Household transmission of SARS-CoV-2 from humans to dogs in Washington and Idaho: burden and risk factors

Short title: Household transmission of SARS-CoV-2 from humans to pets

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ABSTRACT

Background: SARS-CoV-2 is believed to have emerged from an animal reservoir as a zoonotic pathogen. Over the course of the current pandemic, evidence has mounted that infected humans can transmit the virus to animals including household pets, however the frequency of and risk factors for this transmission remain unclear. We carried out a community-based study of pets in households with one or more confirmed SARS-CoV-2 case among the human residents, and report here on interim findings from sampling of dogs.

Methods: Data collection included a survey of human and animal demographic and clinical variables, features of their shared environment, and human-animal contact; blood collection from animals for serology for anti-SARS-CoV-2 antibodies; and nasopharyngeal sampling for PCR testing for SARS-CoV-2.

Results: Sampling consisted of 67 dogs from 46 households. Nasopharyngeal PCR testing results were available for 58 dogs, and serological testing results were available for 51. Clinical signs consistent with COVID-19 were reported in 14 dogs (23.7%, 95% CI 0.13, 0.35), and SARS-CoV-2 antibody testing using viral receptor binding domain ELISA was positive in 22 dogs (43.1%, 95% CI 0.30, 0.57). All PCR tests of nasopharyngeal swabs were negative. Survey respondents commonly reported close human-animal contact, and the majority of households were aware of and adopted measures to mitigate human-to-animal transmission of SARS-CoV-2 following diagnosis. While no statistically significant associations were detected between human-animal contact variables and either seropositivity or COVID-19 like illness in dogs, positive trends were found for sharing beds with humans and the number of SARS-CoV-2 positive humans in the corresponding household. Reported measures taken by the household to mitigate transmission

showed a protective trend, and COVID-19 like illness in a dog was positively associated with seropositivity in that dog.

Discussion: These data indicate that human-to-animal transmission of SARS-CoV-2 in households is common, in a study population characterized by close human-animal contact. They also indicate that infected pets often manifest signs of COVID-like illness. While nasopoharyngeal sampling of dogs in this study has not to date demonstrated positive PCR results, this could be due to delays in sampling. Household members reported taking precautions to protect pets from SARS-CoV-2 infection, indicating an opportunity for further measures to reduce transmission of SARS-CoV-2 between people and animals sharing households.

Keywords: SARS-CoV-2; COVID-19; zoonoses; One Health; anthropozoonoses; household transmission

Abbreviations: DAG: directed acyclic graph

BACKGROUND

Coronaviruses occur in multiple mammalian species, and SARS-CoV-2 virus, the etiological agent of COVID-19 infection, is thought to have jumped to humans from a mammalian source [1]. While currently the virus is spreading person to person, the ACE2 receptor involved in SARS-CoV-2 transmission is present in multiple species and there are numerous anecdotal reports of companion animals becoming infected, including dogs and cats. At the date of this writing, 76 cats and 51 dogs in the USA have been reported by USDA-APHIS to have confirmed SARS-CoV-2 infection based on PCR or antibody testing. Workplace transmission of SARS-CoV-2 between humans and animals has also been documented, including in zoos (felids and non-human primates) and on mink farms [2,3]. This is consistent with previous reports of SARS-CoV-1 infecting cats and ferrets, as well as laboratory studies demonstrating experimental SARS-CoV-2 infection of non-human primates, ferrets, hamsters, and rabbits [4]. Less is known, however, about the frequency of and risk factors for SARS-CoV-2 transmission between humans and companion animals in a household setting. Furthermore, the natural history of COVID-19 infection in pets is poorly understood.

Given the close contact many people have with their pets and the intimate nature of their shared environment, in particular during periods of quarantine or isolation, it is important to better understand the role of companion animals in community infection patterns, including whether such transmission contributes to virus evolution and emergence of novel strains. In light of evidence from mink farms that animal-origin variants may contain spike mutations and other changes that could affect clinical features of infection[5,6], ongoing monitoring of SARS-

CoV-2 transmission between humans and animals in household and other human-animal contact settings remains critical.

We report interim findings from the COVID and Pets Study (CAPS), a cross-sectional community-based study of animals in households of persons with documented COVID-19 infection conducted from 2020 to 2021 in Washington and Idaho. The goal of the study is to describe the frequency of transmission between humans and animals within a household, and to determine human, animal, and environmental risk factors for that transmission, in a One Health framework.

METHODS

The COHERE [7] and STROBE [8] statements were used to guide reporting of the findings and the preparation of this manuscript.

Study population

We recruited households for this study, defining a household as one or more persons ages 18 or older, co-housing, or co-sheltering in the case of unhoused individuals, with at least one pet that does not live solely outdoors. Pets were defined as dogs, cats, ferrets, and hamsters based on prior research documenting experimental COVID-19 infection in these species [9,10].

