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1 **One Health**

2 Research Article

3

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

7

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21

22

23 **ABSTRACT**

24 Dogs are occasionally susceptible to SARS-CoV-2, developing few or no clinical signs.

25 Epidemiological surveillance of SARS-CoV-2 in dogs requires testing to distinguish it from

26 other canine coronaviruses. In the last year, significant advances have been made in the

27 diagnosis of SARS-CoV-2, allowing its surveillance in both human and animal populations.

28 Here, using ELISA and automated western blotting (AWB) assays, we performed a

29 longitudinal study on 809 apparently healthy dogs from different regions of France to
30 investigate anti-SARS-CoV-2 antibodies. There were three main groups: (i) 356 dogs sampled
31 once before the pandemic, (ii) 235 dogs sampled once during the pandemic, and (iii) 218
32 dogs, including 82 dogs sampled twice (before and during the pandemic), 125 dogs sampled
33 twice during the pandemic and 11 dogs sampled three times (once before and twice during the
34 pandemic). Using ELISA, seroprevalence was significantly higher during the pandemic [5.5%
35 (25/453)] than during the pre-pandemic period [1.1% (5/448)]. Among the 2018 dogs sampled
36 twice, at least 8 ELISA-seroconversions were observed. ELISA positive pre-pandemic sera
37 were not confirmed in serial tests by AWB, indicating possible ELISA cross-reactivity,
38 probably with other canine coronaviruses. A significant difference was observed between
39 these two serological tests (Q=88, p=0.008). A clear correlation was observed between
40 SARS-CoV-2 seroprevalence in dogs and the incidence of SARS-CoV-2 infection in human
41 population from the same area. AWB could be used as a second line assay to confirm the
42 doubtful and discrepant ELISA results in dogs. Our results confirm the previous experimental
43 models regarding the susceptibility of dogs to SARS-CoV-2, suggesting that viral
44 transmission from and between dogs is weak or absent. However, the new variants with
45 multiple mutations could adapt to dogs; this hypothesis cannot be ruled out in the absence of
46 genomic data on SARS-CoV-2 from dogs.

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

48

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54 **1. Introduction**

55 Severe Acute Respiratory Syndrome, caused by SARS-CoV-2 coronavirus, a novel
56 emergent variant involved in epidemic disease, was first identified in November 2019 in
57 Wuhan city (Hubei province), China [1,2]. A few months later, the World Health
58 Organization declared a global pandemic. By early July 2021, more than 183 million cases
59 and 3.96 million deaths had been recorded worldwide [3]. In France, the first human cases
60 were diagnosed in late January 2020. At the beginning of July 2021, the cumulative incidence
61 for France reached almost 5.84 million, including 111,297 deaths [3,4].

62 Phylogenetically, SARS-CoV-2 is closely related to SARS CoV (or SARS-CoV-1),
63 which was already involved in the 2003 epidemic, and to BatCoV RaTG13, a
64 Betacoronavirus naturally occurring in bats [5,6]. The scientific community assumes that
65 SARS-CoV-2 has a zoonotic origin from bats, while the intermediate host between bats and
66 humans is not yet known [2,5,6]. Due to the presence of specific receptors for SARS-CoV-2
67 virus in the respiratory tract of mustelids (i.e. ferrets and minks), they are the most susceptible
68 species under both experimental and natural conditions [7]. Globally, coronaviruses are
69 widely distributed in animal fauna (i.e. birds, pigs, ruminants, dogs, cats, etc.)
70 [2,5,6,7,8,9,10,11]. Since the 1970s, Alpha and Betacoronavirus have been in the forefront as
71 the causative agents of canine enteric coronavirus (CECoV) and respiratory coronavirus
72 (CRCoV), respectively [12,13]. However, dogs are occasionally susceptible to SARS-CoV-2
73 with only 31 cases diagnosed worldwide by specific analysis (RT-qPCR) in Hong Kong,
74 USA, Japan, Argentina and Italy by the end of 2020 [9,14,15]. Dogs infected with SARS-
75 CoV-2 have few or no clinical signs [14]. Epidemiological surveillance of SARS-CoV-2 in
76 dogs requires reliable serological methods to distinguish SARS-CoV-2 from other canine
77 coronaviruses. In the last year, advances have been made in the diagnosis of SARS-CoV-2,
78 and monitoring the circulation of the virus in human and animal populations. Here, we

