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First evidence of human-to-dog transmission of SARS-CoV-2 B.1.160 variant in France

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Abstract

Since the start of the coronavirus disease of 2019 (COVID-19) pandemic, several episodes of human-to-animal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission have been described in different countries. The role of pets, especially domestic dogs, in the COVID-19 epidemiology is highly questionable and needs further investigation. In this study, we report a case of COVID-19 in a French dog living in close contact with its owners who were COVID-19 patients. The dog presented rhinitis and was sampled 1 week after its owners (a man and a woman) were tested positive for COVID-19. The nasal swabs for the dog tested remained positive for SARS-CoV-2 by reverse transcription quantitative real-time PCR (RT-qPCR) 1 month following the first diagnosis. Specific anti-SARS-CoV-2 antibodies were detectable 12 days after the first diagnosis and persisted for at least 5 months as tested using enzyme-linked immunoassay (ELISA) and automated western blotting. The whole-genome sequences from the dog and its owners were 99%–100% identical (with the man and the woman's sequences, respectively) and matched the B.1.160 variant of concern (Marseille-4 variant), the most widespread in France at the time the dog was infected. This study documents the first detection of B.1.160 in pets (a dog) in France, and the first canine genome recovery of the B.1.160 variant of global concern. Moreover, given the enhanced infectivity and transmissibility of the Marseille-4 variant for humans, this case also highlights the risk that pets may potentially play a significant role in SARS-CoV-2 outbreaks and may transmit the infection to humans. We have evidence of human-to-dog transmission of the Marseille-4 variant since the owners were first to be infected. Finally, owners and veterinarians must be vigilent for canine COVID-19 when dogs are presented with respiratory clinical signs.

KEYWORDS
B.1.160 variant, COVID-19, dog, epidemiology, France, genome, Marseille-4 variant, SARS-CoV-2

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1 | INTRODUCTION

Since its emergence in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [the aetiological agent of coronavirus disease of 2019 (COVID-19)] has spread across the entire planet and caused a pandemic resulting, at the end of August 2021, in more than 216 million cases and 4.5 million deaths [www.worldometers.info/coronavirus]. While its origins remain unclear, it is accepted that it emerged from an animal reservoir, possibly bats (Zhou et al., 2020), and may have involved an intermediate host such as an animal in which the virus accumulated mutations making it more suitable for transmission to humans (T. Zhang et al., 2020).

Coronaviruses are widespread in animals (including birds, pigs, ruminants, dogs and cats) (Alluwaimi et al., 2020). Experimentally, researchers have shown that the SARS-CoV-2 multiplies easily in ferrets and hamsters, and several animal species may be susceptible to the infection, including *Rousettus aegyptiacus* (fruit bats), pangolins, felines, mink, dogs and rabbits (do Vale et al., 2021). Other species susceptible to infection are likely to be discovered.

In addition, genetic variants of SARS-CoV-2 have been emerging and circulating around the world throughout the COVID-19 pandemic (Centers for Disease Control & Prevention, 2021). Some of these variants, however, have caught scientists off guard by suddenly moving in an unexpected direction. Is this due to failures in virus surveillance or does it mean that SARS-CoV-2 is able to circulate unnoticed in an animal reservoir before returning to humans? In any case, the circulation of this pathogen must be carefully examined, and it is important to understand the role that animals play in the epidemiology of the disease (Maurin et al., 2021).

