SARS-CoV-2 antibodies seroprevalence in dogs from France using ELISA and an automated western blotting assay

Younes Laidoudi[a,b], Youssouf Sereme[a,b], Hacène Medkour[a,b], Stéphanie Watier-Grillot[c,d], Pierre Scandola[a,b,c,g], Jacques Ginesta[e], Virginie Andréo[f], Claire Labarde[c,g], Loïc Comtet[h], Philippe Pourquier[h], Didier Raoult[a,b], Jean-Lou Marié[c], Bernard Davoust[a,b,c,g*]

[a] *Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France*

[b] *IHU Méditerranée Infection, Marseille, France*

[c] *French military health service, Animal epidemiology expert group, Tours, France*

[d] *French army center for epidemiology and public health, Marseille, France*

[e] *24th Veterinary group, Suippes, France*

[f] *26th Veterinary group, Gramat, France*

[g] *1th Veterinary group, Toulon, France*

[h] *Innovative Diagnostics, Grabels, France*

ABSTRACT

Dogs are occasionally receptive to SARS-CoV-2. They develop few or no clinical signs. Epidemiosurveillance of SARS-CoV-2 in dogs requires testing to distinguish it from other canine coronaviruses. Over the last year, significant progress has been made in the diagnosis of SARS-CoV-2, enabling its surveillance in humans and animals. Here, using ELISA and automated western blotting (AWB) assays, we performed a longitudinal study on 809 apparently healthy dogs from different regions of France to investigate anti-SARS-CoV-2 antibodies. There were three principal groups: (i) 356 dogs sampled once before the

pandemic, (ii) 235 dogs sampled once during the pandemic, and (iii) 218 dogs, including 82 dogs sampled twice (before and during the pandemic), 125 dogs sampled twice during the pandemic and 11 dogs sampled three times (once before and twice during the pandemic). Using ELISA, the seroprevalence was significantly higher during the pandemic [4.9% (22/453)] than in the pre-pandemic period [1.1% (5/448)]. At least 8 ELISA-seroconversions were observed among the 218 dogs sampled twice. ELISA positive sera before the pandemic were not confirmed in serial testing by AWB, which suggests a possible cross-reactivity of the ELISA, probably with other canine coronaviruses. No significant difference was observed between these two serological tests (Q=1.455, p=0.228). Positive correlation was observed between the SARS-CoV-2 seroprevalence in dogs and the incidence of the infection in humans. The AWB could be used as a second line assay to confirm the doubtful and discrepant ELISA results in dogs. Our findings confirm the previous experimental models concerning the receptivity of dogs to SARS-CoV-2. They suggest the weak or absence of the virus transmission from the infected to noninfected dogs or humans. However, the new variants with multiple mutations could adapt to dogs; this hypothesis cannot be ruled out in the absence of canine SARS-CoV-2 genomic data.

Keywords: COVID-19, SARS-CoV-2, Serology, Dog, Epidemiosurveillance, One Health

Corresponding author at: IHU Méditerranée Infection, 19-21 Bd Jean Moulin, 13385 Marseille cedex 05, France.

E-mail address: bernard.davoust@gmail.com (B. Davoust).

1. Introduction

Severe acute respiratory syndrome, caused by SARS-CoV-2 coronavirus, a new emergent variant involved in epidemic disease first identified, in November 2019, in Wuhan city (Hubei province), China [1,2]. A few months later, the World Health Organization declared a worldwide pandemic disease. By the end of February 2021, more than 111 million cases and 2.46 million deaths were recorded worldwide [3]. In France, the first human cases were diagnosed in late January 2020. One year later (February 2021), the cumulative incidence for France reached almost 3.59 million, including 84,147 deaths [3,4].