79 performed a longitudinal study of the seroprevalence of SARS-CoV-2 in apparently healthy
80 dogs from different regions of France to highlight the epidemiological role of these animals in
81 the current SARS-CoV-2 pandemic.

82

83 **2. Materials and methods**

84

85 **2.1. Dogs**

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

98 Serum samples were taken in veterinary clinics for screening by veterinary doctors
99 and with the agreement of the owners of the dogs.

100 **2.2. ELISA assay**

101 All sera were subjected to screening for antibodies to SARS-CoV-2 using ID Screen[®]
102 SARS-CoV-2 Double Antigen Multi-species (Innovative Diagnostics, Grabels, France)
103 according to the manufacturer's instructions. The test consists of an ELISA (enzyme-linked

104 immunosorbent assay), that detects antibodies to the major nucleocapsid protein of SARS-
105 CoV-2 on multispecies (i.e. minks, ferrets, cats, dogs, cattle, sheep, goats, horses and all other
106 receptive species) with a specificity range of 97.8% to 100% as reported by the manufacturer
107 (Supplementary data 1). Plates were sensitized with a purified recombinant N antigen. Optical
108 density (OD) was measured at 450 nm using Multiskan GO software (Thermo Scientific,
109 Waltham, MA, USA). The assay was validated when the optical density of positive control
110 (OD_{PC}) was ≥ 0.35 and a mean of positive control (OD_{PC}) to negative control (OD_{NC}) control
111 ratio was greater than three. The optical density of each sample (OD_N) was used to calculate
112 the S/P ratio value (expressed as %) where $S/P = 100 * (OD_N - OD_{NC}) / (OD_{PC} - OD_{NC})$.
113 Samples tested by ELISA were considered positive if the S/P ratio was greater than 60% and
114 doubtful when the P/S ratio ranged between 50 and 60%, while samples displaying an S/P
115 score lower than 50% in ELISA were considered negative.

116

117 **2.3. SARS-CoV-2 antigen preparation and automated western blotting (AWB) assay**

118 The SARS-CoV-2 IHUMI2 strain (lineage 20a) was used to produce of SARS-CoV-2
119 specific antigens as previously described [16]. Briefly, virions were purified and harvested
120 from in vitro infected cells and then fractionated with TS buffer (7 M Urea, 2 M Thiourea, 4%
121 Chaps) to release SARS-CoV-2 antigens. The released antigens were concentrated using the
122 Amicon 3 kDa filter (Merck KGaA, Darmstadt, Germany) before being used in the
123 Automated Western Blotting (AWB) assay [16,17].

124 The JessTM Simple Western automated nano-immunoassay system (ProteinSimple, San
125 Jose, CA, USA, a Bio-Techne Brand), a capillary-based size separation of proteins [16] was
126 used with an internal system control to evaluate the absolute serological response to viral
127 antigens from all ELISA-positive samples. Canine sera were processed according to the
128 manufacturer's standard method for the 12-230-kDa Jessseparation module (SM-W004). The

