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Household clusters of SARS-CoV-2 Omicron subvariants contemporaneously sequenced from dogs and their owners

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ABSTRACT Monitoring the zoonotic potential of emerging SARS-CoV-2 variants in animals is a critical tool to protect public health. We conducted a longitudinal study in 47 households reporting people with COVID-19 in Texas from January to July 2022, during the first Omicron wave. We evaluated 105 people and 100 of their companion animals for SARS-CoV-2 infection at three sequential sampling events, starting 0–5 days after the first reported diagnosis of COVID-19 in the house. SARS-CoV-2 RNA was detected in 68% of people from 43 households; 95.5% of people had antibodies to SARS-CoV-2. Dogs were the only animal species positive by RT-qPCR (5.4%; 3/55), and their viral loads were consistently lower compared with those from household members. Additionally, infected dogs did not yield infectious virus. Clusters of Omicron BA.1.1, BA.2.3.4, and BA.5.1.1 in people, dogs, and a dog food bowl confirmed human-to-dog transmission within households, with no evidence of onward transmission from the infected dogs. Eleven dogs (n = 55) and two cats (n = 26) had neutralizing antibodies against SARS-CoV-2. Overall, infection was not associated with clinical signs in pets; only two animals that tested negative for SARS-CoV-2 were reported to be sick. Nearly one-third (30.2%) of households with active COVID-19 had pets exposed to SARS-CoV-2, similar to our pre-Omicron studies; however, the incidence of infection in cats was lower compared with pre-Omicron. These differences suggest that the zoonotic transmission dynamics in households may differ based on variants.

IMPORTANCE SARS-CoV-2 infects a broad diversity of mammals, with companion dogs and cats at risk of infection via close contact with infectious owners. Longitudinal studies sampling pets and their owners over time are essential to understanding within-household SARS-CoV-2 transmission dynamics. Our repeated sampling in households with people reporting COVID-19 found that 68% of the people in 43 households had active SARS-CoV-2 infection during at least one of the three sampling events. Although none of the 27 cats were positive, 3/55 dogs had active infections. Household clusters of three different Omicron subvariants were involved in these human-to-dog transmission events, and our data suggest reduced infection in pets during Omicron transmission compared with pre-Omicron waves. Protecting pets from SARS-CoV-2 infection remains important, as viral evolution can be accompanied by changes in the infectiousness of different hosts.

KEYWORDS One Health, longitudinal study, surveillance, COVID-19, zoonosis

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of coronavirus disease 2019 (COVID-19), is a zoonotic virus of worldwide importance to public health and the health of at-risk mammalian species. Spillback infections from

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people to animals are widespread, given the wide range of susceptible mammal species (1). Confirmation of the susceptibility of companion animals, especially cats and dogs, to SARS-CoV-2 via experimental challenges (2, 3) and the detection of natural infections at the early stages of the COVID-19 pandemic across multiple continents (4–6) raised questions about the role of pets in transmission cycles of the virus. However, few human infections resulting from contact with infected pets have been reported (7, 8).

The highly transmissible Omicron variant emerged globally in November 2021 and confirmed predictions that virus variants and subvariants may differ in their host range (9, 10). Because dogs and cats remained susceptible to Omicron in the laboratory (11, 12) and in natural conditions (13, 14), it is essential to survey pets for their involvement in SARS-CoV-2 transmission cycles as new variants continue to emerge. This is critical because dogs experimentally infected with Omicron can sustain onward transmission to naive conspecifics (12).

Pets living in households with active COVID-19 cases are more likely to have been exposed to SARS-CoV-2 compared with pets with no or unknown evidence of exposure to infected people (15–17). Although cross-sectional studies are useful for determining pet infection prevalence, longitudinal studies of pets and household members are needed to understand transmission dynamics within households.

Therefore, we conducted a longitudinal investigation enrolling people and pets living in households with active COVID-19 cases during the emergence of Omicron in Texas to understand inter-species transmission patterns under natural conditions. We confirmed through RT-qPCR that 43 households, out of 47 enrolled, had people with active SARS-CoV-2 and detected evidence of human-to-dog transmission in three households. Whole genome sequencing revealed that all clusters involved Omicron variants. Overall, we found no evidence of onward transmission from dogs to other pets or humans.

RESULTS

General results

In total, we sampled 105 people and 100 animals from 47 households where at least one person self-reported COVID-19, with 1–7 people per house and 1–12 pets per house (Table 1). Thirty-five households had dogs, 19 had cats, and nine had both dogs and cats. An average of 1.63 dogs (n = 57; 0.88 standard deviation) and 1.38 cats (n = 29; 0.59 SD) were tested per household. The mean age for dogs and cats was 6.7 (4.58 SD) and 6.4 (3.99 SD) years, respectively. The cohort also included five goats, three horses, two pigs, a donkey, a rabbit, a gecko, and a tortoise.

We collected swabs at three consecutive sampling events 0–33 days after the self-reported date of the first COVID-19 diagnosis within each household (referred to as "days after diagnosis"; Table 1). The average number of days after diagnosis was 2.22 (1.6 SD) for the first sampling, 8.9 (3.7 SD) for the second, and 15.7 (5 SD) for the third.

Detection of SARS-CoV-2 in people and pets

We confirmed at least one positive result among people via RT-qPCR in 43 of the 47 households; households with no SARS-CoV-2 detection in humans were excluded from analyses below. Positivity rate by RT-qPCR in humans (samples positive by both N1 and N2 tests) was 63.9% (n = 97), 52.1% (n = 96), and 22.6% (n = 93) at the first, second, and third sampling events, respectively. Overall, 68.4% (67/98) of the people tested positive at least once. Our linear mixed-effect models showed that estimated viral copies at the log10 scale, based on Ct values from N1 and N2 assays separately, decreased with the number of days after diagnosis (P < 0.001; Fig. 1), between the first and the second sampling events (P < 0.001), and between the second and the third sampling events (P < 0.01; Fig. S1).

