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Identification of virulent *Capnocytophaga canimorsus* isolates by capsular typing

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1 **“Identification of virulent *Capnocytophaga canimorsus* isolates by capsular**

2 **typing”**

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17 **Running title:** Capsular typing of *C. canimorsus*

18

19 **Abstract**

20 *Capnocytophaga canimorsus* is a dog oral commensal that causes rare but severe
21 infections in humans. *C. canimorsus* was recently shown to be endowed with a capsular
22 polysaccharide implicated in the resistance to the innate immune system of the host.
23 Here we developed the first *C. canimorsus* capsular serotyping scheme. We describe
24 nine different serovars (A to I), which allowed to type 25/25 isolates from human
25 infections but only 18/52 isolates from dog mouths, indicating that the repertoire of
26 capsules in the species is vast. However, three serovars only (A, B, and C) covered 88
27 % of the human isolates tested (22/25) while they covered only 7.7 % of the dog isolates
28 (4/52). Serovars A, B, and C were found 22.9, 14.6, and 4.2 fold respectively more often
29 among human isolates than among dog isolates, with no geographical bias, implying
30 that isolates endowed with these three capsular types are more virulent for humans than
31 other isolates. Capsular serotyping would thus allow to identify virulent isolates in dogs,
32 which could contribute to the prevention of these infections. To this end, we developed a
33 PCR typing method based on the amplification of specific capsular genes.

34

35 **Introduction**

36 *Capnocytophaga canimorsus* are agents of septicemia that often evolve to a septic
37 shock in spite of an adequate treatment (1). Since their discovery in 1961 (2) more than
38 480 cases of infections were reported in the literature (for a recent review see (3)). With
39 a mortality rate of 30 % and significant morbidity, the prognosis of *C. canimorsus* sepsis
40 is poor (4, 5). Although less frequently reported, meningitis and endocarditis are also
41 associated with *C. canimorsus* infections (3). The genus *Capnocytophaga*, which
42 belongs to the family of *Flavobacteriaceae* in the phylum of *Bacteroidetes* comprises

43 capnophilic species found in the oral cavities of human and domestic animals. The dogs
44 and cats mouth hosts *C. canimorsus* (6), formerly dysgonic fermenter-2 (DF-2), *C.*
45 *cynodegmi* (1, 6) and the newly described *C. canis* (7) and “*C. stomatis*” (8) but only *C.*
46 *canimorsus* is associated with severe human infections (1, 7, 8). According to studies
47 carried out in different countries, the prevalence of *C. canimorsus* ranges from 19 to
48 74% in dogs and 21 to 57% in cats (9-13). However, these figures may include *C. canis*
49 and “*C. stomatis*” that were separated recently from the *C. canimorsus* species.
50 Transmission to humans mostly occurs through dog (97%) or cat (3%) bites, scratches,
51 licks, or simple contact (3, 14). The prevalence of *C. canimorsus* infections was
52 estimated at 0.5 and 0.63 case per million inhabitants per year in Denmark (5) and in the
53 Netherlands (15) respectively but a recent study in the Helsinki area (Finland) estimated
54 the prevalence as high as 4.1 cases per million inhabitants per year (16). *C. canimorsus*
55 infections could thus be under-diagnosed due to the fastidious and slow growth of *C.*
56 *canimorsus* in culture (1, 17). In addition, the initial clinical manifestations of *C.*
57 *canimorsus* infections are not specific and their onset can be as late as 8 days after
58 contact with a dog (3, 5). The median age of patients is comprised between 52 and 59
59 years and a male to female ratio of 3/2 is generally observed (3, 5, 16). Splenectomy
60 and alcohol abuse are common predisposing factors but up to 40% of patients
61 presented no obvious risk factor (18) implying that *C. canimorsus* cannot solely be
62 considered as an opportunistic pathogen.

63 *C. canimorsus* strain 5 (Cc5, BCCM/LMG 28512), a strain isolated from a fatal
64 septicemia (19) has a lipooligosaccharide (LOS) and a capsular polysaccharide (CPS)
65 which are genetically and biochemically related (20). The CPS plays a key role in the
66 innate immunity evasion by conferring Cc5 its resistance to phagocytosis by

67 macrophages, to polymyxin B, and to 10 % human serum (20). In addition to being
68 recognized virulence factors for both Gram-negative and -positive bacteria (for review
69 see (21)), CPS are also useful to serotype bacteria and to identify virulent isolates (22,
70 23). Here we show that 25 isolates of *C. canimorsus* out of 25 from a collection of
71 isolates from human infections are endowed with a CPS and that those polysaccharide
72 structures present a limited variability, with 3 dominant capsular serovars. In addition, a
73 clear enrichment of these dominant capsular serovars was found in human isolates
74 (22/25) as compared to isolates from dog mouths (4/52). Finally, we show that PCR
75 typing can be used to detect these serovars more virulent for humans. This study paves
76 the way to prevention of these dramatic infections.

77

78 **Material and methods**

79 **Bacterial strains, isolates, and culture conditions**

80 Bacterial strains and isolates used in this study are listed in the **Supplementary Tables**
81 **S1** and **S2**. *C. canimorsus* were grown on heart infusion agar (HIA; BD Difco, Franklin
82 Lakes, NJ, USA) supplemented with 5% sheep blood (SB; Oxoid, Basingstoke, UK)
83 plates (SB plates) for 48h at 37°C with 5% CO₂. *Escherichia coli* were routinely grown in
84 lysogeny broth (LB; Invitrogen, Waltham, MA, USA) at 37°C. Antibiotics used as
85 selective agents were added at the following concentrations: 100 µg/mL ampicillin
86 (AMP) and 50 µg/mL kanamycin (KAN) for *E. coli* and 20 µg/mL gentamicin (GEN), 10
87 µg/mL erythromycin (ERY), and 10 µg/mL ceftiofur (FOX) for *C. canimorsus*. Unless
88 otherwise stated products were purchased from Sigma-Aldrich (Darmstadt, Germany).

89

90 **Anti-sera production and adsorption**

91 Bacteria were grown for 2 days on SB plates supplemented with GEN, gently scraped
92 from the agar, resuspended and washed in PBS. Bacteria were fixed overnight in 0.3%
93 paraformaldehyde (PFA), washed in PBS and inoculated to a rabbit to generate
94 polyclonal sera. Sera were generated at the University of Namur (Belgium) or at the
95 Centre d'économie rurale (CER Groupe; Aye, Belgium). The respective Animal Welfare
96 Committees approved the animal handling and procedures. Polyclonal sera were
97 adsorbed by incubation with an excess of PFA-fixed non-capsulated mutant bacteria
98 unless stated otherwise in results. Incubations were done on a rotating wheel at room
99 temperature (RT) and repeated four times. Bacteria were removed by repeated
100 centrifugations. Adsorption efficacy was assessed by immunofluorescence as follow.
101 Glass coverslips were coated with poly-D-lysine (10 $\mu\text{g}/\text{mL}$ in PBS, for 1 hour at 37°C),
102 washed and incubated for 30 min at 37°C with 300 μL of a bacterial suspension adjusted
103 to an OD_{600} of 0.25. Coverslips were then washed and bacteria were fixed for 15 min
104 with 4% PFA. Coverslips were washed again and blocked with 1% bovine serum
105 albumin (BSA) for 1 hour at RT. Bacteria were stained with the adsorbed sera (1/1000 in
106 PBS) for 1 hour at RT followed by an incubation with an Alexa Fluor 488-coupled donkey
107 anti-rabbit antibody (1/5000 in PBS; Life technologies, Waltham, MA, USA) or a Texas
108 Red coupled goat anti-rabbit antibody (1/1000 in PBS, Southern Biotech, Birmingham,
109 AL, USA) for 45 min. Coverslips were mounted using mowiol mounting medium and
110 images were acquired with an Axio Imager.Z1 (Zeiss, Oberkochen, Germany) and
111 analyzed using Zen 2012 software (Zeiss).