Phylogenetically, SARS-CoV-2 is closely related to SARS CoV (or SARS-CoV-1), previously involved in the epidemic of 2003, and to the BatCoV, a Betacoronavirus found naturally in bats [5,6]. The scientific community believes that SARS-CoV-2 has a zoonotic origin from bats, while the intermediate host between bats and humans is not yet known [2,5,6]. Due to the presence of specific receptors for SARS-CoV-2 virus within the respiratory tract of mustelids (i.e. ferret and mink), these being the most receptive species under both experimental and natural conditions [7]. Globally, coronaviruses are widespread in animal fauna (i.e. birds, pigs, ruminants, dogs, cats, etc.) [2,5,6,7,8,9,10]. Since the 1970s, Alpha and Betacoronavirus have been highlighted respectively as agents for canine enteritic coronavirus (CECoV) and the respiratory coronavirus (CRCoV) [11,12]. However, dogs are occasionally receptive to SARS-CoV-2 with only 31 cases diagnosed worldwide by specific analyses (RT-qPCR) at the end of 2020 in Hong Kong, USA, Japan and Argentina [9,13]. Dogs infected with SARS-CoV-2 have few or no clinical symptoms [13]. The epidemiological surveillance of SARS-CoV-2 in dogs requires reliable serological methods to distinguish between the SARS-CoV-2 and other canine coronavirus. Advances in the diagnosis of SARS-CoV-2 have been made over the past year and surveillance of its circulation in humans and animals is now possible. Here, we performed a longitudinal study of the seroprevalence of SARS-CoV-2 in

apparently healthy dogs from different regions of France in order to highlight their sentinel role during this pandemic.

2. Materials and methods

2.1. Dogs

A total of 809 dogs from France were included in this study (i.e. Bouches-du-Rhône, Marne, Lot, Var, Vaucluse, Corsica and French Guiana), from which 448 serum samples were sampled prior to the SARS-CoV-2 pandemic (from 2006 to January 2020) and 453 during the pandemic (from February 2020 to February 2021). Of those, 559 (69%) consist of military working dogs (MWD), mainly male Belgian shepherds and German shepherds, aged from one to ten years, and 250 (31%) companion dogs (adults of both sexes, mostly living in shelters). Dogs were allocated into three groups: (i) 356 dogs were sampled once before the pandemic, (ii) 235 dogs were sampled once during the pandemic and (iii) 218 dogs, including 82 dogs sampled twice (before and during the pandemic), 125 dogs sampled twice during the pandemic and 11 dogs sampled three times (once before and twice during the pandemic). A total of 901 blood samples were collected using a 3.5 mL vacuum tube with serum separating gel. Canine sera were harvested and stored at - 20°C or + 4°C until analysis.

2.2. ELISA assay

All sera were subjected to the screening for antibodies against SARS-CoV-2 using ID Screen® SARS-CoV-2 Double Antigen Multi-species (Innovative Diagnostics, Grabels, France) following the manufacturer's instructions. The test consists of an enzyme-linked immunosorbent assay ELISA, targeting multispecies (i.e. minks, ferrets, cats, dogs, cattle, sheep, goats, horses and all other receptive species) antibodies directed against the major

nucleocapsid protein of SARS-CoV-2. Plates were sensitized with a purified recombinant N antigen. Optical density (OD) was measured at 450 nm using Multiskan GO software (Thermo Scientific, Waltham, MA, USA). The test was validated when the optical density of positive control (OD_{PC}) was ≥ 0.35 and a mean ratio of positive (OD_{PC}) and negative (OD_{NC}) control is higher than three. The optical density of each sample (OD_N) was used to calculate the S/P ratio score (expressed as a %) where $S/P = 100 * (OD_N - OD_{NC}) / (OD_{PC} - OD_{NC})$. Samples tested by ELISA were considered positive when the S/P ratio score is higher than 60% and doubtful when the P/S percent ranges between 50 and 60%, while samples displaying an S/P score lower than 50% by ELISA were considered as negative.