Of the 55 dogs sampled, three (5.4%) from different households had respiratory swabs positive by RT-qPCR; positive samples were collected at 2, 5, and 9 days after diagnosis of the first person with COVID-19. Rectal swabs from these dogs were negative

TABLE 1 General statistics for 43 households in Texas with active SARS-CoV-2 infections in humans owning pets[a,b]

Factor	Dogs (*n* = 55)			Cats (*n* = 27)	
	Noninfected (*n* = 42)	Infected (*n* = 13)	Odds ratio (95% CI)	Non-infected (*n* = 25)	Infected (*n* = 2)
Sampling aspects					
Days after diagnosis, 1st sampling[d,e]	1.69 (1.57)	3.31 (1.38)	**2.55 (1.4-6.16)**	2.12 (1.54)	3.5 (2.12)
Days after diagnosis, 2nd sampling[d]	8.21 (3.43)	10.54 (3.62)		8.88 (4.03)	11.5 (3.53)
Days after diagnosis, 3rd sampling[d]	15.14 (4.60)	17.46 (3.23)		16.04 (7.35)	18 (1.41)
Household characteristics					
People per household[c]	2.79 (1.41)	3 (1.58)		2.61 (1.50)	3.5 (0.71)
Pets per household[c]	3.33 (2.38)	3.85 (3.72)		2.72 (1.4)	2.5 (2.12)
Infected people per household[d]	1.55 (0.63)	1.54 (0.52)		1.64 (0.76)	1 (0)
Mean viral load (log10) in humans per household at 1st sampling[d]	5.77 (1.59)	5.36 (1.64)		5.98 (1.35)	4.25 (1.06)
Animal aspects					
Male, count (%)[d,e]	15 (62.5)	9 (37.5)	**5.74 (1.25-34.13)**	10 (40)	1 (50)
Female, count (%)[d,e]	26 (86.7)	4 (13.3)		15 (60)	1 (50)
Mostly indoors, count (%)[d]	36 (85.7)	11 (84.6)		22 (88)	2 (100)
Number of infected people pet share bed during sleep[c]	0.69 (0.81)	0.77 (0.83)		0.96 (0.73)	0.5 (0.71)
Number of infected people pet share room during sleep[d]	0.83 (0.82)	0.92 (0.86)		0.92 (0.76)	0.5 (0.71)
Number of infected people petting the pet[c]	1.43 (0.77)	1.38 (0.65)		1.48 (0.87)	1 (0)
Number of infected people cuddling the pet[c]	1.43 (0.77)	1.38 (0.65)		1.28 (0.74)	1 (0)
Number of infected people kissing the pet[d]	1 (0.96)	1.23 (0.83)		0.84 (0.90)	1 (0)
Number of infected people sharing food with the pet[d]	0.57 (0.83)	0.31 (0.48)		0.28 (0.54)	0.5 (0.71)
Number of infected people administering medicine or giving treats by hand to the pet[d,e]	1.14 (0.84)	0.85 (0.80)	0.37 (0.10-1.04)	0.64 (0.76)	0.5 (0.71)
Human-pet interaction aspects					
Infected people not taking precautions with people in the household[d]	0.33 (0.75)	0.31 (0.75)		0.28 (0.68)	0
Infected people not taking precautions with pets in the household[c]	1.43 (0.80)	1.54 (0.52)		1.64 (0.76)	0.5 (0.71)

[a]Logistic regression models were used to determine the factors associated with the risk of infection in dogs only due to the small numbers of cats sampled and determined as infected with or exposed to SARS-CoV-2. Odds ratios and 95% confidence intervals were included for the factors kept in the final model; significant values are in bold.
[b]Values are mean followed by the standard deviation (in parentheses) unless otherwise stated. Pets were considered infected when positive at least once by RT-qPCR and/or when capable of inhibiting at least 50% of viral plaques in plaque reduction neutralization tests. Precautions to prevent transmission of SARS-CoV-2 to people included isolation and using face masks. Precautions to prevent transmission of SARS-CoV-2 to pets included isolation, using face masks, and avoiding touching the pets.
[c]Explanatory variables were not included in the models because they were highly correlated with other variables analyzed. See Materials and Methods for details.
[d]Variables included in the initial logistic regression models.
[e]Variables kept in the final model after employing backward stepwise selection.

at all sampling events. All swab samples (respiratory and rectal) from cats (*n* = 27) and other pet species were negative by RT-qPCR. A total of 3 of 43 households (7%) with people with COVID-19 had RT-qPCR positive pets. Overall, 33 households with people with COVID-19 owned dogs, indicating dogs in 9.1% of these households became positive following potential exposure. The positivity rate by RT-qPCR is lower for cats when compared with our prior, pre-Omicron study (18) (*n* = 157; 13.4%; Fisher's exact test, *P* = 0.048), but the rates did not differ for dogs (*n* = 396; 4.8%; Fisher's exact test, *P* = 0.7).

The estimated number of viral copies from these dogs was consistently lower than values from humans sampled at the same time using the day of the first COVID-19 diagnosis as a reference (linear mixed-effect model; *P* = 0.009 for both N1 and N2 assays; Fig. 1). The first two positive dog samples were sampled during the first event, whereas the third dog converted from negative to positive between the first and second sampling events. In all cases, the household with a positive dog had a second pet (two with dogs and one with a cat) that remained negative by RT-qPCR in all three sampling events.

