First detection of SARS CoV-2 like antibodies in domestic and wild animals in Kenya

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Abstract

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), etiological agent of the novel coronavirus disease 2019 (COVID-19), has spread since December 2019, resulting in massive health and economic crisis worldwide. While efforts to stop the pandemic are crucial, collecting epidemiological data to help manage current and future pandemics will be important. In addition to humans, serological and molecular based studies have demonstrated SARS CoV-2 exposure in several wild, domestic and farmed animals. For examples Shriner and the team showed serologically an exposure of 40% to the white deer living in close proximity to urban centers. Additional reports have also emerged of susceptibility of animal's species like cats, ferrets, raccoon dogs, cynomolgus macaques, rhesus macaques, white-tailed deer, rabbits, Egyptian fruit bats, and Syrian hamsters to SARS-CoV-2 infection.. It's worth emphasizing that these reports are based on experimental data mostly derived from Europe, USA, South America and parts of Asia. In limited instances natural infections of SARS-CoV-2 have been reported in pet dogs, cats, tigers, lions, snow leopards, pumas, gorillas at zoos and farmed mink and ferrets. The presence of the virus in animal species and an understanding of whether these are natural or recent human to animal transmissions is important. It's possible that such transmission could passage the virus or subject the virus to a different immunological pressure thereby helping with the development of viral variants in addition to being a host for future reservoirs of the virus. In Kenya SARS-CoV-2 was first detected on March 12 th 2020 from imported human cases of persons who had travelled from the United States. This was followed by detection of imported cases majorly from China, Sweden and United Kingdom. Later infections were confirmed in Nairobi and Mombasa suggesting further cases of disease importations through the major ports of entry. However, no comparable data on animal exposure have hitherto been generated in Kenya. To address this key concern, we focused on three objectives; 1) development of a robust antibody ELISA based on crude SARS-CoV-2 lysate. 2) SARS-CoV-2 serology of domestic animals in Kenya. 3) Corroboration of the crude lysate based seroprevalence data and a commercial ELISA kit based on the Spike receptor binding domain (RBD) antigen. Our sample set included camel sera (both pre- & post outbreak sera), as well as sera from cats and dogs collected at the peak of the pandemic. Our results using the ELISA based on crude SARS-CoV-2 lysate indicated SARS-CoV-2 antibodies in camels (71%, N=145), cats 11% (N=16) and dogs (81%, N=36) with varying titer levels.

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KEYWORDS: Animals, Detection, SARS CoV-2,

Abstract/Summary

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In addition to humans, serological and molecular based studies have demonstrated SARS CoV-2 exposure in several wild, domestic and farmed animals. For examples Shriner and the team showed serologically an exposure of 40% to the white deer living in close proximity to urban centers. Additional reports have also emerged of susceptibility of animal's species like cats, ferrets, raccoon dogs, cynomolgus macaques, rhesus macaques, white-tailed deer, rabbits, Egyptian fruit bats, and Syrian hamsters to SARS-CoV-2 infection.. It's worth emphasizing that these reports are based on experimental data mostly derived from Europe, USA, South America and parts of Asia. In limited instances natural infections of SARS-CoV-2 have been reported in pet dogs, cats, tigers, lions, snow leopards, pumas, gorillas at zoos and farmed mink and ferrets. The presence of the virus in animal species and an understanding of whether these are natural or recent human to animal transmissions is important. It's possible that such transmission could passage the virus or subject the virus to a different immunological pressure thereby helping with the development of viral variants in addition to being a host for future reservoirs of the virus.

In Kenya SARS-CoV-2 was first detected on March 12^{th}2020 from imported human cases of persons who had travelled from the United States. This was followed by detection of imported cases majorly from China, Sweden and United Kingdom. Later infections were confirmed in Nairobi and Mombasa suggesting further cases of disease importations through the major ports of entry. However, no comparable data on animal exposure have hitherto been generated in Kenya. To address this key concern, we focused on three objectives; 1) development of a robust antibody ELISA based on crude SARS-CoV-2 lysate. 2) SARS-CoV-2 serology of domestic animals in Kenya. 3) Corroboration of the crude lysate based seroprevalence data and a commercial ELISA kit based on the Spike receptor binding domain (RBD) antigen.

Our sample set included camel sera (both pre- & post outbreak sera), as well as sera from cats and dogs collected at the peak of the pandemic. Our results using the ELISA based on crude SARS-CoV-2 lysate indicated SARS-CoV-2 antibodies in camels (71%, N=145), cats 11% (N=16) and dogs (81%, N=36) with varying titer levels. These findings were comparable to those obtained using the commercial ELISA kit based on the spike RBD antigens.

In summary, the data warrants two key conclusions: (i) we have demonstrated that the crude lysate ELISA allows for SARS-CoV-2 antibody detection, and given its potential to offer robust detection could be applied for initial mass screening (ii) although the current study cannot disentangle the relative contributions of antigenic cross-reactivity, pre-pandemic exposure to SARS-CoV-2 or human-animal transmission, it nonetheless demonstrates for the first time the prevalence of SARS-CoV-2 like antibodies in domestic and wild animals

in Kenya. Our findings set the scene for further research into the prevalence of SARS-CoV-2 in domestic and wild animals to understand their potential epidemiological implications.

Introduction and Background

Initial investigations into the origin of the SARS-CoV-2 virus pointed to the animals sold at the Wuhan seafood market (Huang et al, 2020). In particular, the virus is believed to have originated from either bats, pangolins or a recombination between the virus found in bat and pangolins sold in the market (Zhou et al., 2020; Liu et al 2020). Though it is now highly doubtful whether pangolins are the original source, it is important to note that genetic comparisons have shown that the genome of the coronaviruses found in pangolins and thatof the SARS CoV-2 exhibit a genome sequence similarity of 99% (Cyranoski D 2020). By contrast, it is now highly suspected that SARS CoV-2 has its ecological reservoirs in bats. This is based on similarities in the biological features and the sequence identity between bat-nCoV and SARS-CoV-2 (Chan et al 2013; Nguyen et al, 2020: Ren et al., 2020).

SARS-Cov-2 virus is highly contagious and is transmitted between humans via respiratory droplets or close contact (Li et al., 2020). One important aspect of SARS-CoV-2 epidemiology that has received limited attention, particularly in Africa, is the transmission of the virus between humans and animals. This is important because SARS-CoV-2 is known to infect animals (Prince et al., 2021) and human to animal transmissions have been demonstrated. In addition, SARS-CoV-2 infections have been documented in cats and dogs in the UK (Smith et al., 2021), Belgium and the USA [Garigliany et al., 2020; Newman et al., 2020; Sit et al., 2020). The detection of SARS-CoV-2 infections in animals in the studies outlined above were based on RT-PCR or virus isolation [Barrs et al., 2020; Decaro et al., 2020; Hamers et al., 2021; Ruis-Arrondo et al., 2020 & Sailleau et al., 2020).

The use of sero-detection of the anti-SARS CoV-2 antibodies has also been deployed in a number of countries with the aim of providing evidence of exposure of cats and dogs to SARS CoV-2. Independent studies in Italy have also shown evidence of exposure to SARS-CoV-2 in cats and dogs from households (Patterson et al (2020)), while in France, Frits et al (2020) showed a high prevalence of SARS-CoV-2 antibodies in pets from COVID-19 positive households (Fritzs et al., 2020). In Germany, Croatia and China the presence of anti-SARS-CoV-2 antibodies have also been shown in domestic cats and other pet populations. Collectively, these studies have demonstrated that serosurveys can provide reliable evidence of animal infections (Michelitsch et al., 2020; Stevanovic et al., 2020; Zhang et al., 2020).

A close examination of these studies reveal that most of the animals in which SARS-CoV-2 were detected were either companion or domesticated animals. This evidence has been interpreted to suggest that such domestic and companion animals could reliably predict human to animal transmissions especially post the pandemic outbreak. Additional data in support of this conclusion has also recently become available. For example, cases of experimentally induced infections have recently been documented in cats (Halfmann et al., 2020; Bosco-Lauth et al., 2020) and dogs have also been shown to be susceptible to SARS-CoV-2.

