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1 **Title**

2 First detection of *Mycoplasma wenyonii* in France: identification, evaluation of the clinical
3 impact and development of a new specific detection assay.

4

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20

21 **Abstract**

22 *Mycoplasma wenyonii*, a hemoplasma infecting cattle, was never detected in France. In 2014,
23 evocative inclusions were observed in erythrocytes from cattle presenting milk drops, anemia,
24 and edema in Brittany (France). A survey was then initiated to investigate the epidemiological
25 situation and correlate mycoplasma detection with clinical signs. For this purpose, a new PCR

26 assay targeting *polC* gene was designed. Comparative results with published PCR assays
27 place this new one as more specific, allowing a one-step diagnosis without further sequencing.
28 A total of 181 cows were included in this study and 4.97% (n=9) were positive, resulting in
29 the first molecular identification of *M. wenyonii* in France. All positive animals presented
30 anemia, edema and milk drop. When selecting animals presenting evocative clinical signs, the
31 prevalence of *M. wenyonii* in Brittany was estimated to 25.6%. Further studies are needed to
32 evaluate the importance of the infection, the implication of arthropods and the existence of
33 asymptomatic carriers.

34

35 **Key words:** *Mycoplasma wenyonii*, *Mycoplasma ovis*, hemoplasma, cattle, anemia, *polC*.

36

37 **Introduction:**

38 After a period of confusion, *Haemobartonella* and *Eperythrozoon* species were reclassified as
39 haemotropic mycoplasmas (hemoplasmas) based on molecular phylogenetic evidences
40 (Neimark et al., 2001; Neimark et al. 2002). Haemoplasmas species are commonly infecting
41 erythrocytes of a wide range of animals. Two species are suspected to infect cattle:
42 *Mycoplasma wenyonii*, formerly known as *Eperythrozoon wenyonii*, and *Candidatus*
43 *Mycoplasma haemobos*. Data on *Candidatus Mycoplasma haemobos* are very scarce
44 (Niethammer et al., 2018) and most reports on cattle haemoplasmas only relate to *M. wenyonii*
45 (Tagawa et al., 2008 and 2013).

46 *M. wenyonii* is an acknowledged pathogenic species infecting cattle worldwide. Blood smears
47 reveal that this mycoplasma is found attached at the surface of red blood cells and/or free in
48 the plasma (Neimark et al., 2001). Co-infections have been demonstrated with *A. marginale*,
49 *A. phagocytophilum*, *Babesia* spp. and *Theileria* spp. (Hofmann et al., 2004). In cattle, the
50 majority of *M. wenyonii* infections remains subclinical (Montes et al., 1994; Smith et al.,

51 1990), but signs such as anemia, pyrexia, hind limb edema (hock), swollen teats, decreased milk
52 production and weight loss have been observed (Smith et al., 1990; Genova et al., 2011;
53 Collin et al., 2015). Chronical infection has also been reported in a Charolais bull in the USA
54 (Montes et al., 1994). The use of oxytetracycline is an effective therapeutics in most of the
55 cases. However, no antibiotic protocol has been proven to eliminate infection and infected
56 animals may remain carriers for life (Genova et al., 2011).

57 Modalities of dissemination are unknown but horizontal transmission by blood-sucking
58 arthropods has been basically accepted as the most common route for haemoplasma infection
59 (Hofmann-Lehmann et al., 2004). Abortion in association with *M. wenyonii* detection from
60 dam blood suggested that vertical transmission to the conceptus may be an alternative route
61 (Sasaoka et al., 2015). Some studies have reported that dam-to-fetus transmission is possible,
62 but the importance of this route for infection is unknown too (Hornok et al., 2011; Fujihara et
63 al., 2011). Vertical transmission seems possible for *Candidatus Mycoplasma haemobos* too
64 (Niethammer et al., 2018). Risk factors associated to infection have been poorly explored.
65 Housed cattle seem more susceptible to infection than grazing cattle and cattle between 1 and
66 3 years old appear to be the more susceptible to infection (Tagawa et al., 2012).

67 In cattle presenting clinical signs, the usual diagnostic method relies on blood smears stained
68 with Giemsa, but this technique shows low specificity and sensitivity (i.e. possible Howell-
69 Jolly bodies artifact) (Smith et al., 1990). More recently, methods developed for diagnosis
70 included qualitative PCR (McAuliffe et al., 2006), real-time PCR (Willi et al., 2009; Meli et
71 al., 2010), direct PCR from whole blood sample without DNA extraction (Tagawa et al.,
72 2012), and the loop-mediated isothermal amplification (LAMP) assay (Song et al., 2013). Yet,
73 these methods have one main drawback in that they were unsatisfactory to discriminate easily
74 bovine hemoplasmas from other mycoplasmas of ruminant origin (i.e. *M. ovis*), which is an