129 Edouard's protocol [16] was adapted for the detection of canine antibodies to SARS-CoV-2.
130 Briefly, a mixture of SARS-CoV-2 antigens, fluorescent molecular weight markers and 400
131 mM dithiothreitol (Protein Simple) was prepared at final concentration of 0.25 $\mu\text{g}/\mu\text{l}$, and then
132 denatured at 95°C for 5 minutes. Migration of viral proteins through the separation matrix
133 was performed at 375 volts for both SARS-CoV-2 antigens and Ladder (12-230-kDa PS-
134 ST01EZ). The separated proteins were immobilized using the photoactivated capture
135 chemistry within the ProteinSimple proprietary [16]. Subsequently, 1:2 diluted dog sera were
136 incubated for 60 minutes followed by a wash step and a 30 minute incubation within a multi-
137 species HRP-conjugated anti-Fc fragment of IgG/IgM/IgA antibodies (Innovative
138 Diagnostics, Grabels, France). Peroxide/luminol-S (ProteinSimple) was used for
139 chemiluminescent revelation. The Compass Simple Western software (version 5.0.1,
140 ProteinSimple) was used for the automatic calculation of the heights (chemiluminescence
141 intensity), area and signal/noise ratio as well as to capture the digital image of the capillary
142 chemiluminescence.

143

144 ***2.4. Statistical analysis***

145 Comparison between dog's populations was performed using Fisher's exact and Chi-
146 squared tests. Mc Nemar's test was used to compare between ELISA and AWB assays. The
147 exact p-value was computed, and the significant difference was considered at a p-value
148 ≤ 0.05 . All statistical analysis were performed using Addinsoft software (XLSTAT 2018:
149 Data Analysis and Statistical Solution for Microsoft Excel, Paris, France).

150

151 **2. Results**

152

153 **3.1. ELISA antibody detection**

154 Of the 448 sera sampled before the pandemic, 4 (0.9%) were ELISA positive and 1
155 (0.2%) was inconclusive. While of the 453 sera collected during the pandemic, 22 (4.8%)
156 were positive and 3 (0.6%) were classified as doubtful. The infection rate was significantly
157 higher during the pandemic than in the pre-pandemic period; this was observed for all sera
158 (Table 1). In addition, at least 8 ELISA-seroconversions were observed among the 218 dogs
159 during the pandemic (Table 2). During the pandemic, a total of 17 (4.3%) out of 397 MWD
160 and 7 (12.5%) out of 56 companion dogs reacted in the ELISA test, corresponding to a
161 significant difference ($\chi^2=6.61 - p \leq 0.02$) between these two populations. Fourteen (11.1%)
162 of 126 dogs sampled in February 2021 from the South-East area scored positive. A lower
163 prevalence of 3.1% (3/95) was found in the South-West than in the South-East ($\chi^2=4.7 -$
164 $p \leq 0.05$) (Table 3).

165

166 **3.2. Automated western blot results**

167 Of the selected 44 serum samples listed in table 2, 34 (consisting of 25 positive, 3
168 doubtful and 6 negative sera by ELISA assay). AWB detected 17 (68%) of the 25 ELISA-
169 positive sera herein tested. In addition, all doubtful sera within ELISA test (n=3) were scored
170 positive by the AWB assay. Overall, all AWB-positive sera were sampled between the period
171 ranging from January 2020 to February 2021. While no ELISA-positive sera collected before
172 the pandemic or negative controls were detected by the AWB (Figure 1). Which correspond to
173 a significant difference between these two assays ($Q=8, p=0.008$). Finally, all AWB-positive
174 sera yielded a prominent 56-kDa band interpreted as nucleocapsid proteins, while no bands
175 were detected for the other major dominant proteins, such as the protein S (i.e. 170 kDa), S1
176 (i.e. 110 kDa) and S2 (i.e. 90 kDa) (Figure 1).