We conducted this study in King, Snohomish, Yakima, Whitman, Pierce, Spokane, and Benton counties in Washington, and Latah County in Idaho. Enrollment began in April 2020, and continues at the time of publication.

Study design

CAPS is a cross-sectional study with individual- and household-level data collection, with a longitudinal component for households with PCR positive pets. Study participation involved two components, detailed below: an online survey followed by animal sampling.

Study team

Our study team was comprised of veterinarians, microbiologists, physicians, epidemiologists, environmental health experts, and medical anthropologists from the University of Washington's Center for One Health Research, and Washington State University's College of Veterinary Medicine, Washington Animal Disease Diagnostic Laboratory, and Paul G. Allen School for Global Health.

Recruitment and eligibility

Households were recruited through partnerships with other COVID-19 clinical trials, social media, word of mouth and through community partners. Individuals were screened for eligibility using the UW Research Electronic Data Capture (REDCap) system [11], a HIPAA-compliant web tool for clinical research, with criteria including county of residence, pet ownership, and one or more household member with confirmed SARS-CoV-2 infection.

During eligibility screening participants were asked to confirm that any animals to be sampled were up to date on their rabies vaccination, and were suitable for sampling based on knowledge of that pet's behavior when receiving veterinary care. Animals with known fearful and/or aggressive behavior in response to restraint were excluded from sampling, however the corresponding household was not excluded from completing the REDCap survey, nor from animal sampling if other animals residing in the household were amenable to sampling.

Ethical approvals

143 This study and its protocols received ethical approval from the University of Washington's
144 Institutional Review Board STUDY00010585) and Office of Animal Welfare (PROTO201600308:
145 4355-01). Informed consent was obtained from human subjects via REDCap, or over the phone
146 with the study coordinator if preferred by the participant, after the nature and possible
147 consequences of study involvement had been explained. Once eligibility was confirmed and
148 consent was obtained, individuals then completed the online survey.

149 **Survey**

150 A comprehensive survey was completed by a person living in the same household as the pet(s)
151 prior to scheduling of the sampling visit. Surveys could be completed by the study participant
152 online using the REDCap interface, or on the phone with the study coordinator if preferred.
153 Human items included symptoms, timeline, and severity of COVID-19 infection and illness for
154 any affected household members (including individuals who did not have confirmatory testing),
155 and comorbidities. Animal items were stratified on individual animal, and included veterinary
156 clinical variables, history of COVID-like illness, and contact between individual animals and
157 individual members of the household including questions pertaining to co-sleeping, kissing, and
158 sharing of glassware and other food containers ("utensils"). Environmental items included type
159 and size of home, type of flooring (carpet, wood, etc.), and availability of outdoor space for pets
160 to roam.

161 A second brief survey was completed verbally at the time of sampling to collect data on
162 changes in the clinical status of human and animal household members since the REDCap
163 survey was completed, including new hospitalizations, symptoms, or COVID-19 diagnoses.

Confirmation of COVID-19 positive status and testing date was also performed at this time through review of test results by the sampling team.

Animal sampling

Sampling was performed by a team of two study personnel, one veterinarian and either a second veterinarian or an assistant trained in ethical animal restraint. In most cases sampling was conducted at the participant's home, however several animals were tested at veterinary clinics. No chemical restraint was used, nor muzzles due to biosafety concerns. When possible, sampling was performed outdoors to minimize the study team's exposure, however the same PPE and health and safety protocols were adhered to regardless of whether sampling was indoors or outdoors.

Species-appropriate restraint was employed using standard techniques to allow for venipuncture and collection of 3 mL of blood into a labeled serum separator tube. Following venipuncture, swab samples were collected from both rostral nares/nasal passage and the caudal oropharynx, and placed into one Primestore MTM tube [Longhorn Vaccines and Diagnostics]. If an animal started to exhibit severe signs of stress and/or aggression during restraint, attempts to sample were halted to maintain human and animal safety. All participants received educational information about measures to mitigate household COVID-19 transmission from the field team.

Swab and serum samples were transported on ice within 24 hours to the Washington Animal Disease Diagnostic Laboratory (WADDL) for PCR and antibody testing.