During the recent years, several episodes of human-to-animal transmission have been described in different countries. These episodes were mainly related to cats and also to other species, such as dogs and minks (Decaro, Vaccari, et al., 2021; Oude Munnink et al., 2021). Moreover, several cases of SARS-CoV-2 infection have also been reported worldwide in domestic pets (especially cats and dogs), and it has been suggested that these animals became infected by their owners or handlers. Infections of domestic pets mostly result in no to mild digestive and respiratory symptoms (Klaus et al, 2021; Sit et al., 2020). Infected dogs, generally, have few or no clinical symptoms (Sit et al., 2020), but recently it has been shown that the new British B.1.1.7 variant can infect dogs and cats which developed atypical clinical manifestations, including severe cardiac abnormalities secondary to myocarditis and had a profound impairment of the general health status of the animal, without any primary respiratory signs (Ferasin et al., 2021). Few studies on SARS-CoV-2 in dogs are available, probably due to the focus of research on the human disease. According to the World Organization for Animal Health (OIE, 2021), only 86 canine cases (and 115 feline cases) have been diagnosed worldwide using the specific reverse transcription quantitative real-time PCR (RT-qPCR), in Hong Kong, the United States, Japan and Argentina (American Veterinary Medical Association, 2021; do Vale et al., 2021; Sit et al., 2020). Cases of SARS-CoV-2 infection in dogs have been reported to the OIE (OIE, May 24, 2021) in the following countries: the United States, Mexico, Brazil, Argentina, Japan, Thailand, Hong Kong, Germany, Croatia and Bosnia and Herzegovina (Office International des Epizooties, 2021). In France, only three serological studies have been conducted on dogs (Fritz et al., 2021; Laidoudi et al., 2021; Temmam et al., 2020), which reported prevalence from 0% to 15.4%, in line with the incidence of SARS-CoV-2 in humans.

The possibility of SARS-CoV-2 transmission between humans and animals, especially pets, remains unknown for some variants. In this study, we report a case of SARS-CoV-2 infection in a dog in France after being infected by their owners, with evidence of human-to-dog transmission. We also conducted whole-genome characterization of viruses from the dog and its owners.

2 | METHODS

2.1 | Features of the study

On 6 November 2020, a 58-year-old woman from Marseille presented at the Méditerranée Infection University Hospital Institute (IHU) in Marseille (France), feeling feverish and experiencing headaches for the past 24 h. She was tested positive for COVID-19 by RT-qPCR (Amrane et al., 2020), Ct = 25 in a nasopharyngeal swab. On 13 November 2020, her 53-year-old husband and 25-year-old daughter were referred to the IHU for a COVID-19 nasopharyngeal qPCR test as suspected cases. Both were asymptomatic. The man was tested positive by RT-qPCR at Ct = 20.8, while the daughter was negative. The husband had suffered from allergic asthma with desensitization and his last attack had occurred 30 years ago. All the routinely prescribed analyses in cases of COVID-19 were performed on both patients including blood tests, coagulation and electrocardiography (ECG). No contraindication was reported for receiving treatment with oral hydroxychloroquine and oral azithromycin (Gautret et al., 2020; Million et al., 2020), and they were administered these treatments in association with zinc for the man and sodic enoxaparin (due to the concentration of D-dimers < 0.27 μg/ml) for the woman. The family owns a dog.

2.2 | Dog examination and sampling

The dog is a West Highland White Terrier female of 13-year-old, which suffered from a respiratory distress. On 13 November 2020, the dog was taken to a veterinary clinic by his owner. A nasal swab and blood samples were performed and sent to Scanelis, a veterinary test laboratory (Colomiers, France).

2.3 | Nucleic acids extraction and analysis for SARS-CoV-2 and canine pathogens implicated in canine cough/rhinitis

First, nucleic acids were extracted and purified from nasal swabs collected at different times by a silica-based method (Nucleospin RNA

Virus, Macherey Nagel). The final elution volume was 100 μl and 2.5 μl aliquots were used for the RT-PCR assays. Duplicates of nucleic acid samples were analyzed in 384 wells plates (10 μl final PCR volume). The efficiency of the purification step and the quality of the nucleic acids were controlled, with two exogenous external controls (synthetic RNA and DNA spiked in the lysis buffer) systematically added to the specimen and co-purified with it. These controls were detected by RT-qPCR or PCR. A control sample (nuclease free water) was systematically included in each purification series (23 specimens maximum) and was used as a process negative control. Several positive standards were included in order to check the limit of detection in each series and to calculate the loads.

Analyses were performed on the dog samples for canine pathogens implicated in canine cough or rhinitis (*Bordetella bronchiseptica*, canine distemper virus, respiratory canine adenovirus type 2, canine herpes virus and canine parainfluenza virus) using Scanelis qPCR or PCR assays (http://www.scanelis.com) and, finally, SARS-CoV-2 was tested.