As for the epidemiological significance of these infections, cats have been shown to shed off the virus and able to transmit the virus to other cats [Shi et al., 2020; Bosco Lauth et al 2020) and cases of possible natural infections and transmissions have been detected in farmed minks that have been in contact with cats and dogs (Oude Munnink et al., 2020; van Aart et al., 2020). This data therefore suggest the possibility of human to animal transmission and vice versa. While in the studies especially in cats and dogs there were no clinical symptoms, the studies in farmed Minks whose transmissions were from infected human contact respiratory and gastrointestinal signs, and also sudden death after infection (Molenaar et al., 2020). However there were cases of nondomestic felids at the Bronx Zoo and pet cats in New York and Europe, which showed respiratory disease and/or conjunctivitis.

Although this data by itself already suggests a complex persistent zoonosis, it can be further complicated by the presence of wild animals that interface with domestic animals and humans as is the case in most parts of Africa. In other parts of the world with a domesticated animal- wild animal interface, new evidence of infection of some of these animals has recently been documented. These include: Croatia where fifteen

out of 533 (2.8%) positive ELISA results has been detected in wild boars (3.9%), red foxes (2.9%) and jackals (4.6%) (Jemeršić, et al., 2021). Given these findings, it's only a matter of time before the virus start spreading in the wild. These facts together with the evidences that a certain wild animals like the lion are susceptible to SARS CoV-2 (Koeppel et al., 2022) and develop pneumonia, could mean maintenance of the virus in the wild, with potentially negative consequences on wild populations and biodiversity. These therefore underscore the importance of extending surveillance to animals in the wild in future..

Herein, in pursuit of the broad objective of SARS-CoV-2 surveillance in domestic animals in Africa, we have developed a robust ELISA diagnostic assay based on a crude viral lysate and used it to detect antibodies to SARS-CoV-2 in cats, dogs and camels in Kenya. Our specific goal was to develop a robust ELISA diagnostics assay for SARS-CoV-2 in order to understand the seroprevalence of SARS-CoV-2 in domestic animals.

Methodology

Ethical approval, Consent and Research License

This research was approval by the Institutional Research and Ethics Committee of the Institute of Primate Research, Karen Kenya reference number (ISERC/01/2021).

Samples

Two sets of samples from three geographically distinct sites in Isiolo, Nairobi and Machakos that are 500 km apart were utilized in the present study. These included: (i) randomly collected camel samples from Isiolo and from Athi – River, Machakos County, and (ii) randomly collected animal samples from the veterinary clinics around Nairobi (cats, & dogs) and one veterinary clinic in Syokimau-Machakos County.

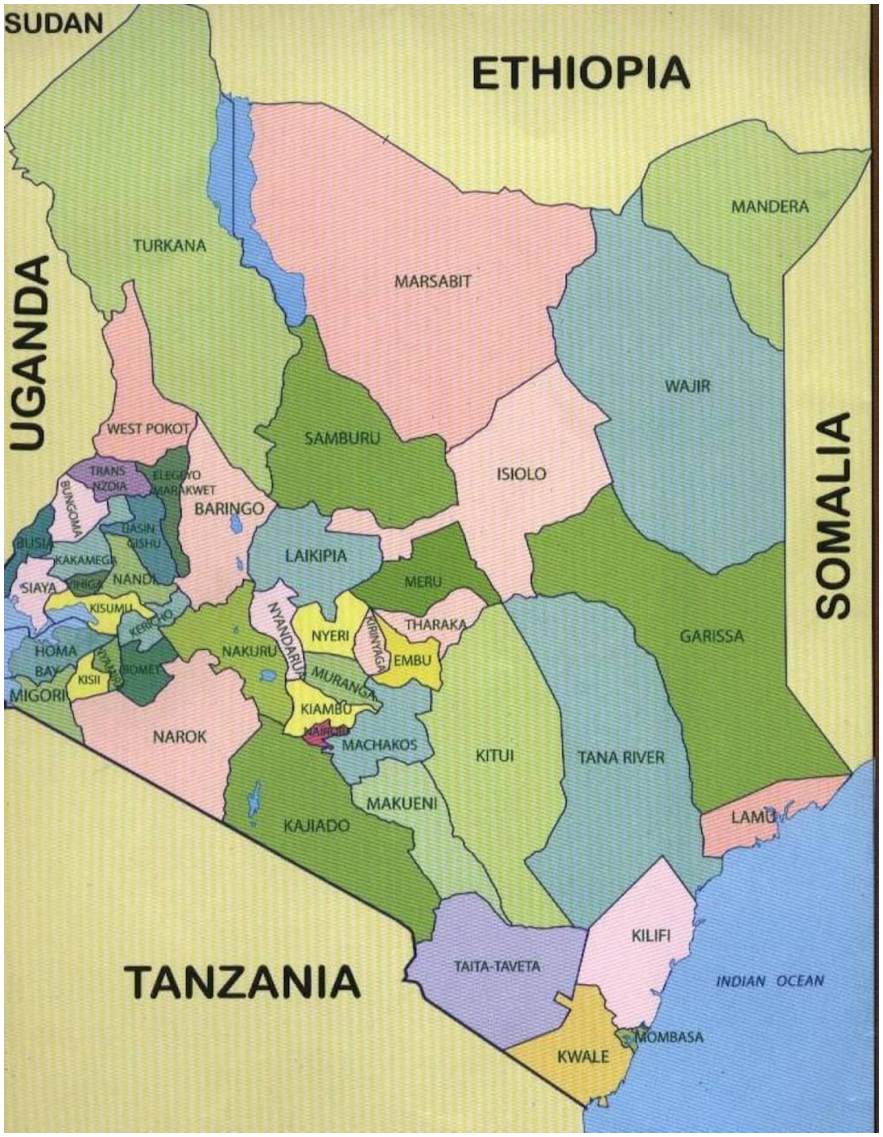

Figure 1 Sampling sites in Isiolo (camels), Nairobi (cats and dogs) & Machakos (camels) are indicated by the red dots.

Camels were sampled randomly as follows: 144 fecal specimens, 144 nasal swabs and 144 blood samples collected into serum vacutainers. The camels were identified through their owners whose consent was initially sought; the animals were restrained using trained animal handlers and samples taken. The blood samples were processed for serum the same day, labeled, aliquoted into two portions and stored at -20^{0}C. The duplicate samples were then transported to the Machakos University, Department of Biological sciences laboratory and the other part transported to the institute of Primate research, Kenya-snake bite research center, Karen Kenya.

Samples from cats (16 samples) and dogs (36 samples) were collected at the veterinary clinics on different dates. The consent was sought from their owners before drawing of the blood. 6 ml of blood was drawn from each animal onto a serum vacutainer and stored at 4^{0}C until they were collected and transported to the Institute of primate Research. The blood sera were transported in vacutainer tubes to the Institute of Primate Research where the sera separation was done. The sera were aliquoted and stored at -20^{0}C till use.

In order to ascertain that the samples were suitable for the ELISA assay, we tested a selected number of samples with a commercial kit based on purified SARS-CoV-2 receptor domain binding site specific ELISA (PISHTAZ Teb) based ELISA. The kit has been optimized for human samples, in our test we modified the kit to be able to detect camel, cats and dogs samples by replacing the kit's anti-Human IgG-HRP with either anti-Llama IgG-HRP (for camels and dogs or Invitrogen anti-human IgG-HRP (for cats samples). To ensure that our modified kits was working well we used the kits confirmed human positive controls to ascertain that the kit was also working on camel, cat and dog samples. The rest of the procedure and reagents used was as described in the kits manual. We eventually compared the results from the commercial kit to those obtained using our crude lysate ELISA designed as described below.