Testing

<u>SARS-CoV-2 RT-PCR</u>

Total nucleic acid was extracted from nasopharyngeal swab samples in 1mL of PrimeStore MTM [LongHorn Diagnostics] using MagMAX™-96 Viral RNA Isolation Kit [ThermoFisher, Waltham, MA 02451], per the manufacturer's instructions. Reverse transcriptase (RT) real-time PCR to the SARS-CoV-2 RNA-dependent RNA polymerase gene (RDRp) was performed as previously described using SARS-CoV-2 primers RdRp_SARSr-F2 5'-GTGARATGGTCATGTGTGGCGG-3' and COVID-410R 5'-CCAACATTTTGCTTCAGACATAAAAAC-3' [12], using TaqMan Fast Virus 1-Step Master Mix Kit [Thermo Fisher]. RNA amplification was done using ABI 7500 Fast (ThermoFisher, Waltham, MA 02451). Controls included positive extraction control (RdRp_GATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACTATATGT TAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTGTCAA GCTGTCACGGCCAATGTTAATGCACTTTTATCTACTGATGGTAACAAAATTGCCGATAAGTATGTCCGCA ATTTAC), negative extraction control (PCR water), positive amplification control (SARS-CoV-2 whole genome RNA), and negative amplification control (No template control). Graphs and tabular Ct results were reviewed on the ABI 7500 FAST program. Unknown samples were considered positive if they rose above the threshold by cycle 45. All others were considered negative.

SARS-CoV-2 Spike Protein ELISA

For dog antibody testing, WADDL developed a SARS-CoV-2 ELISA assay using recombinant SARS-CoV-2 Spike Receptor Binding Domain protein as antigen (S-RBD). The recombinant RBD was obtained from the UW Center for Emerging and Reemerging Infectious Disease (CERID) laboratory of Dr. Wesley Van Voorhis through an institutional Material Transfer Agreement. WADDL used an in-house standard operating procedure for indirect ELISA of SARS-CoV-2 in 96-

well format based up a previous publication in humans. The major components of the assay included: 1) rS-RBD coating of plates as target antigen (2ug/ml in Sigma Carbonate-Bicarbonate Buffer); 2) 1:100 dilution of test sera (diluted in ChronBlock ELISA Buffer-Chondrex Inc.); 3) anti dog IgG-HRP as linker (Southern BioTech goat anti-canine IgG) and 4) Sigma (TMB) liquid substrate system to develop OD. Plates were blocked with ChronBlock ELISA buffer per manufacturer's instructions, washing solution consisted of PBS+0.1% Tween 20 (Sigma), and plates were read on a plate reader at 450 nM. Test samples and controls were run in triplicate. The negative controls consisted of sera from six pre-COVID dogs, archived at WADDL, run in triplicate and the mean utilized as "OD negative controls". The positive cutoff of 2.0 test OD:negative control OD equated to mean of negative controls + 3 standard deviations of the mean.

Statistical analyses

A study database was created using an anonymous identifier to store epidemiological and clinical data through REDCap. All analyses were conducted in R [13].

The primary aim of this study was to estimate the burden of household SARS-CoV-2 transmission from humans to their pets. Secondary aims included describing the nature of human-animal contact within households, and identifying risk factors for household transmission, including human-animal contact.

Outcome

Animal infection with SARS-CoV-2 was defined as an animal meeting one or more of the following criteria: (1) seropositive status, (2) PCR positive status, or (3) COVID-19 like illness, defined as participant answer of "yes" to the survey question: "Since the time of COVID

diagnosis/symptom onset in the household, has this animal had any new issues with difficulty breathing, coughing and/or decreased interest in playing, walking, or eating?"

Descriptive statistics

All descriptive statistics were generated at the animal-level. Key variables included human-animal transmission, animal and human clinical variables, environmental variables, and human-animal contact variables. If there was more than one SARS-CoV-2 positive household member, the index case was defined as the person completing the survey.

Regression models

Outcomes

Outcome was defined as an animal case of SARS-CoV-2, defined above. Separate regression models were fit for each outcome definition.

Exposures

Household-level exposures for animal infection included residence in house versus apartment or condominium (binary), home size in square feet (continuous), and the number of confirmed SARS-CoV-2 cases (continuous).

Animal-level exposures for infection included bedsharing with one or more human household members (binary), sharing eating utensils with humans (binary), and SARS-CoV-2 positive household members taking precautions to prevent transmission to their pets following diagnosis, including not petting or kissing the animal, staying in a different room, and having someone else feed and walk the animal (binary).

We also examined the association between canine seropositivity and COVID-19 like illness in the animal, and between seropositivity and time since the animal was first exposed,

252 defined as 2 days prior to the first date any household member had symptoms of COVID-19 or
253 tested positive, whichever was earlier.

254 *Confounders*

255 We identified possible confounders *a priori* using a directed acyclic graph (DAG; Figure 1). The
256 minimum sufficient adjustment set was defined, using this DAG and DAGitty.net, separately for
257 each exposure [14].

