmed cases were asymptomatic, hence the chances of inapparent transmission to humans and possibly their animals increased and many cases probably went undetected [4,5]. In mid-2021, a seroprevalence study using a SARS-CoV-2 specific Enzyme Linked ImmunoSorbent Assay (ELISA) detected

* Corresponding authors at: Institute of International Animal Health, One Health, Friedrich-Loeffler-Institut, Suedufer 10, 17493 Greifswald, Insel Riems, Germany.
E-mail addresses: Valerie.Allendorf@fli.de (V. Allendorf), Anja.Globig@fli.de (A. Globig).

https://doi.org/10.1016/j.onehlt.2024.100709
Received 5 December 2023; Accepted 11 March 2024
Available online 12 March 2024

antibodies in almost 80% of the human serum samples [6], indicating a much higher level of circulation of the virus than estimated by the daily case counts published by the Nigerian Center for Disease Control (NCDC).

Even though the exact origin of SARS-CoV-2 is yet unclear, a zoonotic origin is widely assumed. The virus closest relatives were found in bats of the genus *Rhinolophus* in China [7] and Laos [8], suggesting the spill-over of its progenitor to humans, possibly either directly or via an intermediate host. In fact, phylogenetic and molecular clock analyses suggest that today's *Alpha-* and *Betacoronavirus* emerged from the host order *Chiroptera* [9]. Various species of the genus *Betacoronavirus* are meanwhile well adapted to their particular host species, but may sometimes also cross species barriers. Examples include human coronavirus OC43 (HCoV-OC43) in cattle and humans [10], Rabbit coronavirus HKU14 (RbCoV-HKU14) in rabbits [11], canine respiratory coronavirus (CRCoV) in dogs [12] or Middle East respiratory syndrome-related (MERS) coronavirus in dromedary camels [13]. The subgenus *Sarbecovirus* within the *Betacoronavirus* genus is thought to be one of the youngest subgenera, from which the most recently emerged species Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and SARS-CoV-2 jumped somehow from their original bat host to humans [14]. In both events, intermediate hosts are thought to have enabled this jump, serving as incubators and propagators for the further evolution and adaptation of the virus to new host species [15,16].

Generally, zoonotic animal-to-human and human-to-animal spill-over events that contribute to the emergence of diseases are complex to follow and are therefore rarely studied. Hence, the link between specific interactions and spillover risk is poorly understood. In 2002, the emergence of SARS-CoV in Southern China from wildlife then spread through human-to-human transmission and subsequent spread to 31 other countries, has been associated with contact and interaction with intermediate hosts at the live animal markets [17]. Evidently, SARS-CoV has a zoonotic origin and horseshoe bats seem to be its natural reservoir [18], but it has also been detected in palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*) as possible intermediate hosts [19]. Whereas the SARS pandemic was rapidly brought under control and was declared over only nine months after its onset, the global spread of SARS-CoV-2 among the human population led to further adaptation and evolution of the virus [20]. Facilitated by close human-animal-environment interactions, anthropozoonotic transmissions have also been reported worldwide [21], giving the virus opportunities to establish in further host species, potentially establishing a new reservoir. The most prominent example is the co-circulation of the Alpha, Delta, Gamma and Omicron variants of SARS-CoV-2 within the white-tailed deer population in North America [22,23]. Apart from these wild ungulates, transmission to different carnivore species were reported globally, among others to ferrets, cats and dogs due to their close interaction with humans [24]. No significant onward transmission of the virus and establishment within these new host species could be detected [25]. Fortunately, livestock animals like pigs, chickens and goats [26] are not efficiently susceptible under experimental conditions [27]. Nevertheless, there is documentation of occasional spillover to animals like cattle [28].

In experimental challenge experiments, rabbits were also shown to be susceptible to SARS-CoV-2 and to excrete infectious virus particles via the upper respiratory tract [29], potentially leading to circulation in farms. Natural infections were retrospectively confirmed by serological investigations in France [30].

In general, there is limited information on SAR-CoV-2 spillover events from humans to animals in Africa and particularly Nigeria [31]. Here, rapid population growth, changes in land use and urbanization are some of the most important drivers for the zoonotic transmission of pathogens in both directions. Wild animals probably highly susceptible to sarbecoviruses, such as bats and pangolins [32], share their habitat with humans, or are hunted and sold at live animal markets. Dogs are kept as companion animals in many households or are abundant in peri-urban and rural settings as feral or stray animals. In the livestock sector, rabbits play an important role in backyard farming, where they are often kept in small, crowded pens that provide the opportunity for pathogens to circulate in the population.

In this study, we attempted to investigate the level of antibodies reactive against SARS-CoV-2 and related SARS-CoV in wildlife, companion animals and farmed animals in Nigeria, namely bats of the migratory species *Eidolon helvum*, pangolins of the species *Phataginus tricuspis*, companion and feral dogs as well as rabbits kept on farms in Nigeria.

2. Methods

2.1. Ethical statement

This study was approved by the Animal Use and Care Committee, National Veterinary Research Institute (NVRI), Vom, Nigeria (NVRI/AEC/03/118/22).

2.2. Sampling

Sampling was conducted at different time periods between September 2020 to April 2022 (see Fig. 1). A total of 504 individual blood samples of mammals were collected in different federal states in Nigeria (see Fig. 2) and transported to the NVRI, for processing. In February/March 2022 as part of pest control measures to reduce bat roosts on the campus of the Federal College of Agriculture in Akure, Ondo State, convenience samples from 87 straw-coloured fruit bats (*Eidolon helvum*) were collected by puncture of the aortic ventricle shortly after death. In addition, convenience blood samples of 26 pangolins (*Phataginus tricuspis)* rescued between February and April 2022 from Lagos live animal market and transferred to a pangolin sanctuary were obtained by puncture of the tail vein. In Plateau State, blood from 95 feral dogs (local breed of dogs, commonly seen on the streets and dump sites feeding on various food sources, while returning to the owners on a regularly basis) from Jos South and 54 companion dogs attending the Veterinary Teaching Hospital in Jos were collected from June to September 2021 and in April 2022, respectively. The non-heparinized blood samples were centrifuged (Eppendorf AG centrifuge 5427) at 1200 $\times g$ at 4 °C for 10 min and the sera were collected. In order to adhere to biosecurity measures and to eliminate contamination, the sera were subsequently heat-inactivated at 56 °C for 2 h in a water bath (Certomat ® WR, B. Braun Biotech International) before being stored at -20 °C until the laboratory analyses were performed. Additionally, 242 archived rabbit sera collected during rabbit hemorrhagic disease (RHD) outbreak in a cross-sectional survey in 19 federal states from September to December 2020, were retrieved.