2.3. Western blot assay

2.3.1. SARS-CoV-2 antigen preparation

The strain SARS-CoV-2 IHUMI2 (lineage 20a) was used in the production of SARS-CoV-2 specific antigens as previously described [14]. Briefly, one liter of infected cells was collected and centrifugated at 700 x g for 10 min. The supernatant was then filtrated twice using 0.45 µm and 0.2 µm pore sized filter. Virions were aggregated by overnight precipitation at 4ºC with 10% polyethylene glycol 8000 white flake type (PEG-8000, BioUltra, Sigma-Aldrich, USA) and 2.2% crystalline NaCl, with gentle swirling. Precipitated virus particles were then centrifuged at 10,000 x g for 30 min using a Sorvall Evolution centrifuge with SLA-3000 Recent 1 fixed angle rotor pre-cooled at 4ºC (Kendro Laboratory Products, Newtown, USA). The pellet was resuspended with Hepes-saline (0.9% NaCl, 10 mL of 1 M Hepes, 990 mL purified water) previously vacuum-sterilized through a 0.2 µm pore size membrane; swirled in the cold Hepes-saline until dissolved to avoid using pipette as it may hurts viral spikes at this step. Resuspended pellet was then applied to a 30% sucrose cushion in 25 x 89 mm centrifuge tubes (Ultra-Clear, Beckman Coulter, CA, USA). Final

purification was achieved by ultracentrifugation at 100,000 x g for 90 min at 4°C, followed by two 30-minute washes with HBSS using Sorvall Discovery 90SE with Surespin 630 rotor (Kendro Laboratory Products). Final pellet was resuspended in 400 µL of Hepes-buffered saline and heat-inactivated at 65°C for 1 hour. Finally, the virions were fractionated with TS buffer (7 M Urea, 2 M Thiourea, 4% Chaps) to release the antigen. The released antigens were then concentrated with the Amicon 3 kDa filter (Merck KGaA, Darmstadt, Germany) before being analyzed by western blot.

2.3.2. Automated western blotting (AWB) assay

All samples showing positive results on ELISA were further assessed using the Jess™ Simple Western system (ProteinSimple, San Jose, CA, USA, a Bio-Techne Brand), an automated capillary-based size separation and nano-immunoassay system. To quantitate the absolute serological response to viral antigens, we followed the manufacturer's standard method for 12-230-kDa Jess separation module (SM-W004). SARS-CoV-2 antigens (1 µg/µL) were mixed with 0.1X Sample buffer and Fluorescent 5X Master mix (ProteinSimple) to achieve a final concentration of 0.25 µg/µL in the presence of fluorescent molecular weight markers and 400 mM dithiothreitol (ProteinSimple). This preparation was denatured at 95°C for 5 minutes. Ladder (12-230-kDa PS-ST01EZ) and SARS-CoV-2 proteins were separated in capillaries as they migrated through a separation matrix at 375 volts. A ProteinSimple proprietary photoactivated capture chemistry was used to immobilize separated viral proteins on the capillaries. Patients' serum diluted at a 1:2 was added and incubated for 60 minutes. After a wash step, goat HRP-conjugated anti-Fc fragment of IgG/IgM/IgA antibodies (Jackson Immuno-Research) diluted 1:500 was added for 30 minutes. The chemiluminescent revelation was established with peroxide/luminol-S (ProteinSimple). Digital image of chemiluminescence of the capillary was captured with Compass Simple Western software

(version 4.1.0, ProteinSimple) that automatically calculated heights (chemiluminescence intensity), area and signal/noise ratio. Results were visualized as electropherograms representing peaks of chemiluminescence intensity and as lane view from signal of chemiluminescence detected in the capillary. An internal system control was included in each run.

2.4. Statistical analysis

Comparison between dog's populations was performed using Fisher's exact and Chi-squared tests. The Mc Nemar test was used to compare between ELISA and AWB assays. All statistical analysis was performed using Addinsoft software (XLSTAT 2018: Data Analysis and Statistical Solution for Microsoft Excel, Paris, France). A p-value < 0.05 was considered statistically significant.