Development of a robust in-house ELISA for SARS-CoV-2 antibodies in cats, dogs and camels

The SARS CoV-2 virus was isolated from a COVID-19 patient via nasopharyngeal swab. The swab was then inoculated onto vero cells cultured in DMEM media with 15%FBS & Pen/Strep and allowed to infect the and grow in the cells. The presence of SARS-CoV-2 was confirmed with RT-PCR reactions. The virus infected vero cells were then cultured in DMEM media with 15%FBS & Pen/Strep. The cells were monitored and harvested at 80% CPE. The harvested infected cells were broken down by freeze thawing and under 5% SDS detergent to inactivate them and viral particles was separated on sucrose gradient sedimentation solution and resuspended in 100µl of DMEM media. The harvested virus was further inactivated by three cycles of freeze thawing and then at 55 degree Celsius for 1 hour and then stored at -20^{0}C. The crude viral lysate was re- suspended in 400 µl ml of PBS and taken as the neat lysate. The viral antigen titration curve was done by diluting the viral lysate in the range of 5 x 10^{-1} to 5 x 10^{-10} with PBS. The 50 µl of titrated viral lysate was used to coat the plates overnight at 4^{0}C for the titration and determination of the appropriate dilution assay. Finally a dilution of of 5 x 10^{-7} was adopted and used for subsequent plate coating and further assays. Wells without coating (No coats) were also included to compensate for nonspecific interactions of the serum with other factors other than the antigen. Before addition of the sera the excess antigen flicked out and the plate tapped upside down on the soft tissue to remove the excess of the antigen.

In developing the assay further ELISA protocol developed byAmanat et al., 2020 was adopted with a few modifications as described below. First, in order to identify the positive and negative camel control sera a aselected pre-COVID-19 and post COVID-19 camel samples were screened using PISHTAZ Teb commercial kit. The camel sera was selected, titrated in the range of 1/100, 1/500, 1/1000, 1/5000, 1/10 000 and 1/50 000, and finally 1/1000 was adopted. Cat & dogs sera was titrated in the 1/5, 1/10, 1/50, 1/100, 1/500 and 1/1000 and finally 1/50 was adopted. Wells with antigen coating but no sera (No Sera) were included to check on nonspecific cross-reactivities. Positive controls sera from camel (C0874 & C1191), cats (CAT 2 & 4) and dogs (DOG 1 & 2) were identified and used to check if the serum is binding on anything other than the antigens. Serum dilutions were done in PBS /1% skimmed milk/0.1% SDS and incubation was done for 1hr at 37^{0}C. For the safety of technologist the animal samples/sera were inactivated at 55^{0}C and stored at -20^{0}C till used for the ELISA assay.

The secondary step antibody used was either a reacting anti-Human IgG HRP for Cats sera or anti-Llama IgG –HRP for camel and dogs samples, the conjugate incubation was done for 1hr at 37^{0}C. Washing of the wells was done five times with 200µl per well of PBS/0.1% SDS. 100µl of substrate was used to develop the assay and incubated for 30 minutes at 37^{0}C after which the reaction was stopped with sulphuric acid and the plate read at 450nm.

To test if our crude lysate ELISA assay could be used for surveillance, we collected a pool of randomly sampled animal sera from three different animal species (Camel, n=144, cats, n= 16 and dogs, n=36) and three different locations (Fig.1). The samples were screened with our crude lysate ELISA assay for the presence of cross reacting antibodies and compared with a commercial kits based on the purified recombinant spike RBD antigen. The camel sera samples were used at 1/1000, while the cats and dogs sera samples were used at 1/50.

Results

Development of a robust ELISA for SARS-CoV-2 antibodies in cats, dogs and camels.

Titrating the crude lysate

The crude lysate used to coat the ELISA plates was titrated in the range of $5x10^{-2}$ and $5x10^{-10}$ of the viral lysate of 80% CPE viral harvest. Wells without coating (No coats) were included and a positive serum (C0874) was used to check if the serum is binding on anything other than the antigen. First pre-COVID-19 and post COVID-19 camel sera were screened using a commercial kit to identify reacting samples. The positively reacting camel sera was selected and used at a dilution of 1/1000, cat & dogs sera was used at 1/50. Wells with antigen coating but no sera (No Sera) were included to check for nonspecific cross-reactions.

The crude lysate antigen reactivity with camel, cats and dogs sera was optimal at a dilution range of $5x10^{-7}$ and $5x10^{-8}$ of the lysate of 80% CPE viral harvest. Beyond $5x10^{-8}$ dilution, the lysate couldn't pick or distinguish between a SARS CoV-2 negative from a positive sera (Fig.2).

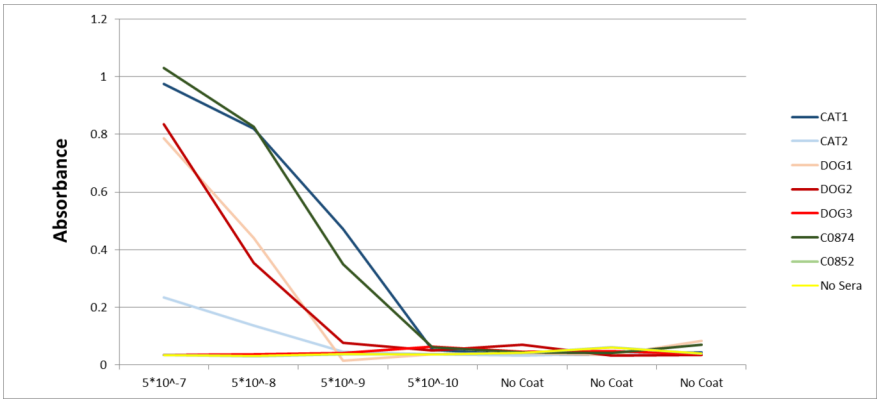

Figure 2: Titration of Crude SARS COV-2 Lysate antigen. Shown on the y-axis is the ELISA OD scores, while the X-axis shows the crude lysate titrations. The serum for the o titration gradient was used at 1/10 for Cat, 1/10 for Dogs sera and 1/1000 for the Camel sera. The camel and cats titres were higher than those of the dogs.

b) Screening of positive and negative controls against best performing Secondary HRP conjugated antibodies To check if anti-Llama IgG conjugated with HRP could also cross react with dogs and cats sera and also if anti-human IgG conjugated with –HRP could cross react with camel, cats and dogs sera, we coated the ELISA plates with the crude lysate. The wells were then probed with sera from camel, cats and dogs. The sera were then detected with both Anti-Llama IgG-HRP and anti-Human IgG-HRP. The rest of the conditions were as described in Amanat et al., 2020. Anti-Llama IgG Conjugated – HRP detects /cross reacts with dogs antibodies whereas Anti-human IgG Conjugated – HRP with dogs antibodies do not. Using crude lysate ELISA, it's possible to discriminate positive & negatives on dog samples: D1, D2, D3 and D5 dogs' samples are positive (figure 3). Crude lysate ELISA can pick positive & negatives camel sera: Camel D8-874 react positively with both anti-human IgG-HRP and anti-Llama IgG- HRP-conjugated antibody while the negative camel sera (D0) 0852 remains negative.

Figure 3: Bar graph showing positive cat, dogs and camel Sera detected either with Secondary second step anti-human IgG HRP or anti-Llama IgG HRP conjugated antibodies. The selected anti-human IgG conjugated with HRP can discriminate between positive sera (CAT1 & 2) and negative cat sera (CAT3, 4 &5) samples, while anti-Llama IgG-conjugated HRP cannot discriminate between positives and negative cats sera. cat sera dogs sera (D1, 2, 3 & 5). Anti-human IgG conjugated HRP does not detect Camel sera therefore doesn't distinguish between positive and negatives (C0852-Antihuman IgG HRP, C874 anti-Human) while anti-Llama IgG HRP does detect camel sera and can discriminate between a SARS CoV-2 positive sera (C874 –Anti-Llama IgG-HRP from the negative sera(C0852-Anti-Llama IgG HRP)

c) Titration of serum samples (camel, dogs and cats) against Crude SARS-CoV-2 lysate antigen

To determine the right sera dilution to use for assay and to help avoid non-specific antibody-antigen interactions, we titrated the three serum samples (camel, cats and dogs sera). Camel sera was titrated in the range of 1/100, 1/500, 1/1000, 1/5000, 1/10 000 and 1/50 000, cats and dogs sera was titrated in the 1/5, 1/10, 1/50, 1/100, 1/500 and 1/1000. The diluted sera was then used to perform the ELISA assay and detection of the antibodies was achieved by either Anti-Llama IgG –HRP for Camels and dogs samples while the cats antibodies were detected using anti-Human IgG–HRP.