2.3. SARS-CoV and -2 antibody detection by RBD ELISA

An indirect multi-species enzyme-linked immunosorbent assay (ELISA) based on the receptor- binding domain (RBD) of SARS-CoV-2 [33] was employed to screen the serum samples on the potential presence of SARS-CoV-2 antibodies. In a similar manner, ELISA was also performed on the same serum samples to screen them for the potential presence of SARS-CoV [34] reactive antibodies as the ELISA is valid for several species because of the multi-specie conjugate used. Briefly, reaction wells of Nunc Immuno Maxisorp flat bottom plates were coated with 100 ng/well SARS-CoV-2 or SARS-CoV RBD respectively in 0.1 M carbonate buffer while the control-wells were filled with 100 µl/well 0.1 M carbonate, incubated overnight at 4 °C and washed thrice with 200 µl wash buffer (Tris-buffered saline (TBS)/ 0.05% Tween 20). The plates were blocked with 100 µl/well 5% skim milk in phosphate-buffered saline (PBS), incubated at 37 °C for 1 h and washed 3 times with 200 µl wash buffer. After washing, serum (50 µl/well) diluted 1:100 in wash buffer was added, incubated at 37 °C for 1 h and washed 3×

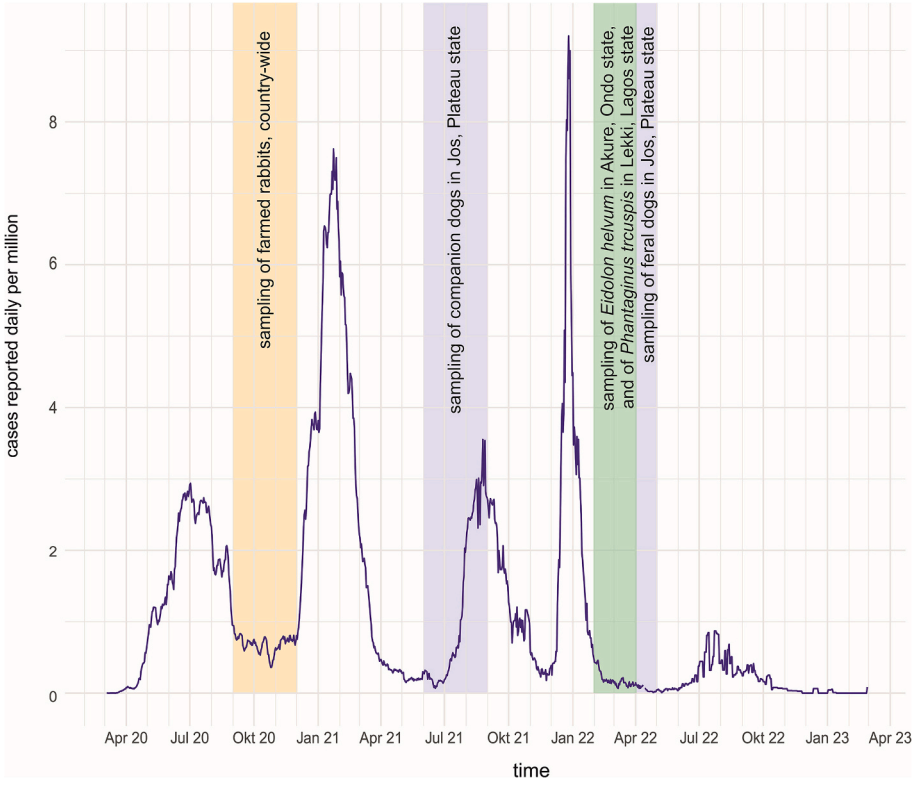

Fig. 1. Periods of sample collection, aligned with the daily new confirmed cases per million people in Nigeria as reported on ourworldindata.com [46]; highlighted in orange, is the time period of the rabbit sampling; highlighted in blue, the periods of sampling of dogs; and highlighted in green, the period of sampling of *Eidolon helvum*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

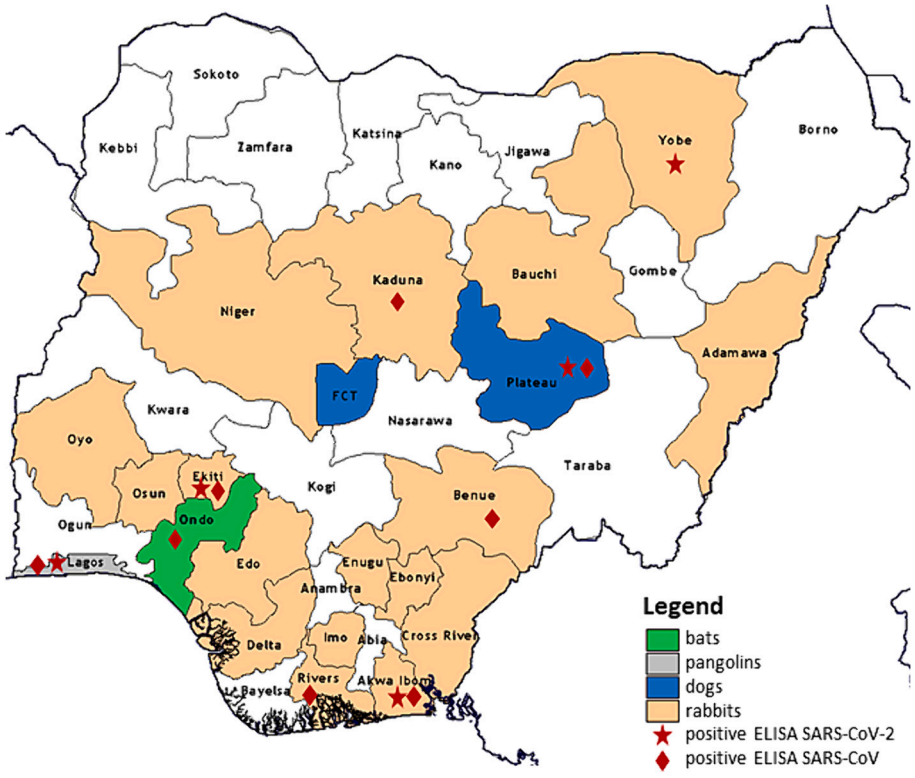

Fig. 2. Map of Nigeria, indicating the federal states in which sampling took place by species (colors), and where at least one of the investigated sera yielded in a positive result in the RBD ELISA (star / diamond).

with 200 µl wash buffer. Then, 50 µl/well of multi-species conjugate (diluted 1:80 in wash buffer) were added, incubated at room temperature for 1 h and washed 3× with 200 µl wash buffer. TetraMethylBenzidine (TMB) (100 µl/well) were added and incubated in the dark at room temperature for 10 min and the reactions were immediately stopped by the addition of 1 M sulfuric acid/stop solution (100 µl/well). The optical density (OD) of each sample in the plates were measured at 450 nm wavelength on a Tecan-Microplate Reader Infinit 200 pro. The absorbance was calculated as the OD values obtained from the protein-coated wells minus the OD values measured for the uncoated control wells for individual samples. Serum samples with OD values of ≥0.3 were determined as positive, while serum samples with OD values of 0.2–0.3 were considered borderline and serum samples with OD values of <0.2 were regarded as negative [33]. To further confirm the ELISA-positive samples, they were subsequently tested by indirect immunofluorescence assay (iIFA) and a virus neutralization test (VNT).