258 For house type, the minimum sufficient set was {SES}; for indoor-only status, the
259 minimum sufficient set was {number of positive household members, house size, house type,
260 precautions taken, bedsharing, and sharing eating utensils}; for house size, the minimum
261 sufficient set was {SES, house type}; for sharing eating utensils, the minimum sufficient set was
262 {number of positive household members, house type, house size, indoor-only status,
263 precautions taken, and bedsharing}; for number of positive household members, the minimum
264 sufficient set was {SES, house size}; for bedsharing, the minimum sufficient set was {number of
265 positive household members, house size, house type, indoor-only status, precautions taken,
266 and sharing eating utensils}; and for precautions taken, the minimum sufficient set was
267 {number of positive household members, house size, house type, indoor-only status,
268 bedsharing, and sharing eating utensils}.

269

270 **[Figure 1: Directed acyclic graph for human-animal SARS CoV2 transmission.** Variables
271 outlined with a square are the exposures of interest, while outcome (approximated by
272 serostatus, PCR result, and COVID-19 like illness in separate models) is outlined with a circle.
273 HAB: human-animal bond; SES: socioeconomic status; took precautions: SARS-CoV-2 positive

household member(s) took precautions to prevent transmission to pet; indoor-only: animal does not go outdoors; bedshare: animal shares a bed with one or more household members.**]**

Models

For each exposure of interest we implemented a generalized estimating equation (GEE) approach with an exchangeable working correlation structure, household as the clustering variable, and binomial models with a logit link, using the geepack package in R [15]. For regression of serostatus on COVID-19 like illness and time since first exposure, we performed logistic regression using the glm() function in R.

RESULTS

Recruitment

Out of 70 households enrolled to date, 54 had completed the REDCap survey. Out of these 54 households, 35 were in King County, 6 in Whitman County, 2 in Pierce County, 1 in Spokane County, 1 in Benton County (all Washington), 8 in Latah County, Idaho, and one unknown (no sample visit conducted). There were four households in which the index case's date of diagnosis was not confirmed by the study team during sampling. No unhoused households have been recruited to date. After subsetting to households containing dogs, 67 dogs from 46 households were available for analyses. The data for cats are undergoing a separate analysis, and no ferrets or hamsters have been enrolled or sampled.

Recruitment flow is detailed in Figure 2. The two households removed in the final stage correspond to a dog which was moved from the participant's home to a family member's home

296 immediately after the onset of the participant's COVID-19 symptoms. That family member
297 subsequently tested positive, thus the dog and corresponding households were removed from
298 analyses as it was difficult to determine which household should be assigned to this dog for
299 analysis. This dog was seropositive.

300 Sample collection is detailed in Figure 3. Out of 67 dogs corresponding to households
301 with completed surveys, two belong to one household which had not yet been sampled, and a
302 third belongs to a household which was unable to be sampled. Six dogs belonged to households
303 in which other animals were sampled but these dogs were judged unsafe to be sampled for PCR
304 or serology, while an additional seven dogs were judged safe to restrain for swab samples but
305 not for serum collection.

306

307 **[Figure 2: Flowchart depicting study recruitment.** Two households were removed at the final
308 step as the corresponding dog was moved, immediately after onset of the resident's illness,
309 from the household of residence to a family member's household.**]**

310

311 **[Figure 3: Flowchart depicting serological and PCR sampling.** Out of 67 dogs corresponding to
312 5 households with completed surveys, PCR testing is complete for 58 dogs, and serological
313 testing is complete for 51 dogs. The remaining dogs were not sampled due to safety concerns.**]**

314

315 <u>Descriptive statistics</u>

316 Descriptive statistics are presented in Table 1; note the unit of analysis in this table is a dog.
317 PCR results were available for 58 dogs and serology results were available for 51 dogs. Of these,

22 (43.1%, 95% CI 0.30, 0.57) of dogs were seropositive and 14 (23.7%, 95% CI 0.13, 0.35) had COVID-19 like illness reported. All dogs in the study were SARS-CoV-2 PCR negative from nasopharyngeal swab samples. There were 6 households with more than one seropositive dog: 5 households with two dogs each who were both seropositive, and 1 household with 5 dogs, two of whom were seropositive.

Nearly one-third of dogs engaged in activities outside of the household during periods of isolation or quarantine, 42 (63%) resided in households whose residents reported awareness of CDC guidelines to prevent human-animal transmission of SARS-CoV-2, and 33 (51%) resided in households which reported taking precautions to prevent such transmission to household pet(s) following diagnosis. With regards to human COVID-19 illness in household residents, only one dog resided in a household in which the case was hospitalized, however 28% resided in households in which the case had pre-existing conditions. Nearly all dogs had access to yards or gardens (85%) and were allowed on furniture (89%), and the majority were kissed by (69%) and shared beds with (72%) human household members. Almost all dogs' eating utensils were washed in the kitchen (95%).