The SARS-CoV-2 test was performed using the SARS-CoV-2 Scanelis test adapted from RT-qPCR designed by the Centers for Disease Control and Prevention, USA to obtain a performant multiplex RT-qPCR assay. The primers and probes were as follows: 2019-nCoV_N1-F: 5′-GAC CCC AAA ATC AGC GAA AT-3′; 2019-nCoV_N1-R: 5′-TCT GGT TAC TGC CAG TTG AAT CTG-3′; 2019-nCoV_N1-P: 5′-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3′ and 2019-nCoV_N3-F: 5′-GGG AGC CTT GAA TAC ACC AAA A-3′; 2019-nCoV_N3-R: 5′-TGT AGC ACG ATT GCA GCA TTG-3′; 2019-nCoV_N3-PSCA: 5′-FAM-ATC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3′. The RT-qPCR was run on Applied Biosystems QuantStudio 6 Flex Real-Time PCR System QS6 following the thermal programme: 20 min at 50°C, 15 min at 95°C and 50 cycles of 15 s at 92°C then 30 s at 60°C. Each sample was analyzed in duplicate. The mean of the duplicate results was calculated for quantitative results. The detection limit (95%) of the assay was determined in probit analysis as seven copies equivalent viral genome per reaction and the limit of quantification as 10 copies equivalent genome per analysis in duplicate. The linearity range of the multiplex assay is 10–10^7 copies equivalent genome per analysis.

2.4 | Dog's follow-up

The dog was followed up, examined and sampled, by nasal swabs and/or blood, 12, 19 and 28 days later. The virus concentrations were measured at these stages using the RT-qPCR test cited above. All the RNA extracts (days 0, +12, +19 and +28) were sent to the IHU laboratory for further analysis.

For further analysis, dog sera were collected at days +12 and +19 and +146 after the first consultation. The sera were tested using enzyme-linked immunoassay (ELISA) (Laidoudi et al., 2021) and automated western blotting (WB) assays as recently described (Edouard, Jaafar, et al., 2021; Edouard, Colson, et al., 2021). For ELISA, we used ID Screen SARS-CoV-2 Double Antigen Multi-species (Innovative Diagnostics, Grabels, France) following the manufacturer's instructions. The test targets 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 was higher than three. The optical density of each sample (OD_N) was used to calculate the sample to positive (S/P) ratio (expressed as a %) where $S/P = 100 \times (OD_N - OD_{NC})/ (OD_{PC} - OD_{NC})$. When the S/P score was lower than 50% by ELISA, samples were considered negative. They were considered as positive when it was higher than 60% and doubtful when 50 < P/S score <60%.

For WB, the strain SARS-CoV-2 IHUMI2 (lineage 20a) was used to produce SARS-CoV-2 antigens, as previously described (Edouard, Jaafar, et al., 2021). The Jess Simple Western automated nano-immunoassay system (ProteinSimple, San Jose, CA, USA, a Bio-Techne Brand), a capillary-based size separation of proteins (Edouard, Jaafar, et al., 2021) was used with an internal system control to evaluate the absolute serological response to viral antigens from sera. Canine sera were processed according to the manufacturer's standard method for 12-230-kDa Jess separation module (SM-W004). Briefly, a mixture of SARS-CoV-2 antigens, fluorescent molecular weight markers and 400 mM dithiothreitol (ProteinSimple) was prepared at a final concentration of 0.25 μg/μl, then denatured at 95°C for 5 min. Viral protein migration was performed through the separation matrix at 375 V for both SARS-CoV-2 antigens and Ladder (12-230-kDa PS-ST01EZ). Separated proteins were immobilized using photoactivated capture chemistry within the ProteinSimple proprietary system (Edouard, Jaafar, et al., 2021). Finally, canine sera diluted at 1:2 were incubated for 60 min followed by a wash step and underwent 30 min incubation within a multi-species HRP-conjugated anti-Fc fragment of IgG/IgM/IgA antibodies (Innovative Diagnostics). The peroxide/luminol-S (ProteinSimple) was used for the chemiluminescent revelation. The Compass Simple Western software (version 5.0.1, ProteinSimple) was used for the automatic calculation of the heights (chemiluminescence intensity), area and signal/noise ratio as well so as to capture the digital image of the capillary chemiluminescence.