2.4. Indirect immunofluorescence assay

Vero76 (CCLV-RIE 0228) cells were seeded on a 96 well plate format and infected with $10^{2.5}$ $TCID_{50}$/ml of either SARS-CoV, BavPat1 (D614G SARS-CoV-2 variant) or SARS-CoV-2 Omicron BA.2. After 24 h plates were fixed with 4% Paraformaldehyde (PFA) and permeabilized with triton X-100. Finally, cells were washed with PBS and the serum samples were applied in 1:100 and 1:500 dilutions. After 1 h incubation, cells were washed and the secondary FITC-labeled antibody respective of the species was applied (bat 1:100, dog 1:100, rabbit 1:1000). Due to the lack of a pangolin reactive secondary antibodies, these sera were not applied in iIFA. Following another 1 h incubation the immunofluorescence readout was done using a fluorescence microscope (Nicon Eclipse Ti with cool LED pE 300 lite laser l).

2.5. Virus neutralization test (VNT)

To detect SARS-CoV or SARS-CoV-2 antibodies that are capable of neutralizing virus replication, the ELISA positive sera were subjected to VNT in VeroE6 (CCLV-RIE 0929) cells. Briefly, the positive serum samples were pre-diluted 1:16 with Dulbecco's modified Eagle's medium (DMEM) in a 96-well plate. Next, 100 µl of the pre-diluted sera were transferred into three wells of a new 96-well plate, representing three technical replicates. A two-fold dilution was conducted by serially transferring 50 µl of the serum in 50 µl DMEM, leaving 50 µl of sera dilution in each well up to 1:4096 dilutions. Subsequently, 50 µl of SARS-CoV or the respective SARS-CoV-2 (BavPat1 or omicron BA.2) virus dilution (100 $TCID_{50}$/well) was added to each well and incubated at 37° C for 1 h. Lastly, 100 µl VeroE6 cells in DMEM with 1% penicillin/streptomycin supplementation was added to each well and incubated at 37° C, 5% CO_2 for 72 h. After the incubation period, the cells were evaluated by using an inverted light microscope (Motic AE20) to assess specific cytopathic effect (CPE). A serum dilution was considered as neutralizing if there was no specific CPE detectable. The virus titre (100 $TCID_{50}$ used for each well) was confirmed by virus back titration in triplicate; positive and negative control serum samples were included.

3. Results

A total of 504 serum samples (87 bats, 149 dogs (companion and feral), 26 pangolins and 242 rabbits) were initially screened for the presence of SARS-CoV-2 antibodies by the SARS-CoV-2 RBD ELISA as described. Subsequently, 497 out of the same 504 samples had sufficient volume and quality left to continue with a screening for the presence of SARS-CoV antibodies (Table 1).

We detected SARS-CoV-2 RBD reactive sera by ELISA with OD values higher than 0.3 in none of the sera of the companion dogs but 17 (17.9%) of the feral dogs, in 7 (2.9%) of the rabbits, and 2 (7.7%) pangolins. All sera from fruit bats were negative for SARS-CoV-2 reactive antibodies. In the SARS-CoV ELISA, again no companion dog, but 15 (15.8%) of the feral dogs reacted positive, further on 7 (2.97%) rabbits, 2 (7.7%) pangolins and 2 (2.3%) bat samples showed reactivity with the RBD. Nine of the sera (six dogs, two rabbits, one pangolin) reacted positive in both the SARS-CoV and the SARS-CoV-2 ELISA (Fig. 3).

All samples that reacted positive in the SARS-CoV-2 RBD-based ELISA ($n = 28$) and within the borderline range ($n = 12$) were subsequently tested by iIFA and most ($n = 35$) also by VNT against the SARS-CoV-2 ancestral strain (BavPat1) and the Omicron strain (BA.2), as displayed in Fig. 4. Two dog sera reacted by immune fluorescence against BavPat1 SARS-CoV-2. From all 35 samples analyzed in the SARS-CoV-2 VNT, one pangolin serum tested positive against SARS-CoV-2 ancestral strain BavPat1 with 32 neutralizing dose 100 (ND100). The selected samples ($n = 43$) that reacted positive in the SARS-CoV RBD-based ELISA were further subjected to VNT and iIFA using the SARS-CoV virus. None of the tested sera had neutralizing antibody titres to SARS-CoV, by testing pre-dilutions between 1:16–1:128. Nevertheless, six dog sera and five rabbit sera reacted specifically with SARS-CoV at 1:100 dilutions in the immune fluorescence test (Fig. 4).

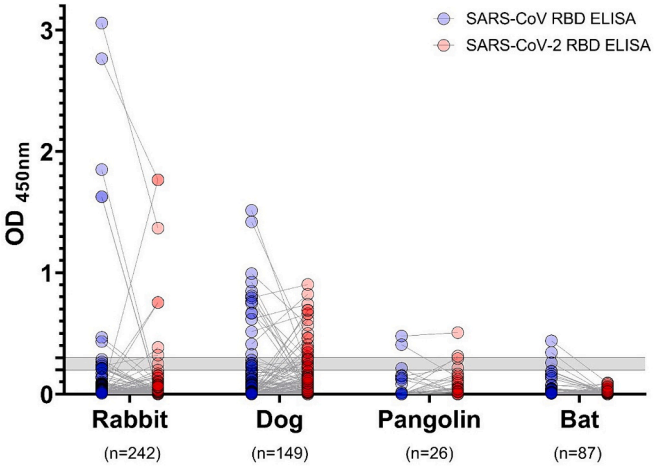

Fig. 3. Results of the indirect multi-species ELISA of SARS-CoV and SARS-CoV-2 per species and individuum. Results of the individual sera for each of the tests are connected with a thin grey line. OD values of 0.2 to 0.3 (grey horizontal bar) are regarded as borderline, values >0.3 are interpreted as positive.

Table 1
SARS-CoV and SARS-CoV-2 receptor-binding domain (RBD) ELISA results of animals tested within this study.

Animals sampled	SARS-CoV-2 ELISA			SARS-CoV ELISA			Positive in both assays
	Total	Positive	Borderline	Total	Positive	Borderline	
Straw-coloured fruit bats *(Eidolon helvum)*	87	0	0	87	2	1	0
Companion dogs *(Canis lupus familiaris)*	54	0	0	54	0	0	0
Feral dogs (Basenji) *(Canis lupus familiaris)*	95	17	8	95	15	6	6
Tree pangolins *(Phataginus tricuspis)*	26	2	2	26	2	1	1
Rabbits *(Oryctolagus cuniculus)*	242	7	1	235	7	9	2