112

113 **Mutagenesis by allelic exchange**

114 Mutagenesis of Cc6, Cc9, and Cc12 strains was performed as previously described (24).
115 The *C. canimorsus* deletion mutants and the *E. coli* strains used are listed in the
116 **Supplementary Table S2**. Briefly, replacement cassettes with flanking regions spanning
117 approximately 500 base pairs (bp) homologous to regions directly framing targeted
118 genes were constructed with a three-fragment overlapping PCR strategy. First, two
119 PCRs were performed on 100 ng of Cc6, Cc9, or Cc12 genomic DNA with primers 1.1
120 and 1.2 for the upstream flanking regions and with primers 2.1 and 2.2 for the
121 downstream regions (**Supplementary Table S3**). Primers 1.2 and 2.1 contained an
122 additional 5' 20-nucleotide extension homologous to the *ermF* insertion cassette. The
123 *ermF* resistance cassettes were amplified from plasmid pMM13 (24) DNA, with primers
124 3.1 and 3.2. All three PCR products were cleaned and then mixed in equal amounts for
125 PCR using Phusion polymerase (Finnzymes, Espoo, Finland). The initial denaturation
126 was at 98°C for 2 min, followed by 12 cycles without primers to allow annealing and
127 elongation of the overlapping fragments (1 cycle consists of 98°C for 30 s, 50°C for 40 s,
128 and 72°C for 2 min). After the addition of external primers (primers 1.1 and 2.2), the
129 program was continued with 20 cycles (1 cycle consists of 98°C for 30 s, 50°C for 40 s,
130 and 72°C for 2 min 30 s) and finally 10 min at 72°C. Final PCR products consisting of
131 *locus::ermF* insertion cassettes were then digested with *Pst*I and *Spe*I (New England
132 Biolabs, Ipswich, MA, USA) for cloning into the appropriate sites of the *C. canimorsus*
133 suicide vector pMM25 (24). The resulting plasmids were transferred by RP4-mediated
134 conjugative DNA transfer from *E. coli* S17-1 to the corresponding *C. canimorsus* strains
135 to allow integration of the insertion cassette. Transconjugants were then selected for the
136 presence of the *ermF* cassette on erythromycin-containing plates and checked for
137 sensitivity to ceftiofuran. Deletion of the appropriate regions was verified by PCR.

138

139 Western blotting of polysaccharide structures

140 Bacteria were harvested by gently scraping colonies off the agar surface of GEN SB
141 plate and resuspended in PBS. Bacteria suspensions were adjusted to an OD₆₀₀ of 1 in
142 PBS. 750 µL of the suspension were pelleted and resuspended in 125 µL loading buffer
143 (1% sodium dodecyl sulphate (SDS), 10% glycerol, 50mM dithiothreitol, 0.02%
144 bromophenol blue, 45mM Tris (pH6.8)). Samples were heated for 10 min at 99°C.
145 Proteinase K (VWR Chemicals, Radnor, PA, USA), was added to a final concentration of
146 50 µg/mL and samples were incubated overnight at 37°C. Subsequently, samples were
147 heated for 10 min at 99°C and proteinase K was added again at the same final
148 concentration. Samples were incubated for 3 hours at 55°C, heated for 5 min at 99°C
149 and loaded on a 12% polyacrylamide gel. After SDS-PAGE (polyacrylamide gel
150 electrophoresis), proteinase K resistant structures were transferred on a nitrocellulose
151 membrane (GE Healthcare, Chicago, IL, USA). Membranes were blocked and incubated
152 with polyclonal crude or adsorbed sera (dilutions ranging from 1/400 to 1/8000) followed
153 by incubation with a horseradish peroxidase (HRP)-coupled goat anti-rabbit polyclonal
154 antibody (1/2000; Dako Agilent Technologies, Santa Clara, CA, USA). Membranes were
155 revealed using a chemiluminescent substrate (KLP, Gaithersburg, MD, USA) on an
156 Amersham Imager 600 (GE Healthcare). Blocking and all incubations were conducted in
157 5% non-fat dry milk diluted in PBS 0.05% Tween.

158

159 Capsular serotyping by ELISA

160 Bacteria suspensions were adjusted to an OD₆₀₀ of 0.5 and were killed by an incubation
161 of 30 min at 70°C. Heat-killed bacteria suspensions were used to coat 96 well plates

7

162 (ThermoScientific, Waltham, MA, USA) overnight at 4°C. The next day plates were
163 washed to remove unfixed bacteria and blocked for 1 hour at RT with 1% BSA in PBS.
164 Plates were washed and incubated with adsorbed polyclonal serum (1/1000 to 1/5000 in
165 PBS) for 1 hour at RT. Plates were washed again and incubated with HRP-coupled goat
166 anti-rabbit polyclonal antibody for 1 hour at RT (Dako Agilent Technologies; 1/2000 in
167 PBS). Plates were then washed and revealed using 3,3',5,5'-Tetramethylbenzidine
168 (TMB) as a chromogenic substrate.

169

170 **Capsular serotyping by PCR**

171 Bacteria were grown on SB plates supplemented with GEN and a single colony was
172 resuspended in 100 µL ddH₂O and heated for 15 min at 98 °C. Two microliters were
173 used as template for amplification. PCR detection was performed using the Promega Go
174 Taq® G2 polymerase (Madison, WI, USA) under the following conditions: an initial
175 denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s,
176 annealing at 52°C for 45 s, extension at 72°C for 1 min and 30 s, and a final extension at
177 72°C for 7 min.

178

179 **Synteny analysis**

180 Synteny statistics were obtained using the MicroScope PkGDB synteny statistics tool
181 (<https://www.genoscope.cns.fr/agc/microscope/home/index.php>) (25). Putative
182 orthologous relations based on the bi-directional best hit (BBH) criterion were
183 considered for at least 35% of sequence identity on 80% of the length of the smallest
184 protein. For the synteny analysis, all possible kinds of chromosomal rearrangements are
185 allowed (inversion, insertion/deletion) and the gap parameter, representing the

186 maximum number of consecutive genes which are not involved in a synteny group, is set
187 to five genes.

188

189 **Statistical analysis**

190 Statistical significance was evaluated by Fisher's exact tests using the BiostaTGV
191 website (<https://marne.u707.jussieu.fr/biostatgv>).