A total of 39 dogs and 21 cats from 32 households had their food and water bowls tested, and a food bowl utilized by an RT-qPCR-positive dog likewise tested positive (2.6%) by RT-qPCR. This household also had a cat that tested negative by RT-qPCR at all three sampling events, and whose food bowl also consistently tested negative.

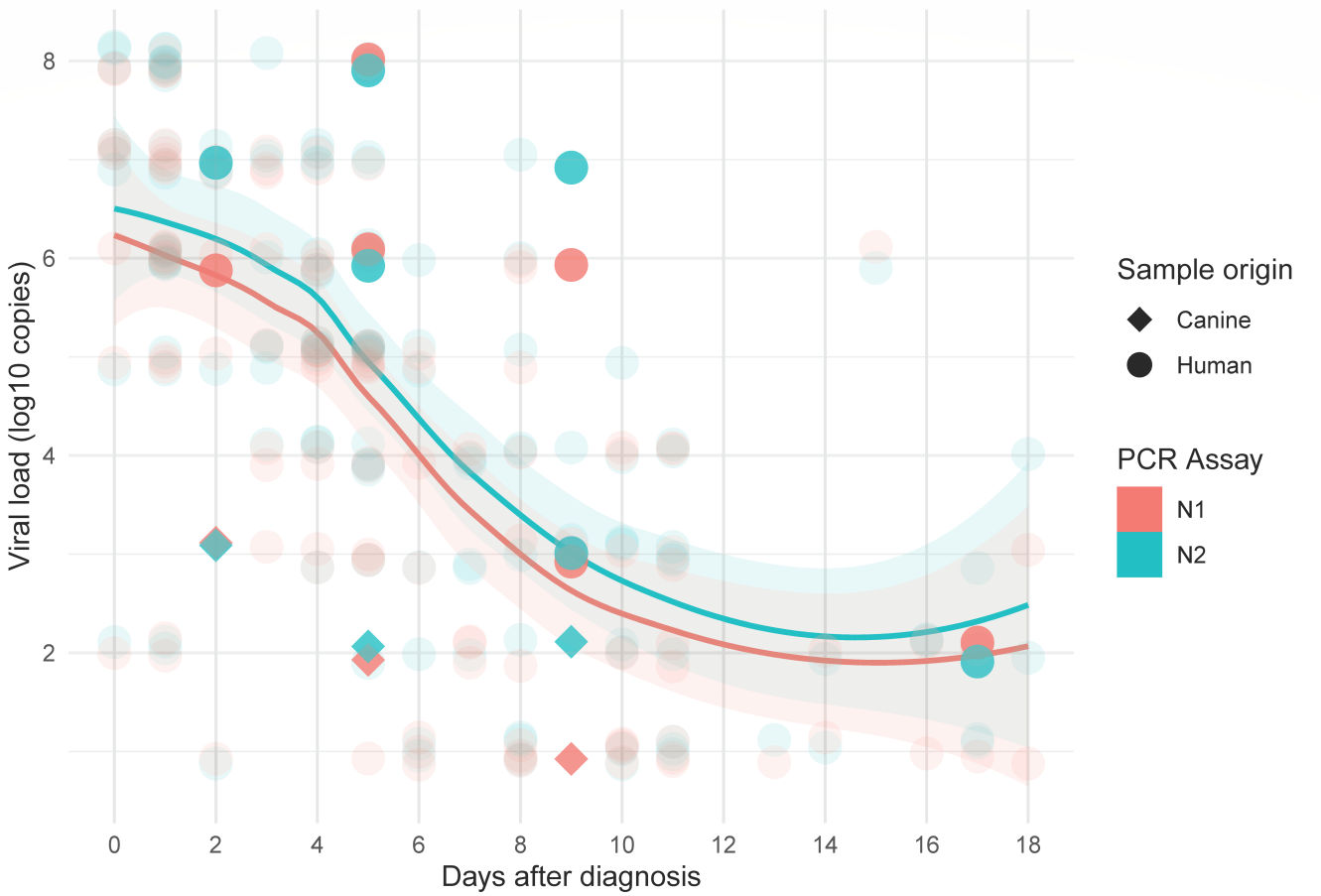

FIG 1 Distribution of SARS-CoV-2 estimated viral copies at the log10 scale from positive people (68 testing positive at least once, with a total of 133 positive samples) and dogs (n = 3) in relation to the number of days after diagnosis of first COVID-19 case in the household. Locally estimated scatterplot smoothing ("loess" function) was fitted for data from each RT-qPCR assay for human samples, displaying 95% confidence intervals. Viral copies from household members sampled on the same day as positive dogs and a positive food bowl (at day 17) are in bold colors.

We attempted to isolate infectious virus from RT-qPCR-positive samples collected from dogs and the food bowl by passaging them on Vero E6-TMPRSS2-T2A-ACE2 cells, but no viable virus was recovered. Ct values for these samples ranged from 32.5 to 36.9, with an estimated viral load of 100 copies/50 µL of sample or lower.

Whole genome sequencing reveals Omicron transmission between people and their pets

Whole-genome sequencing revealed that viruses detected in humans, dogs, and a food bowl from each of the three households clustered in monophyletic clades by household (Fig. 2).

The subvariant BA.1.1 was detected in samples collected from a dog (GISAID accession EPI_ISL_18065574, GenBank accession OR398175) and from two people (EPI_ISL_18065564, EPI_ISL_18065569; OR398179, OR398183) living in the same household (HH 9A). Sequencing the virus collected at the second sampling event from one of these two people revealed the same virus sequence (EPI_ISL_18065567; OR398182). Sequences obtained from humans and from the dog had 99.6%–99.7% and 95.8% coverage of the SARS-CoV-2 genome, respectively, being identical to each other when excluding gaps in coverage.