The camel sera C1191 and C0874 had high titers such that even at a higher dilution series they were still positive for SARS-CoV-2 and it titrated out confirming the positive detection of SARS COV-2, while C0852 remained constantly low confirming its negativity (figure 4a).. The positive SARS CoV-2 cats samples (CAT2, & CAT4) also titrated out while the negative cat samples remained constant low suggesting that the test is capable of discriminating between cats SARS CoV-2 positive and negative sera (figure 4b). The same dynamics was seen with the dogs samples where DOG 1 & 2 shown positive titration trend in detection of SARS COV-2 while DOG4 sera remained negative suggesting the capability of the system to positively detect dogs samples (figure 4c) .

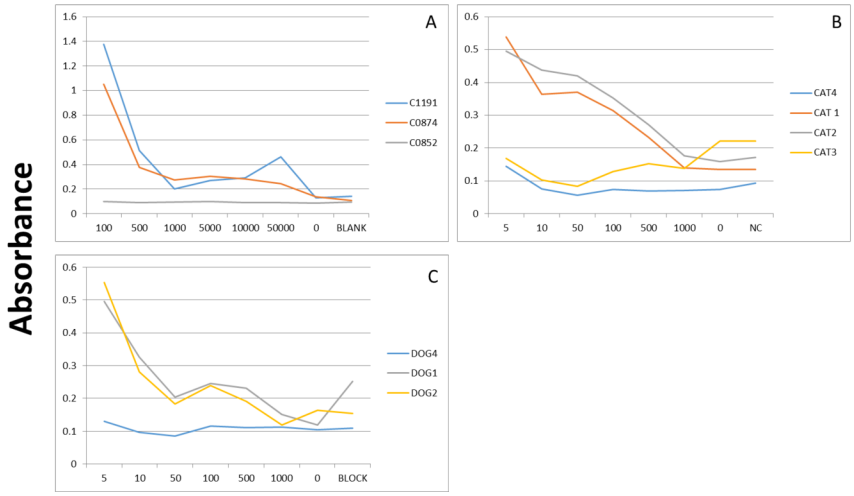

Figure 4: Titrating Serum against Crude SARS COV-2 Lysate antigen. Samples in panel A: C1191, C0874 and C0852 are Camel samples titrated from 1/100 to 1/50,000. Samples in panel B: CAT1, CAT2, CAT3 and CAT4 are CAT samples titrated from 1/5 to 1/1000 and Samples in panel C: DOG1, Dog2 and Dog4 are selected dogs sera titrated from 1/5 to 1/1000.

2) Assessment of the SARCoV-2 sero-status of domestic and wild animals in Kenya.

Using the validated crude lysate ELISA, we found that 71% of camel samples, 11% cat samples and 83% of dog samples were positive.

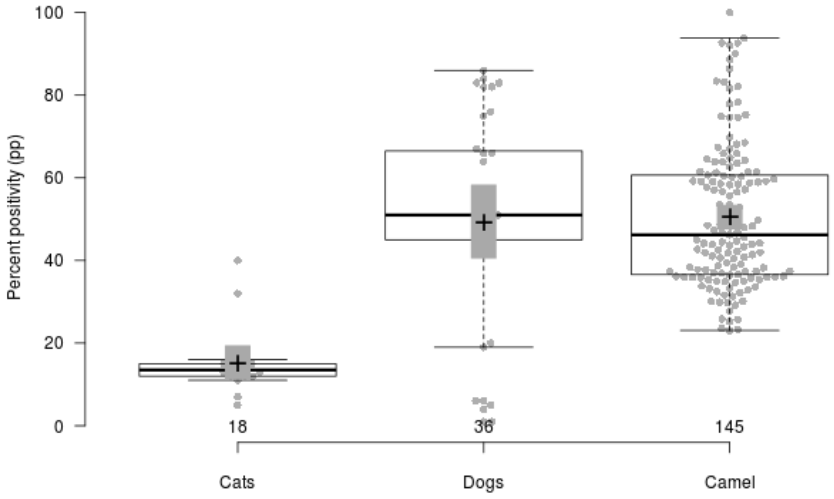

Figure 5: Distribution of SARS COV-2 antibodies among cats, dogs and camels in Kenya as assessed using the crude lysate ELISA developed herein. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 95% confidence intervals of the means; data points are plotted as open circles. Results are given as a percentage of the positive control serum (percent positivity, PP). The data interpretation was based on the stringent cut-offs (20% PP) for negative or positive ELISA results.

SAR CoV-2 positive Camel samples are strongly reacting to SARS CoV-2

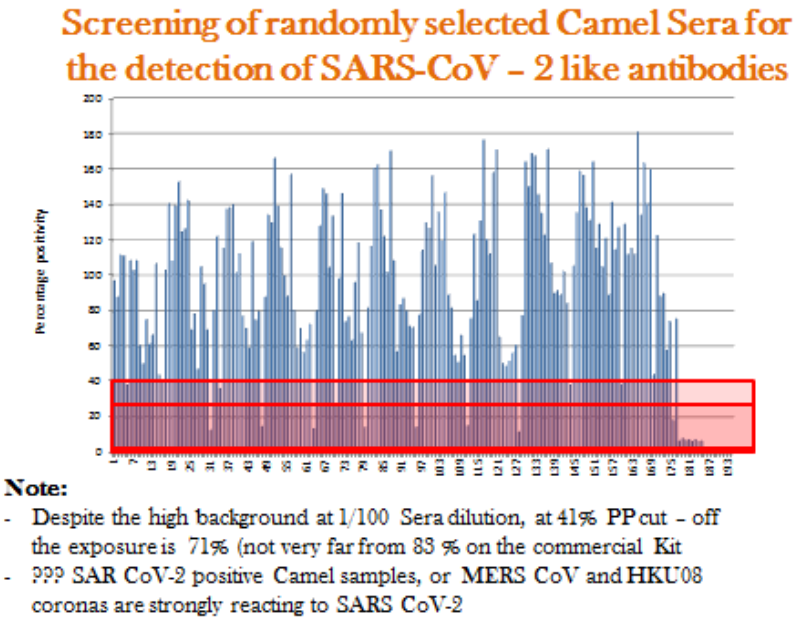

Figure 6: Screening of randomly selected Camel Sera for the detection of SARS-CoV – 2 like antibodies. Despite the high background at 1/100 Sera dilution, at 41% PP cut – off the exposure is 71% (comparable

to the 83 % on the commercial Kit.

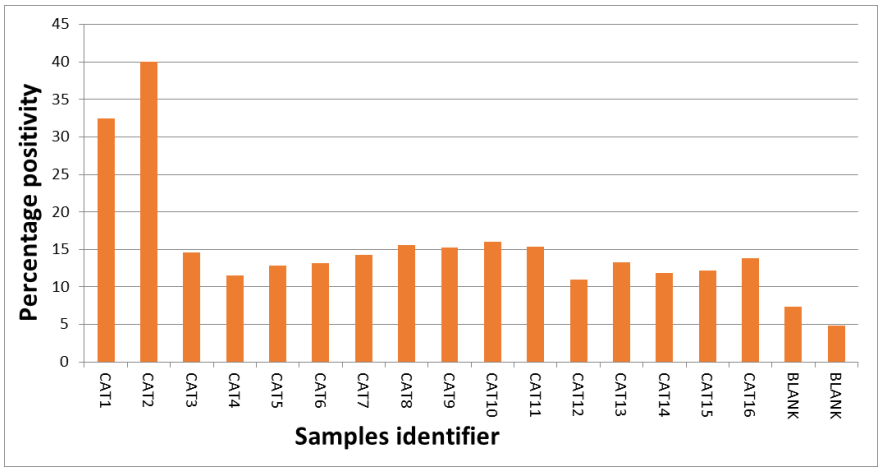

Figure 7: Screening of randomly selected Cats Sera for the Detection of SARS-CoV – 2 like antibodies. 1/50 sera dilution