192

193 **Accession numbers**

194 Accession numbers of genes used in this study are listed in **Supplementary Table S4**.

195

196 **Results**

197 **Capsular serotyping identifies 5 serovars in a collection of *C. canimorsus* isolated** 198 **from human infections**

199 The prevalence of the capsular serovar of strain Cc5 was tested in a collection of 25 *C.*
200 *canimorsus* isolated from human infections (**Supplementary Table S1**). Whole bacteria
201 were digested with proteinase K and bacterial polysaccharides were analyzed by
202 western blot using an anti-serum directed against Cc5 bacteria and adsorbed with the
203 non-capsulated Cc5 transposon mutant Y1C12 (20, 26). The serum recognized a high
204 molecular weight (MW) band (>250 kDa) in the extracts from Cc5 and from ten other
205 isolates, namely Cc1 (BCCM/LMG 11511; CCUG 17234; strain P810; strain SSI P810),
206 Cc2, Cc3, Cc10 (BCCM/LMG 11541, CCUG 24741, ATCC 35978, CDC C8936), Cc13,
207 Cc15, Cc21 (CCUG 60839), Cc22 (CCUG 20318), Cc24 (CCUG 67384), and Cc25
208 (CCUG 66222) (**Figure 1A**). Since this band was identified as the CPS of Cc5 (20), we
209 concluded that the capsular serovar of Cc5 was shared with these 10 isolates

210 representing 44% of our collection of human isolates. We named this capsular serovar
211 A.

212 To determine the capsular serovar of the 14 non-A human isolates, 9 new anti-sera were
213 raised and tested by western blot on polysaccharide extracts from these 14 isolates. The
214 antisera raised against Cc6, Cc9 (BCCM/LMG 11510, CCUG 12569, CDC A3626), Cc12
215 (type strain, ATCC 35979, CDC 7120, CCUG 53895), and Cc4 allowed detecting a high
216 MW polysaccharide, most likely corresponding to a CPS (**Figure 1**) in all the 14 isolates.
217 The anti-Cc6 serum recognized a high MW polysaccharide structure in Cc6 but also in
218 Cc8, Cc11, Cc16, Cc17, Cc18, and Cc23 (CCUG 48899) (**Figure 1B**). This serovar,
219 named B, had thus a prevalence of 28% in our collection of human isolates, with 7
220 isolates positive out of 25. The anti-Cc9 serum recognized a high MW polysaccharide
221 structure in Cc9, Cc14, Cc19, and Cc20 (CCUG 55909) (**Figure 1C**). This serovar,
222 named C had thus a prevalence of 16%. The anti-Cc12 serum recognized a high MW
223 polysaccharide structure in Cc12 and Cc7 (**Figure 1D**). The prevalence of this serovar,
224 named D, was of 8%, thus more limited than that of serovars A, B, and C. Finally, the
225 anti-Cc4 serum recognized a high MW polysaccharide band only in Cc4. This serovar
226 had thus a prevalence of only 4% and was named E (**Figure 1E**).

227 In order to confirm that the high MW bands recognized are CPS, we next attempted to
228 generate non-capsulated deletion mutants of Cc6, Cc9, Cc12, and Cc4 (**Supplementary**
229 **Table S2**). Since the capsule of Cc5 is made of the same sugars as the LOS O-chain,
230 we decided to generate rough non-capsulated mutants. To this aim we sequenced the
231 genomes of the isolates Cc6, Cc9, and Cc4 and used the previously published genome
232 of Cc5 (GenBank: CP002113) (27) and draft genome of Cc12 (GenBank:
233 CDOE00000000.1) (28). Homologs of Cc5 *wbuB* gene (*Ccan_23370*), which is the gene

234 mutated in the LOS/CPS mutant Y1C12 and encodes a N-acetyl-fucosamine (FucNAc)
235 transferase (20), were found in the genomes of Cc6 (*Cc6_1430029*) and Cc9
236 (*CCAN9_740038*) but not in those of Cc12 and Cc4. In the latter genomes we identified
237 homologs of Cc5 *wbtA* (*Ccan_23400*) that is mutated in the LOS/CPS mutant Y1D1 (20)
238 of Cc5 and encodes an UDP-N-acetylglucosamine 4,6-dehydratase (*CCAN12_760057*,
239 and *CC4_530070* respectively) (20). The *wbuB* genes were thus mutated in Cc6 and
240 Cc9 while gene *wbtA* was mutated in Cc12. The polysaccharide extracts from the
241 mutants of Cc6, Cc9, and Cc12, analyzed by western blot with the anti-Cc6, anti-Cc9,
242 and anti-Cc12 sera, did not contain the high MW band indicating that it was indeed a
243 CPS (**Figure 1B, C and D**). Gene *wbtA* from Cc4 could not be mutated despite several
244 attempts and hence we could not formally prove that the high MW polysaccharide is a
245 CPS related to the LOS. Nevertheless, the presence of a *wza* homolog, encoding for the
246 capsular transporter across the outer membrane, suggests that Cc4 is indeed endowed
247 with a capsule.

248 We thus conclude that the 25 *C. canimorsus* human isolates of our collection are all
249 endowed with a CPS and that the antigenic repertoire of these CPS is limited since 88%
250 of the isolates (22/25) belong to serovars A, B, and C. Interestingly, the distribution of
251 serovars A, B, and C is not affected by a geographical bias since each serovar was
252 found in isolates from at least three different countries (**Supplementary Table S5**).

253

254 **Prevalence of capsular serovars A, B, and C among *C. canimorsus* isolated from** 255 **dog mouths**

256 We next assessed the prevalence of the capsular serovars A to E among a collection of
257 52 isolates of *C. canimorsus* from dog mouths (**Supplementary Table S1**) (7). To this

258 aim we set up an ELISA screening using entire heat-killed bacteria. Since we needed
259 sera that were specifically recognizing the CPS, for serovar A, we used the Y1C12-
260 adsorbed anti-Cc5 serum; for serovars B, C, and D, we adsorbed the crude anti-Cc6,
261 anti-Cc9, and anti-Cc12 sera with Cc6 *wbuB*, Cc9 *wbuB*, and Cc12 *wbtA* mutant
262 bacteria respectively. Due to the lack of a non-capsulated Cc4 mutant strain, we
263 adsorbed the anti-Cc4 serum (serovar E) with the 24 other human isolates belonging to
264 different capsular serovars (**Figure 1E**). The efficacy of the different adsorptions was
265 validated by immunofluorescence staining and microscopy analysis (**Supplementary**
266 **Figure S1**). The five adsorbed sera were then used to test our collection of dog isolates
267 by ELISA. The reactivity of each isolate was calculated with respect to that of the type
268 strain of each serovar (Cc5 for A, Cc6 for B, Cc9 for C, Cc12 for D, and Cc4 for E). The
269 non-capsulated mutant strains were used as negative controls. The results of the
270 screening are summarized in **Table 1**. Only two isolates, CcD68 and CcD105, were
271 positive for serovar A with a reactivity of $43\% \pm 7$ and $107\% \pm 28$ respectively. The high
272 MW polysaccharidic structures of these isolates were analyzed by western blot and only
273 the strongly reacting CcD105 displayed a serovar A capsule (**Supplementary Figure**
274 **S2A**). For serovar B, only isolate CcD68 was found to be positive ($110\% \pm 11$) by ELISA
275 and by western-blot (**Supplementary Figure S2B**). For serovar C, isolates CcD43 and
276 CcD130 were positive by ELISA ($86\% \pm 5$ and $108\% \pm 26$ reactivity respectively) and
277 western blot (**Supplementary Figure S2C**). For serovar D, three isolates were strongly
278 recognized by ELISA and confirmed by western blot: CcD16 ($86\% \pm 14$), CcD89 ($95\% \pm$
279 9), and CcD117 ($99\% \pm 12$) (**Supplementary Figure S2D**). Finally for serovar E, isolate
280 CcD96 displayed a high reactivity of $118\% \pm 37$ and isolates CcD20 and CcD106
281 displayed intermediate reactivities of respectively $57\% \pm 24$ and $59\% \pm 24$ while some