In a second household (HH 21A), a dog and a person were infected by the Omicron lineage BA.2.3.4 (EPI_ISL_18065573; OR398173 and EPI_ISL_18065570; OR398184, respectively). We successfully sequenced the same virus genome from this person at the

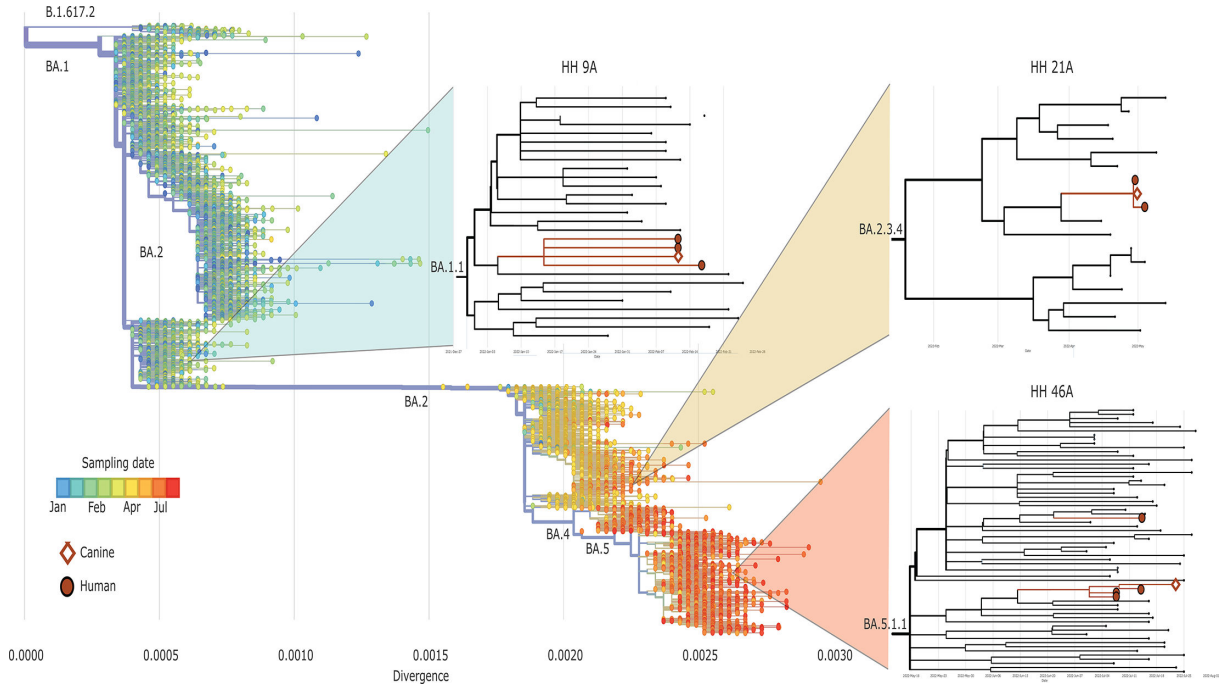

FIG 2 Phylogenetic context of SARS-CoV-2 from dogs in relation to people from the same households. Genome sequences of SARS-CoV-2 from each household with an infected dog were analyzed alongside 15,305 sequences from community surveillance in Texas collected within 2 weeks before or after the start of sampling at each household. The main phylogeny at the top left shows sequence divergence with branch labels for the major PANGO lineages and color-coding for the time of specimen collection. Insets show specimens from each household displayed with the most closely related surveillance samples and positioned by collection date. Viral hosts are indicated with markers: circle for human; diamond for canine (including water bowl in HH 46A).

second sampling event (EPI_ISL_18065566; OR398181). Sequence coverage was 99.5%–99.6% for human and 98.2% for dog samples, and viral genomes were identical to each other.

In the third household (HH 46A), the Omicron lineage BA.5.1.1 was sequenced from two people during the first sampling event (EPI_ISL_18065565, EPI_ISL_18065562; OR398180, OR398177; sequencing coverage = 99.6%); a second viral sequence was obtained from one of the people during the second sampling event (EPI_ISL_18065563; OR398178; sequencing coverage = 99.6%) and from the other person during the third sampling event (EPI_ISL_18065561; OR398174; sequencing coverage = 90.1%). The virus sequences obtained during the first two sampling events were identical to the one obtained from a dog food bowl that was positive in the third sampling event (EPI_ISL_18065572; OR398176; sequencing coverage = 93.1%). The virus sequenced from one person sampled in the third event, 14 days after the first sampling, had six single nucleotide polymorphisms (SNP) and a six-nucleotide deletion compared with the other sequences obtained from this household. One of these mutations (C27532A), which was not present in the first sample sequenced from this person, placed this sequence into a separate clade within BA.5.1.1 from Texas, suggesting an independent infection event. The virus detected from the dog was not successfully sequenced. Shotgun metagenomic sequencing was conducted to verify the species origin of samples from the positive food bowl from this household, which showed a mixture of not only mitochondrial DNA matching human and canine but also chicken, cow, and pig, likely reflecting dog food components.