2.5 | Virus culture

Virus cell culture was performed for samples of the dog's owners as previously described (La Scola et al., 2020). Briefly, 500 μl of the liquid collected from the nasopharyngeal swab was first passed through a 0.22-μm pore sized centrifugal filter (Merck Millipore, Darmstadt, Germany) and then inoculated in wells of 96-well culture microplates, each well contained Vero E6 cells (American type culture collection ATCC CRL-1586) maintained in minimal essential medium with 4% of foetal bovine serum and 1% glutamine (complete medium). After centrifugation at 4000 *g*, the microplates were incubated at 37°C. The plates were observed daily for evidence of cytopathogenic effect.

TABLE 1 Sampling and results for the analysis [clinical examination, reverse transcription quantitative real-time PCR (RT-qPCR) and serology] performed for the dog

Sampling date	Type of sample	Concentration (RNA copy/μl)	ELISA (OD %)	WB	Clinical phase
13-November-2020	Day 0: nasal swab + blood	1.60E+04	N/A	N/A	Clinical phase (rhinitis)
25-November-2020	Day +12: nasal swab + serum	9.50E+01	Doubtful (14.5%)	+	Asymptomatic phase
02-December-2020	Day +19: nasal swab + serum	1.19E+02	Positive (38.3%)	+++	
11-December-2020	Day +28: nasal swab	<4	N/A	N/A	
10-April-2021	Day +146: nasal swab + serum	Negative	Positive (31.1%)	++	

Abbreviation: ELISA, enzyme-linked immunoassay; WB, western blotting.

2.6 | SARS-CoV-2 whole-genome sequencing

SARS-CoV-2 RNA was extracted from nasopharyngeal swabs of the dog and its owners, and also from the positive culture isolate of the man owner sample, using MagMax Viral/Pathogen kit (Thermo Fisher Scientific, Woodward St. Austin, USA) with Kingfisher Flex System instrument (Thermo Fisher Scientific) according to the manufacturer's instructions. Then, next-generation sequencing (NGS) was performed using the Illumina COVIDSeq Test kit (Illumina Inc., San Diego, CA, USA) that allows a highly accurate detection of SARS-CoV-2, followed by indexation using IDT for PCR Indexes Sets 1–4 (Illumina) according to the manufacturer's instructions. The pool and denaturation of libraries were performed using the protocol B of the 'NovaSeq 6000 System Denature and Dilute Librairies Guide' (Document #1000000106351 v03, Illumina). Sequencing reaction was run on the NovaSeq 6000 (Illumina) for 15 h.

The genomic consensus sequences were generated through mapping on the SARS-CoV-2 genome using the sequence from the Wuhan-Hu-1 reference strain (GenBank accession no: NC_045512.2) with Minimap2 software (Li, 2018). Then, Samtools software was used to allow both soft-clipping PCR primers and removal PCR duplicates, and Freebayes software was used to detect the variant mutants with a minimum mapping quality of 20.

2.7 | SARS-CoV-2 genotyping and phylogeny

The genomic study was performed for all obtained sequences and hallmarks of mutations were retrieved to identify the variant.

Phylogenetic reconstruction was performed using the IQ-TREE software with the GTR Model and 1000 ultrafast bootstrap repetitions after alignment of genomes using MAFFT v.7 (Colson et al., 2021). The tree was visualized with the iTOL (Interactive Tree Of Life) software as previously described (Katoh & Standley, 2013).

3 | RESULTS

3.1 | Dog's clinical examinations, analysis and follow-up

The main clinical sign observed on the dog was rhinitis (a severe bout of acute rhinitis). Except for SARS-CoV-2, all the other tests for canine pathogens implicated in canine cough or rhinitis were negative. The dog was positive for SARS- CoV-2 with a viral load of 16,000 SARS-CoV-2 copy genome equivalent per 1 μl extracted RNA (3.19E+06 copies of viral RNA per swab) using RT-qPCR (Table 1). This viral load is equivalent to viral loads usually detected in humans during the symptomatic phase of the disease (Amrane et al., 2020).