15% PP cutoff: 89 % exposure. 20% PP cut off: 11 % exposure

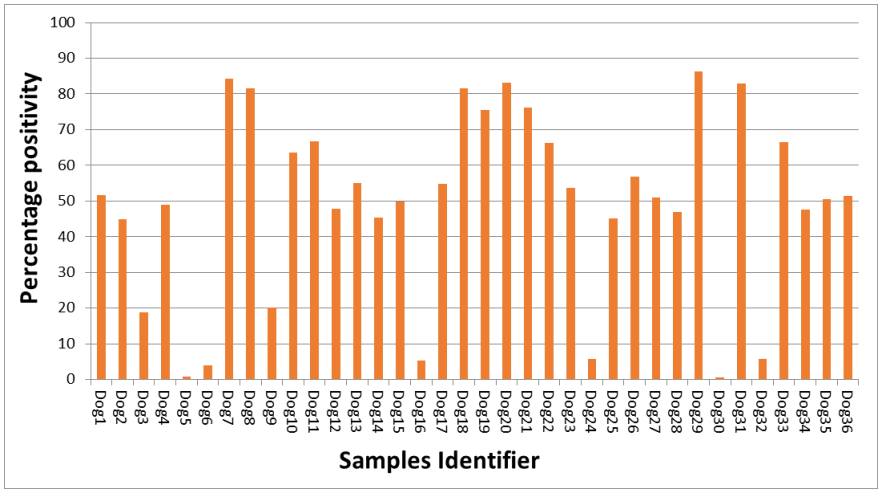

Figure 8: Screening of randomly selected Dogs Sera for the detection of SARS-CoV – 2 like antibodies. Sera dilution 1/50, (antigen at 5 x 10ˆ-6). 15% PP cutoff: 83% exposure; 20%, cutoff point: 81% exposure to COVID-like antibodies

3) Corroboration of results using a commercial kit ELISA based on the spike RBD antigen s

This finding of the presence of SARS COV-2 like antibodies in Camels in Kenya based on our crude lysate ELISA, was corroborated by the results obtained using the commercial kit based on the Spike specific RBD antigen (Figure 9 & 10). One potential explanation for the generally higher OD scores in crude lysate ELISA relative to the commercial kit is that the crude lysate ELISA could include up to 4 (NP, S1 & S2 and M) major antigens as opposed to the single antigen (Spike RBD) in the commercial kit.

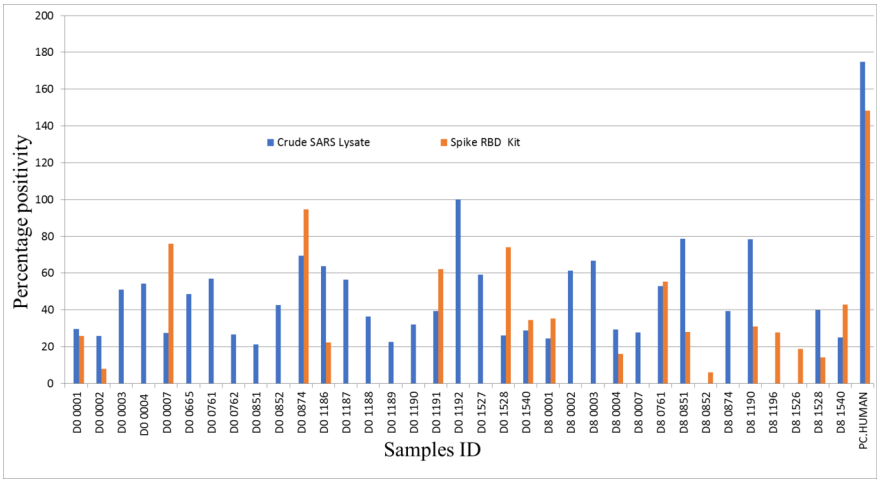

Figure 9: ELISA detection of SARS-CoV – 2 like antibodies in animal samples using modified human Diagnostics Commercial Kit. The assay is a modification of the ELISA test described by Amanat et al, (2020) by replacing the anti-human IgG conjugate antibody with (anti-Llama IgG). D0 – samples collected before COVID-19 outbreak announcement in Kenya while D8 samples were collected 8 days after the COVID-19 outbreak announcement in the country. The 10% PP cut off: 83 % exposed / cross reacting, 20% PP cut off: 57% exposed

The mean OD absorbance values generated in Figure 9 above were used to calculate the percent positivity scores relative to the absorbance values for the positive control serum. On the basis of the stringent pp threshold of 20%, we found evidence of SARS COV-2 like antibodies in all the three species studied.

The plot in figure 10 enables clearer visual comparisons to be drawn between the results of the analysis of SARS COV-2 like antibody titers based on the crude lysate ELISA and the commercial kit based on the Spike specific RBD antigen. In the figure, the individual camel antibody titers are shown as small white lines in a one-dimensional scatter plot. The estimated density of the distributions of the percent positivity values is visible as a polygon and the average is also shown as a black line. It is clear that the results based on the crude lysate are corroborated by commercial kit ELISA and as already mentioned, the higher pp scores in the crude lysate ELISA relative to the commercial kit could be due to the additional antigens present in the crude lysate.

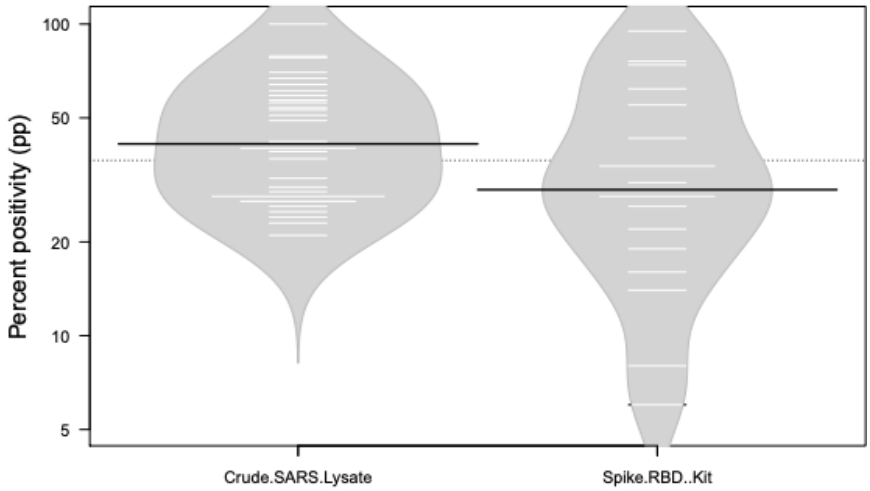

Figure 10: A comparison of the distribution of SARS CoV-2 like antibodies in camel sera determined both by a commercial kit (n=18) and by the crude lysate ELISA (n=32), and expressed as a percentage of the positive control serum (percent positivity). Black lines show the means; white lines represent individual data points; polygons represent the density of the data. The plot was generated using the R package beanplot (Kampastra 2009).

Discussions

Our aim was twofold: (1) Develop and validate a SARS-CoV-2 crude lysate ELISA that could be routinely applied for surveying the presence of SARS-CoV-2 like antibodies in wild and domestic animals (2) utilize the validated SARS-CoV-2 crude lysate ELISA to analyses antibody responses in camels, cats and dogs from geographically widely separated areas in Kenya.

The SARS-CoV-2 crude lysate ELISA that we developed represent a major advance, particularly with respect to the ability to conduct broad analyses of SARS-CoV-2 like antibody responses in livestock and wildlife. To validate our SARS-CoV-2 crude lysate ELISA, we modified a commercial (PISHTAZ TEB) kit coated with Spike specific -RBD antigen and used an ELISA protocol developed by Amanat et al., (2020 to detect SARS-CoV-2 antibodies in camels, cats and dogs (Fig. 1, 2 & 3). By modification of the commercial kit by replacing anti-human IgG-HRP with anti-Llama IgG HRP for camel samples and dogs samples (with anti-human IgG-HRP) we were able to positively detect SARS CoV-2 like antibodies in those animal species. The modified commercial kit gave 83% exposure for camel samples, at the 10% PP cutoff recommended by the manufacturers for human samples. Using the more stringent 20% PP cut off we were still able to record a 57% exposure and the negative controls were convincing. As already mentioned, these high titers diminish concerns that positive samples could largely be accounted for by antigenic cross reactions and not actual SARS-CoV-2 viral exposures.