2. Results

3.1. ELISA antibody detection

In total, of the 448 pre-pandemic sera collected, 4 (0.9%) were ELISA positive and 1 (0.2%) was inconclusive. This confirms a measured specificity of 99.1% [97.7 – 99.7] for the ELISA. While of the 453 sera collected during the pandemic, 20 (4.4%) were positive and 2 (0.5%) were considered doubtful. The infection rate was significantly higher during the pandemic compared to the period before the pandemic; this was observed for all sera samples (Table 1). Furthermore, at least 8 ELISA-seroconversions among the 218 dogs during the pandemic were observed (Table 2). During the pandemic, a total of 17 (4.3%) out of 397 MWD and 6 (10.7%) out of 56 companion dogs were reacted within ELISA test, which

corresponds to a significant difference (Khi2=4.213 - p=0.04) between these two populations. Fourteen (11.1%) out of 126 dogs sampled in February 2021 from the South-East area scored positive. A lower prevalence of 3.1% (3/95) was recorded in the South-West compared to that recorded in the South-East (Khi2=4.7 - p≤0.05) (Table 3).

3.2. Automated western blot results

Among the 41 serum samples listed in table 2, 31 of them were assessed by the AWB, including 27 ELISA-positive sera, one doubtful serum and 3 ELISA-negative sera. In addition, three other ELISA-negative sera were also tested. AWB yielded the detection of 17 (63%) out of the 27 ELISA-positive sera (including doubtful sera). In addition, 3 ELISA-negative sera were found positive within AWB. One of them was a MWD (D14) which exhibited an S/P ratio of 49%, and the two other sera collected at one week apart (D26). Globally, all AWB-positive sera were sampled between the period ranging from January 2020 to February 2021. While no ELISA-positive sera collected before the pandemic or negative controls were detected by the AWB (Figure 1). No significant difference was observed between these two assays (Q=1.455, p=0.228). Finally, all AWB-positive sera yielded a prominent 56-kDa band interpreted as the nucleocapsid, while no bands were detected for the other major dominant proteins, such as the protein S (i.e. 170 kDa), S1 (i.e. 110 kDa) and S2 (i.e. 90 kDa) (Figure 1).

4. Discussion

To date, studies investigating SARS-CoV-2 in dogs are scarce, probably due to the lower susceptibility of dogs to this infection and the focus of research on the human disease. In France, only two serological studies have been carried out on dogs. One study involved 12 dogs of SARS-CoV-2-positive owners. In this study, no positive dog was detected using the

luciferase immuno-precipitation assay [15]. The second study was carried out using the microsphere immunoassay. Authors reported 2 (15.4%) seropositive dogs among 13 of SARS-CoV-2-positive owners, while no positive-dog was found within 22 other dogs of owners with an unknowing SARS-CoV-2 status [16]. In Italy, the antibody neutralization assay was used for the surveillance of SARS-CoV-2 infection in 451 dogs during the pandemic, and 15 (3.3%) dogs were found seropositive [17]. In Wuhan city (China), 16 (1.7%) positive dogs were detected among the 946 tested during the pandemic using a newly developed double-antigen sandwich ELISA assay [18]. In Croatia, a survey reported that 7.6% of dogs (13/172) were positive by ELISA test [19]. In Texas, USA, 15.3% of 59 dogs were positive for SARS-CoV-2 by RT-PCR and genome sequencing or neutralizing antibodies, in homes where at least one human case of COVID-19 was diagnosed [20]. In Spain, canine seroprevalence (ELISA) was overall 16.7% (10/60) but it was higher (25% - 5/60) in dogs living in COVID-19-positive households, indicating their susceptibility to SARS-CoV-2 infection [21]. These discrepancies in results between the different studies may be related to the sensitivity of the different assays. The results of this comprehensive study of SARS-CoV-2 infection in companion and military working dogs sampled before and during the pandemic in areas of active human viral transmission made it possible to evaluate the specificity of the ELISA and AWB tests. The same ELISA test used in our study detected anti-SARS-CoV-2 antibodies in the serum of a cat with PCR positive, living in a household in Chile, where a human was infected [22].