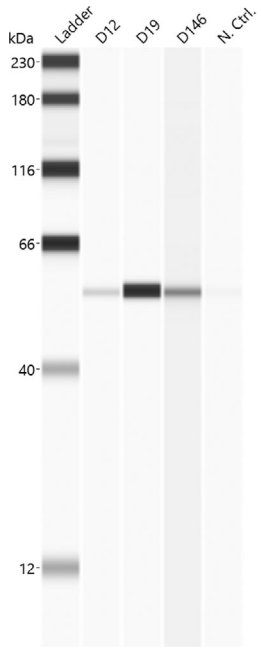

FIGURE 1 Results of the automated western blotting assay of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the dog from France at 12, 19 and 146 days following the presentation of the clinical sign (rhinitis)

The virus concentrations detected at 12, 19 and 28 days later to the first test were 95, 119 and <4 SARS-CoV-2 copy genome equivalent per 1 μl of extracted RNA, respectively, using the same RT-qPCR. No clinical signs were observed during these consultations (Table 1).

For ELISA, when the S/P score was lower than 50%, samples were considered negative. They were considered positive when it was higher than 60% and doubtful when it ranged from 50% to 60%. All dog sera were tested positive (Table 1). Simultaneously, the entire dog's sera were tested positive by WB (Table 1) (Figure 1).

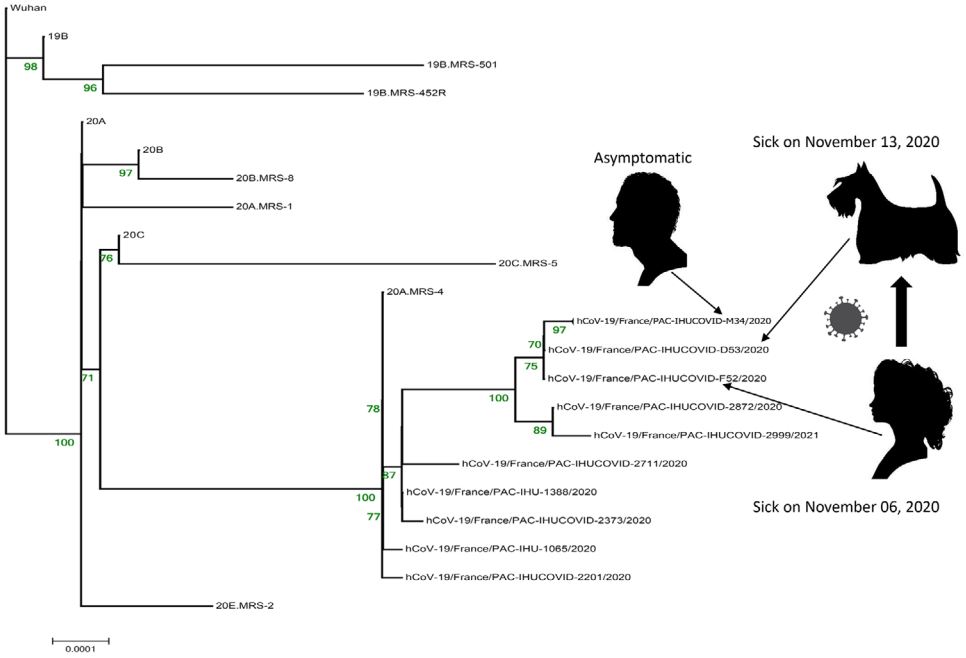

FIGURE 2 Phylogeny tree of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes. This tree includes one representative of each variant, eight samples of Marseille-4 and all the other samples of this study. All sequences are available through GISAID

3.2 | Virus culture

Virus isolation was only positive from the man owner sample, and it was confirmed by both specific RT-qPCR and whole genome sequencing.

3.3 | SARS-CoV-2 genotyping, genome sequencing and phylogenetic tree

Although 100% of whole genome was covered from both nasopharyngeal swab and culture isolate of the man owner, only 83.4% and 51.93% were covered from the first collected dog sample (corresponds to the clinical phase) and woman owner sample, respectively.