Our comparative validation analysis revealed that the SARS-CoV-2 crude lysate ELISA corroborated the results obtained using the commercial kit based on the Spike specific RBD antigen (Fig. 9 and 10), albeit with a few more positive samples. This however is not surprising and one plausible explanation could be that SARS-CoV-2, like many pathogens induce antibody responses to multiple antigens. In this respect, it is important to recognize that there could be additional epitopes in the crude lysate that are potentially identified by the antibodies (whole spike S1 and the RBD, nucleocapsid protein and the membrane protein). Although, the spike S1 and RBD portions could be the most immunogenic (Meyer et al., 2014; Tai et al., 2020), it is conceivable that in the crude lysate, the other two antigens present could help in boosting the overall antibodies titers and hence increase the chance of positivity. On the basis of these findings, we suggest that the crude lysate can be used for initial screening as it avoids false negatives while simultaneously taking into account potential background cross-reactivity.

The data presented here, based both on our SARS-CoV-2 crude lysate ELISA and the modified commercial kit, indicate that SARS-CoV-2 antibodies or antibodies cross-reacting with the SARS-CoV-2 virus, are present in camels, cats and dogs in Kenya. An obvious question arising from these findings on the presence of SARS-CoV-2 in camel, cats and dogs in Kenya concerns how these infections relates to the human SARS-CoV-2 that has triggered an ongoing crises of public health, and economic wellbeing worldwide, including Kenya and continue to cause deaths daily. It is important to emphasize here that some of the camel samples that tested positive had been collected prior to the first reported human case in Kenya. Although this observation in itself does not suffice to rule out the possibility that the suggested exposure might have been a case of human to animal transmission, it suggests that this is an issue worthy of further investigation. This is because one possibility suggested by these results is that, the virus or a closely related virus had been in existence in Kenya before the pandemic. If that were to be the case, it would mean that the animals and or human have been previously exposed and so could reasonably be expected to have developed a certain level of protection against the SARS CoV-2 virus. If this were the case, understanding the mechanism leading to such protection would be the next logical step and the findings could have far reaching implications.

It is now known that the origin of SARS-CoV was in bats and that it was transmitted to humans via

civet cats (Wang and Eaton 2007) while MERS virus is known to have originated in camels (Dudas et al., 2018). As already mentioned, the early evidences had suggested that pangolins were the mammals originating the pandemic in humans; possibly after a failed attempt to sneak Malaysian pangolin into China and subsequent single cell sequencing suggesting that SARS-CoV-2 could infect pangolin cell types (Chen et al., 2020). The probable cause of the infections in their case could have been from human transmissions during the shipment or rather from the other animals being traded together at the illegal market. More recently, through experimental studies cats (Shi et al., 2020; Chen et al., 2020), ferrets (Kim et al., 2020), dogs (Shi et al., 2020), hamsters (Chan et al., 2020; Osterrieder et al., 2020), bats (Schlottau, et al., 2020) and non-human primates (Lu et al., 2020; Denis, et al., 2020; Hartmann et al., 2020; Munster, et al 2020) can be infected with SARS-CoV-2 and transmission can occur (Damas et al., 2020). Dogs appear to have limited susceptibility to SARS-CoV-2, while other domestic species including pigs and poultry do not appear susceptible (Shi et al., 2020; Suarez et al., 2020). While most of these studies have relied on experimental infection, natural SARS-CoV-2 infections in animals haven't been confirmed. There are a few cases of 14 pets, 8 captive big cats and farmed mink thought to have been positive for SARS-CoV-2 (Hobbs & Reids 2020). Thus our data is a valuable addition to the efforts aimed at ultimately confirming possible viral exposure in domesticated animals (Camel, Cats and the Dogs) (Fig 3,5,6,7 8 &).

Though the camels showed no clinical symptoms of COVID-19, cats and dogs were brought for treatments and thus it might have been possible that they were somehow symptomatic. Furthermore, infections in dogs are typically asymptomatic (Hobbs and Reids 2020) and hence it might have been hard to suspect SARS-CoV-2. Whether the seropositivity were as a result of natural exposure or human to animal transmission is still unclear, though the animal numbers 18, 19, 20, 21 and 22 have been traced to owners who were exposed to COVID-19, while dog number 22 and 23 came from owners with confirmed SARS-CoV-2 (Fig. 8). Furthermore like in the case of the camel samples, the fact that the commercial antigen based on spike RBD was used to screen the sample and the result gave an exposure level of 83% is reinforces the possibility that some of the animals testing positive are as a result of SARS-CoV-2 exposure rather than non- specific cross-reactions. It's important to note that some of the samples (n=23) which tested positive (Fig.9) had been collected 1 week before the first confirmed human case in Kenya. This might confirm the fact that the disease could have been in the country much earlier or overlaps from other coronaviruses such as MERS COV, SARS COV-2 or HKU8 is a possibility. SARS COV-2 antibodies have been shown to be present in cat samples, taken after the SARS-CoV-2 outbreak in Wuhan China (Qiang et al., 2020) with titers ranging 1/20 to 1/1080.

The presence of SARS COV-2 antibodies in animal might not be limited to Kenya alone, in the UK they have shown the presence of the antibodies in sera collected during the second wave, 1.4% (n=4) of dogs and 2.2% (n=2) cats tested positive for neutralizing antibodies (Smith et al., 2021). There was a strong reaction and high titers in camel samples, while dogs and cats showed lower titers. The high titers and strong reaction in camels might be due to either co-infection with MERS CoV or HKU8, as these viruses have been shown serologically to co-infect the dromedary camels in the same region where we collected the samples (Wei Zhang et al 2019) and therefore their cross reaction especially with the crude lysate ELISA (Fig 6) cannot be ruled out. The fact that both MERS COV and HKU8 which is a bat Coronaviruses has been previously found in camel and also the fact that coronaviruses have been shown to undergo recombination could elicit questions relating to whether that recombination has led to a more human infective SARS-CoV-2 . This data should be supported by molecular screening of the camel, cat, dog and bat samples for possible presence of SARS-CoV-2 viruses. Bat samples need to be tested, especially those totally in the wild. Bats are suspected as the key reservoir of the viruses and show close similarity with SARS-CoV-2 with as much as 96.2% identical in genome sequencing of SARS-CoV-2 with bat CoV RaTG13 (Zhou et al.,2020).

In summary the present study demonstrated for the first time the presence of SARS-CoV-2 antibodies in camels, cats, bats and dogs. Studies aimed at validating these findings will most likely benefit from the array of molecular tools for virus characterization. Serological detection of other coronaviruses in the same blood samples is also a priority area for further research. More broadly, the implication of our data on the current pandemic cannot be understated as the following key question need to be addressed: (1) If the

present results are attributable to specific cross-reaction with other corona viruses such as MERS COV or HKU8, then would the antibodies neutralize, block or even improves immunity against SARS-CoV-2 and (2) If indeed the animals examined were SARS-CoV-2 positive then did the viruses exist in the country before the pandemic or is it the case of human to animal transmissions. If it's the latter, an additional question related to the duration for which the virus has been in humans in Kenya prior to detection and what the correlates of immunity in such a symptomatic human hosts might be important to scrutinize.

