Dogs and Cats Are Less Susceptible to the Omicron Variant of Concern of SARS-CoV-2: A Field Study in Germany, 2021/2022

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a betacoronavirus of the subgenus Sarbecovirus, is the causative agent of COVID-19 (coronavirus disease 2019), which was reported for the first time in late 2019 in Wuhan, China [1]. Thereafter, the novel pathogen very rapidly spread globally, driven by direct human-to-human virus transmission via aerosolized particles, and led to millions of human deaths worldwide [2, 3]. Since the first detection of the virus, SARS-CoV-2 has evolved, leading to the emergence of numerous variants, some of which represent so-called variants of interest (VOIs) or variants of concern (VOCs) that are continuously monitored by, e.g., the World Health Organization [4]. VOCs could spread more easily, escape the host’s immune response, cause more severe disease in humans, change the clinical presentation, decrease the effectiveness of vaccines, treatments, or diagnostic tools, or display an altered host range [5].

Besides humans, several animal species can be infected with SARS-CoV-2, including nonhuman primates, felines, canines, mustelids, some ruminant species, and several rodents [6, 7], but their susceptibility might vary depending on the virus variant. As an example, house mice (Mus musculus) were excluded as amplifying host for the wild-
type virus by experimental infection [8], but they are susceptible to some VOCs, in particular the beta (B.1.351) and gamma (P1) variants [9, 10]. Ferrets (Mustela putorius) are susceptible to wild-type SARS-CoV-2 and some of the VOCs, particularly alpha (B.1.1.7) and delta (B.1.617.2) [11–15]. However, a BA.1 strain of the omicron (B.1.1.529) VOC failed completely to induce productive infections in these animals [11].

Among the animal species that are in principle susceptible to SARS-CoV-2, those are of particular concern which have frequently close contact to humans. Examples for animals in close contact to humans include companion animals like cats (Felis catus) and dogs (Canis lupus). Therefore, pets were included in surveillance studies early in the course of the human pandemic and, indeed, anthropo-zoonotic SARS-CoV-2 transmissions from infected owners to their cats or dogs were noticed and included multiple VOCs [16–19]. In various seroprevalence studies, varying proportions of seropositive animals were found, which depended on the location and especially the study period. During the first months of the pandemic, relatively small proportions of seropositive animals (<5%) were found in randomly selected samples, while higher seroprevalences were observed in pets as the pandemic progressed and the number of cases in humans increased sharply worldwide [20–25]. Particularly high seroprevalences were recorded when cats and dogs from households with human COVID-19 patients were sampled [26–28].

Although a few infections of companion animals with the omicron VOC were published [29, 30], often reporting low viral loads, there are considerably fewer reports than those during the periods at which the earlier VOCs were circulating. So far, it is not known whether cats and dogs are less susceptible to omicron, especially as data from experimental infection studies are not yet available, or whether only frequency of testing and reporting has decreased. Therefore, we investigated cats and dogs from COVID-19 households during the transition period from delta as the dominating VOC to omicron in the human population, in order to investigate the frequency of virus transmission of both variants from infected owners to their pets.

### 2. Materials and Methods

We aimed to investigate dogs and cats from households with confirmed human COVID-19 cases by serological methods. Households were recruited through information letters distributed by associations of independent veterinarians, social media, online platforms, and word of mouth. Serum or plasma samples of the animals were taken between three weeks and three months after the first SARS-CoV-2 detection in a pet owner. The study period started in November 2021, when delta was the dominant variant detected in the human population in Germany, and went through April 2022, thereby covering the period at which omicron BA.1 and BA.2 strains dominated in humans [31] (Figure 1). A total of 241 cats and 290 dogs could be recruited for the study. The number of samples per species and month is given in Figures 2 and 3.