In our study, the ELISA we used detected 1.1% of 448 pre-pandemic sera. This highlights the possible cross reactivity with other canine coronaviruses, probably the Betacoronavirus of dogs [23]. On the other hand, the seroconversion of 8, as well as the significant increase in seroprevalence in dogs during the pandemic (i.e., 4.9% out of 453 dogs tested), particularly in the Bouches-du-Rhône region, a high endemic area for human SARS-CoV-2 infection

(www.cascoronavirus.fr), could explain the occurrence of SARS-CoV-2 infection in dogs. On the other hand, the AWB assay yielded the detection of 62.7% ELISA-positive sera. However, all of them were sampled between the periods ranging from January 2020 to February 2021, which is in line with the outbreak of the pandemic in France. In addition, some inconsistencies were also observed between these two assays. For example, some dogs with high ELISA S/P ratio sampled before the pandemic (i.e. dog D1 and D2) or even during the pandemic (i.e. dog D6 and D7) gave a negative AWB result, whereas some ELISA-negative or doubtful sera with low ELISA S/P ratio (i.e. dog D14, D22, D26 and D29) were positive using AWB assay (Fig.1). Though few canine sera were herein tested by the AWB, which may represent a limitation of the assay, all AWB-positive sera were sampled during the pandemic which suggests the specific detection of antibodies to SARS-CoV-2 in dogs. The discrepancy between these two assays could be explained by the type of antigens used for each assay. ELISA test was developed on the basis of a truncated N recombinant antigen from the viral nucleocapsid which probably provided the detection of conformational epitopes that could also be shared with the other coronaviruses. In contrast, the AWB was based on the integral SARS-CoV-2 nucleocapsid antigens which may react only with the linear epitopes [24]. However, the clear-cut decision regarding the specificity of the AWB assay cannot be ruled out in the absence of a reliable gold standard, since the possible cross-reaction has already been described with other human Betacoronavirus within the AWB assay [25].

The AWB assay based on the purified virus antigens was first adapted for the diagnosis and the evaluation of the human immune-response against SARS-CoV-2 antigens. The assay proved to be effective principally in detecting antibodies to nucleocapsid proteins [25]. Our results showed that the AWB yielded only the detection of antibodies against the nucleocapsid proteins from all positive dogs. However, we do not know whether this is related to the lower sensitivity of AWB to spike virus proteins in dogs.

Despite the receptivity of dogs to SARS-CoV-2 infection under experimental conditions [26], they were unable to transmit the virus [7,9,10]. Our results indicated that, in spite of the presence of positive dogs in kennels, there were most probably few infected animals. Thereby, this suggests that dogs do not transmit the virus, which may be due to the poor viral replication in dogs [26]. On the other hand, previous studies have demonstrated the presence of a few differences between human and canine angiotensin-converting enzyme 2 (ACE2), the interactive receptor within the spike protein of the SARS-CoV-2 [9]. However, recent studies have demonstrated the continuous emergence of new SARS-CoV-2 with multiple spike protein mutations. It is not known whether dogs infected with these new variants could transmit the virus to other animals or to humans [27,28,29]. In March 2021, a study carried out on British dogs reported for the first time canine and feline infections with the SARS-CoV-2 B.1.1.7 variant in addition to some of these pets suffering from myocarditis [30].

5. Conclusion

The AWB assay, previously standardized as first or second line method to confirm the diagnosis of SARS-CoV-2 from human patients, could also be used as a second line assay to confirm negative, doubtful and discrepant ELISA results in dogs. These findings along with the results from the previous experimental models of SARS-CoV-2 in dogs confirm the receptivity of dogs to SARS-CoV-2 infection. They also suggest the absence of the virus transmission from infected to non-infected dogs as well as to humans. In the absence of genomic data on SARS-CoV-2 in dogs, the hypothesis that new SARS-CoV-2 variants with multiple mutations in the spike protein could induce adaptation of the virus to dogs cannot be ruled out.

Authors' contributions

BD and YL conceived the original paper. BD, YL and HM wrote the initial draft. BD, YL, PS, SWG, JG, VA, CL and JLM collected the blood samples. BD, YL, PS, HM and YS carried out analyzes in the laboratory. BD, YL, HM, SWG, LC, PP, JLM and DR extensively revised and approved the final version of the manuscript.

Ethics approval and consent to participate

All applicable international, national and military guidelines for the care and use of dogs were followed. The owners of the dogs have given their consent for the samples to be taken.