282 other isolates presented a limited reactivity. All the isolates with a value equal or higher
283 than 30 % were checked by western blot and only one isolate, CcD96, was confirmed to
284 belong to serovar E (**Supplementary Figure S2E**). The results from the ELISA and the
285 western blot analyses are summarized in **Figure 2**. While all the human isolates
286 belonged to serovars A, B, C, D, or E, 84.6% of the dog isolates were left non-typeable.
287 In conclusion, the prevalence of serovar A was 22.9 fold higher in human isolates than in
288 dog isolates (Fisher's exact test, $p=6.45.10^{-6}$) while the prevalence of serovar B was
289 14.6 fold higher (Fisher's exact test, $p=0.00123$). A 4.2 fold increase was found for the
290 serovar C, but it was not statistically significant (Fisher's exact test, $p=0.0831$). Finally,
291 there was no significant difference in the prevalence of serovars D and E (p values of
292 0.657 and 0.547 respectively in Fisher's exact test).

293

294 **There is a high capsular variability among the isolates from dog mouths**

295 To investigate the variability of the capsular serovars in the 44 untyped dog isolates, we
296 generated sera against 4 isolates randomly chosen (CcD37, CcD63, CcD101, and
297 CcD129). Since we could not generate uncapsulated mutants because the genomes of
298 these isolates are not available, the anti-sera were adsorbed using a mix of the 25 *C.*
299 *canimorsus* human isolates. After validating the adsorption efficacy by
300 immunofluorescence (**Supplementary Figure S1**) we screened the 52 dog isolates by
301 ELISA (**Table 1**). The adsorbed anti-CcD37 serum reacted not only with CcD37 but also
302 with CcD13, CcD52, CcD113, CcD118, and CcD124 with reactivities comprised between
303 83 and 111%. All these reactions were confirmed by western blot (**Supplementary**
304 **Figure S2F**). This serovar, named F, had thus a prevalence of 11.5% among dog
305 isolates (6/52). The adsorbed anti-CcD63 reacted with the CPS of CcD63 but with no

306 other isolate (**Supplementary Figure S2G**). This serovar, G, had thus a reduced
307 prevalence of 1.9% (1/52). The adsorbed anti-CcD101 serum reacted with only one
308 other isolate, (CcD53) but this isolate did not show any CPS (**Supplementary Figure**
309 **S2H**). This serovar, H, had thus a prevalence of 1.9 % (1/52). Finally the adsorbed anti-
310 CcD129 serum reacted by ELISA and western blot with the CPS of CcD129 and CcD33
311 (**Supplementary Figure S2I**). This serovar, I, had thus a prevalence of 3.8% (2/52).
312 There were no significant differences in the prevalence of serovars F, G, H, and I
313 between dog and human isolates (p values of 0.169 for F and 1 for G, H, and I in
314 Fisher's exact test), but while five serovars covered the 25 human isolates (100 %), nine
315 serovars covered only 18 dog isolates (34.6 %) (**Figure 2**). This result indicates there is
316 a higher variability of capsular serovars among dog isolates than among human isolates.

317

318 **Detection of the capsular serovars A to E by PCR**

319 Our data so far clearly show that the capsular serotyping could help identifying dogs
320 hosting *C. canimorsus* isolates that are more virulent for humans than others. Since
321 immunological screening methods are somehow difficult to implement in diagnostics
322 laboratories, we tried to develop a PCR-based method using different oligonucleotides
323 couples that would allow the identification of the 5 serovars found among human
324 isolates.

325 We thus first compared the capsule and LOS biosynthesis loci in the seven available
326 genomes of *C. canimorsus* isolates belonging to the five serovars (Cc5, Cc2, Cc6, Cc11,
327 Cc9, Cc12, and Cc4) (**Figure 3**). Looking for a gene that was specific to serovar A
328 isolates (Cc5, Cc2), we identified an A4GalT-like glycosyltransferase gene (*Ccan_23210*
329 and *CCAN2_1920004* in Cc5 and Cc2 respectively) (20). Two amplimers were designed

330 and our complete *C. canimorsus* collection was tested by PCR. As shown in **Figure 4**
331 and **Table 2**, this analysis detected all serovar A isolates (11 human- and one dog-
332 isolates) and no other isolate.

333 Regarding serovar B, we could not identify any gene that was unique to the Cc6 and
334 Cc11 genomes (**Figure 3**). However, while genes *CC6_1430035* and *CCAN11_10027*,
335 both encoding a putative family 1 glycosyltransferase, were exactly conserved in Cc5
336 (serovar A), they were not in Cc2 (also serovar A). Aligning *CC6_1430035* and
337 *CCAN11_10027* with their homologs from Cc2 (*CCAN2_1430008*) and Cc9 (serovar C)
338 (*CCAN9_740032*) (20) revealed a difference in the 16 base pairs immediately
339 downstream of the start codon (**Supplementary Figure S3**). Since both serovar B
340 isolates (Cc6 and Cc11) had the exact same gene sequence, shared by only one of the
341 two serovar A isolates (Cc5), we tested whether the exact same gene sequence would
342 not be shared by all serovar B isolates. We thus designed two oligonucleotides to
343 amplify this specific gene region and, as shown in **Figure 4** and **Table 2**, by this PCR,
344 we could indeed detect all the 7 serovar B human isolates as well as the only serovar B
345 dog isolate (CcD68). As expected we could also detect Cc5 but two other serovar A
346 isolates, namely Cc15 and Cc24, as well. Surprisingly, the PCR gave a positive result for
347 one dog isolate (CcD57) that did not belong to any of the 5 serovars (**Table 1**,
348 **Supplementary Figure S2E** and **Supplementary Figure S4A**) and thus represents a
349 false positive. Nevertheless, with this PCR we could detect all serovar B isolates of our
350 collection and this analysis, if combined with the one specific for the serovar A, allowed
351 the discrimination between serovars A and B. Indeed, serovar B isolates are positive for
352 PCR B but negative for PCR A.

353 Regarding serovar C, we could not identify any gene unique to the Cc9 genome but
354 *CCAN9_740031*, encoding a putative O antigen polymerase (*wzy*) had an homolog only
355 in one serovar A isolate, namely Cc2 (**Figure 3**). We thus tested by PCR whether this
356 gene would be shared by all serovar C isolates. As shown in **Figure 4** and **Table 2**, we
357 could detect all serovar C isolates namely the 4 from humans (Cc9, Cc14, Cc19) and
358 Cc20) as well as the two from dogs (CcD43 and CcD130). This PCR thus allows the
359 detection of the serovar C isolates and, if combined with the PCR for the serovar A, to
360 discriminate between these two serovars. Indeed, serovar C isolates are positive for
361 PCR C but negative for PCR A.