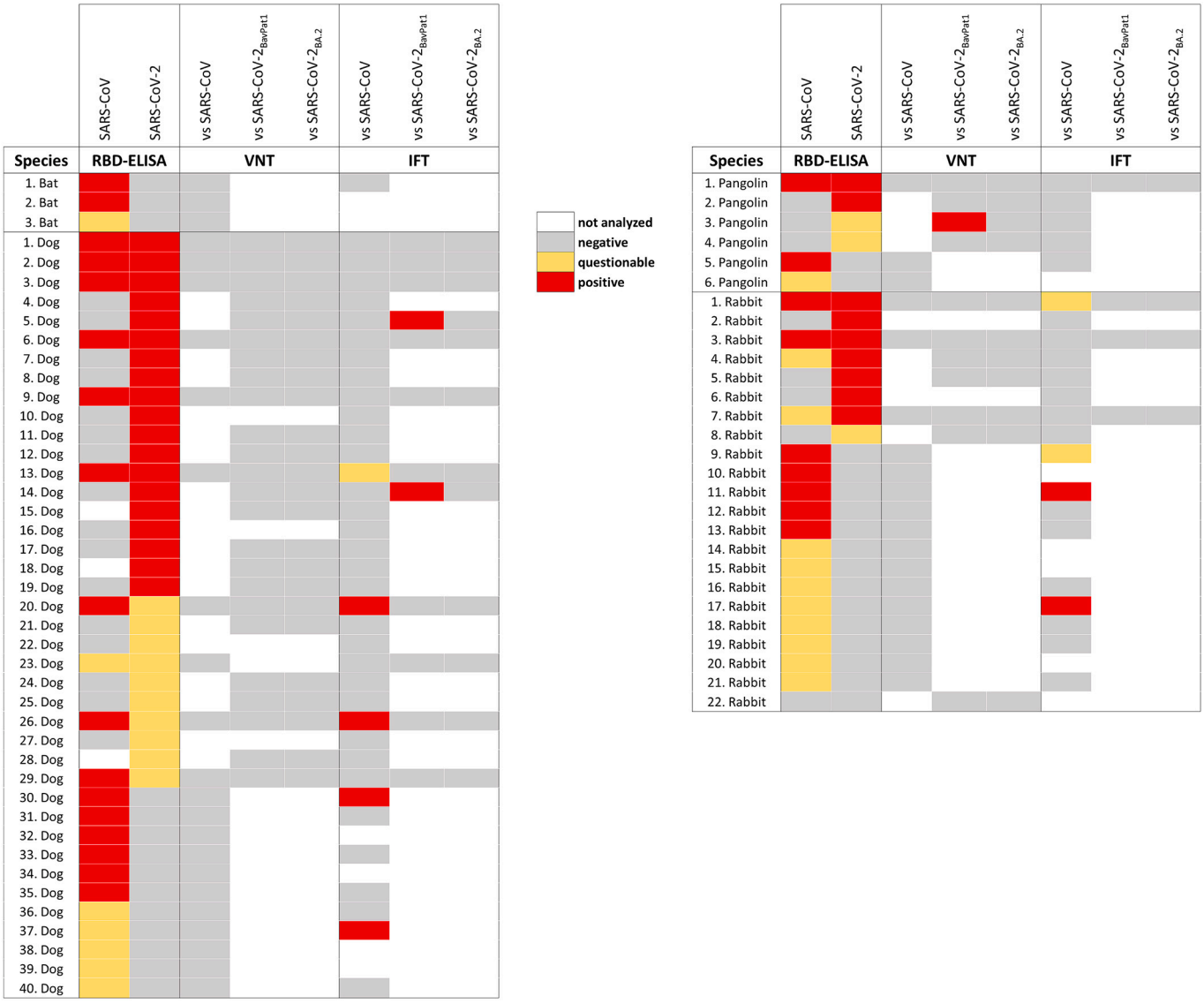

Fig. 4. Results of ELISA, iIFA and VNT to SARS-CoV and SARS-CoV-2 in comparison. Each line represents the serum of one individual, ordered by species. Colors show the reactiveness of the sera in each test (columns). White space means that the respective serum was not analyzed in the specific test.

4. Discussion

To understand the ecology and epidemiology of potentially zoonotic pathogens in Nigeria and elsewhere, focused One Health monitoring of possible animal reservoirs in addition to those animals that may have been exposed are important steps in pandemic preparedness [35]. In the present study, SARS-CoV-2 RBD ELISA reactive sera were found in feral dogs, rabbits, and two pangolins. In the SARS-CoV RBD ELISA, the same was observed. In addition, two (2.3%) bat samples were ELISA-positive. Notably, some of these ELISA-positive samples (three sera from bats, eleven sera from feral dogs and four sera from rabbits) also exhibited positive results in the iIFA to SARS-CoV showing further confirmation. One of the two SARS-CoV-2 ELISA-positive pangolin samples had a neutralizing antibody titre of 32. To our knowledge, this is the first research in Nigeria documenting the detection of SARS-CoV and SARS-CoV-2 reactive antibodies in pangolins and farmed rabbits [36]. The positive reactions with the RBD of two known zoonotic sarbecoviruses in the ELISA indicate circulating antibodies against this subgenus of betacoronaviruses within the animal species investigated. With VNT and iIFA, further confirmation of the specificity of the antibodies detected was intended. However, the majority of sera showed neither neutralizing antibodies nor antibodies reacting with the virus in the iIFA, pointing towards an immunoreaction of the animals against a possibly related sarbecovirus.

Three out of the 26 pangolin samples were positive by RBD ELISA for SARS-CoV or SARS-CoV-2, indicating possible exposure to circulating sarbecoviruses. In the case of SARS-CoV-2, neutralizing antibody to the ancestral strain was only detected in one pangolin. The animal may have been exposed to SARS-CoV-2 possibly through various sources including the hunter, at the market when kept together with other animal species, or by the people handling it at the animal rescue center. Pangolins have been shown to host a variety of coronaviruses [37,38], making them a potential reservoir species in the transmission cycle and in the emergence of zoonotic coronaviruses. However, the ability of the individual to develop neutralizing antibodies indicates an effective immunoreaction of the animal to eliminate the virus, preconditions unlikely leading to the formation of a potential reservoir in this case. But this coincidental finding underlines the risk of transmission of zoonotic pathogens between humans and wildlife in both directions when interaction occurs.

Almost 3% of the rabbit samples collected from farms within the first peak of COVID-19 (September to December 2020) showed reactivity

against the RBD of SARS-CoV-2, but could not be confirmed in either iIFA or VNT. A similar ELISA-based study by Fritz et al. [30] observed a seroprevalence between 0.7% and 1.4%, which is comparable to our results. ELISA-positive animals were found in farms of four out of the 19 different federal states in Nigeria (Akwa Ibom, Ekiti, Plateau, and Yobe). Interestingly, >2% of the rabbit sera originating from the states Akwa Ibom, Benue, Ekiti, Kaduna and Rivers reacted with the RBD of SARS-CoV, two individual sera from two different states (Akwa Ibom and Ekiti) even in both assays. As none of the ELISA results were confirmed in iIFA or VNT, and positive sera were not evenly distributed over all geographic locations as seen in Fig. 2, this may indicate the circulation of a related but yet unknown sarbecovirus. Cross-reactivity with other betacoronaviruses cannot be entirely excluded, but previous investigations showed a high specificity for SARS-CoV-2 of the here used assay against BCoV and FCoV [33,39,40]. Moreover, Evans et al. [41] showed that seropositivity against the SARS-CoV-2 RBD followed by VNT is a valuable way to get an impression of serology against several sarbecoviruses.