To additionally cover the period when further omicron subvariants were predominantly spreading, randomly selected feline serum or plasma samples taken in Germany between calendar weeks 11 and 23 of the year 2022 (n = 172) or in weeks 31/32 of the year 2022 (n = 200) were serologically tested (Figure 1). The samples were obtained from domestic cats during clinical examination by the attending veterinarian and were sent to a clinical diagnostic laboratory (LABOKLIN GmbH & Co. KG) for non-SARS-CoV-2-related testing (e.g., hematology testing). Superfluous sample material was kindly provided to the Friedrich-Loeffler-Institut for SARS-CoV-2 serology. The health status of the cats is not known to the authors, and the only data given was the postal code of the veterinarian who had taken the sample; the veterinary practices were spread all over Germany. The infection status of the specific animal owners was likewise unknown. However, during the sampling period, a very high prevalence was recorded in the human population [32]. Therefore, a certain proportion of cats with contact to infected owners could be expected.

All sera were tested by a commercial, species-independent surrogate virus neutralization test (sVNT) (cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, GenScript, the Netherlands). The test was performed as prescribed by the manufacturer using a cutoff of ≥30% for positivity and <30% for negativity. The sVNT in its original composition allows for the detection of antibodies against the wild-type virus and diverse SARS-CoV-2 VOCs including delta, except omicron. For omicron and its subvariants, a specific horseradish peroxidase- (HRP-) conjugated receptor-binding domain (RBD) protein is provided by the test manufacturer. The suitability to detect and discriminate antibodies against the delta and omicron variants was proven by testing sera obtained from goats experimentally delta-infected [33] and from mice infected with an omicron BA.1 strain (kindly provided by the Institute of Virology, Medical Center University of Freiburg, Germany).

Feline and canine samples collected in November 2021, i.e., prior to the first detection of the omicron VOC in the human population of Germany [31] (Figure 1), were tested only by the original composition of the sVNT. Samples collected from December 2021 onwards were tested by the sVNT using the original RBD and, in a parallel approach, using the omicron-specific RBD.

Sera that reacted positive in the sVNT were subsequently tested by an indirect immunofluorescence assay (iIFA) for confirmation. The test was performed as described previously [13, 24] with a starting dilution of 1/16 and four log2 dilution steps. As secondary antibody, FITC-labelled anti-cat IgG (dilution 1/600; Sigma-Aldrich, Steinheim, Germany) and anti-dog IgG (1/100; Sigma-Aldrich), respectively, were used.

### 3. Results and Discussion

#### 3.1. Analysis of Samples from COVID-19 Households from November 2021 through April 2022 Suggests a Lower Susceptibility of Dogs and Cats to the Omicron VOC

To control for the suitability of detecting and discriminating antibodies against the delta and omicron variants, sera obtained from
goats experimentally delta-infected and from mice infected with an omicron BA.1 strain were tested. Every control sample reacted in the sVNT as expected, i.e., the goat sera tested positive when using the original RBD, and the mouse samples reacted positive against the omicron RBD (Figure 4).

Of the 290 dogs with contact to SARS-CoV-2-infected owners, 20 animals tested positive by sVNT (6.9%, 95% confidence interval (CI): 4.0%–9.8%). However, there were marked differences between the months of the study period and, related thereto, between the virus variants the dogs had contact to. While antibodies against SARS-CoV-2 could be detected in 18 of 36 dogs (50.0%, 95% CI: 33.7%–66.3%) that had contact to an infected owner in November 2021, only 2 of the 254 animals that were sampled from December 2021 through April 2022 tested positive (0.8%, 95% CI: 0.0%–1.9%) (Figure 2). In all cases, the positive reaction was recorded against the original RBD. When using the omicron RBD, every canine sample tested negative (Figure 2). All positive sVNT results could be confirmed by the iIFA as every sample that tested positive in the sVNT also gave a positive result in the iIFA; the titers ranged from 1/32 to >1/128.