Limited seropositivity in pets during the initial Omicron wave

We used an ELISA assay targeting IgG against SARS-CoV-2 spike protein (Inbios, Seattle, WA, USA) in eluates from dried blood spots (DBS) collected from people in the first sampling event. This serological test detects IgG in response to either natural exposure

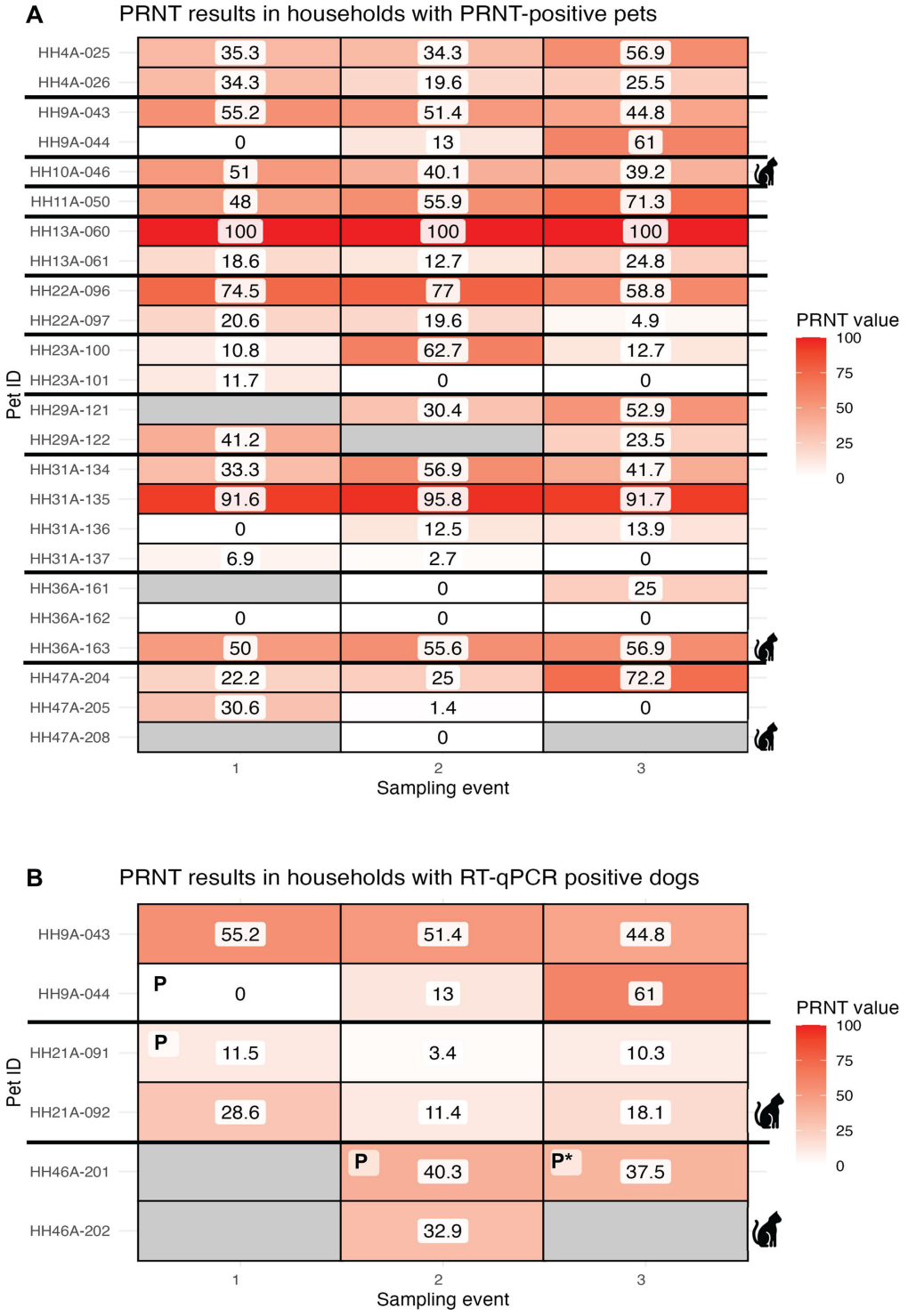

FIG 3 Plaque reduction neutralization assay (PRNT) results over time for dogs and cats sampled in households with a human COVID-19 case. Numbers inside boxes represent the proportion of viral plaques neutralized per pet per sampling event. (A) All pets from households with seropositive pets at one or more time points are displayed. (B) $PRNT_{50}$ for pets in households with RT-qPCR-positive dogs, which are identified with a "P". The food bowl from dog HH46A-201 was RT-qPCR-positive at the third sampling point ("P*"). Households are separated by thick horizontal lines; tiles in grey indicate missing data. Cats are indicated by icons; all other individuals are dogs.

to SARS-CoV-2 or vaccination. Overall, 95.3% of the people (82/86) were seropositive. Five people with serology data (5.8%) reported never having been vaccinated against COVID-19, three of whom were seropositive and tested positive by RT-qPCR, at days 3 and 5 after the initial COVID-19 diagnosis in their households. Only one person was vaccinated yet tested negative by serology.

Eleven out of 55 dogs (20%) had antibody titers capable of neutralizing the formation of at least 50% of viral plaques ($PRNT_{50}$-positive), two of which (18.2%) neutralized 90% or more virus plaques ($PRNT_{90}$) at 1:10 and at 1:20 dilutions during all three sampling events. Two cats (7.7%; n = 26) were $PRNT_{50}$-positive (Fig. 3A). Five goats, three horses, and one donkey tested negative for PRNT. Two households had two pets each that were seropositive, whereas five other multi-pet households had only a single seropositive animal (Fig. 3A). Two households had only one pet, which was seropositive in both cases.

SARS-CoV-2 active infection and/or past exposure in pets, as demonstrated by positivity by RT-qPCR and by PRNT, respectively, was not statistically different between dogs (23.6%) and cats (7.4%; Fisher's exact test, P = 0.13). Seroprevalence is lower for cats when compared to our pre-Omicron study (n = 146; 35.7%; Fisher's exact test, P = 0.005), but the rates are similar for dogs (n = 382; 24.9%; χ2 with Yates correction = 0.4, P = 0.54) (18).