[Table 1: Descriptive statistics for 67 dogs corresponding to 46 households. *mean (standard deviation). [a]Activity defined as going to a veterinary clinic or groomer, being walked off-leash, or visiting an off-leash park, dog park, kennel, or daycare facility. [b]Precautions to prevent human-animal SARS-CoV-2 transmission following diagnosis: not petting or kissing the animal, staying in a different room, and having someone else feed and walk the animal. [c]Guidelines to prevent human-animal SARS-CoV-2 transmission. [d]First diagnosis: earliest SARS-CoV-2 diagnosis

in the household; final diagnosis: last SARS-CoV-2 diagnosis in the household. [e]Prexisting conditions: diabetes, kidney disease, heart disease, hypertension, immunosuppression. [f]Household members who had COVID-19 symptoms but did not get tested.**]**

Regression models

Results of regression models are presented in Table 2 as prevalence odds ratios, reflecting the cross-sectional design of this study. With the exception of house size, which was adjusted for house type as the minimum sufficient adjustment set was very small for this exposure, confounders were not adjusted for due to concerns regarding overfitting arising from the small sample size. Effect modification, e.g. by animal age or sex, was not explored for the same reason.

No effect estimates reached statistical significance, however there were positive trends across both outcome definitions for bed sharing with humans and the number of SARS-CoV-2 positive humans in the corresponding households, and a negative effect for precautions taken to prevent SARS-CoV-2 transmission following diagnosis. We also found serostatus was associated with COVID-19 like illness.

[Table 2: Regression model results. House size was adjusted for house type, but no other models were not adjusted for confounders due to overfitting concerns. [a]Results available for 51 dogs. [b]House versus apartment or condominium. [c]Animals and humans share eating utensils. [d]Precautions taken to prevent human-animal SARS-CoV-2 transmission following diagnosis: not petting or kissing the animal, staying in a different room, and having someone else feed and

walk the animal. [e]First exposure defined as 2 days prior to first positive diagnosis in the household or onset of symptoms, whichever was earlier. POR: prevalence odds ratio; 95% CI: 95% confidence interval.**]**

DISCUSSION

We present the results of a cross-sectional, One Health study of dogs and humans sharing households where at least one human was infected with SARS-CoV-2. The study results indicate that household transmission of SARS-CoV-2 from humans to animals occurs frequently, and that these animals commonly display signs of COVID-19 like illness. Notably, in the vast majority of cases with multiple dogs in a household, all the dogs shared the same serostatus. We furthermore show that close human-animal contact is common among people and their pets in this study population, that this contact appears to facilitate SARS-CoV-2 transmission, and that pet owners in this population are familiar with and willing to adopt measures to protect their pets from COVID-19.

There are several limitations to our approach. First, several weeks had elapsed from first reported exposure to household sample collection from animals in most households, limiting our ability to detect viral shedding by PCR testing if nasal shedding is short-lived, but perhaps strengthening our ability to detect seroconversion. Second, we report here on the findings of the cross-sectional (baseline) component of our study. Were any pets to test PCR positive, a longitudinal component would follow. As the outcomes are common, our prevalence odds ratios do not approximate prevalence ratios. Third, our study is subject to residual confounding due to inability to adjust for confounders without risking over-fitting, with the exception of

house size, which was adjusted for house type. While we believe the confounders examined, most of which are also exposures of interest, are likely strong risk factors for the outcome, they are only strong confounders if they also have strong relationships with the exposure of interest. We do not expect this association to be strong for confounders that do not represent latent (and therefore difficult to measure and model) constructs, such as socioeconomic status, strength of the human-animal bond, and level of concern about zoonotic disease transmission.

With the exception of PCR testing, mentioned above, we do not expect strong measurement error in any of the variables examined. As no gold-standard for canine anti-SARS-CoV-2 serology exists we could not estimate sensitivity of our serological test, however all pre-COVID-19 samples evaluated were negative, indicating specificity approaches 100%. While our primary aim—to estimate the burden of human-animal SARS-CoV-2 transmission—was estimated with reasonable precision, as we were not able to estimate sensitivity of our serological test, we could not propagate uncertainty arising from imperfect sensitivity in our prevalence estimates. Furthermore, due to our small sample size variance was high for our estimated prevalence odds ratios. Finally, by nature of our recruitment methods and study population, generalizability of our findings is likely limited to highly-educated, higher-income individuals residing in urban and suburban communities.