177

178 **4. Discussion**

179 To date, studies investigating SARS-CoV-2 in dogs are scarce, probably due to the
180 lower susceptibility of dogs to this infection and the focus of research on human disease. In
181 France, only two serological studies have been carried out on dogs. One study based on the
182 luciferase immuno-precipitation assay and involving 12 dogs from SARS-CoV-2-positive
183 owners. None of these dogs was scored positive [18]. The second study was performed using
184 microsphere immunoassay on 13 dogs from SARS-CoV-2-positive owners and 22 dogs from
185 owners with an unknowing SARS-CoV-2 status. Only two dogs were reported positive by the
186 authors from positive owners [19]. In Italy, the antibody neutralization assay was used to
187 monitor SARS-CoV-2 infection in 451 dogs during the pandemic, and 15 (3.3%) dogs were
188 found seropositive [20]. In Wuhan city (China), 16 (1.7%) positive dogs were detected among
189 the 946 using a newly developed double-antigen sandwich ELISA assay [21]. In Croatia, an
190 investigation revealed that 7.6% of dogs (13/172) were positive by ELISA assay [22]. In
191 Texas, USA, 15.3% of 59 dogs were positive for SARS-CoV-2 by RT-PCR and genome
192 sequencing or neutralizing antibodies, in homes where at least one human case of COVID-19
193 was diagnosed [23]. The overall prevalence of canine SARS-Cov-2 infection in Spain was
194 16.7% (10/60), particularly in dogs from COVID-19-positive households, indicating their
195 susceptibility to SARS-CoV-2 infection [24]. These discrepancies in results between the
196 different studies may be related to the sensitivity of the different assays. The results of this
197 comprehensive study of SARS-CoV-2 infection in companion and military working dogs
198 sampled before and during the pandemic in areas of active human viral transmission allowed
199 the evaluation of the specificity of the ELISA and AWB assays. The same ELISA test used in
200 our study detected anti-SARS-CoV-2 antibodies in the serum of PCR-positive cats, living in a
201 household in Chile, where a human was infected [25].

202 In our study, the ELISA we used detected 1.1% of 448 pre-pandemic sera. This
203 highlights the possible cross reactivity with other canine coronaviruses, probably the
204 Betacoronavirus of dogs [26]. On the other hand, the seroconversion of 8, as well as the
205 significant increase in seroprevalence in dogs during the pandemic (i.e., 5.5% out of 453 dogs
206 tested), particularly in the Bouches-du-Rhône region, a high endemic area for human SARS-
207 CoV-2 infection (www.casccoronavirus.fr), could explain the occurrence of SARS-CoV-2
208 infection in dogs. On the other hand, the AWB assay yielded the detection of 66% ELISA-
209 positive sera. However, all of them were sampled between the periods ranging from January
210 2020 to February 2021, which is in line with the outbreak of the pandemic in France. In
211 addition, some inconsistencies were also observed between these two assays. For example,
212 some dogs with high ELISA S/P ratio sampled before the pandemic (i.e. dog D1 and D2) or
213 even during the pandemic (i.e. dog D6 and D7) gave a negative AWB result, whereas some
214 ELISA-negative or doubtful sera with low ELISA S/P ratio (i.e. dog D14, D22, D26 and D29)
215 were positive using AWB assay (Fig.1). Though few canine sera were herein tested by the
216 AWB, which may represent a limitation of the assay, all AWB-positive sera were sampled
217 during the pandemic which suggests the specific detection of antibodies to SARS-CoV-2 in
218 dogs. The discrepancy between these two assays could be explained by the type of antigens
219 used for each assay. ELISA test was developed on the basis of a truncated N recombinant
220 antigen from the viral nucleocapsid which probably provided the detection of conformational
221 epitopes that could also be shared with the other coronaviruses. In contrast, the use of the
222 integral SARS-CoV-2 nucleocapsid antigens in AWB assay may led the detection of linear
223 epitopes only [17]. However, the clear-cut decision regarding the specificity of the AWB
224 assay cannot be ruled out in the absence of a reliable gold standard, since the possible cross-
225 reaction has already been described with other human Betacoronavirus within the AWB assay
226 [17].

227 The AWB assay based on the purified virus antigens was first adapted for the diagnosis
228 and the evaluation of the human immune-response against SARS-CoV-2 antigens. The assay
229 proved to be effective principally in detecting antibodies to nucleocapsid proteins [17]. Our
230 results showed that the AWB yielded only the detection of antibodies against the
231 nucleocapsid proteins from all positive dogs. However, the lower sensitivity of AWB to spike
232 proteins in dogs may due to the use of the integral, which may give rise hidden epitopes.