362 Concerning serovar D, the Cc12 LOS/CPS locus was previously shown to be very
363 divergent from the ones of serovars A, B, and C isolates with a limited number of
364 conserved genes (20) (**Figure 3**). We chose to amplify gene *CCAN12_760043* encoding
365 a putative lipopolysaccharide biosynthesis O-acetyl transferase (*WbbJ*) that had no
366 homologs in all the other serovars loci. As shown in **Figure 4** and **Table 2**, this PCR
367 exclusively detected the serovar D isolates and it detected them all (Cc12, Cc7, CcD16,
368 CcD89, and CcD117).

369 Finally, as for Cc12, the serovar E strain Cc4 LPS/CPS locus strongly diverged from the
370 ones of all the other serovars (**Figure 3**). We thus chose as target gene a Cc4 unique
371 gene, namely *CC4_530066*, encoding a glycosyltransferase 1 family protein. As shown
372 in **Figure 4** and **Table 2**, this PCR detected the Cc4 and CcD96 serovar E isolates.
373 Among the other isolates, only CcD10 gave a positive result although it did not react with
374 the E antiserum (**Table 1** and **Supplementary Figure S4B**) and could thus be
375 considered as a false positive. In summary, in order to determine the serovar of a *C.*
376 *canimorsus* isolate, the five (A, B, C, D, and E) PCR should be performed and the

377 results interpreted as follows: i) all isolates that are positive for PCR A belong to serovar
378 A; ii) isolates that are positive for PCR B belong to serovar B if they are not positive for
379 PCR A; iii) isolates that are positive for PCR C are serovar C if they are not positive for
380 PCR A; iv) isolates that are positive for PCR D are serovar D; v) isolates that are
381 positive for PCR E are serovar E (**Table 2** and **Table 3**)

382 In conclusion, capsular serotyping can be done by PCR (**Table 2** and **Table 3**) with a
383 very limited margin of error (2 false positive dog isolates).

384 Next, given the higher prevalence of serovars A, B, and C (22/25) among human
385 isolates, we decided to develop a PCR that would allow to detect all serovar A, B, and C
386 isolates. To this aim, taking advantage of the high similarity among the LOS/CPS loci of
387 the isolates belonging to serovars A, B, and C, we designed two amplimers specific to
388 the conserved region of the putative glycosyltransferase *wfdR* orthologs genes of
389 serovar A (*Ccan_23240* in Cc5 and *CCAN2_1430002* in Cc2), serovar B (*CC6_1430040*
390 in Cc6 and *CCAN11_2010013* in Cc11), and serovar C (*CCAN9_740027*). As shown in
391 **Figure 4** and **Table 2**, by this PCR we could detect all the isolates belonging to serovars
392 A, B, and C. Among the non-A, -B, or -C isolates, only CcD77 gave an amplification but
393 not of the same size (**Figure 4**). This PCR, allowing to identify fast and specifically all
394 the *C. canimorsus* isolates belonging to serovars A, B, or C (**Table 3**) could thus be a
395 valuable tool in terms of prevention.

396

397 **Discussion**

398 Here we show that all 25 out of 25 *C. canimorsus* isolated from human infections and 18
399 dog isolates out of 18 tested are endowed with a CPS. We thus confirm our previous
400 observation where a capsular-like polysaccharide structure was found in ten human

401 isolates (20). This result further reinforces the commonality of the presence of a CPS in
402 *C. canimorsus*. In addition, we developed a serotyping scheme based on the capsular
403 antigens and we described nine serovars (A to I). The LOS and CPS synthesis are
404 genetically linked in strain Cc5, resulting in similar polysaccharide units compositions in
405 both structures (20). For serovars B to I, we also found shared epitopes between the
406 CPS and LOS (data not shown). Even more, the antiserum directed against the
407 CPS/LOS from serovar C recognized the LOS but not the CPS from some serovar A
408 isolates (data not shown), revealing some complexity in the CPS/LOS relation. Because
409 of this complexity and because it is the CPS rather than the LOS that impacts the host-
410 pathogen interaction (20), we based our typing scheme on the CPS only. However,
411 because of this cross-reaction, the distinction between serovars A and C must be done
412 by western blotting and not by immuno-fluorescence or ELISA. Because western blotting
413 is a tedious technique for clinical laboratories, we set up a PCR method for the capsular
414 serotyping. The cross-reaction between the LOS of serovar A and some strains of
415 serovar C also appeared when the typing was done by PCR but combining the two PCR
416 reactions allows to determine the serovar without any ambiguity. Further work will be
417 required to understand the molecular mechanisms underlying these LOS cross-reactions
418 but carbohydrate chemistry always represents a long-term project.

419 The nine serovars described covered only 18 dog isolates out of 52 tested while five
420 serovars only covered the 25 human isolates. Thus, there was a high variety of capsular
421 serovars among dog isolates. In contrast, only three serovars (A, B, and C) covered 88
422 % of the human isolates tested (22/25) while they covered only 4 dog isolates (7.7 %).
423 There was thus a very strong enrichment of serovars A, B, and, to a lesser extend C in
424 human isolates as compared to dog ones. Interestingly, these three dominant capsular

425 serovars were not restricted to a geographical area but were rather distributed
426 worldwide. This observation clearly indicates that the strains belonging to serovars A, B,
427 and, possibly C, are more virulent for humans than strains from the other serovars. This
428 sets the bases for the prevention of these severe infections. To this aim, one could
429 envision the detection of potentially more dangerous dogs using a PCR reaction carried
430 out directly on the dog's saliva and monitoring simultaneously the three more virulent
431 serovars. Our results on collection isolates have indeed shown that PCR is reliable with
432 a very limited number of false positives and, in our experience, no false negative.
433 Owners of a dog hosting a serovar A, B, or C strain should be educated to limit the
434 contact with the dog's saliva and if a bite or a lick occurs, to apply strict hygiene
435 measures. In addition, splenectomized and more generally immunocompromized
436 persons should not consider adopting a dog hosting a virulent *C. canimorsus* strain.
437 Ideally, more human isolates should be serotyped to reinforce the correlation between
438 some capsular serovars and human infections but their collection is very tedious due to
439 the rarity of the disease and the fastidious character of these bacteria.
440 There was no significant difference in the distribution of capsular serovars D and E
441 among dog (4/52) and human isolates (3/25), which suggests that they are probably not
442 more virulent than most dog strains. This observation leads to the conclusion that, while
443 a majority of the patients (88 % of our sample) are infected with virulent strains (A, B,
444 possibly C), a minority of patients (12 % of our sample) could have been infected by
445 strains that belong to a less virulent serovar (D and E). This is consistent with the fact
446 that some patients were obviously at risk while others had no history of immune
447 deficiency. In agreement with this hypothesis, the patient infected with Cc4 (serovar E)
448 was highly immunocompromised (29) and the patient infected with Cc12 (serovar D)

449 was splenectomized (2) (**Supplementary Table S1**). Hence, splenectomized and more
450 generally immunocompromised persons should be extremely cautious when interacting
451 with dog hosting *C. canimorsus* regardless of the serovar of the latter.