Regarding the genomic study, all samples were identified as variant Marseille-4 (20A.EU2; B.1.160). The hallmarks of mutations of this variant were confirmed for most positions except for position C22572T, which was present in the man but not in the dog or the woman sequences. The phylogenetic tree was reconstructed by integrating all samples, as well of other SARS-CoV-2 variants (Figure 2). Within the Marseille-4 clades, all samples (woman, man and dog) were closely related and belonged to the same cluster. Both results (genotyping and phylogeny) suggested a human-to-dog transmission since the owner (the woman) was the first to be infected.

4 | DISCUSSION

More than a year after the COVID-19 pandemic began, it is accepted today that pets are not involved in the transmission of SARS-CoV-2 to humans (Decaro, Balboni, et al., 2021). Cats are more susceptible than dogs and cat-to-cat SARS-CoV-2 transmission has been demonstrated (Halfmann et al., 2020; Hosie et al., 2021). In France, in April 2020, a cat, whose owner had been infected with the virus 17 days prior, presented clinical signs with anorexia, vomiting and coughing (Sailleau et al., 2020). Eight days after the onset of symptoms, molecular analysis (SARS-CoV-2 qPCR) showed that the nasopharyngeal swab was negative, but the rectal swab was positive (Ct: 29). PCRs were negative 28 days after the appearance of the clinical signs. Sequencing was performed and showed that SARS-CoV-2 from the cat was comparable to that circulating in humans at the same time. The genome shows the D614G amino acid mutation in the spike glycoprotein, specific to the A2 clade. This confirmed feline case is the first reported in France.

Cases of SARS-CoV-2 infection in dogs are considered very rare (86 described worldwide compared to more than 216 million cases reported in human). To date, dog-to-dog SARS-CoV-2 transmission (e.g. in a family home or kennel) and dog-to-human transmission have not been demonstrated (Maurin et al., 2021). Dogs are therefore assumed to be infected by their owners. This has been well described, particularly, in Italy and Brazil in large population surveys of canines living in households and in contact with COVID-19 infected or uninfected people (Calvet et al., 2021; Patterson et al., 2020). The SARS-CoV-2 infected dogs have very few clinical signs, most of which are respiratory (Michael et al., 2021). In Texas, as part of a longitudinal household transmission study of pets living with persons with COVID-19, two pets were confirmed to be infected with the SARS-CoV-2 B.1.1.7 variant of concern (VOC). The pets were a dog and a cat from the same household, sampled two days after their owner was tested positive for COVID-19. The oral, nasal and fur swabs for both pets were tested positive for SARS-CoV-2 by qRT-PCR, and consensus whole-genome sequences from the dog and cat were 100% identical and

matched the B.1.1.7 VOC. Sneezing by both pets was noted by the owner in the weeks between initial and follow-up testing (Hamer et al., 2022). Serological investigations show that dogs in contact with the virus produce antibodies without having any symptoms (Laidoudi et al., 2021).

In the laboratory, of five dogs inoculated experimentally by the intranasal route, only one had a positive PCR (rectal swab) 6 days post infection (Shi et al., 2020). In another study, virus shedding was not demonstrated after the experimental infection of three dogs (Bosco-Lauth et al., 2020). In the canine case we studied, the first viral load was high when the dog was symptomatic and the viral RNA-carriage period was longer than in the French cat case because the dog was still positive (by qPCR) 28 days after the first positive diagnosis. However, no virus culture was performed on the dog samples, so we cannot confirm that the dog was infectious or even contagious for 1 month. It is important to note that the 25-year-old daughter of the COVID-19 positive patients lives with her parents and had close contact with the infected dog without becoming positive herself.

For the development of the infection, it is necessary for the receptor encoding angiotensin-converting enzyme 2 (ACE2) to be present in the host. In dogs and cats, as in humans, it binds to the envelope of SARS-CoV-2 through the S-glycoprotein of the virus that allows viral endocytosis (Hernandez et al., 2020; Luan et al., 2020; Zhai et al., 2020; H. L. Zhang et al., 2021). The interaction between the ACE2 receptor and the virus is critical for virus replication. The penetration, multiplication and persistence of the virus could make the dog an epidemiological reservoir of COVID-19 which is even more insidious, due to its low expression of or absence of clinical signs. At this stage of the pandemic and considering the variants of SARS-CoV-2 that are currently circulating, experience shows that the dog does not play this role in the COVID-19 epidemiology. In fact, the virus replicates poorly in dogs, particularly due to the fact that they have few ACE2-carrying cells in the respiratory tracts (Shi et al., 2020; Zhai et al., 2020).