In feral dogs, SARS-CoV and SARS-CoV-2 specific antibodies were detected in 20 and 16 of the 95 analyzed sera, respectively, whereas no ELISA-reactive sera were seen in companion dogs. None of the ELISA-positive sera were able to neutralize the respective virus though, but the sera of two feral dogs were reactive to the ancestral SARS-CoV-2 in the iIFA. The discrepancy between samples collected from companion dogs that are kept as household pet and free-ranging feral dogs sampled in villages and peri-urban centres may be explained by the different risk of exposure. Pet dogs in Nigeria rarely leave their home compound, and contact with other dogs, humans or any other animals is fairly restricted to their household members. Feral dogs on the opposite due to their increased range of motion and their behavior may come in contact with many different people and their waste, as well as other animals, which may play a role in the spread of coronavirus [42].

In contrast, out of the 87 straw coloured fruit bat sera tested, none showed reactivity with the RBD of SARS-CoV-2, but two were SARS-CoV reactive. Though the closest relative of SARS-CoV described in Nigeria was a SARS-CoV-like virus in a leaf-nosed bat (*Hipposideros commersoni*) named Zaria bat coronavirus (ZBCoV) [43], detected in gastrointestinal tissue obtained from bats roosting in bat caves that were frequently entered by humans. In another pre-COVID-19 study, in six of 79 fecal samples of *E. helvum* collected from an urban colony in south-west Nigeria, sequences of the RNA-dependent RNA polymerase (*RdRp*) coding region of a betacoronavirus clustering in lineage D were obtained [44]. While bats are intensively studied for coronaviruses and are believed to be the ancestral host of many alphacoronaviruses and betacoronaviruses [45], higher diversity has been found in insectivorous species than in fruit bats, suggesting that insects and other species may also be the source of infection [46]. Thus, the likelihood of coronavirus diversity in other species may be underestimated and underreported [47,48].

Of particular interest is the simultaneous detection of antibodies to the RBD of both SARS-CoV and SARS-CoV-2 (7.7%) in two rabbits, six feral dogs and one pangolin investigated within this study. Whereas antibodies reacting with only the RBD of SARS-CoV-2 may point to an exposure of the animals to this virus, the unexpectedly large proportion of sera of especially feral dogs suggests the circulation of one or more probably yet unknown sarbecoviruses. Since sarbecoviruses have been shown to cross-react with one another [41,49], could it be that an as-yet unidentified coronavirus (sarbecovirus) is circulating in Nigeria? Sera of humans infected with endemic human coronaviruses like OC43, 229E and NL63 failed to recognize the RBD of SARS-CoV-2 [50]. Even though the ELISA used in our study as well as the majority of ELISAs established as rapid serological diagnostic tools for humans were shown to be specific for SARS-CoV-2, they were often only tested against pre-pandemic sera from humans or animals from countries in the Global North and it would worthwhile to investigate human samples. To what extent these diagnostics serve the same purpose in countries of Sub-Saharan Africa was to our knowledge never investigated, but would be highly interesting, as our results suggest the cross-reactivity with antibodies of other circulating sarbecoviruses in animals. The sampling period bias and the convenience sample approach adopted in this study may constitute a limitation. Notwithstanding, this study has demonstrated the immunological response to the receptor binding domain of both SARS-CoV and SARS-CoV-2 or likely a close relative in some wild and domestic animals in Nigeria, suggesting the exposure to these agents or to related sarbecoviruses. There are also reports about the presence of SARS-CoV and SARS-CoV-2 cross-reactive antibodies in different deer species, also indicating an unknown circulating sarbecovirus [28]. Further research regarding molecular detection and virus isolation from animals and potentially humans is needed to obtain insights into the epidemiology and abundance of sarbecoviruses in Nigeria. Such in-depth data will better explain the risk of cross-infection, viral evolution and genetic changes that may affect pandemic prevention and preparedness.

Funding

This research was conducted within the project “Nigeria Addressing COVID-19 Through a One Health Approach”, funded by the German Federal Ministry of Health on the basis of a decision by the German Bundestag within the “Global Health Protection Programme” (grant number ZMI1-2521GHP904).

CRediT authorship contribution statement

Ebere R. Agusi: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jacob Schön:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. **Valerie Allendorf:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Emmanuel A. Eze:** Supervision, Writing – review & editing. **Olayinka Asala:** Investigation, Methodology, Writing – review & editing. **Ismaila Shittu:** Investigation, Methodology, Writing – review & editing. **Anne Balkema-Buschmann:** Methodology, Resources, Writing – review & editing. **Kerstin Wernike:** Methodology, Resources, Writing – review & editing. **Ishaya Tekki:** Resources, Writing – review & editing. **Mark Ofua:** Investigation, Writing – review & editing. **Omowunmi Adefegha:** Investigation, Resources, Writing – review & editing. **Oluwatoyin Olubade:** Resources, Writing – review & editing. **Oluyemi Ogunmolawa:** Investigation, Writing – review & editing. **Klaas Dietze:** Supervision, Writing – review & editing, Funding acquisition. **Anja Globig:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Donata Hoffmann:** Formal analysis, Methodology, Resources, Validation, Writing – review & editing. **Clement A. Meseko:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interest.

Data availability

Data will be made available on request.

Acknowledgement

The Federal College of Agriculture, Akure, and David Omoniwa and Asabe Dzikwi-Emennaa from the Veterinary Teaching Hospital Jos, we thank for the collection and provision of samples. We acknowledge the Federal Department of Veterinary and Pest Control Services (FDVPCS) for the nationwide collection of rabbit sera used in this study. Andrea

Aebischer and Anna Herrmann, we appreciate for their valuable input and advice with regard to the methodology. We would also like to thank Simone Lüert and Mareen Grawe for their excellent technical assistance.

References

[1] World Health Organization, WHO Coronavirus (COVID-19) Dashboard, Available online: https://covid19.who.int, 2023 (accessed 2023-09-08).

[2] K.O. Elimian, C.L. Ochu, E. Ilori, J. Oladejo, E. Igumbor, L. Steinhardt, J. Wagai, C. Arinze, W. Ukponu, C. Obiekea, O. Aderinola, E. Crawford, A. Olayinka, C. Dan-Nwafor, T. Okwor, Y. Disu, A. Yinka-Ogunleye, N.E. Kanu, O.A. Olawepo, O. Aruna, C.A. Michael, L. Dunkwu, O. Ipadeola, D. Naidoo, C.D. Umeokonkwo, A. Matthias, O. Okunromade, S. Badaru, A. Jinadu, O. Ogunbode, A. Egwuenu, A. Jafiya, M. Dalhat, F. Saleh, G.B. Ebhodaghe, A. Ahumibe, R.U. Yashe, R. Atteh, W.E. Nwachukwu, C. Ezeokafor, D. Olaleye, Z. Habib, I. Abdus-Salam, E. Pembi, D. John, U.J. Okhuarobo, H. Assad, Y. Gandi, B. Muhammad, C. Nwagwogu, I. Nwadiuto, K. Sulaiman, I. Iwuji, A. Okeji, S. Thliza, S. Fagbemi, R. Usman, A. A. Mohammed, O. Adeola-Musa, M. Ishaka, U. Aketemo, K. Kamaldeen, C. E. Obagha, A.O. Akinyode, P. Nguku, N. Mba, C. Ihekweazu, Descriptive epidemiology of coronavirus disease 2019 in Nigeria, Epidemiology & Infection 148 (2020) E208, https://doi.org/10.1017/S095026882000206X.