Funding

This study was supported by the Health Service of the French Army and the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program "Investissements d'avenir", reference ANR-10-IAHU-03, the Region Provence-Alpes-Côte d'Azur and European funding FEDER PRIMI.

Declaration of competing interest

There are no competing interests. Two authors (Loïc Comtet and Philippe Pourquier) are currently employees of Innovative Diagnostics company, however there were no conflicting interests that may have biased the work reported in this paper.

Acknowledgements

Authors would like to thank the company Innovative Diagnostics which provide them the ELISA ID Screen® SARS-CoV-2 kits.

References

[1] A. Awadasseid, Y. Wu, Y. Tanaka, W. Zhang, Initial success in the identification and management of the coronavirus disease 2019 (COVID-19) indicates human-to-human transmission in Wuhan, China. Int. J. Biol. Sci. 16(11) (2020) 1846–1860, doi:10.7150/ijbs.45018.

[2] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E.C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395(10224) (2020) 565–574, doi.org/10.1016/S0140-6736(20)30251-8.

[3] COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU) . ArcGIS. Johns Hopkins University. Retrieved 21 February 2021.

https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6

[4] S. Bernard Stoecklin, P. Rolland, Y. Silue, A. Mailles, C. Campese, A. Simondon, M. Mechain, L. Meurice, M. Nguyen, C. Bassi, E. Yamani, S. Behillil, S. Ismael, D. Nguyen, D. Malvy, F.X. Lescure, S. Georges, C. Lazarus, A. Tabaï, M. Stempfelet, V. Enouf, B. Coignard, D. Levy-Bruhl, Investigation Team, First cases of coronavirus disease 2019 (COVID-19) in France: surveillance, investigations and control measures, January 2020. Euro Surveill. 25(6) (2020) 2000094, doi:10.2807/1560-7917.ES.2020.25.6.2000094.

[5] A.A. Latif, S. Mukaratirwa, Zoonotic origins and animal hosts of coronaviruses causing human disease pandemics: A review. Onderstepoort J. Vet. Res. 87(1) (2020) e1-e9, doi:10.4102/ojvr.v87i1.1895.

[6] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798) (2020) 270–273, doi.org/10.1038/s41586-020-2012-7.

[7] M.A.A. Mahdy, W. Younis, Z. Ewaida, An overview of SARS-CoV-2 and animal infection. Front. Vet. Sci. 7 (2020) 596391, doi:10.3389/fvets.2020.596391.

[8] M. Hernández, D. Abad, J.M. Eiros, D. Rodríguez-Lázaro, Are animals a neglected transmission route of SARS-CoV-2? Pathogens. 9(6) (2020) 480, doi:10.3390/pathogens9060480.

[9] B. do Vale, A.P. Lopes, M. da C. Fontes, M. Silvestre, L. Cardoso, A.C. Coelho, Bats, pangolins, minks and other animals - villains or victims of SARS-CoV-2? Vet. Res. Commun. 45(1) (2021) 1-19, doi:10.1007/s11259-021-09787-2.

[10] A.S. Abdel-Moneim, E.M. Abdelwhab, Evidence for SARS-COV-2 infection of animal hosts. Pathogens 9(7) (2020) 529, doi:10.3390/pathogens9070529.

[11] B.N. Licitra, G.E. Duhamel, G.R. Whittaker, Canine enteric coronaviruses: Emerging viral pathogens with distinct recombinant spike proteins. Viruses 6(8) (2014) 3363–3376, doi:10.3390/v6083363.

[12] K. Erles, C. Toomey, H.W. Brooks, J. Brownlie, Detection of a group 2 coronavirus in dogs with canine infectious respiratory disease. Virology 310(2) (2003) 216–223. doi:10.1016/s0042-6822(03)00160-0.

[13] T.H.C. Sit, C.J. Brackman, S.M. Ip, K.W.S. Tam, P.Y.T. Law, E.M.W. To, V.Y.T. Yu, L.D. Sims, D.N.C. Tsang, D.K.W. Chu, R.A.P.M. Perera, L.L.M. Poon, M. Peiris, Infection of dogs with SARS-CoV-2. Nature 586(7831) (2020) 776–778, doi.org/10.1038/s41586-020-2334-5.