233 Despite the receptivity of dogs to SARS-CoV-2 infection under experimental conditions
234 [27], they were unable to transmit the virus [7,9,10]. Our results indicated that, in spite of the
235 presence of positive dogs in kennels, there were most probably few infected animals.
236 Thereby, this suggests that dogs do not transmit the virus, which may be due to the poor viral
237 replication in dogs [28]. On the other hand, previous studies have demonstrated the presence
238 of a few differences between human and canine angiotensin-converting enzyme 2 (ACE2), the
239 interactive receptor within the spike protein of the SARS-CoV-2 [9]. However, recent studies
240 have demonstrated the continuous emergence of new SARS-CoV-2 with multiple spike
241 protein mutations. It is not known whether dogs infected with these new variants could
242 transmit the virus to other animals or to humans [29,30,31]. In March 2021, a study carried
243 out on British dogs reported for the first time canine and feline infections with the SARS-
244 CoV-2 B.1.1.7 variant in addition to some of these pets suffering from myocarditis [32].

245

246 **5. Conclusion**

247 The AWB assay, previously standardized as first or second line method to confirm the
248 diagnosis of SARS-CoV-2 from human patients, could also be used as a second line assay to
249 confirm negative, doubtful and discrepant ELISA results in dogs. These findings along with
250 the results from the previous experimental models of SARS-CoV-2 in dogs confirm the
251 receptivity of dogs to SARS-CoV-2 infection. They also suggest the absence of the virus

252 transmission from infected to non-infected dogs as well as to humans. In the absence of
253 genomic data on SARS-CoV-2 in dogs, the hypothesis that new SARS-CoV-2 variants with
254 multiple mutations in the spike protein could induce adaptation of the virus to dogs cannot be
255 ruled out.