For cats, a similar pattern was seen. Overall, 26 of the 241 feline sera (10.8%, 95% CI: 6.9%–14.7%) tested seropositive. Sixteen of the positive reacting sera were collected in households that had human COVID-19 patients in November 2021 (16/42; 38.1%, 95% CI: 23.4%–52.8%). Of the 199 cats that had contact to a SARS-CoV-2-positive owner between December 2021 and March 2022, only 10 scored positive (10/199; 5.0%, 95% CI: 2.0%–8.1%). From these 10 samples, five showed a stronger reaction against the original RBD and five against the omicron RBD (Figure 3). Again, all positive sVNT results could be confirmed by the iIFA. The titers ranged from 1/64 to >1/128.

The serological results of cats and dogs, specifically the markedly reduced rate of seropositive animals in the period of omicron circulation in comparison to the delta period, suggest a considerable reduction in the susceptibility of these animal species to the omicron VOC. In November 2021, i.e., when delta represented the dominant VOC in the human population of Germany [31], a high proportion of cats and dogs were infected by contact to their virus-positive owners. This is in line with previous household studies conducted.
Figure 2: Serological results of dogs kept in COVID-19 households. In the upper panel, the shares of positive (red) and negative (grey) results are given; the animals are sorted into the month in which their owner tested SARS-CoV-2 positive. In the lower panel, the values as measured in the surrogate virus neutralization test are shown individually for each canine sample. In November 2021, before the omicron variant of concern was detected for the first time in the human population of Germany, the canine sera were tested only against the original RBD. From December 2021 onwards, the samples were tested in parallel using the original as well as the omicron RBD, and the results of individual samples are connected by a black line. The cutoff is indicated by a horizontal dashed line.

Figure 3: Serological results of cats kept in COVID-19 households. In the upper panel, the shares of positive (red) and negative (grey) results are given; the animals are sorted into the month in which their owner tested SARS-CoV-2 positive. In the lower panel, the values as measured in the surrogate virus neutralization test are shown individually for each feline sample. In November 2021, before the omicron variant of concern was detected for the first time in the human population of Germany, the feline sera were tested only against the original RBD. From December 2021 onwards, the samples were tested in parallel using the original as well as the omicron RBD, and the results of individual samples are connected by a black line. The cutoff is indicated by a horizontal dashed line.
prior to 2022, where seroprevalences well over 10% were consistently found, independent of the study area, time, and VOC circulating at that time [26, 28, 34, 35]. Therefore, cats and dogs appear to be susceptible to the wild-type virus and the earlier VOCs up to delta [16–18, 36]. The susceptibility for the wild-type virus was additionally confirmed by experimental infection [14, 37]. In contrast, from December 2021 onwards, when omicron took over dominance in humans, we detected antibodies against SARS-CoV-2 in only a considerably lower proportion of cats, and, among the dogs, not even a single animal seroreactive against the omicron RBD was found. Hence, cats and dogs seem to be much less receptive to omicron, which mirrors the situation in ferrets. Ferrets are like cats and dogs to the order Carnivora and can be infected with the wild-type virus, some of the earlier VOCs, but not with the omicron BA.1 VOC [11–15]. This susceptibility pattern in carnivores is in stark contrast to humans, where the secondary attack rate of the omicron variant in households was even higher than that of the delta variant [38].

Ever since the first reporting of omicron in South Africa, its origin was up to debate, with hypotheses about an animal origin and those about continuous evolution in humans being raised [7, 39–41]. The markedly reduced susceptibility or even unresponsiveness of carnivores contradicts the theory of omicron’s origin in an animal reservoir, at least for carnivores, and strongly supports the second hypotheses, i.e., that omicron and its subvariants evolved in and adapted to humans. The emergence of omicron’s subvariant BA.1 in the human population represents a remarkable evolutionary leap, as the omicron VOC has acquired up to 50 mutations.