CONCLUSIONS

These limitations aside, our study contributes important and novel findings to the literature on cross-species transmission of SARS-CoV-2, with relevance to other zoonoses and anthropozoonoses transmitted in a household setting. Furthermore, we collected human,

405 animal, and environmental data, representing a true One Health approach to this critical
406 research question. Finally, our findings indicate households in this population are willing to
407 adopt measures to protect their pets from SARS-CoV-2 infection, and that these measures may
408 be effective, indicating an opportunity to prevent household transmission of zoonoses and
409 anthropozoonoses through health education and policy. As vaccine roll-out continues and
410 human-to-human transmission wanes, and in preparation for the next pandemic of zoonotic
411 origin, rigorous characterization of the nature of human-animal contact within households, and
412 the implications of this contact for disease transmission, is critical.
413

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422

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DATA STATEMENT

De-identified data and code will be made available in a GitHub repository prior to publication.

DECLARATIONS OF INTEREST

None.

AUTHOR CONTRIBUTIONS

J Meisner: conceptualization, data curation, formal analysis, methodology, software, visualization, writing – original draft, writing – review & editing. T Baszler: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, writing – original draft, writing – review & editing. K Kuehl: conceptualization, data curation, writing – original draft, writing – review & editing. V Ramirez: conceptualization, data curation, formal analysis, funding acquisition, project administration, resources, software, supervision, writing – review & editing. A Baines: data curation, formal analysis, software, writing – review & editing. L Frisbie: data curation, formal analysis, software, writing – review & editing. E Lofgren: conceptualization, formal analysis, methodology, writing – review & editing. D DeAvila: formal analysis, methodology, validation. R Wolking: formal analysis, methodology, validation. D

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452

REFERENCES

[1] M. Konda, B. Dodda, V.M. Konala, S. Naramala, S. Adapa, Potential Zoonotic Origins of SARS-CoV-2 and Insights for Preventing Future Pandemics Through One Health Approach, Cureus. (2020). https://doi.org/10.7759/cureus.8932.

[2] R.J. Molenaar, S. Vreman, R.W. Hakze-van der Honing, R. Zwart, J. de Rond, E. Weesendorp, L.A.M. Smit, M. Koopmans, R. Bouwstra, A. Stegeman, W.H.M. van der Poel, Clinical and Pathological Findings in SARS-CoV-2 Disease Outbreaks in Farmed Mink (Neovison vison), Vet. Pathol. (2020). https://doi.org/10.1177/0300985820943535.

[3] B.E.E. Martina, B.L. Haagmans, T. Kuiken, R.A.M. Fouchier, G.F. Rimmelzwaan, G. Van Amerongen, J.S.M. Peiris, W. Lim, A.D.M.E. Osterhaus, SARS virus infection of cats and ferrets, Nature. (2003). https://doi.org/10.1038/425915a.

[4] American Veterinary Medical Association, SARS-CoV-2 in Animals, (n.d.). www.avma.org/resources-tools/animal-health-and-welfare/covid-19/sars-cov-2-animals-including-pets.

[5] P. F, SARS-CoV-2 variants lacking ORF8 occurred in farmed mink and pangolin, Gene. 784 (2021) 145596. https://doi.org/10.1016/j.gene.2021.145596.

[6] S. Guo, K. Liu, J. Zheng, The Genetic Variant of SARS-CoV-2: Would it matter for Controlling the Devastating Pandemic?, Int. J. Biol. Sci. (2021). https://doi.org/10.7150/ijbs.59137.

[7] M.F. Davis, S.C. Rankin, J.M. Schurer, S. Cole, L. Conti, P. Rabinowitz, G. Gray, L. Kahn, C. Machalaba, J. Mazet, M. Pappaioanou, J. Sargeant, A. Thompson, S. Weese, J. Zinnstag, Checklist for One Health Epidemiological Reporting of Evidence (COHERE), One Heal.

(2017). https://doi.org/10.1016/j.onehlt.2017.07.001.

[8] STROBE statement - Checklist of items that should be included in reports of observational studies (© STROBE Initiative), Int. J. Public Health. (2008). https://doi.org/10.1007/s00038-007-0239-9.

[9] M. Imai, K. Iwatsuki-Horimoto, M. Hatta, S. Loeber, P.J. Halfmann, N. Nakajima, T. Watanabe, M. Ujie, K. Takahashi, M. Ito, S. Yamada, S. Fan, S. Chiba, M. Kuroda, L. Guan, K. Takada, T. Armbrust, A. Balogh, Y. Furusawa, M. Okuda, H. Ueki, A. Yasuhara, Y. Sakai-Tagawa, T.J.S. Lopes, M. Kiso, S. Yamayoshi, N. Kinoshita, N. Ohmagari, S.I. Hattori, M. Takeda, H. Mitsuya, F. Krammer, T. Suzuki, Y. Kawaoka, Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development, Proc. Natl. Acad. Sci. U. S. A. (2020). https://doi.org/10.1073/pnas.2009799117.