256

257 **Authors' contributions**

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

262

263 **Ethics approval and consent to participate**

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

267

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273

274 **Declaration of competing interest**

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

278

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282

283 **References**

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415 **Legends**

416

417 **Table 1**

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

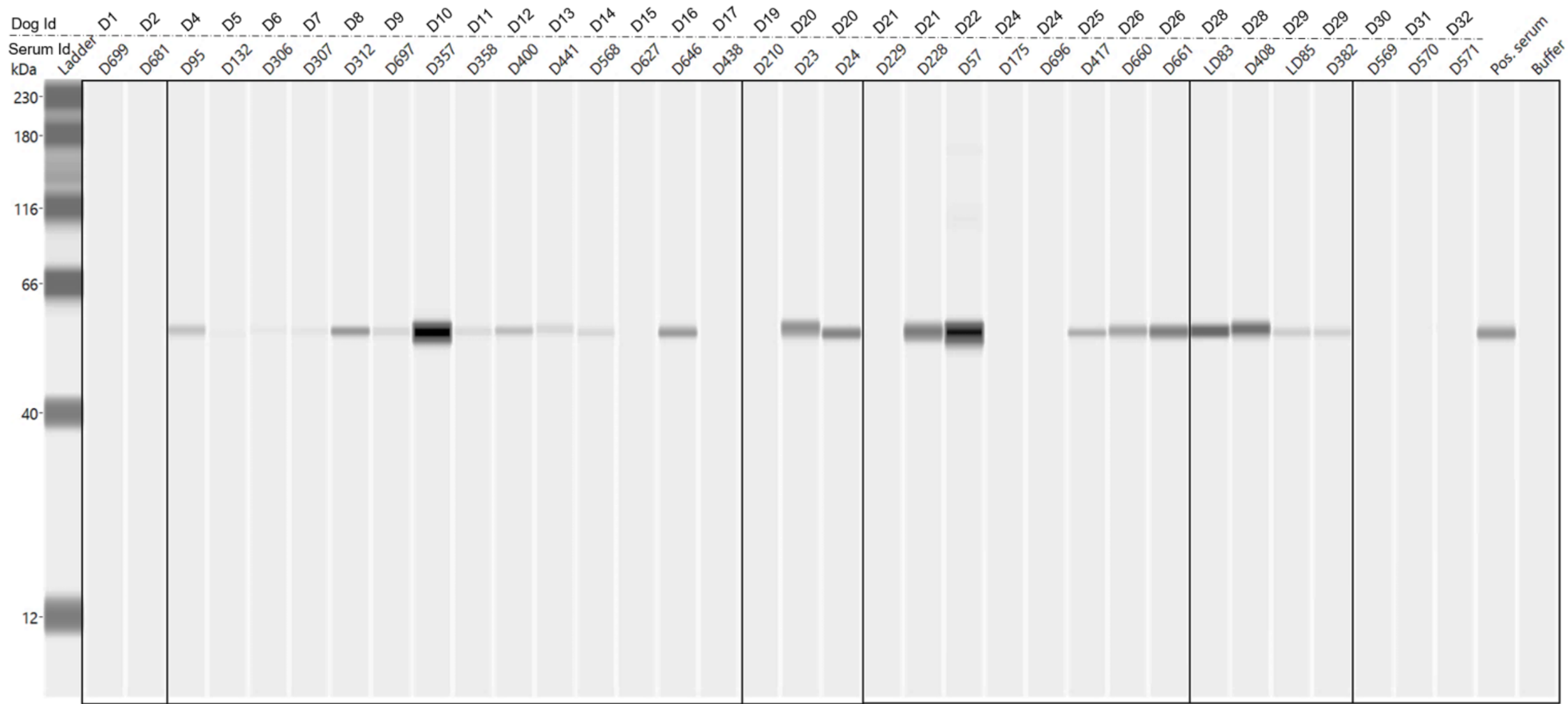
420 **Table 2**

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

423 **Table 3**

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

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



Block a

Block b

Block c

Block d

Block e

Block f: Controls

Block a: Dogs collected once before the pandemic

Block b: Dogs collected once during the pandemic

Block c: Dogs collected twice, once before and once during the pandemic

Block d: Dogs collected twice during the pandemic

Block e: Dogs collected three times, once before and twice during the pandemic

Block f: Controls

	Samples of dogs	No. of positive (%)	No. of doubtful (%)	No. of sera reacted with ELISA (%)
Total sera	Before the pandemic (N=448)	4 (0.9%)	1 (0.2%)	5 (1.1%)
	During the pandemic (N=453)	22 (4.8%)	3 (0.6%)	25 (5.5%)
	Statistics	Khi2=13.56; p<0.001		
Sera sampled one time	Before the pandemic (N=356)	3 (0.8%)	0 (0%)	3 (0.8%)
	During the pandemic (N=235)	14 (5.9%)	0 (0%)	14 (5.9%)
	Statistics	Khi2=13.26; p<0.001		
Dogs' activity	MWD (N=397)	15 (3.7%)	2 (0.5%)	17 (4.3%)
	SD and PD (N=56)	7 (12.5%)	0 (0%)	7 (12.5%)
	Statistics	Khi2=6.61; p<0.02		