452 It is likely that it is the capsule itself that confers an enhanced virulence to serovars A, B,
453 and C, as is classical for other pathogens (21). In support of this, the capsule of the type
454 strain Cc5 has been recently shown to provide resistance to phagocytosis by
455 macrophages, to killing by 10% human serum and to killing by the cationic antimicrobial
456 peptide polymyxin B (20). These results suggest that the serovar A CPS could indeed
457 participate to the innate immune evasion in humans. Ideally, these *in vitro* data should
458 be reinforced by *in vivo* studies but the lack of a relevant sepsis animal model to study
459 *C. canimorsus* infections prevents such confirmation. Further *in vitro* work could
460 determine if the capsular serovars A, B, and C provide the strains a higher resistance to
461 the innate immune system. However, we cannot exclude that other virulence factors
462 could be genetically linked to some capsular serovars. It would thus be interesting to
463 compare the whole genomes looking for genes that would be shared in serovars A, B,
464 and C strains and absent in serovars F, G, H, and I strains.

465

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475

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561

562 **Figure legends**

563 **Figure 1. Capsular serotyping of *C. canimorsus* isolates from human infections.**

564 Western blot analysis of proteinase-K treated lysates of *C. canimorsus* human isolates
565 using the following sera: Y1C12 adsorbed anti-Cc5 (A), anti-Cc6 (B), anti-Cc9 (C), anti-
566 Cc12 (D), and anti-Cc4 (E). Non-capsulated mutants Cc5 Y1C12, Cc6 $\Delta wbuB$, Cc9
567 $\Delta wbuB$, and Cc12 $\Delta wbtA$ were used as controls in panels A, B, C, and D respectively.
568 Numbers correspond to molecular mass markers in kDa.

569

570 **Figure 2. Prevalence of capsular serovars A to I in *C. canimorsus* isolated from**
571 **human infections and dog mouths.** Summary of capsular serovars A to I prevalence
572 in human (A) or dogs (B) isolates.

573

574 **Figure 3. Synteny analysis of LOS/CPS loci in the A, B, C, D, E capsular serovars.**

575 Comparison of the LOS/CPS-biosynthesis and transport genetic loci of the seven *C.*
576 *canimorsus* isolates whose genomes were sequenced. The boxes indicate different
577 genomic loci. Homologs of the Cc5 genes are indicated in grey. The genes amplified by
578 the A, B, C, D, and E serovar specific PCR are indicated in green, blue, orange, black,
579 and yellow respectively. The target genes amplified by the ABC serovars specific PCR
580 are in red. Genes indicated in white are isolate specific genes likely involved in
581 LOS/CPS biosynthesis. The hatched pattern indicates genes likely unrelated to
582 LOS/CPS biosynthesis and transport. Fragmented genes are marked with (f). Note that
583 the genomes of Cc2, Cc4, Cc6, Cc9, Cc11, and Cc12 are draft genomes. For the sake
584 of simplicity genes are not represented to scale.

585

586 **Figure 4. Capsular typing by PCR.** PCR detection of capsular serovars A, B, C, D, and
587 E in *C. canimorsus* human and dog isolates using the oligonucleotides given in **Table**
588 **S3.** *C. canis* (type strain CcD38, LMG 29146, DSM 101831) and *C. cynodegmi* (type
589 strain Ccyn ATCC 49044) were used as negative controls.
590

591
592Table 1: Capsular serotyping of *C. canimorsus* dog isolates by ELISA

Strain/ Isolate	Capsular serovar								
	A	B	C	D	E	F	G	H	I
Cc5	100 ± 0	20 ± 8	27 ± 11	24 ± 6	17 ± 2	13 ± 4	14 ± 5	10 ± 4	13 ± 5
Cc5 Y1C12	14 ± 6	nd	nd	nd	nd	nd	nd	nd	nd
Cc6	32 ± 7	100 ± 0	24 ± 12	20 ± 4	13 ± 2	14 ± 6	12 ± 3	11 ± 4	14 ± 7
Cc6 <i>ΔwbuB</i>	nd	14 ± 7	nd	nd	nd	nd	nd	nd	nd
Cc9	15 ± 3	17 ± 7	100 ± 0	22 ± 4	17 ± 5	14 ± 5	13 ± 4	10 ± 3	14 ± 4
Cc9 <i>ΔwbuB</i>	nd	nd	20 ± 7	nd	nd	nd	nd	nd	nd
Cc12	19 ± 7	15 ± 5	23 ± 8	100 ± 0	18 ± 0	14 ± 5	15 ± 5	10 ± 3	13 ± 5
Cc12 <i>ΔwbtA</i>	nd	nd	nd	20 ± 5	nd	nd	nd	nd	nd
Cc4	16 ± 3	14 ± 3	30 ± 10	26 ± 5	100 ± 0	13 ± 5	13 ± 4	10 ± 2	13 ± 5
CcD3	18 ± 7	11 ± 4	18 ± 5	19 ± 3	14 ± 6	10 ± 3	12 ± 1	15 ± 4	12 ± 6
CcD5	17 ± 4	13 ± 8	16 ± 4	22 ± 4	13 ± 6	11 ± 5	13 ± 2	14 ± 6	14 ± 6
CcD6	18 ± 10	11 ± 5	20 ± 7	18 ± 3	15 ± 7	11 ± 3	14 ± 2	17 ± 4	16 ± 10
CcD10	17 ± 8	12 ± 5	18 ± 3	17 ± 2	17 ± 5	10 ± 1	14 ± 2	15 ± 6	14 ± 6
CcD13	16 ± 7	11 ± 5	21 ± 4	17 ± 2	12 ± 5	99 ± 1	12 ± 1	13 ± 4	12 ± 5
CcD16	18 ± 7	10 ± 4	17 ± 4	86 ± 14	11 ± 5	10 ± 3	13 ± 1	15 ± 5	12 ± 5
CcD18	19 ± 9	12 ± 6	17 ± 5	15 ± 2	28 ± 12	10 ± 2	15 ± 1	17 ± 6	13 ± 6
CcD20	17 ± 6	11 ± 6	19 ± 3	17 ± 3	57 ± 24	11 ± 3	12 ± 1	14 ± 4	12 ± 5
CcD25	15 ± 6	11 ± 5	18 ± 4	17 ± 2	12 ± 5	9 ± 2	11 ± 1	14 ± 4	12 ± 3
CcD33	20 ± 9	11 ± 6	17 ± 6	22 ± 2	13 ± 5	10 ± 3	12 ± 2	13 ± 4	106 ± 30
CcD34	16 ± 9	10 ± 4	16 ± 3	14 ± 2	13 ± 6	10 ± 2	12 ± 1	13 ± 4	13 ± 6
CcD35	14 ± 7	12 ± 4	15 ± 5	12 ± 1	12 ± 4	11 ± 3	12 ± 2	14 ± 3	12 ± 5
CcD37	15 ± 4	9 ± 3	19 ± 2	16 ± 0	12 ± 4	100 ± 0	11 ± 1	12 ± 4	11 ± 5
CcD39	14 ± 4	10 ± 5	18 ± 3	22 ± 2	14 ± 6	9 ± 2	11 ± 1	12 ± 3	12 ± 5
CcD40	16 ± 8	11 ± 5	19 ± 4	19 ± 4	12 ± 5	10 ± 2	13 ± 2	16 ± 5	13 ± 6
CcD43	20 ± 10	24 ± 14	86 ± 5	17 ± 1	13 ± 5	10 ± 3	12 ± 1	16 ± 1	14 ± 2
CcD44	15 ± 8	9 ± 4	25 ± 7	16 ± 0	12 ± 5	8 ± 1	11 ± 1	12 ± 4	11 ± 5
CcD47	16 ± 6	11 ± 6	17 ± 3	18 ± 0	12 ± 5	8 ± 2	12 ± 1	14 ± 3	11 ± 4
CcD51	15 ± 7	14 ± 5	18 ± 4	20 ± 4	11 ± 5	9 ± 2	12 ± 0	14 ± 4	12 ± 5
CcD52	16 ± 8	11 ± 7	16 ± 5	20 ± 6	11 ± 5	83 ± 4	13 ± 2	14 ± 4	12 ± 5
CcD53	19 ± 8	12 ± 6	17 ± 2	18 ± 2	12 ± 5	9 ± 2	14 ± 2	41 ± 7	11 ± 4
CcD57	17 ± 6	28 ± 18	21 ± 4	23 ± 12	32 ± 10	9 ± 2	13 ± 1	13 ± 4	12 ± 5
CcD58	18 ± 7	11 ± 5	17 ± 3	22 ± 3	34 ± 11	11 ± 2	14 ± 1	15 ± 5	13 ± 5
CcD63	15 ± 9	11 ± 5	17 ± 1	17 ± 0	29 ± 11	11 ± 5	100 ± 0	14 ± 5	12 ± 5
CcD68	43 ± 7	110 ± 11	16 ± 5	18 ± 0	13 ± 5	9 ± 2	13 ± 1	14 ± 5	11 ± 5
CcD69	14 ± 7	11 ± 6	16 ± 3	13 ± 2	12 ± 5	8 ± 3	12 ± 1	12 ± 4	11 ± 5
CcD71	15 ± 6	11 ± 5	17 ± 6	16 ± 2	13 ± 6	10 ± 2	13 ± 1	14 ± 5	13 ± 5
CcD73	19 ± 7	12 ± 7	17 ± 0	23 ± 3	15 ± 6	15 ± 7	13 ± 5	18 ± 7	13 ± 5
CcD76	13 ± 6	16 ± 8	14 ± 1	15 ± 3	15 ± 7	15 ± 7	13 ± 4	12 ± 4	13 ± 4
CcD77	14 ± 9	13 ± 4	17 ± 7	14 ± 2	15 ± 7	14 ± 6	12 ± 5	10 ± 2	13 ± 4
CcD80	16 ± 11	13 ± 4	19 ± 5	17 ± 3	30 ± 9	13 ± 5	13 ± 5	10 ± 4	13 ± 5
CcD81	16 ± 5	12 ± 4	23 ± 7	19 ± 4	22 ± 5	12 ± 5	12 ± 5	9 ± 3	11 ± 4
CcD84	17 ± 8	14 ± 7	13 ± 1	18 ± 2	29 ± 16	12 ± 5	12 ± 3	14 ± 4	13 ± 4
CcD89	17 ± 6	15 ± 1	20 ± 6	95 ± 9	14 ± 9	14 ± 5	13 ± 4	13 ± 5	13 ± 5