[14] S. Edouard, P. Colson, C. Melenotte, F. di Pinto, L. Thomas, B. La Scola, M. Million, H. Tissot-Dupont, P. Gautret, A. Stein, P. Brouqui, P. Parola, J.C. Lagier, D. Raoult, M. Drancourt, Evaluating the serological status of 367 COVID-19 patients using an indirect immunofluorescent assay, France. Eur. J. Clin. Microbiol. Infect. Dis. (2020) 1-11, https://doi.org/10.1007/s10096-020-04104-2.

[15] M. Fritz, B. Rosolen, E. Krafft, P. Becquart, E. Elguero, O. Vratskikh, S. Denolly, B. Boson, J. Vanhomwegen, M.A. Gouilh, A. Kodjo, C. Chirouze, S.G. Rosolen, V. Legros, E.M. Leroy, High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health 11 (2021) 100192, doi:10.1016/j.onehlt.2020.100192.

[16] S. Temmam, A. Barbarino, D. Maso, S. Behillil, V. Enouf, C. Huon, A. Jaraud, L. Chevallier, M. Backovic, P. Pérot, P. Verwaerde, L. Tiret, S. van der Werf, M. Eloit, One Health 10 (2020) 100164, doi:10.1016/j.onehlt.2020.100164.

[17] E.I. Patterson, G. Elia, A. Grassi, A. Giordano, C. Desario, M. Medardo, S.L. Smith, E.R. Anderson, T. Prince, G.T. Patterson, E. Lorusso, M.S. Lucente, G. Lanave, S. Lauzi, U.

Bonfanti, A. Stranieri, V. Martella, F. Solari Basano, V.R. Barrs, A.D. Radford, U. Agrimi, G.L. Hughes, S. Paltrinieri, N. Decaro, Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat. Commun. 11 (2020) 6231, doi:10.1038/s41467-020-20097-0.

[18] Y. Zhao, Y. Yang, J. Gao, K. Huang, C. Hu, X. Hui, X. He, C. Li, W. Gong, C. Lv, Y. Zhang, H. Chen, Z Zou, Q Zhang, M. Jin M, A serological survey of severe acute respiratory syndrome coronavirus 2 in in Wuhan. Transbound. Emerg. Dis. (2021), doi:10.1111/tbed.14024.

[19] V. Stevanovic, T. Vilibic-Cavlek, I. Tabain, I. Benvin, S. Kovac, Z. Hruskar, M. Mauric, L. Milasincic, L. Antolasic, A. Skrinjaric, V. Staresina, L. Barbic, Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. Transbound. Emerg. Dis. 15 (2020)10.1111/tbed.13924, doi:10.1111/tbed.13924.

[20] S.A. Hamer, A. Pauvolid-Corrêa, I.B. Zecca, E. Davila, L.D. Auckland, C.M. Roundy, W. Tang, M. Torchetti, M.L. Killian, M. Jenkins-Moore, K. Mozingo, Y. Akpalu, R.R. Ghai, J.R. Spengler, C.B. Behravesh, R.S.B. Fischer, G.L. Hamer GL, Natural SARS-CoV-2 infections, including virus isolation, among serially tested cats and dogs in households with confirmed human COVID-19 cases in Texas, USA. bioRxiv [Preprint]. (2020) 8:2020.12.08.416339, doi:10.1101/2020.12.08.416339.

[21] A.J. Perisé-Barrios, B.D. Tomeo-Martín, P. Gómez-Ochoa, P. Delgado-Bonet, P. Plaza, P. Palau-Concejo, J. González, G. Ortiz-Díez, A. Meléndez-Lazo, M. Gentil, J. García-Castro, A. Barbero-Fernández, Humoral responses to SARS-CoV-2 by healthy and sick dogs during the COVID-19 pandemic in Spain. Vet. Res. 52(1) (2021) 22, doi:10.1186/s13567-021-00897-y.