SD: shelter dogs, MWD: military working dogs, PD: pet dogs

	Serum Id.	Dog Id.	Dog category	Location Department	Date of sample	ELISA OD	(S/P) %	ELISA result	WB result
Dogs collected once before the pandemic (N=356)	D699	D1	SD	French Guiana	01.2016	0.558	231.1	Positive	Negative
	D681	D2	SD	Corsica	03.2018	1.012	424.3	Positive	Negative
	D662	D3	MWD	Bouches-du-Rhône	10.2018	0.214	84.7	Positive	Not tested
Dogs collected once during the pandemic (N=235)	D95	D4	MWD	Marne	06.2020	3.89	278.1	Positive	Positive
	D132	D5	MWD		09.2020	0.901	61.2	Positive	Negative
	D306	D6	SD		02.2021	0.78	166.8	Positive	Negative
	D307	D7	SD		02.2021	0.764	162.9	Positive	Negative
	D312	D8	SD		02.2021	0.62	127.8	Positive	Positive
	D697	D9	SD	Bouches-du-Rhône	02.2021	0.197	77.4	Positive	Positive
	D357	D10	SD		02.2021	0.739	156.8	Positive	Positive
	D358	D11	SD		02.2021	0.52	103.4	Positive	Positive
	D400	D12	MWD		02.2021	1.648	435.2	Positive	Positive
	D441	D13	MWD		02.2021	0.82	207.7	Positive	Positive
	D568	D14	MWD	Lot	02.2021	0.332	49.0	Positive	Positive
	D627	D15	MWD		02.2021	0.41	61.2	Positive	Negative
	D646	D16	MWD		02.2021	0.421	62.9	Positive	Positive
	D438	D17	MWD	Bouches-du-Rhône	02.2021	0.29	62.1	Positive	Negative
	Dogs collected twice, once before and once during the pandemic (82)	D560	D18	MWD	Bouches-du-Rhône	06.2018	0.043	0.7	Negative
CR10		MWD		10.2020		0.344	55.3	Doubtful	Not tested
D211		D19	MWD	Marne	11.2019	0.065	1.4	Negative	Not tested
D210			MWD		05.2020	1.166	96.0	Positive	Negative
D23			MWD		01.2020	1.31	83.0	Positive	Positive
D24			MWD		06.2020	1.373	87.2	Positive	Positive
Dogs collected twice during the pandemic (N=125)	D229	D21	MWD	Marne	03.2020	0.21	13.8	Negative	Negative
	D228		MWD		07.2020	0.821	66.4	Positive	Positive
	D57		MWD		04.2020	0.435	25.0	Negative	Not tested
	D58	D22	MWD	Marne	09.2020	0.89	55.2	Doubtful	Positive
	D96		MWD		06.2020	0.292	17.0	Negative	Not tested
	D97	D23	MWD	Marne	10.2020	0.704	46.9	Negative	Not tested
	D175		MWD		07.2020	0.069	0.8	Negative	Negative
	D696		MWD		01.2021	0.106	38.7	Negative	Negative
	LD82	D24	MWD	Bouches-du-Rhône	10.2020	0.314	27.1	Negative	Not tested
	D417		MWD		02.2021	0.78	196.7	Positive	Positive
	D660		PD		11.2020	0.049	14.5	Doubtful	Positive
D661	D26	PD	Bouches-du-Rhône	12.2020	0.105	38.3	Positive	Positive	
Dogs collected three times, once before and twice during the pandemic (N=11)	LD84	D28	MWD	Bouches-du-Rhône	06.2018	0.101	3.7	Negative	Not tested
	LD83		MWD		10.2020	1.503	157.6	Positive	Positive
	D408		MWD		02.2021	0.512	123.1	Positive	Positive
	LD86	D29	MWD	Bouches-du-Rhône	06.2018	0.111	4.8	Negative	Not tested
	LD85		MWD		10.2020	0.592	57.6	Doubtful	Positive
	D382		MWD		02.2021	0.549	133.2	Positive	Positive
Negative controls	D569	D30	MWD	Lot	02.2021			Negative	Negative
	D570	D31	MWD		02.2021			Negative	Negative
	D571	D32	MWD		02.2021			Negative	Negative

SD: shelter dog, MWD: military working dog, PD: pet dog

OD: optical density

WB: western-blot

Location Department	Number of dogs	Number of ELISA negative dogs	Number of ELISA positive dogs	Canine seroprevalence (%)	Human COVID-19 incidence rate per 100,000 inhabitants as of 02/16/2021	Seropositivity rate of COVID-19 virological tests carried out in humans (%) as of 02/16/2021
Bouches-du-Rhône (South-East)	126	113	14	11.1	332	6.6
Lot (South-West)	95	92	3	3.1	100	2.9
Total	221	205	17	7.7		
Statistical comparisons				Chi-square = 4.7 between these two groups - significant difference $p \leq 0,05$	Chi-square = 124 between these two groups - very significant difference	