CcD96	20 ± 8	14 ± 1	19 ± 7	15 ± 1	118 ± 37	12 ± 4	11 ± 4	9 ± 3	13 ± 5
CcD101	18 ± 5	12 ± 3	13 ± 4	16 ± 1	13 ± 7	12 ± 5	10 ± 3	100 ± 0	12 ± 4
CcD104	16 ± 9	14 ± 1	18 ± 7	21 ± 4	31 ± 11	11 ± 4	10 ± 3	9 ± 3	11 ± 4
CcD105	107 ± 28	14 ± 2	16 ± 8	17 ± 5	14 ± 8	13 ± 5	11 ± 4	9 ± 3	12 ± 4
CcD106	13 ± 8	16 ± 1	21 ± 11	17 ± 2	59 ± 24	13 ± 5	11 ± 4	9 ± 3	13 ± 5
CcD113	15 ± 11	16 ± 2	19 ± 9	14 ± 0	14 ± 6	108 ± 6	11 ± 4	9 ± 4	12 ± 4
CcD115	15 ± 9	16 ± 2	19 ± 9	21 ± 4	16 ± 8	12 ± 4	10 ± 4	9 ± 3	12 ± 5
CcD116	14 ± 8	14 ± 2	19 ± 5	18 ± 2	15 ± 7	12 ± 5	12 ± 5	9 ± 3	13 ± 5
CcD117	19 ± 10	16 ± 1	19 ± 8	99 ± 12	14 ± 7	13 ± 6	13 ± 5	9 ± 3	13 ± 5
CcD118	15 ± 7	14 ± 1	20 ± 9	19 ± 4	15 ± 7	111 ± 5	12 ± 4	10 ± 3	12 ± 5
CcD119	15 ± 8	16 ± 2	16 ± 9	28 ± 22	15 ± 9	12 ± 4	11 ± 4	12 ± 5	12 ± 4
CcD120	15 ± 8	17 ± 3	17 ± 5	23 ± 7	16 ± 8	13 ± 5	12 ± 4	10 ± 3	12 ± 4
CcD122	12 ± 8	14 ± 1	17 ± 6	13 ± 1	14 ± 8	13 ± 5	12 ± 4	9 ± 2	12 ± 4
CcD124	15 ± 8	16 ± 1	19 ± 8	19 ± 4	15 ± 7	109 ± 9	11 ± 4	9 ± 3	12 ± 4
CcD126	13 ± 7	14 ± 1	16 ± 6	21 ± 3	15 ± 7	13 ± 6	11 ± 4	11 ± 4	12 ± 4
CcD129	13 ± 9	15 ± 2	21 ± 6	20 ± 2	16 ± 8	12 ± 5	14 ± 6	10 ± 2	100 ± 0
CcD130	13 ± 7	29 ± 11	108 ± 26	19 ± 2	11 ± 6	15 ± 6	12 ± 4	10 ± 4	13 ± 5
CcD131	12 ± 7	14 ± 2	17 ± 6	19 ± 3	14 ± 6	13 ± 6	11 ± 4	10 ± 3	12 ± 4

593
594

595 Capsular serotyping was determined by ELISA on entire heat-killed bacteria. The
596 following sera were used: Y1C12 adsorbed anti-Cc5 (A), Cc6 $\Delta wbuB$ adsorbed anti-Cc6
597 (B), Cc9 $\Delta wbuB$ adsorbed anti-Cc9 (C), Cc12 $\Delta wbtA$ adsorbed anti-Cc12 (D), anti-Cc4
598 adsorbed with all human isolates except Cc4 (E), anti CcD37 adsorbed with all human
599 isolates (F), anti CcD63 adsorbed with all human isolates (G), anti CcD101 adsorbed
600 with all human isolates (H), and anti CcD129 adsorbed with all human isolates (I). The
601 readout of the ELISA was absorbance but results are expressed here as percentage of
602 reactivity calculated with respect to the absorbance value obtained for the capsular type
603 strain. Values are the mean (\pm standard deviation, SD) of at least 3 independent
604 experiments. The type strains for each capsular serovar and the strains with strong
605 reactivities (>80%) are highlighted in dark grey. The strains presenting intermediate
606 reactivities comprised between 30 and 60% are highlighted in light grey.