[3] O.W. Akande, K.O. Elimian, E. Igumbor, L. Dunkwu, C. Kaduru, O.O. Olopha, D. O. Ohanu, L. Nwozor, E. Agogo, O. Aruna, M.S. Balogun, O. Aderinola, A. Ahumibe, C. Arinze, S.O. Badaru, W. Nwachukwu, A.O. Dada, C. Erameh, K. Hamza, T. B. Mohammed, N. Ndodo, C. Obiekea, C. Ofoegbunam, O. Ogunbode, C. Ohonsi, E. A. Tobin, R. Yashe, A. Adekaiyaoja, M.C. Asuzu, R.A. Audu, M.B. Bello, S.O. Bello, Y.Y. Deeni, Y. Disu, G. Joseph, C. Ezeokafor, Z.G. Habib, C. Ibeh, F. Ike, E. Iwara, R. K. Luka-Lawal, G. Namara, T. Okwor, L. Olajide, O.O. Ilesanmi, S. Omonigho, F. Oyiri, K. Takpa, N.U. Ugbogulu, P. Ibekwe, J. Oladejo, E. Ilori, C.L. Ochu, C. Ihekweazu, Epidemiological comparison of the first and second waves of the COVID-19 pandemic in Nigeria, February 2020-April 2021, BMJ Glob Heal. 6 (11) (2021) 1–14, https://doi.org/10.1136/bmjgh-2021-007076.

[4] T.K. Burki, Undetected COVID-19 cases in Africa, Lancet Respir. Med. 9 (12) (2021) e121, https://doi.org/10.1016/S2213-2600(21)00504-X.

[5] S. Frazzini, M. Amadori, L. Turin, F. Riva, SARS CoV-2 infections in animals, two years into the pandemic, Arch. Virol. 167 (12) (2022) 2503–2517, https://doi.org/10.1007/s00705-022-05609-1 (Epub 2022 Oct 7. PMID: 36207554; PMCID: PMC9543933).

[6] O.M. Kolawole, O. Tomori, D. Agbonlahor, E. Ekanem, R. Bakare, N. Abdulsalam, O. Okojie, E. Braide, B. Uzochukwu, A. Rafindadi, S. Bello, S. Shehu, O. Gureje, M. Lecky, O. Onwujekwe, C. Onyedum, A. Ezike, D. Bukbuk, G. Ashir, B. Anyaehie, U. Amazigo, A. Habib, J. Ufere, N. Azodoh, SARS CoV-2 Seroprevalence in selected states of high and low disease burden in Nigeria, JAMA Netw. Open 5 (10) (2022) E2236053, https://doi.org/10.1001/jamanetworkopen.2022.36053.

[7] M. Konda, B. Dodda, V.M. Konala, S. Naramala, S. Adapa, Potential zoonotic origins of SARS-CoV-2 and insights for preventing future pandemics through one health approach, Cureus 12 (6) (2020), https://doi.org/10.7759/cureus.8932.

[8] S. Temmam, K. Vongphayloth, E. Baquero, S. Munier, M. Bonomi, B. Regnault, B. Douangboubpha, Y. Karami, D. Chrétien, D. Sanamxay, V. Xayaphet, P. Paphaphanh, V. Lacoste, S. Somlor, K. Lakeomany, N. Phommavanh, P. Pérot, O. Dehan, F. Amara, F. Donati, T. Bigot, M. Nilges, F.A. Rey, S. van der Werf, P. T. Brey, M. Eloit, Bat coronaviruses related to SARS-CoV-2 and infectious for human cells, Nature 604 (7905) (2022) 330–336, https://doi.org/10.1038/s41586-022-04532-4.

[9] P.C. Woo, S.K. Lau, C.S. Lam, C.C. Lau, A.K. Tsang, J.H. Lau, R. Bai, J.L. Teng, C. C. Tsang, M. Wang, B.J. Zheng, K.H. Chan, K.Y. Yuen, Discovery of seven novel mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavi, J. Virol. 86 (7) (2012) 3995–4008, https://doi.org/10.1128/jvi.06540-11.

[10] Z.W. Ye, S. Yuan, K.S. Yuen, S.Y. Fung, C.P. Chan, D.Y. Jin, Zoonotic origins of human coronaviruses, Int. J. Biol. Sci. 16 (10) (2020) 1686–1697, https://doi.org/10.7150/ijbs.45472.

[11] S.K. Lau, P.C. Woo, C.C. Yip, R.Y. Fan, Y. Huang, M. Wang, R. Guo, C.S. Lam, A. K. Tsang, K.K. Lai, K.H. Chan, X.Y. Che, B.J. Zheng, K.Y. Yuen, Isolation and characterization of a novel betacoronavirus subgroup A coronavirus, rabbit coronavirus HKU14, from domestic rabbits, J. Virol. 86 (10) (2012) 5481–5496, https://doi.org/10.1128/jvi.06927-.

[12] S.L. Priestnall, Canine respiratory coronavirus : A naturally occurring model of COVID-19 ? Vet. Pathol. 57 (4) (2020) 467–471, https://doi.org/10.1177/0300985820926485.

[13] C. Reusken, B.L. Haagmans, M.P. Koopmans, Dromedaris en 'Middle East respiratory syndrome': MERS-coronavirus in het 'schip van de woestijn'. Dromedary camels and Middle East respiratory syndrome: MERS coronavirus in the 'ship of the desert', Ned. Tijdschr. Geneeskd. 158 (2014) A7806.

[14] C. Meseko, I. Shittu, A. Adedeji, The bush meat trade thrives in Nigeria despite anxiety over coronavirus, Trans. R. Soc. Trop. Med. Hyg. 114 (9) (2020) 639–641, https://doi.org/10.1093/trstmh/traa060.

[15] G. Tang, Z. Liu, D. Chen, Human coronaviruses: origin, host and receptor, J. Clin. Virol. 155 (July) (2022) 105246, https://doi.org/10.1016/j.jcv.2022.105246.

[16] B.G. Schindell, M. Allardice, J.A.M. McBride, B. Dennehy, J. Kindrachuk, SARS-CoV-2 and the missing link of intermediate hosts in viral emergence - what we can learn from other betacoronaviruses, Front Virol. 2 (July) (2022) 1–20, https://doi.org/10.3389/fviro.2022.875213.

[17] R.K. Plowright, C.R. Parrish, H. McCallum, P.J. Hudson, A.I. Ko, A.L. Graham, J. O. Lloyd-Smith, Pathways to zoonotic spillover, Nat. Rev. Microbiol. 15 (8) (2017) 502–510, https://doi.org/10.1038/nrmicro.2017.45.

[18] Y. Guan, B.J. Zheng, Y.Q. He, X.L. Liu, Z.X. Zhuang, C.L. Cheung, S.W. Luo, P.H. Li, L.J. Zhang, Y.J. Guan, K.M. Butt, K.L. Wong, K.W. Chan, W. Lim, K.F. Shortridge, K. Y. Yuen, J.S. Peiris, L.L. Poon, Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China, Science (80-) 302 (5643) (2003) 276–278, https://doi.org/10.1126/science.1087139.