[22] V. Neira, B. Brito, B. Agüero, F. Berrios, V. Valdés, A. Gutierrez, N. Ariyama, P. Espinoza, P. Retamal, E.C. Holmes, A.S. Gonzalez-Reiche, Z. Khan, A. van de Guchte, J. Dutta, L. Miorin, T. Kehrer, N. Galarce, L.I. Almonacid, J. Levican, H. van Bakel, A. García-Sastre, R.A. Medina, A household case evidences shorter shedding of SARS-CoV-2 in naturally infected cats compared to their human owners. Emerg. Microbes Infect. 10(1) (2021) 376-383, doi:10.1080/22221751.2020.1863132.

[23] I.M. Donnik, I.V. Popov, S.V. Sereda, I.V. Popov, M.L. Chikindas, A.M. Ermakov, Coronavirus infections of animals: Future risks to humans. Biol. Bull. Russ. Acad. Sci. 48(1) (2021) 26-37, doi:10.1134/S1062359021010052.

[24] J.S. Terry, L.B. Anderson, M.S. Scherman, C.E. McAlister, R. Perera, T. Schountz, B.J. Geiss, Development of a SARS-CoV-2 nucleocapsid specific monoclonal antibody. Virology 558 (2021) 28-37, doi:10.1016/j.virol.2021.01.003.

[25] S. Edouard, R. Jaafar, N. Orain, P. Parola, P. Colson, B. La Scola, P.-E. Fournier, D. Raoult, M. Drancourt, Automated western immunoblotting detection of anti-SARS-CoV-2 serum antibodies. medRxiv 2020.11.26.20238733, doi:https://doi.org/10.101101/2020.11.26.20238733.

[26] J. Deng, Y. Jin, Y. Liu, J. Sun, L. Hao, J. Bai, T. Huang, D. Lin, Y. Jin, K. Tian, Serological survey of SARS-CoV-2 for experimental, domestic, companion and wild animals excludes intermediate hosts of 35 different species of animals. Transbound. Emerg. Dis. 67(4) (2020) 1745–1749, doi: 10.1111/tbed.13577.

[27] N. Decaro, A. Balboni, L. Bertolotti, P.A. Martino, M. Mazzei, F. Mira, U. Pagnini, SARS-CoV-2 infection in dogs and cats: Facts and speculations. Front. Vet. Sci. 10(8) (2021) 619207, doi:10.3389/fvets.2021.619207.

[28] H.A. de Morais, A.P. dos Santos, N.C. do Nascimento, L.B. Kmetiuk, D.S. Barbosa, P.E. Brandão, A.M.S. Guimarães, C. Pettan-Brewer, A.W. Biondo, Natural infection by

SARS-CoV-2 in companion animals: A review of case reports and current evidence of their role in the epidemiology of COVID-19. Front. Vet. Sci. 27(7) (2020) 591216. doi:10.3389/fvets.2020.591216.

[29] M. Kiros, H. Andualem, T. Kiros, W. Hailemichael, S. Getu, A. Geteneh, D. Alemu, W.E. Abegaz, COVID-19 pandemic: current knowledge about the role of pets and other animals in disease transmission. Virol. J. 17(1) (2020) 143. doi:10.1186/s12985-020-01416-9.

[30] L. Ferasin, M. Fritz, H. Ferasin, P. Becquart, V. Legros, E.M. Leroy, Myocarditis in naturally infected pets with the British variant of COVID-19. bioRxiv (2021) 03.18.435945, doi:https://doi.org/10.1101/2021.03.18.435945.

Legends

Table 1

Seroprevalence of SARS-CoV-2 antibodies, detected with a double antigen ELISA test, in dogs from France before and during the COVID-19 pandemic (N=901).

Table 2

Individual positive results of serological detection of SARS-CoV-2 infection by the double antigen ELISA test (N=28 dogs).

Table 3

Comparison of seroprevalences (ELISA) of SARS-CoV-2 infection in dogs from the French departments of Bouches-du-Rhône (South-East) and Lot (South-West) in February 2021, and the correlation with the COVID-19 incidence in humans.

Fig. 1. Results of the automated western blotting assay of SARS-CoV-2 infection in dogs from France, before and during the COVID-19 pandemic (N=32).