607 nd, not determined

608

609
610**Table 2: Summary of capsular typing of human isolates by PCR**

Strain/Isolate	PCR A Primers 8244- 8245	PCR B Primers 8246- 8247	PCR C Primers 8274- 8275	PCR D Primers 8276- 8277	PCR E Primers 8278- 8279	PCR ABC Primers 8296- 8297	Serovar
Cc1	X		X			X	A
Cc2	X		X			X	A
Cc3	X		X			X	A
Cc5	X	X				X	A
Cc10	X		X			X	A
Cc13	X		X			X	A
Cc15	X	X				X	A
Cc21	X		X			X	A
Cc22	X		X			X	A
Cc24	X	X				X	A
Cc25	X		X			X	A
Cc6		X				X	B
Cc8		X				X	B
Cc11		X				X	B
Cc16		X				X	B
Cc17		X				X	B
Cc18		X				X	B
Cc23		X				X	B
Cc9			X			X	C
Cc14			X			X	C
Cc19			X			X	C
Cc20			X			X	C
Cc7				X			D
Cc12				X			D
Cc4					X		E

611

612 PCR positive results are represented by X.

613

614 **Table 3: Interpretation of PCR typing results**

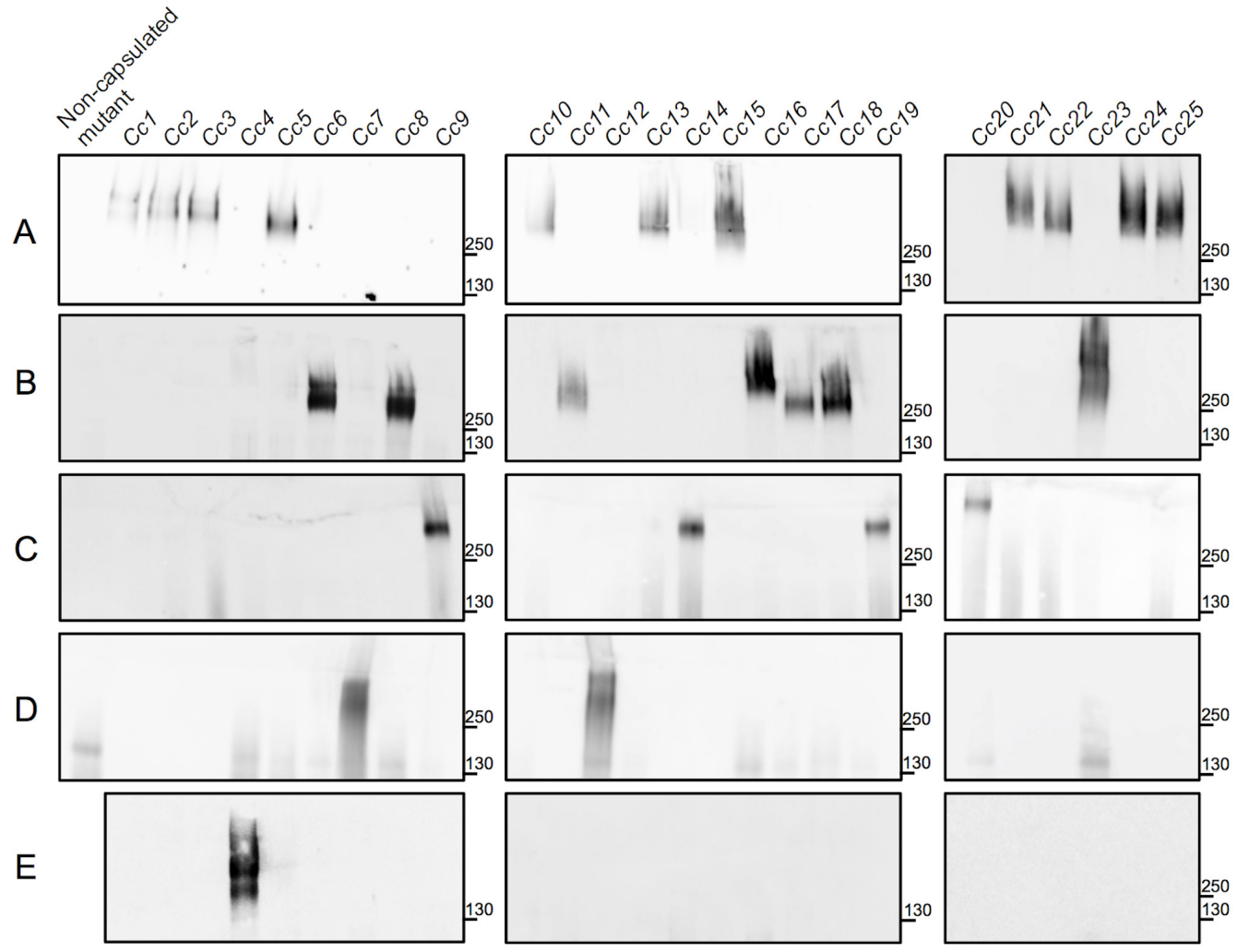
615

	PCR A Primers: 8244-8245	PCR B Primers: 8246-8247	PCR C Primers: 8274-8275	PCR D Primers: 8276-8277	PCR E Primers: 8278-8279	PCR ABC Primers: 8296-8297
Serovar A	X					X
	X	X				X
	X		X			X
Serovar B		X				X
Serovar C			X			X
Serovar D				X		
Serovar E					X	

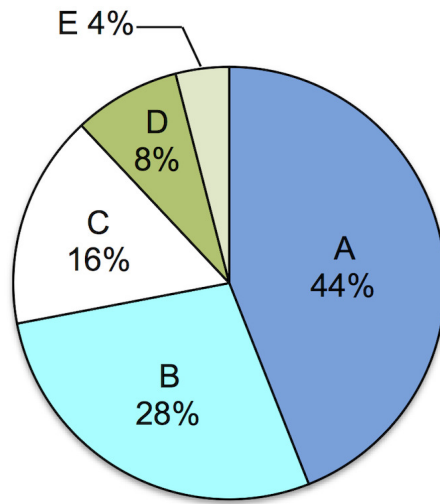
616

617 PCR positive results are represented by X.

618

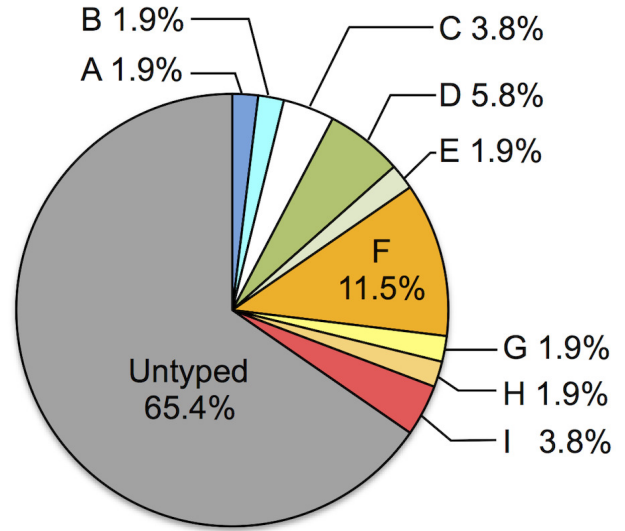


A



Serovars A to E
in human isolates

B



Serovars A to I
in dog isolates