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Declarations of conflicts of interest:

None

References

1. Barrs VR, Peiris M, Tam KWS, et al. SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. Emerg Infect Dis 2020; 26:3071–4. [PMC free article] [PubMed] [Google Scholar]
2. Bosco-Lauth M. A, Hartwig, A.E, Porter, A.M, Gordy, P.W,Nehring, M, Byas A.D, Vande Woude, S, Ragan, I.K, Maison, R.M., and Bowen, R.A.,(2020). Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. PNAS | 117 (42) 26382-26388 |September 29, 2020 https://doi.org/10.1073/pnas.2013102117
3. Chan, J.- F.-W., Zhang, A. J., Yuan, S., Poon, V.- K.-M., Chan, C.- C.-S., Lee, A.- C.-Y., Chan, W.-M., Fan, Z., Tsoi, H.-W., Wen, L., Liang, R., Cao, J., Chen, Y., Tang, K., Luo, C., Cai, J.-P., Kok, K.-H., Chu, H., Chan, K.-H., ... Yuen, K.-Y. (2020). Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: Implications for disease pathogenesis and transmissibility. Clinical Infectious Diseases. [in press]. 10.1093/cid/ciaa325. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
4. Chan, J. F., To, K. K., Tse, H., Jin, D. Y., and Yuen, K. Y. (2013). Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. 21, 544–555. doi: 10.1016/j.tim.2013.05.005
5. Chen, D., Sun, J., Zhu, J., Ding, X., Lan, T., Zhu, L., Xiang, R., Ding, P., Wang, H., Wang, X., Wu, W., Qiu, J., Wang, S., Li, H., An, F., Bao, H., Zhang, L., Han, L., Zhu, Y., ... Xu, X. (2020). Single-cell screening of SARS-CoV-2 target cells in pets, livestock, poultry and wildlife. bioRxiv. [preprint]: p. 2020.06.13.149690. 10.1101/2020.06.13.149690. Available from: https://www.biorxiv.org/content/biorxiv/early/2020/06/14/2020.06.13.149690.full.pdf [CrossRef] [Google Scholar] [Ref list]
6. Cyranoski D. Did pangolins spread the China coronavirus to people [Internet]. Heidelberg: Springer Nature; 2020 [cited 2020 Feb 16]. Available from: https://www.nature.com/articles/d41586-020-00364-2 4
7. Damas, J., Hughes, G. M., Keough, K. C., Painter, C. A., Persky, N. S., Corbo, M., Hiller, M., Koepfli, K.-P., Pfenning, A. R., Zhao, H., Genereux, D. P., Swofford, R., Pollard, K. S., Ryder, O. A., Nweeia, M. T., Lindblad-Toh, K., Teeling, E. C., Karlsson, E. K., & Lewin, H. A. (2020). Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. bioRxiv. [preprint]: p. 2020.04.16.045302. 10.1101/2020.04.16.045302. Available from: https://www.biorxiv.org/content/biorxiv/early/2020/04/18/2020.04.16.045302.full.pdf [PMC free ar-

ticle] [PubMed] [CrossRef] [Google Scholar] [Ref list]

8. Decaro N, Vaccari G, Lorusso A, et al. Possible Human-to-Dog Transmission of SARS-CoV-2, Italy, 2020. Emerg Infect Dis 2021; 27. [PMC free article] [PubMed] [Google Scholar]
9. Denis, M., Vandeweerd, V., Verbeeke, R., Laudisoit, A., Reid, T., Hobbs, E. C., & Van der Vliet, D. (2020). COVIPENDIUM: Information available to support the development of medical countermeasures and interventions against COVID-19. Transdisciplinary Insights. [Living paper]. 10.5281/zenodo.4025278. Available from: https://rega.kuleuven.be/if/corona_covid-19 [CrossRef] [Google Scholar] [Ref list]
10. Dudas G., Carvalho L.M., Rambaut A., Bedford T. MERS-CoV spillover at the camel-human interface. Elife. 2018;7 doi: 10.7554/eLife.31257. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
11. Fatima Amanat, Thi H.O. Nguyen, Veronika Chromikova, Shirin Strohmeier, Daniel Stadlbauer, Andres Javier, Kaijun Jiang, Guha Asthagiri Arunkumar, Jose Polanco, Maria Bermudez-Gonzalez, Daniel Caplivski, Allen Cheng, Katherine Kedzierska, Olli Vapalahti, Jussi M. Hepojoki, Viviana Simon, Florian Krammer (2020). A serological assay to detect SARS-CoV-2 seroconversion in humans. medRxiv 2020.03.17.20037713; doi: https://doi.org/10.1101/2020.03.17.20037713
12. Fritz M, Rosolen B, Krafft E, et al. High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health 2021; 11:100192. [PMC free article] [PubMed] [Google Scholar
13. Garigliany M, Van Laere AS, Clercx C, Giet D, Escriou N, Huon C, van der Werf S, Eloit M, Desmecht D (2020). SARS-CoV-2 Natural Transmission from Human to Cat, Belgium, March 2020. Emerg Infect Dis. 2020 Dec; 26(12):3069-3071. [PubMed] [Ref list]
14. Halfmann PJ, Hatta M, Chiba S, et al. Transmission of SARS-CoV-2 in Domestic Cats. N Engl J Med 2020. [PubMed] [Google Scholar]
15. Hamer SA, Pauvolid-Correa A, Zecca IB, et al. SARS-CoV-2 Infections and Viral Isolations among Serially Tested Cats and Dogs in Households with Infected Owners in Texas, USA. Viruses 2021; 13. [PMC free article] [PubMed] [Google Scholar]
16. Hartman, A. L., Nambulli, S., McMillen, C. M., White, A. G., Tilston-Lunel, N., Albe, J. R., Cottle, E. L., Dunn, M. D., Frye, L. J., Gilliland, T. H., Olsen, E. L., O'Malley, K. J., Schwarz, M. M., Tomko, J. A., Walker, R. C., Xia, M., Hartman, M. S., Klein, E., Scanga, C., ... Duprex, W. P. (2020). SARS-CoV-2 infection of African green monkeys results in mild respiratory disease discernible by PET/CT imaging and prolonged shedding of infectious virus from both respiratory and gastrointestinal tracts. bioRxiv. [preprint]: p. 2020.06.20.137687. 10.1101/2020.06.20.137687. Available from: https://www.biorxiv.org/content/biorxiv/early/2020/06/21/2020.06.20.137687.full.pdf [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
17. Hobbs, E. C., & Reid, T. J. (2020). Animals and SARS-CoV-2: Species susceptibility and viral transmission in experimental and natural conditions, and the potential implications for community transmission. Transboundary and Emerging Diseases, 68, 1850–1867. 10.1111/tbed.13885 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
18. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497–506.
19. Jemeršić, L., Lojkić, I., Krešić, N., Keros, T., Zelenika, T. A., Jurinović, L., Skok, D., Bata, I., Boras, J., Habrun, B., & Brnić, D. (2021). Investigating the Presence of SARS CoV-2 in Free-Living and Captive Animals. Pathogens (Basel, Switzerland), 10(6), 635. https://doi.org/10.3390/pathogens10060635
20. Kim, Y.-I., Kim, S.-G., Kim, S.-M., Kim, E.-H., Park, S.-J., Yu, K.-M., Chang, J.-H., Kim, E. J., Lee, S., Casel, M. A. B., Um, J., Song, M.-S., Jeong, H. W., Lai, V. D., Kim, Y., Chin, B. S., Park, J.-S., Chung, K.-H., Foo, S.-S., ... Choi, Y. K. (2020). Infection and rapid transmission of SARS-CoV-2 in ferrets. Cell Host & Microbe, 27, 10.1016/j.chom.2020.03.023. Available from: http://www.sciencedirect.com/science/article/pii/S1931312820301876 [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
21. Koeppel KN, Mendes A, Strydom A, Rotherham L, Mulumba M, Venter M. SARS-CoV-2 Reverse Zoonoses to Pumas and Lions, South Africa. Viruses. 2022; 14(1):120. https://doi.org/10.3390/v14010120