The dynamics for RT-qPCR and $PRNT_{50}$ tests for pets positive by RT-qPCR and for other pets from the same household are shown in Fig. 3B. Only one RT-qPCR-positive dog had neutralizing antibodies ($PRNT_{50}$-positive) against SARS-CoV-2, which were first detected at the third sampling event. The other dog from this same household was $PRNT_{50}$-positive at the first and second sampling events. None of the other RT-qPCR-positive dogs or pets with which they were co-housed were $PRNT_{50}$-positive.

We examined viral plaque neutralization capacity over time among samples that did not meet the $PRNT_{50}$ threshold. At the first, second, and third sampling events, mean PRNT values were 21.9% (SD = 21.8), 24.2% (SD = 24.2), and 27.1% (SD = 23.6) for dogs, and 20.4% (SD = 16.3), 21.2% (SD = 14.6), and 16.2% (SD = 14.4) for cats, respectively, with no difference over time (GLMM, P = 0.29; Fig. S2).

Combining both RT-qPCR and serostatus data, infection rates are lower for cats when compared with our pre-Omicron study (n = 157; 35.7%; Fisher's exact test, P = 0.002) but are similar for dogs (n = 396; 27.3%; χ2 with Yates correction = 0.6, P = 0.68). Overall, 13 out of 43 (30.2%) households with active COVID-19 cases had pets infected/exposed to SARS-CoV-2, similar rates to pre-Omicron (n = 281; 39.1%; χ2 with Yates correction = 1, P = 0.31) (18).

Survey and odds of infection in pets

The only two pets reported by household members to have clinical signs of disease (coughing and lethargy) were dogs that tested negative by both diagnostic methods at all three time points. Logistic regression models indicated that the odds of detecting infected or exposed dogs were correlated with the number of days after human diagnosis in the first sampling event (Table 1; P = 0.01). Specifically, for each additional day after human diagnosis, the odds of being positive increased by a factor of 2.55 (95% CI: 1.40–6.16). No correlation was observed for the second and third sampling events. Male dogs had 5.74 higher odds of being positive compared with females (95% CI: 1.25–34.14; P = 0.03). Although the number of infected people administering medicine or providing treats to dogs showed a trend toward reducing the odds of positivity (odds ratio = 0.37, 95% CI: 0.10–1.05), this effect was not statistically significant (P = 0.09). None of the other explanatory factors included in the initial models were retained in the final model (Table 1).

DISCUSSION

We conducted a longitudinal study examining humans and their pets for SARS-CoV-2 infection during the peak of the BA.1 Omicron wave to the BA.2/BA.5 wave. By obtaining full virus genomes from people, from pets, and from a food bowl utilized

by an RT-qPCR-positive dog, we confirmed horizontal transmission from humans to their pets within households. We detected SARS-CoV-2 RNA and neutralizing antibodies at low rates in pets, particularly cats, despite multiple samples starting shortly after human COVID-19 diagnosis (average of 2.2 days). We show transmission dynamics within houses, with the conversion of a dog from RT-qPCR-negative to positive between consecutive sampling events 6 days apart. Additionally, late seroconversion was observed in most pets that were seropositive at least once, emphasizing the importance of longitudinal sampling in SARS-CoV-2 studies.

SARS-CoV-2 infection in cats was less frequent in this study compared with our pre-Omicron cross-sectional survey conducted in Texas during the first 1.5 years of the pandemic (18). Similarly, higher infection rates in cats were observed in other pre-Omicron studies, including a longitudinal survey conducted in Brazil during the initial year of the pandemic (19) and a cross-sectional study performed in the Netherlands between July 2020 and April 2021 (17). In contrast, infection rates in dogs did not differ significantly before and after the emergence of Omicron among these studies. However, an epidemiological study in Germany reported a marked reduction in seropositivity in both cats and dogs after the emergence of the Omicron variant (20). Continued studies investigating SARS-CoV-2 infections are warranted to assess human-to-pet transmission risk in cats and dogs, as different companion animal species may have different levels of susceptibility and/or exposure to new variants emerging in human populations.

The estimated number of viral copies in dogs was consistently below the lower limit of the confidence interval of the values from human samples collected at the same reference time, indicating lower viral loads in dogs. Within the three households with RT-qPCR-positive dogs, all co-housed pets remained negative despite sequential sampling. Ct values (as a proxy for viral copies) may or may not be correlated with the likelihood of detecting infectious virus in biological samples (reviewed by Puhach et al. [21]), but our results provide epidemiological data showing that the low infectability of dogs during the high exposure time period, and lack of onward transmission to other pets, may be associated with low viral copies during active infections. Failure to isolate virus from RT-qPCR-positive swab samples from pets further confirms that they were not shedding or shedding extremely low levels of infectious virus at the time of sampling. We, therefore, suggest that Omicron variants are not efficiently transmitted from humans to pets or between pets under natural conditions. Factors such as increased levels of immunity due to vaccines and prior infection in humans could have contributed to reduced human-to-pets transmission.

Of note, 49% of dogs and 48% of cats were living with between two and four people with active COVID-19 who were not taking precautions to prevent SARS-CoV-2 transmission to their pets in most cases, revealing high chances of natural exposure. However, none of the dog-human interaction factors were associated with increased odds of infection. For instance, sharing food with pets has been linked to increased human-to-animal transmission of SARS-CoV-2 in Ecuador (22), but we did not detect this effect. The small number of households and pets tested may have limited the power of our analysis.