[10] J. Shi, Z. Wen, G. Zhong, H. Yang, C. Wang, B. Huang, R. Liu, X. He, L. Shuai, Z. Sun, Y. Zhao, P. Liu, L. Liang, P. Cui, J. Wang, X. Zhang, Y. Guan, W. Tan, G. Wu, H. Chen, Z. Bu, Z. Bu, Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2, Science (80-.). (2020). https://doi.org/10.1126/science.abb7015.

[11] P.A. Harris, R. Taylor, B.L. Minor, V. Elliott, M. Fernandez, L. O'Neal, L. McLeod, G. Delacqua, F. Delacqua, J. Kirby, S.N. Duda, The REDCap consortium: Building an international community of software platform partners, J. Biomed. Inform. (2019). https://doi.org/10.1016/j.jbi.2019.103208.

[12] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K.W. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, D.G.J.C. Mulders, B.L. Haagmans, B. Van Der Veer, S. Van Den Brink, L. Wijsman, G. Goderski, J.L. Romette, J. Ellis, M. Zambon, M. Peiris, H.

Goossens, C. Reusken, M.P.G. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Eurosurveillance. (2020). https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[13] R.F. for S. Computing., R Core Team 2020, R A Lang. Environ. Stat. Comput. (2020).

[14] J. Textor, B. van der Zander, M.S. Gilthorpe, M. Liśkiewicz, G.T. Ellison, Robust causal inference using directed acyclic graphs: The R package "dagitty," Int. J. Epidemiol. (2016). https://doi.org/10.1093/ije/dyw341.

[15] S. Højsgaard, U. Halekoh, J. Yan, The R Package geepack for Generalized Estimating Equations Journal of Statistical Software, CRAN. (2006).

[16] A.C. Walls, Y.J. Park, M.A. Tortorici, A. Wall, A.T. McGuire, D. Veesler, Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein, Cell. (2020). https://doi.org/10.1016/j.cell.2020.02.058.

[17] I.Q. Phan, S. Subramanian, D. Kim, M. Murphy, D. Pettie, L. Carter, I. Anishchenko, L.K. Barrett, J. Craig, L. Tillery, R. Shek, W.E. Harrington, D.M. Koelle, A. Wald, D. Veesler, N. King, J. Boonyaratanakornkit, N. Isoherranen, A.L. Greninger, K.R. Jerome, H. Chu, B. Staker, L. Stewart, P.J. Myler, W.C. Van Voorhis, In silico detection of SARS-CoV-2 specific B-cell epitopes and validation in ELISA for serological diagnosis of COVID-19, Sci. Rep. (2021). https://doi.org/10.1038/s41598-021-83730-y.

Figure 1: Directed acyclic graph for human-animal SARS CoV2 transmission. Variables outlined with a square are the exposures of interest, while outcome (approximated by serostatus, PCR result, and COVID-19 like illness in separate models) is outlined with a circle. HAB: human-animal bond; SES: socioeconomic status; took precautions: SARS-CoV-2 positive household member(s) took precautions to prevent transmission to pet; indoor-only: animal does not go outdoors; bedshare: animal shares a bed with one or more household members.

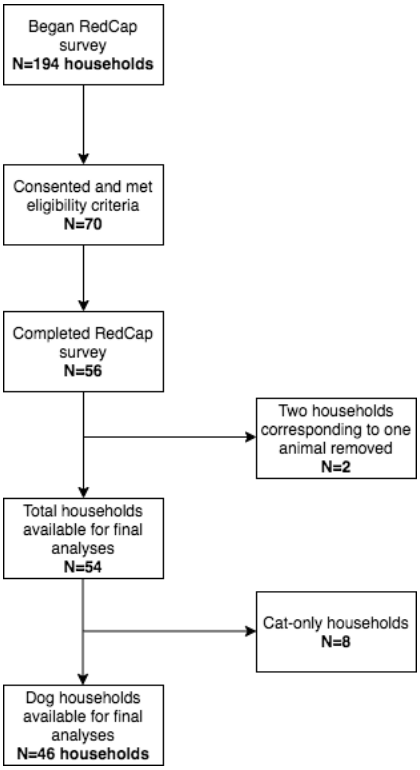

Figure 2: Flowchart depicting study recruitment. Two households were removed at the final step as the corresponding dog was moved, immediately after onset of the resident's illness, from the household of residence to a family member's household.