[19] C. Drosten, S. Günther, W. Preiser, S. van der Werf, H. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R.A.M. Fouchier, A. Berger, A. Burguière, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J. Manuguerra, S. Müller, V. Rickerts, M. Stürmer, S. Vieth, H. Klenk, A. Osterhaus, H. Schmitz, H. Doerr, Identification of a novel coronavirus in patients with severe acute respiratory syndrome, N. Engl. J. Med. 348 (20) (2003) 1967–1976, https://doi.org/10.1056/nejmoa030747.

[20] B. Hu, H. Guo, P. Zhou, Z.L. Shi, Characteristics of SARS-CoV-2 and COVID-19, Nat. Rev. Microbiol. 19 (3) (2021) 141–154, https://doi.org/10.1038/s41579-020-00459-7.

[21] B. Pickering, O. Lung, F. Maguire, P. Kruczkiewicz, J.D. Kotwa, T. Buchanan, M. Gagnier, J.L. Guthrie, C.M. Jardine, A. Marchand-Austin, A. Massé, H. McClinchey, K. Nirmalarajah, P. Aftanas, J. Blais-Savoie, H. Chee, E. Chien, W. Yim, A. Banete, B.D. Griffin, L. Yip, M. Goolia, M. Suderman, M. Pinette, G. Smith, D. Sullivan, J. Rudar, O. Vernygora, E. Adey, M. Nebroski, G. Goyette, A. Finzi, G. Laroche, A. Ariana, B. Vahkal, M. Côté, A.J. McGeer, L. Nituch, S. Mubareka, J. Bowman, Divergent SARS-CoV-2 variant emerges in white-tailed deer with deer-to-human transmission, Nat. Microbiol. 7 (2022) 2011–2024, https://doi.org/10.1038/s41564-022-01268-9.

[22] K.J. Vandegrift, M. Yon, M. Surendran Nair, A. Gontu, S. Ramasamy, S. Amirthalingam, S. Neerukonda, R.H. Nissly, S.K. Chothe, P. Jakka, L. Labella, N. Levine, S. Rodriguez, C. Chen, V.S. Boorla, T. Stuber, J. Boulanger, N. Kotschwar, S.G. Aucoin, S. Richard, K. Toal, R. Olsen, J. Davis, D. Bold, N. Gaudreault, K.D. Perera, Y. Kim, K. Chang, C. Maranas, J.A. Richt, J. Musser, P. Hudson, V. Kapur, S. Kuchipudi, SARS-CoV-2 omicron (B.1.1.529) infection of wild white-tailed deer in new York City, Viruses 14 (12) (2022), https://doi.org/10.3390/v14122770.

[23] L.C. Caserta, M. Martins, S.L. Butt, N.A. Hollingshead, L.M. Covaleda, S. Ahmed, M. R.R. Everts, K.L. Schuler, D.G. Diel, White-tailed deer (*Odocoileus virginianus*) may serve as a wildlife reservoir for nearly extinct SARS-CoV-2 variants of concern, Proc. Natl. Acad. Sci. U. S. A. 120 (6) (2023) e2215067120, https://doi.org/10.1073/pnas.2215067120.

[24] WOAH, SARS-CoV-2 in Animals. Situation Report 20, World Organisation for Animal Health, 2022, pp. 2–3. May, https://www.woah.org/app/uploads/2023/01/sars-cov-2-situation-report-20.pdf.

[25] O. Calatayud, F. Esperón, S. Cleaveland, R. Biek, J. Keyyu, E. Eblate, E. Neves, T. Lembo, F. Lankester, Carnivore parvovirus ecology in the Serengeti ecosystem: vaccine strains circulating and new host species identified, J. Virol. 93 (13) (2019) 1–18, https://doi.org/10.1128/jvi.02220-18.

[26] L. Fernández-Bastit, N. Roca, M. Romero-Durana, J. Rondon, G. Cantero, O. Garcia, C. Lopez, M. Perez, R. Lopez, J. Carillo, I. Nuria, B. Julia, C. Bonaventura, J. Pujols, J. Vergara, J. Segales, C. Lorca-Oro, Susceptibility of Domestic Goat (*Capra aegagrus* hircus) to Experimental Infection with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) B.1.351/Beta Variant, Viruses 14 (9) (2022), https://doi.org/10.3390/v14092002.

[27] K. Schlottau, M. Rissmann, A. Graaf, J. Schön, J. Sehl, C. Wylezich, D. Höper, T. C. Mettenleiter, A. Balkema-Busc, T. Harder, C. Grund, D. Hoffmann, A. Breithaupt, SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study, Lancet Microbe. 1 (5) (2020) e218–e225, https://doi.org/10.1016/S2666-5247(20)30089-6.

[28] K. Wernike, J. Böttcher, S. Amelung, K. Albrecht, T. Gärtner, K. Donat, M. Beer, Antibodies against SARS-CoV-2 suggestive of single events of spillover to cattle, Germany, Emerg Infect Dis. 28 (9) (2022) 1916–1918, https://doi.org/10.3201/eid2809.220125.

[29] A.Z. Mykytyn, M.M. Lamers, N.M.A. Okba, T.I. Breugem, D. Schipper, P.B. van den Doel, P. van Run, G. van Amerongen, L. de Waal, M.P.G. Koopmans, K.J. Stittelaar, J.M.A. van den Brand, B.L. Haagmans, Susceptibility of rabbits to SARS-CoV-2, Emerg Microbes Infect. 10 (1) (2021) 1–7, https://doi.org/10.1080/22221751.2020.1868951.

[30] M. Fritz, D. de Riols, D. de Fonclare, S. Beurlet Garcia, P. Becquart, S.G. Rosolen, A. Briend-Marchal, E.M. Leroy, First evidence of natural SARS-CoV-2 infection in domestic rabbits, Vet Sci. 9 (2) (2022) 2–5, https://doi.org/10.3390/vetsci9020049.

[31] A.N. Happi, A.O. Ayinla, O.A. Ogunsanya, A.E. Sijuwola, F.M. Saibu, K. Akano, U. E. George, A.E. Sopeju, P.M. Rabinowitz, K.K. Ojo, L.K. Barrett, W.C. Van Voorhis, C.T. Happi, Detection of SARS-CoV-2 in terrestrial animals in southern Nigeria: Potential Cases of Reverse Zoonosis, Viruses 15 (5) (2023), https://doi.org/10.3390/v15051187.

[32] L.F. Wang, Z. Shi, S. Zhang, H. Field, P. Daszak, B.T. Eaton, Review of bats and SARS, Emerg. Infect. Dis. 12 (12) (2006) 1834–1840, https://doi.org/10.3201/eid1212.060401.

[33] K. Wernike, A. Aebischer, A. Michelitsch, D. Hoffmann, C. Freuling, A. Balkema-Buschmann, A. Graaf, T. Muller, N. Osterrieder, M. Rissmann, D. Rubbenstroth, J. Schön, C. Schulz, J. Trimpert, L. Ulrich, A. Volz, T. Mettenleiter, M. Beer, Multi-species ELISA for the detection of antibodies against SARS-CoV-2 in animals, Transbound. Emerg. Dis. 68 (2021) 1779–1785, https://doi.org/10.1111/tbed.13926.