22. Lau, S., Luk, H., Wong, A., Li, K., Zhu, L., He, Z. . . .Woo, P. (2020). Possible Bat Origin of Severe Acute Respiratory Syndrome Coronavirus 2. Emerging Infectious Diseases, 26(7), 1542-1547. https://doi.org/10.3201/eid2607.200092
23. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med [Preprint]. 2020 [cited 2020 Feb 16]: [9 p.]. Available from: https://doi.org/10.1056/NEJMoa2001316
24. Lu, S., Zhao, Y., Yu, W., Yang, Y., Gao, J., Wang, J., Kuang, D., Yang, M., Yang, J., Ma, C., Xu, J., Qian, X., Li, H., Zhao, S., Li, J., Wang, H., Long, H., Zhou, J., Luo, F., . . . Peng, X. (2020). Comparison of SARS-CoV-2 infections among 3 species of non-human primates. bioRxiv. [preprint]: p. 2020.04.08.031807. 10.1101/2020.04.08.031807. Available from: https://www.biorxiv.org/content/biorxiv/early/2020/04/12/2020.04.08.031807.full.pdf [CrossRef] [Google Scholar] [Ref list]
25. Liu P, Jiang JZ, Wan XF, Hua Y, Li L, et al. (2021) Correction: Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)?. PLOS Pathogens 17(6): e1009664. https://doi.org/10.1371/journal.ppat.1009664 View correction
26. Meyer B , Drosten C, Müller MA. Serological assays for emerging coronaviruses: challenges and pitfalls. Virus Res 2014; 194:175–83. Google ScholarCrossrefPubMed
27. Michelitsch A, Hoffmann D, Wernike K, Beer M. Occurrence of Antibodies against SARS-CoV-2 in the Domestic Cat Population of Germany. Vaccines (Basel) 2020; 8. [PMC free article] [PubMed] [Google Scholar]
28. Michelitsch A, Schon J, Hoffmann D, Beer M, Wernike K. The Second Wave of SARS-CoV-2 Circulation-Antibody Detection in the Domestic Cat Population in Germany. Viruses 2021; 13. [PMC free article] [PubMed] [Google Scholar]
29. Molenaar RJ, Vreman S, Hakze-van der Honing RW., Zwart R, de Rond J, Weesendorp E, Smit, LAM, Koopmans M, Bouwstra, R, Stegeman, A, van der Poel WHM (2020).Clinical and pathological findings in SARS-CoV-2 disease outbreaks in farmed mink. (Neovison vison). Vet. Pathol. 57, 653–657 (2020).
30. Munster, V. J., Feldmann, F., Williamson, B. N., van Doremalen, N., Pérez-Perez, L., Schulz, J., Meade-White, K., Okumura, A., Callison, J., Brumbaugh, B., Avanzato, V. A., Rosenke, R., Hanley, P. W., Saturday, G., Scott, D., Fischer, E. R., & de Wit, E. (2020). Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. Nature, 585(7824), 268–272. 10.1038/s41586-020-2324-7 [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Re
31. Newman A, Smith D, Ghai RR, et al. First Reported Cases of SARS-CoV-2 Infection in Companion Animals - New York, March-April 2020. MMWR Morb Mortal Wkly Rep 2020; 69:710–3. [PMC free article] [PubMed] [Google Scholar]
32. Nguyen, T. T., Abdelrazek, M., Nguyen, D. T., Aryal, S., Nguyen, D. T., and Khatami, A. (2020). Origin of novel Coronavirus (COVID-19): a computational biology study using artificial intelligence. [Preprint]. doi: 10.1101/2020.05.12.091397
33. Osterrieder, N., Bertzbach, L. D., Dietert, K., Abdelgawad, A., Vladimirova, D., Kunec, D., Hoffmann, D., Beer, M., Gruber, A. D., & Trimpert, J. (2020). Age-dependent progression of SARS-CoV-2 infection in Syrian hamsters. bioRxiv. [preprint]: p. 2020.06.10.144188. 10.1101/2020.06.10.144188. Available from: https://www.biorxiv.org/content/biorxiv/early/2020/06/10/2020.06.10.144188.full.pdf [CrossRef] [Google Scholar] [Ref list]
34. Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science 2021; 371:172–7. [PMC free article] [PubMed] [Google Scholar]
35. Patterson EI, Elia G, Grassi A, et al. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat Commun 2020; 11:6231. [PMC free article] [PubMed] [Google Scholar]
36. Prince T, Smith SL, Radford AD, Solomon T, Hughes GL, Patterson EI Viruses. (2021). Review: SARS-CoV-2 Infections in Animals: Reservoirs for Reverse Zoonosis and Models for Study. 2021 Mar 17; 13(3):. [PubMed] [Ref list]
37. Qiang Zhang, Huajun Zhang, Jindong Gao, Kun Huang, Yong Yang, Xianfeng Hui, Xinglin He,

Chengfei Li, Wenxiao Gong, Yufei Zhang, Ya Zhao, Cheng Peng, Xiaoxiao Gao, Huanchun Chen, Zhong Zou, Zheng-Li Shi & Meilin Jin (2020) A serological survey of SARS-CoV-2 in cat in Wuhan, Emerging Microbes & Infections, 9:1, 2013-2019, DOI: 10.1080/22221751.2020.1817796

38. Ren, L. L., Wu, C., Guo, L., Yao, J. C., Wang, C. H., Xiao, Y., et al. (2020b). Single-cell transcriptional atlas of the Chinese horseshoe bat (Rhinolophus sinicus) provides insight into the cellular mechanisms which enable bats to be viral reservoirs. [Preprint]. doi: 10.1101/2020.06.30.175778
39. Ruiz-Arrondo I, Portillo A, Palomar AM, et al. Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: A case of an asymptomatic cat with SARS-CoV-2 in Europe. Transbound Emerg Dis 2020. [PMC free article] [PubMed] [Google Scholar]
40. Sailleau C, Dumarest M, Vanhomwegen J, et al. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis 2020; 67:2324–8. [PMC free article] [PubMed] [Google Scholar]
41. Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., Liu, R., He, X., Shuai, L., Sun, Z., Zhao, Y., Liu, P., Liang, L., Cui, P., Wang, J., Zhang, X., Guan, Y., Tan, W., Wu, G., ... Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. Science. p. eabb7015. 10.1126/science.abb7015. Available from: https://science.sciencemag.org/content/sci/early/2020/04/07/science.abb7015.full.pdf [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
42. Sit THC, Brackman CJ, Ip SM, et al. Infection of dogs with SARS-CoV-2. Nature 2020. [PMC free article] [PubMed] [Google Scholar]
43. Smith, S. L., Anderson, E. R., Cansado-Utrilla, C., Prince, T., Farrell, S., Brant, B., Smyth, S., Noble, P. M., Pinchbeck, G. L., Marshall, N., Roberts, L., Hughes, G. L., Radford, A. D., & Patterson, E. I. (2021). SARS-CoV-2 neutralising antibodies in Dogs and Cats in the United Kingdom. bioRxiv : the preprint server for biology, 2021.06.23.449594. https://doi.org/10.1101/2021.06.23.449594
44. Stevanovic V, Vilibic-Cavlek T., Tabain I., Benvin I., Kovac S., Hruskar Z., Mauric M., Milasincic L., Antolasic L., Skrinjaric A., Staresina V. and Barbic L. Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. Transbound Emerg Dis 2020. [PMC free article] [PubMed] [Google Scholar]
45. Suarez, D. L., Pantin-Jackwood, M. J., Swayne, D. E., Lee, S. A., Deblois, S. M., & Spackman, E. (2020). Lack of susceptibility of poultry to SARS-CoV-2 and MERS-CoV. bioRxiv. [preprint]: p. 2020.06.16.154658. 10.1101/2020.06.16.154658. Available from: https://www.biorxiv.org/content/biorxiv/early/2020/06/16/2020.06.16.154658.full.pdf [CrossRef] [Google Scholar] [Ref list]
46. Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol2020; 17:613–20. Google ScholarCrossrefPubMed
47. Wang LF, Eaton BT (2007). Review Bats, civets and the emergence of SARS. Curr Top Microbiol Immunol. 2007; 315():325-44. [PubMed] [Ref list]
48. Van Aart AE VF, Fischer EAJ, Broens EM, Egberink H, Zhao S, Engelsma M, Hakze-van der Honing RW, Harders F, de Rooij MMT, Radstake C, Meijer PA, Oude Munnink BB, de Rond J, Sikkema RS, van der Spek AN, Spierenburg M, Wolters WJ, Molenaar RJ, Koopmans MPG, van der Poel WHM, Stegeman A, Smit LAM. SARS-CoV-2 infection in cats and dogs in infected mink farms. Transboundary and Emerging Diseases 2021. [PMC free article] [PubMed] [Google Scholar]
49. Zhang Q, Zhang H, Gao J, et al. A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg Microbes Infect 2020; 9:2013–9. [PMC free article] [PubMed] [Google Scholar]
50. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579:270–273. doi: 10.1038/s41586-020-2012-7 [Crossref], [Web of Science (r)], [Google Scholar]