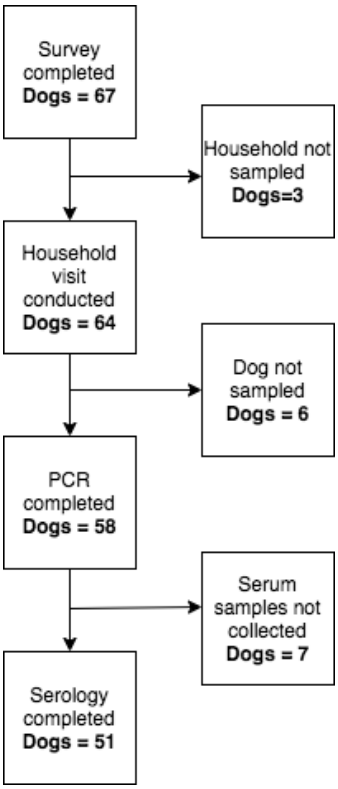

Figure 3: Flowchart depicting serological and PCR sampling. Out of 67 dogs corresponding to 5 households with completed surveys, PCR testing is complete for 58 dogs, and serological testing is complete for 51 dogs. The remaining dogs were not sampled due to safety concerns.

	n (%)
Animal	
Seropositive	22 (43%)
COVID-19 like illness	14 (24%)
Activity[a] during quarantine	20 (31%)
Took precautions[b]	33 (51%)
Age	6.74 (3.85)*
Male	36 (55%)
Respondent aware of CDC guidelines[c]	42 (63%)
Time from first diagnosis[d] to sampling (days)	43.06 (33.37)*
Time from last diagnosis[d] to sampling (days)	37.92 (40.9)*
Human	
Index case age	42.06 (13.6)*
Index case male	19 (28%)
Index case preexisting condition[e]	19 (28%)
Index case was hospitalized	1 (1%)
Number of SARS-CoV-2 positive household members	1.72 (1.28)*
Number of household members with COVID-19 symptoms[f]	0.31 (0.72)*
Number of household residents	3.4 (1.54)*
Environment	
Reside in a house	53 (79%)

Reside in an apartment or condominium	30 (21%)
Square footage of housing	1901 (974)*
Number of bedrooms	3.34 (1.46)*
Number of floors	1.82 (0.67)*
Access to outdoor space where pets can roam	57 (85%)
Human-animal contact	
Animal eating utensils cleaned in the kitchen	63 (95%)
Humans and animals share eating utensils	9 (13%)
Humans wash hands before handling animals	8 (12%)
Humans wash hands after handling animals	23 (34%)
Animal bedshares with humans	47 (72%)
Animal shares a bedroom but not a bed with humans	22 (34%)
Animal goes indoors and outdoors	26 (40%)
Animal sleeps outdoors	0 (0%)
Humans pet the animal	65 (100%)
Humans kiss the animal	45 (69%)
Animal is allowed on furniture	58 (89%)

Table 1: Descriptive statistics for 67 dogs corresponding to 46 households. *mean (standard deviation). [a]Activity defined as going to a veterinary clinic or groomer, being walked off-leash, or visiting an off-leash park, dog park, kennel, or daycare facility. [b]Precautions to prevent human-animal SARS-CoV-2 transmission following diagnosis: not petting or kissing the animal, staying in a different room, and having someone else feed and walk the animal. [c]Guidelines to

prevent human-animal SARS-CoV-2 transmission. [d]First diagnosis: earliest SARS-CoV-2 diagnosis in the household; final diagnosis: last SARS-CoV-2 diagnosis in the household. [e]Prexisting conditions: diabetes, kidney disease, heart disease, hypertension, immunosuppression. [f]Household members who had COVID-19 symptoms but did not get tested.

	COVID-19 like illness	Seropositive[a]
Exposure	POR (95% CI)	
Indoor-only	1.41 (0.41, 4.83)	0.7 (0.2, 2.4)
House type[b]	0.42 (0.12, 1.45)	1.92 (0.56, 6.59)
House square footage	1 (0.29, 3.43)	1 (0.29, 3.44)
Share eating utensils[c]	0.91 (0.27, 3.14)	2.08 (0.61, 7.16)
Bedsharing	2.82 (0.82, 9.68)	1.91 (0.56, 6.55)
Took precautions[d]	0.95 (0.28, 3.27)	0.38 (0.11, 1.3)
# SARS-CoV-2 infected humans	1.2 (0.35, 4.13)	1.11 (0.32, 3.83)
Canine COVID-19 like illness	-	1.89 (0.48, 7.37)
Time since first exposure (days)[e]	-	1.00 (0.98, 1.02)

Table 2: Regression model results. House size was adjusted for house type, but no other models were not adjusted for confounders due to overfitting concerns. [a]Results available for 51 dogs. [b]House versus apartment or condominium. [c]Animals and humans share eating utensils. [d]Precautions taken to prevent human-animal SARS-CoV-2 transmission following diagnosis: not petting or kissing the animal, staying in a different room, and having someone else feed and walk the animal. [e]First exposure defined as 2 days prior to first positive diagnosis in the household or onset of symptoms, whichever was earlier. POR: prevalence odds ratio; 95% CI: 95% confidence interval.