[34] K. Wernike, L. Fischer, M. Holsteg, A. Aebischer, A. Petrov, K. Marquart, U. Schotte, J. Schön, D. Hoffmann, S. Hechinger, A. Neubauer-Juric, J. Blicke, T. C. Mettenleiter, M. Beer, Serological screening in wild ruminants in Germany, 2021/2022: no evidence of SARS-CoV-2, bluetongue virus or pestivirus spread but high seroprevalences against Schmallenberg virus, Transbound. Emerg. Dis. 69 (5) (2022) e3289–e3296, https://doi.org/10.1111/tbed.14600.

[35] E.R. Agusi, V. Allendorf, E.A. Eze, O. Asala, I. Shittu, K. Dietze, F. Busch, A. Globig, C.A. Meseko, SARS-CoV-2 at the human–animal interface: implication for global public health from an African perspective, Viruses 14 (11) (2022) 1–14, https://doi.org/10.3390/v14112473.

[36] T.T.Y. Lam, N. Jia, Y.W. Zhang, M.H. Shum, J.F. Jiang, H.C. Zhu, Y.G. Tong, Y. X. Shi, X.B. Ni, Y.S. Liao, W.J. Li, B.O. Jiang, W. Wei, T.T. Yuan, K. Zheng, X. M. Ciu, J. Li, G.Q. Pei, X. Qiang, W.Y.M. Cheung, L.F. Li, F.F. Sun, S. Qin, J. C. Huang, G.M. Leung, E.C. Holmes, Y.L. Hun, Y. Guan, W.C. Cao, Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins, Nature 583 (7815) (2020) 282–285, https://doi.org/10.1038/s41586-020-2169-0.

[37] G.Z. Han, Pangolins Harbor SARS-CoV-2-related coronaviruses, Trends Microbiol. 28 (7) (2020) 515–517, https://doi.org/10.1016/j.tim.2020.04.001.

[38] K. Xiao, J. Zhai, Y. Feng, N. Zhou, X. Zhang, J.J. Zou, N. Li, Y. Guo, X. Li, X. Shen, Z. Zhang, F. Shu, W. Huang, Y. Li, Z. Zhang, R.A. Chen, Y.J. Wu, S.M. Peng, M. Huang, W.J. Xie, Q.H. Cai, F.H. Hou, W. Chen, L. Xiao, Y. Shen, Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins, Nature 583 (7815) (2020) 286–289, https://doi.org/10.1038/s41586-020-2313-x.

[39] A. Michelitsch, D. Hoffmann, K. Wernike, M. Beer, Occurrence of antibodies against SARS-CoV-2 in the domestic cat population of Germany, Vaccines (Basel). 8 (4) (2020) 772, https://doi.org/10.3390/vaccines8040772. PMID: 33348613; PMCID: PMC7766304.

[40] L. Ulrich, K. Wernike, D. Hoffmann, T.C. Mettenleiter, M. Beer, Experimental infection of cattle with SARS-CoV-2, Emerg. Infect. Dis. 26 (12) (2020) 2979–2981, https://doi.org/10.3201/eid2612.203799.

[41] T.S. Evans, C.W. Tan, O. Aung, S. Phyu, H. Lin, L.L. Coffey, A.T. Toe, P. Aung, T. H. Aung, N.T. Aung, C.M. Weiss, K.Z. Thant, Z.T. Htun, S. Murray, L. Wang, C. K. Johnson, H.M. Thu, Exposure to diverse sarbecoviruses indicates frequent zoonotic spillover in human communities interacting with wildlife, Int. J. Infect. Dis. 131 (2023) 57–64, https://doi.org/10.1016/j.ijid.2023.02.015.

[42] F. Carraturo, C. Del Giudice, M. Morelli, V. Cerullo, G. Libralato, E. Galdiero, M. Guida, Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces, Environ. Pollut. 265 (2020) 115010, https://doi.org/10.1016/j.envpol.2020.115010.

[43] P.L. Quan, C. Firth, C. Street, J.A. Henriquez, A. Petrosov, A. Tashmukhamedova, S.K. Hutchison, M. Eg-holm, M.O. Osinubi, M. Niezgoda, A.B. Ogunkoya, T. Briese, C.E. Rupprecht, W.I. Lipkin, Identification of a severe acute respiratory syndrome coronavirus-like virus in a leaf-nosed bat in Nigeria, MBio 1 (4) (2010) 1–9, https://doi.org/10.1128/mBio.00208-10.

[44] S. Leopardi, D. Oluwayelu, C. Meseko, S. Marciano, L. Tassoni, S. Bakarey, I. Monne, G. Cattoli, P. De Benedictis, The close genetic relationship of lineage D Betacoronavirus from Nigerian and Kenyan straw-colored fruit bats (Eidolon helvum) is consistent with the existence of a single epidemiological unit across sub-Saharan Africa, Virus Genes 52 (4) (2016) 573–577, https://doi.org/10.1007/s11262-016-1331.

[45] B.A. Han, A.M. Kramer, M. Drake, Global patterns of zoonotic disease in mammals, Trends Parasitol. 32 (2016) 565–577.

[46] A.J. Bennett, T. Bushmaker, K. Cameron, A. Ondzie, F.R. Niama, H.J. Parra, J. V. Mombouli, S.H. Olson, V.J. Munster, T.L. Goldberg, Diverse RNA viruses of arthropod origin in the blood of fruit bats suggest a link between bat and arthropod viromes, Virology 528 (2019) 64–72, https://doi.org/10.1016/j.virol.2018.12.009.

[47] D. Vijaykrishna, G.J.D. Smith, J.X. Zhang, J.S.M. Peiris, H. Chen, Y. Guan, Evolutionary insights into the ecology of coronaviruses, J. Virol. 81 (8) (2007) 4012–4020, https://doi.org/10.1128/jvi.02605-06.

[48] Z. Ma, P. Li, Y. Ji, A. Ikram, Q. Pan, Cross-reactivity towards SARS-CoV-2: the potential role of low-pathogenic human coronaviruses, Lancet Microbe. 1 (4) (2020) e151, https://doi.org/10.1016/S2666-5247(20)30098-7.

[49] T.J. Hancock, P. Hickman, N. Kazerooni, K. Melissa, S.A. Kania, M. Dennis, N. Szafranski, R. Gerhold, C. Su, T. Masi, S. Smith, T.E. Sparer, Possible cross-reactivity of feline and white-tailed deear antibodies against the SARS-CoV-2 recptor binding domain, J. Virol. 96 (8) (2022), https://doi.org/10.1128/jvi.00250-22 e00250–22.

[50] M. Edouard, R. Hannah, R. Lucas, A. Cameron, G. Charlie, H. Joe, M. Boobie, D. Saloni, B. Diana, O.O. Esteban, R. Max, et al., Coronavirus Pandemic (COVID-19), Published online at, OurWorldInData.org, 2020. Retrieved from: https://ourworldindata.org/coronavirus;. accessed on 2023-03-01.