More than 30 of them are located within the spike gene [42], which among others mediates binding of the virus to the cellular surface protein angiotensin-converting enzyme 2 (ACE2) [43, 44]. This high number of alterations in the spike protein might lead to substantial changes in antigenicity and/or receptor binding compared to earlier virus variants and, as a consequence, could lead to an altered host range. As an example, it has been demonstrated that the Syrian hamster is a highly susceptible animal model for wild-type SARS-CoV-2 and multiple VOCs from alpha to delta [15, 45–50]. However, competitive infection and transmission experiments using the omicron VOC in the hamster model did not reflect the epidemiological situation seen in the human population any more [11], which hints to an adaptation of the virus to the human host. Accordingly, also carnivores appear less susceptible to the omicron VOC. It cannot productively replicate in ferrets [11], and, in our study, we observed a markedly reduced rate of seropositive cats and dogs in the period of omicron circulation, strongly supporting the theory that omicron and its subvariants evolved in and adapted to humans.

3.2. No Detection of Antibodies against the Omicron VOC in Cats Randomly Sampled in Weeks 11 to 23 and 31/32 of 2022. Sample collection from cats and dogs from COVID-19 households ended in April 2022. Nevertheless, to also examine the effect of further omicron subvariants on cats, sera of randomly selected animals were tested. For this part of the study, only cats were chosen as this species showed, in contrast to dogs, single seropositive reactions against the omicron RBD in the household study during the time of BA.1/BA.2 circulation. During the first sampling period (calendar weeks 11 to 23 of the year 2022), 172 sera were analyzed and four of them tested positive in the sVNT (2.3%, 95% CI: 0.1% to 4.6%). During the second period (calendar weeks 31/32 of 2022), 200 samples were collected and seven scored positive in the sVNT (3.5%, 95% CI: 1.0%–6.0%). All sera showed a stronger reaction against the original RBD than against the omicron ortholog (Figure 5), and, again, all positive sVNT results were confirmed by the iIFA.

Hence, the results of the random sampling confirmed those of the household study, that is, a lower ratio of seroconversions against the omicron VOC. Overall, several studies demonstrated lower prevalences in companion animals with unknown household status [26, 27], but given the high prevalence of infections with omicron’s subvariants in the human population during the first half of the year 2022 [31, 32], at least single seropositive cats are to be expected. Indeed, antibodies against earlier VOCs could be detected, even though the circulation of non-omicron-VOCs was some time ago and although previous studies demonstrated that serum antibody levels decline in cats to the limit of detection within only a few months [51, 52]. The latter makes it even more surprising that we could detect antibodies directed against the original RBD, indicating that the animals had contact to delta or one of the earlier virus variants, while none of the samples showed a stronger reaction against the omicron RBD. Therefore, we conclude that cats are less...
susceptible to omicron, presumably with no or only marginal differences between omicron’s subvariants. Nevertheless, dogs and cats should be included in monitoring studies or epidemiological investigations also in the future, especially when new virus variants emerge for which the degree of susceptibility of companion animals is not known.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

The serum samples taken from dogs and cats from COVID-19 households were collected by the responsible veterinarians in the context of diagnostic testing; no permissions were needed to collect the specimens. The randomly selected samples were left-over sera collected in a laboratory for veterinary diagnostics. The samples were taken by the responsible veterinarians in the context of health monitoring of the respective animal; no permissions were needed to collect the specimens.

Disclosure

A preprint has previously been published [53].

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

Martin Beer, Nicolai Denzin, and Kerstin Wernike conceptualized the study. Constantin Klein and Kerstin Wernike were responsible for investigation, formal analysis, and visualization. Anna Michelitsch, Valerie Allendorf, Franz Josef Conraths, and Nicolai Denzin provided resources. Kerstin Wernike was responsible for supervision, original draft preparation, and methodology. All authors reviewed and edited the manuscript. All authors have read and agreed to the published version of the article. Nicolai Denzin and Kerstin Wernike contributed equally to this study.

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