75 important objective (Aktas and Ozubek, 2017). To overcome this issue, we developed a
76 highly specific PCR assay including a plasmid for positive control.
77 We report hereafter the first clinical cases due to *M. wenyonii* (MW) observed in a dairy herd
78 Brittany, France. Suspected cases, based on visual examination of blood smears or imprecise
79 PCR assays have been presented as oral presentation in cattle medicine congresses, but this is
80 subsequently the first identification of MW in France. The clinical signs consisted in
81 hyperthermia (up to 40°C), anemia, and lower limbs and teat edema, and the disease affected
82 up to 40% of the cows. Blood smears and molecular evidence using protocols as described by
83 Tagawa *et al.* (2008) and Sasaoka *et al.* (2015), confirmed the diagnosis. Our second
84 objective was to evaluate the feasibility and performances of an original one step detection
85 based on gene *polC*.

86

87 **Materials and Methods**

88 **DNA samples**

89 DNA was extracted from 200 µl of EDTA-blood samples collected from 2 cattle with clinical
90 signs and positive smear evocating infection by *Mycoplasma wenyonii* (sample 141145 and
91 141146, collected in France (Collin *et al.*, 2015) using a QIAamp DNA Blood Mini Kit
92 (QIAGEN), eluted with 100 µl of buffer AE and stored at -20°C until use. DNA extract from
93 sample known as positive from previous investigation on *M. wenyonii* in Japan[16] was used
94 as positive control for PCR detection. For defining the specificity of the PCR assay, genomic
95 DNA from other mycoplasma species (*Mycoplasma ovis*, *M. fermentans*; *M. hominis*; *M.*
96 *orale*; *Achleplasma laidlawi*; *M. capricolum* subsp. *capricolum*; *M. mycoides* subsp. *capri*;
97 *M. leachi*; *M. putrefaciens*; *M. ovipneumoniae*; *M. bovigentialium*; *M. conjunctivae*;
98 *M. arginini*; *M. gallisepticum*; *M. canis*; *U. diversum*; *M. bovis*; *M. agalactiae*) were used and
99 extracted from liquid culture as described elsewhere (Sambrook and Russel, 2001; Chen and

100 Kuo, 1993) except for *M. ovis* for which it was extracted from whole blood of a positive
101 animal. The *Mycoplasma* species have been chosen either for their renown existence in
102 ruminants (n=13) or for their existence in common domestic animals or humankind (n=5)

103 **PCR detection assays**

104 Four set of primers were used for PCR assays (Table 1). Primers for 16S_rRNA and primers
105 for *rnpB* were designed according to Tagawa et al.2008 and Sasaoka et al. 2015 respectively.
106 Based on the alignment *polC* sequences available in databases for MW and MO, forward and
107 reverse primers were designed to amplify a 500 bp product. Using the Primer blast software,
108 these primers were predicted *in silico* to specifically amplify MW. The same strategy was
109 used to designed MO specific primers.

110 PCR assays were performed using an Eppendorf Mastercycler ep-Gradient thermocycler in 25
111 μ L reaction mixtures containing 0.4 mM of each primer, 1X PCR reaction buffer (with
112 MgSO₄, New England Biolabs [NEB], Evry, France), 200 mM dNTPs and 2.5 U Taq DNA
113 polymerase (NEB). Reaction mixtures were subjected to 2 min at 94°C, 30 cycles (except for
114 16S rRNA: 35) of 30s at 94 °C, 30s at 58°C (except for 16S rRNA: 62°C), 30s at 72 °C, and a
115 final elongation step of 5 min at 72°C. Annealing temperature was determined based on data
116 obtained from a gradient of temperature. PCR products were analyzed by gel electrophoresis
117 in 2% agarose for 16S RNA (Tagawa et al.2008) and *rnpB* (Sasaoka et al. 2015) assays or in
118 1% agarose for MW-*polC* and MO-*polC* assays (Molecular weight standard: Smart Ladder,
119 Eurogentec, Liège, Belgium).

120 **PCR sensitivity and design of replicative plasmid as positive controls**

121 PCR products were purified (QIAquick PCR Purification Kit, QIAGEN, Courtaboeuf, France)
122 and cloned into pGEMT-easy plasmid vector (Promega, Charbonnières les bains, France).
123 Recombinant plasmid vectors were transformed in *Escherichia coli* strain DH5a
124 (Invitrogen/Life-Technologies, Courtaboeuf, France) grown in LB medium supplemented

125 with ampicillin, and purified with QIAprep Spin Miniprep kit (QIAGEN). DNA fragment
126 insertion was monitored by PCR with the appropriate specific primers, followed by
127 sequencing. Plasmid concentration was determined by Qubit Fluorometric Quantitation
128 (ThermoFischer, Villebon sur Yvette, France). PCR assays were performed with serial
129 dilution of the purified plasmid as templates to determine the minimal concentration for which
130 amplification is detected. Each experiment was repeated three times to assess repeatability
131 and we obtained every time the same results. For comparison, the PCR assay was run in
132 parallel with serial dilution of positive samples.