Genomic epidemiology has inferred only two and four cases of transmission of SARS-CoV-2 from dogs and cats to humans, respectively (23). However, the inferred number of transmissions from humans to pets was at least 13 times higher (23). Our results suggest that pets may not transmit the Omicron variants efficiently intra- and inter-species under natural conditions, which may explain the low likelihood of pet-to-human transmission.

Whole genome sequencing confirmed that dogs and people from the same household were infected by the same virus in two cases, with no variation in the sequences recovered from the animals and people. Additionally, the same virus infecting people was present in the food bowl from a household with a positive dog. One human sample from this household contained a viral sequence with six SNPs and a deletion relative to other viral sequences from the household, including one obtained from the

same individual 2 weeks earlier. This number of mutations is at the extreme end of what is typically observed among viruses collected from the same household (24) or in persistent infections (25), suggesting a separate introduction of SARS-CoV-2 into the household. This inference is supported by one of these mutations being shared with sequences obtained from community surveillance activities in Texas in the weeks prior. Reinfections within a period of a few weeks are rare but have been reported (25).

Our detection of an RT-qPCR-positive dog food bowl may reflect use by a positive dog and/or contamination by a positive household member. People experimentally infected with the wild-type SARS-CoV-2 contaminated household items with the virus (26). Moreover, contaminated surfaces may be correlated with increased within-household transmission risk (27). Both people from the household with the positive food bowl were positive at the first sampling event. However, the food bowl tested positive only at the third sampling, when only one person was still positive, whereas the dog was positive at the second sampling event only. This suggests that viral RNA detected in the food bowl was derived from the positive dog and is further supported by the fact that the food bowl used by a cat from this household was negative during all three sampling events.

Experimentally infected cats shed lower virus loads of an Omicron variant when compared with pre-Omicron variants (11). Additionally, a recent study did not isolate infectious viruses from cats infected with a low dose of Omicron, despite recovering infectious viruses from cats infected with the ancestral, Gamma and Delta strains (28). This may explain the lack of SARS-CoV-2 detection in cats in this study, and the lower detection rates when compared with our similar pre-Omicron study in Texas (18).

In experimental challenges, cats seroconvert at 7 days post-challenge with pre-Omicron strains, and dogs seroconvert 7–14 days (2, 11). However, Omicron-infected cats display delayed seroconversion between 7 and 14 days post-infection (11) or do not seroconvert when exposed to low viral doses (28). The repeated nature of our study allowed the detection of late seroconversion in seven out of 11 seropositive dogs. We also observed that most seropositive pets had low antibody titers ($PRNT_{50}$-positive at 1:10 serum dilution only). These low titers could explain why 30.1% of these pets were seronegative in a subsequent sampling event, suggesting waning neutralizing antibodies within 1–2 weeks. All but one of these pets were negative by RT-qPCR, indicating that the low titers may alternatively reflect exposures to SARS-CoV-2 earlier in the pandemic. Lack of seroconversion in two of the three infected dogs, including one that was resampled 5 and 11 days after the positive RT-qPCR diagnosis and another resampled 8 days after RT-qPCR positivity, suggests late seroconversion or undetectable titers of neutralizing antibodies.

In conclusion, multiple Omicron variants were detected in household clusters among dogs and household members; however, infection rates in cats were lower than those in pre-Omicron studies. The low viral load (high Ct values) and lack of infectious virus in samples from dogs, associated with a lack of evidence of onward transmission between pets, indicate that dogs and cats were unlikely to act as amplifying hosts for early Omicron variants. As host-breadth and virus fitness change with the evolution of new variants, continued surveillance using One Health approaches may be critical as new waves driven by a diversity of viruses are expected for years to come.

MATERIALS AND METHODS

Recruiting and sampling

Between January and July 22, 2022, we recruited people and pets living in the same household as a person with a SARS-CoV-2 infection via the COVID-19 portal of Texas A&M University (TAMU). Participating persons responded to a short questionnaire by phone to tally the number of humans and pets living in the house; human COVID-19 vaccination

history, date of positive human test result, pet species and signalment (breed, age, and sex), and any pet clinical signs of disease.

We visited each house three times over an approximately 2-week period for sample collection. We collected nasal swabs from people during all three sampling visits. From the pets, we collected nasal and oral swabs that were combined into a single vial containing 3 mL of viral transport media (VTM; made following CDC SOP#: DSR-052-02), whereas rectal swabs were stored in a separate vial with 3 mL of VTM. Additionally, in a subset of households, we collected swab samples from food and water bowls utilized by the pets. Swabs in VTM were stored in a cooler with ice packs until arrival in the laboratory, where samples were stored in a −80°C freezer.

From humans, we collected blood onto Whatman protein saver cards (Sigma-Aldrich, St. Louis, MO, USA) via finger prick at the first visit only. These samples were air-dried and stored at room temperature. From pets, blood was collected from either jugular, cephalic, or saphenous veins into clot activator tubes and kept in a cooler until centrifugation with serum aliquots stored at −80°C. Upon completion of the third visit, we issued an incentive of a $100 gift card to H-E-B grocery store or Amazon to each participating household.

Molecular testing

VTM aliquots from humans, pets, food, and water bowls were shipped to the Wisconsin Veterinary Diagnostic Laboratory for RT-qPCR targeting nucleocapsid gene region 1 (N1) and nucleocapsid gene region 2 (N2) (29, 30) of SARS-CoV-2. Cycle threshold (Ct) values were reported as the average of the values for regions N1 and N2. Absolute quantification of the viral load was not performed during the testing of the clinical samples. However, the semi-quantification of viral copies was inferred from the standard curve generated during assay validations and expressed as an estimated log10 viral copies/50 µL of sample for N1 and N2 assays separately.