A new 20A variant emerged in June 2020 in agricultural workers in northeast Spain and France in July 2020 (Hodcroft et al., 2020). In Marseille, the first COVID-19 wave (from March to May 2020) was due to the initial virus of Chinese origin. A second wave began in August 2020 with new variants. Sequencing showed that the Marseille-4 variant (Nexstrain clade 20A.UE2) caused almost all of the diagnosed infections in Marseille in late September and also in November 2020 (Hodcroft et al., 2020). This was when the human cases and the canine case described here occurred. The Marseille-4 variant harbours 13 characteristic mutations (Colson et al., 2021). This variant could have originated from SARS-CoV-2 passing through an animal, possibly a mink (Fournier et al., 2021; Fenollar et al., 2021). Based on previous serological studies from France, it was also concluded that transmission from humans to pets (including dogs) was most likely (Fritz et al., 2021; Laidoudi et al., 2021), but these studies could not confirm on the transmission between humans and animals. In this study, genotyping and phylogeny results both highlight virus transmission between humans and dogs, with evidence of human-to-dog transmission since the woman owner was the first to be infected.

5 | CONCLUSION

We describe here the first confirmed case of COVID-19 in a dog in France. Transmission of the virus from human to dog is therefore possible (Leroy et al., 2020). Dogs appear to be susceptible to the Marseille-4 variant and can develop mild clinical signs such as rhinitis. However, it is unknown whether this variant is more infectious for dogs as compared to the original strain used in previous experimental infections. Further studies will be needed to determine whether this variant is indeed more infectious to dogs. Moreover, given the enhanced infectivity and transmissibility of the Marseille-4 variant for humans, this case also highlights the risk that pets can potentially play a significant role in SARS-CoV-2 outbreaks and may transmit the infection to humans. Owners and veterinarians must be aware of this and suspect COVID-19 in dogs presenting respiratory clinical signs, especially when owners have been tested positive for SARS-Cov-2.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

All the human data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille university hospitals), and this study results from routine standard clinical management. This study has been approved by the ethics committee of our institution (No. 2020–029). Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019–73.

All applicable international and national guidelines for the care of dogs were followed. The owners of the dog gave their consent for the samples to be taken by a veterinarian.

AUTHOR CONTRIBUTIONS

Conceptualization: Hacène Medkour, Sébastien Catheland, Corine Boucraut-Baralon and Bernard Davoust. *Methodology*: Hacène Medkour, Sébastien Catheland, Corine Boucraut-Baralon, Younes Laidoudi, Youssouf Sereme, Anthony Levasseur and Bernard Davoust. *Validation*: Anthony Levasseur and Corine Boucraut-Baralon. *Formal*

analysis: Hacène Medkour, Jean-Luc Pingret, Younes Laidoudi, Youssouf Sereme, Linda Houhamdi and Bernard Davoust. *Clinical investigations*: Matthieu Million and Sébastien Catheland. *Resources*: Julien Cabassu, Corine Boucraut-Baralon and Bernard Davoust. *Data curation*: Anthony Levasseur. *Writing—original draft preparation*: Hacène Medkour, Younes Laidoudi, Corine Boucraut-Baralon, Anthony Levasseur, Youssouf Sereme and Bernard Davoust. *Writing—review and editing*: Hacène Medkour and Bernard Davoust. *Supervision and project administration*: Bernard Davoust. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Data underlying the study are available from the GISAID database (https://www.gisaid.org/) or from the corresponding author upon request.

DECLARATION TO THE AUTHORITIES

The World Organisation for Animal Health (OIE) guidelines requires notification of SARS-CoV-2 animal cases. The doctor in veterinary medicine B. Davoust declared the confirmed canine case in Marseille to the local veterinary authority (Direction départementale de la protection des populations des Bouches-du-Rhône) on 06 March 2021.

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