133 **DNA sequencing**

134 Sequences of PCR product and plasmid were obtained by Sanger direct sequencing method
135 using specific primers at the sequencing facility of UMR 5165 (CNRS, UPS, CHU Purpan,
136 Toulouse, France). Sequence analysis was done with Chromas lite (available at
137 http://www.technelysium.com.au/chromas_lite.html) and NCBI blast tools
138 (<https://blast.ncbi.nlm.nih.gov/>).

139 **Case detection study**

140 A case detection study was implemented in 2016 with the help of veterinarians in French
141 Brittany. Cases were included in the study on the basis of cattle presenting: anemia without
142 hemoglobinuria, edema of the limbs and/or the udder, transient milk drop, excluding
143 anaplasmosis and babesiosis, medical history was recorded. A total of 181 cows in 6 herds
144 were included in the study: 34 suspected clinical cases were collected and 147 samples from
145 animals without clinical signs were also collected at the same time and from the same region.
146 Animal blood samples were collected (5 mL, EDTA) for blood-smear primary detection using
147 Diff Quick® coloration and, DNA extraction (QIAamp DNA Blood Mini Kit, Qiagen) for
148 PCR detection using MW-*polC* amplification.

149 **Nucleotide sequence accession numbers.**

150 Nucleotide sequences have been submitted to GenBank under accession numbers MH001426,
151 MH001427, MH001428 and MH001429 respectively.

152

153 **Results**

154 **First detection of hemoplasmas in French ruminants**

155 Using the PCR assay developed by Tagawa *et al.* in 2008, the presence of MW was detected
156 in 9 blood samples (i) collected in France. We selected only 2 after the sequencing process
157 (due to the 100% level of similarity between the 9) from two cows suspected to be infected
158 by MW, namely 141145 and 141146, (see Material and methods) and (ii) from an animal
159 known as being infected by MW, as positive control. More specifically, with these samples, a
160 PCR product of the expected size, 193-bp, was detected. Regardless of the stringency of the
161 PCR conditions, the same PCR assay yielded a similar size product with DNA extracted from
162 *M. bovis*, another ruminant mycoplasma species known to infect ruminant and initially
163 included in our study as a negative control (Figure 1A). The 193-bp PCR product of 141145,
164 141146 and *M. bovis* were sequenced. Data showed that 141145 and 141146 sequences are
165 identical and closely matched sequences belonging to the 16s rRNA of MW and MO, with 0
166 to 2 SNPs, depending on the strains used for comparison (Figure 1B). For *M. bovis*, the
167 sequence was identical to the of *M. bovis* 16s rRNA. All together these data suggested that the
168 PCR developed by Tagawa *et al.* (2008), while detecting hemoplasmas, does not discriminate
169 between MO, MW and MB. When we tested the PCR assay developed by Sasaoka *et al.*
170 (2015), which one targets the *rnpB* gene, *M. bovis* DNA gave a negative result while 141145
171 and 141146 yield the expected size product which sequences match specifically the MW. Yet,
172 MO was positive (Figure 1C).

173

174 **Development of a specific PCR for MW and MO**

175 Overall, these results reflect the lack of a proper diagnostic assay that would allow
176 unambiguously the detection of MW in one single step. To fill this gap, we designed a PCR
177 assay targeting the *polC* gene to amplify specifically a 500 bp product. This was further
178 confirmed *in vitro* using genomic DNA extracted from MW, and MB with a 55°C optimum
179 temperature (Figure 1D). The same strategy was used to designed MO specific primers. The
180 specificity of these PCR assays, further designated as MW-*polC* or MO-*polC*, was then tested
181 against 19 *Mycoplasma* species, of which 15 are known in ruminants (Table 2). Results
182 shown in Table 2 and illustrated in Figure 1 were compared to PCR assays previously
183 developed. All species but two, *M. canis* and *U. diversum*, were detected in our hands with the
184 assay targeting the 16S rRNA (Tagawa et al; 2008). The PCR assay developed by Sasaoka *et*
185 *al.* (2015) was more specific; yet amplification was obtained with MO. Overall, in our hands,
186 comparison of the different PCR assays currently available points towards MW-*polC* as being
187 the more specific for MW detection.