Virus isolation

Aliquots of VTM samples from the three pets and the food bowl that tested positive for RT-qPCR were transferred to a BSL-3 laboratory at Texas A&M University. For virus isolation, 100 µL of VTM with 900 µL of 1× Dulbecco's modified Eagle medium (DMEM) via syringe filtration using a 0.2 µm pore size onto Vero E6-TMPRSS2-T2A-ACE2 (BEI Resources, NR-54970) cells expressing both endogenous cercopithecine ACE2 and TMPRSS2 as well as transgenic human ACE2 and TMPRSS2. Plates were incubated for 72 h, and the presence of cytopathic effects was evaluated using a brightfield microscope following published protocols (31).

SARS-CoV-2 whole genome sequencing, metagenomics, and phylogenetic analysis

Aliquots of VTM from infected pets, food bowls, and the humans living with infected pets were sent to the CDC for SARS-CoV-2 whole genome sequencing according to established protocols (32). Phylogenetic trees were inferred using RAxML in the NextStrain pipeline (v7.1.0 [33]) with all 15,305 SARS-CoV-2 genomes from the GISAID database that were detected in Texas 2 weeks before or after the start of sampling at each household. PANGO lineages were assigned using "pangolin" (software v4.3.1; data v1.29 [34]). Mammalian mitochondrial DNA was identified by untargeted metagenomic sequencing as described previously (35), followed by mapping reads to a database of mtDNA sequences representing clusters with 93% sequence identity (36).

Serologic testing

At the TGen North laboratory, human dried blood spots were eluted to 1:100 in dilution buffer. Anti-SARS-CoV-2 antibodies were detected using a qualitative SARS-CoV-2 IgG

ELISA assay (InBios, Seattle, WA, USA). All assays, including controls, were used as per the manufacturer's recommendations.

Pet serum samples were tested by plaque neutralization tests (PRNT) at Texas A&M University Global Health Research Complex to quantify neutralizing antibodies against SARS-CoV-2 in BSL-3 following the protocol described by Roundy et al. (37). Briefly, we used Vero CCL-81 cell cultures in 6-well plates and SARS-CoV-2 isolate USAIL1/2020, NR 52381 (BEI Resources, Manassas, VA, USA) for an initial screening using serum samples at a dilution of 1:10 to test their ability to reduce virus plaques by at least 50% when compared with the virus control, a well-established method (38), with a sensitivity of 97% (39), which reduced the chances of assigning pets as false-negative in our study. The subset of positive serum samples with antibody titers able to reduce more than 90% of virus plaques was further tested at 2-fold serial dilutions to determine 90% endpoint titers.

Statistical analysis

We used either χ^2 test with Yates' correction or Fisher's exact test to compare positivity rates by RT-qPCR and $PRNT_{50}$ between this study and a previous one conducted pre-Omicron in the same region in Texas (18).

We employed linear mixed-effect models (using the *lme4* package [40]) to analyze changes in the number of estimated viral copies from positive samples using species (humans or dogs), days after diagnosis and sampling event as fixed effects, and host ID as random effects. We used separate models for N1 and N2 genes, which produced similar results. To assess changes in PRNT values over time, we employed a GLMM using the *lme4* package, adding species as an explanatory variable and also using animal ID as a random effect. We used a Tweedie distribution with a log link function because PRNT values presented a non-normal distribution and had values equal to zero. We performed backward stepwise selection using the Akaike Information Criterion (AIC) to identify significant predictors.

We built a logistic regression model to determine factors associated with the risk of dogs becoming infected with SARS-CoV-2. Using the *car* package (41), we detected high multicollinearity (correlation values above or below 0.85 and −0.85, respectively) among some explanatory variables. For example, the number of infected people petting, cuddling, and not taking precautions to not transmit SARS-CoV-2 was highly correlated with the number of infected people per household. Therefore, only the latter variable was kept. Similarly, the number of infected people sharing the bed and room with pets was also correlated, and only the latter was included. The initial model included the number of days after diagnosis, pet sex, number of infected people in household, geometric mean of viral copies in people in household, number of infected people interacting with dogs (sleeping in the same room, kissing, sharing food, giving medicine, and treats by hand), and whether the pet stayed >75% indoors. We performed backward stepwise selection using AIC to identify significant predictors in these models using the package *MASS* (42). The coefficients from the final model were used to calculate odds ratios (OR) and their 95% confidence intervals (CI). We did not build models for cats because only two individuals tested positive. We performed all analyses using R 4.2.2 (43).

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The authors declare no competing interests.

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DATA AVAILABILITY

The data reported in this study are available in OAKTrust (http://hdl.handle.net/1969.1/1582327). Virus genome sequences are available in GISAID (https://gisaid.org/; accession numbers EPI_ISL_18065561-67, EPI_ISL_18065569-70 and EPI_ISL_18065572-74) and in GenBank (https://www.ncbi.nlm.nih.gov/genbank/; accession numbers OR398173–OR398184). The following reagent was obtained through BEI Resources, National Institute of Allergy and Infectious Diseases, National Institutes of Health: Cercopithecus aethiops Kidney Epithelial Cells Expressing Transmembrane

Protease, Serine 2 and Human Angiotensin-Converting Enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2), NR-54970.

ETHICS APPROVAL

The TAMU Institutional Animal Care and Use Committee (2018-0460 CA) and Clinical Research Review Committee approved animal sampling. In accordance with 45 CFR 46.102(1)(2), the TAMU Institutional Review Board issued a determination of 'not human research' on 24 September 2021, as the activities met the public health surveillance requirements.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental material (mSphere00074-25-s0001.docx). Supplemental figures.

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