188 **Development of molecular positive controls**

189 Since MW and MO are not cultivable, the use of a field sample as positive control which
190 availability is limited poses the question of the reproducibility and of the estimation of the
191 sensitivity of the assay. To address these issues, we engineered a set of 4 replicative plasmids
192 carrying sequences targeted by the PCR assays and corresponding to: the MW- or MO- 530
193 bp or 545 bp *polC* sequence respectively, the 189 bp MW *rnpB* sequence and the MW 16s
194 rRNA. Replicates of PCR assays using serial dilution of known plasmid concentration
195 indicates that MW-*polC* is less sensitive than other PCR assays, with that targeting the two
196 16s rRNA gene copies being as expected the most sensitive (Table 3 and Figure 2A).
197 Replicates of PCR assays using serial dilution of a field sample (Figure 2B) permit to confirm
198 these sensitivity differences and estimate the bacterial load of a clinically infected animal to

199 10⁷ bacteria/ml of blood assessing that the sensitivity of MW-*polC* assay is sufficient enough
200 to detect clinical cases.

201 **Case detection study**

202 Cases were recruited on basis of clinical signs evocating infection by *M. wenyonii* as detected
203 by veterinarians in Brittany. Animals without clinical signs were also sampled at the same
204 time. A total of 34 samples from adult dairy cattle presenting clinical signs were collected and
205 147 from animals without clinical signs. All were analyzed with MW-*polC* PCR assay. Of the
206 34 animals presenting signs evocating *M. wenyonii* infection, 9 were positive while the 147
207 samples from asymptomatic animals were all negative (Figure 3).

208 **Discussion**

209 This study presents the molecular and clinical identification of a hemoplasma in cattle in
210 France. To the authors' knowledge, this is the first report of a molecular investigation of *M.*
211 *wenyonii* in this country. Hemoplasma organisms (named before 2001 *Eperythrozoon*) have
212 been identified in a variety of mammals (including cattle) and the infection by these
213 organisms has been associated with extravascular hemolytic anemia, reproductive disorders,
214 milk drop syndrome, edema of dependent parts, growth retardation... of varying intensity:
215 subclinical infection seems common (Messik, 2004). In Brittany (France), evocative
216 inclusions in bovine erythrocytes have been observed for the first time in 2014, associated
217 with clinical signs in adult cows (Collin et al., 2015).

218 Our study confirms the presence of *Mycoplasma wenyonii* in France. Surprisingly, in
219 our survey 'Candidatus *M. haemobos*' was not detected unlike in other European countries
220 (Ade et al., 2018; Niethammer et al. 2018), but this species is even less documented than MW.
221 As expected, the number of infected animals by MW is much higher when selecting them on
222 the basis of a clinical suspicion (9/34: 25.6 %) than when sampling them at random in the
223 same area (9/181: 4.9 %). The selected significant clinical signs were: anemia without

224 hemoglobinuria, edema of the limbs and/or the udder, transient milk drop. Of course clinical
225 anaplasmosis and babesiosis were excluded by optical microscopy and PCR (data not shown).
226 No coinfection was observed in our study but is likely to occur from time to time since their
227 potential vectors share the same biotope (Hornok et al., 2011, Messik, 2004).

228 Additionally, the purpose of our study was to address the specificity of previously
229 published PCR assays, and to develop a specific molecular diagnosis a one-step assay,
230 without any subsequent amplification. We demonstrated that no other assay is as discriminant
231 as the one we developed: using MW-*polC* amplification (with the probes we developed)
232 allows detecting only *Mycoplasma wenyonii*, and no other mycoplasma of ruminant origin
233 (Figures 4 and 5). Moreover, the amplicon size is Mw-specific and quite different from *M.*
234 *ovis* amplicons, an important objective (Aktas and Ozubek, 2017) we fulfilled. At first, the
235 suitability of this assay in a real-time PCR was not the purpose of this study.

236 Sensitivity of the existing and new test has been evaluated. The detection threshold is as low
237 as $1.7 \cdot 10^6$ copies of the genome per mL total blood, but it is less sensitive than PCR assays
238 targeting the 16S rRNA gene copies which are, as expected, the most sensitive. The future
239 development of a real-time PCR will be the way to increase the sensitivity of the detection.

240 Sensitivity study was made possible thanks to the engineering of recombinant plasmids. These
241 have several advantages over positive controls from the field infected animals whose
242 reproducibility is poor and/or variable: they can be produce in large amount and offer
243 reproducible and comparative data across labs.

244 Further studies are needed to evaluate the importance of MW infection. The systematic
245 exploration of anemia in cattle could be the first step, as this symptom appears to be the more
246 consistent in cattle of all ages. Studies on coinfections with *Theileria* spp., *Babesia* spp.,
247 *Anaplasma* spp. and *Bartonella* spp. are to be initiated too, because infectivity and disease

248 could be associated with coinfection, apart from other factors to determine. Correlations of
249 MW infection with productivity and growth might be evaluated subsequently.

250 As there is no clear consensus either on the potential vectors, or on transmission, arthropod
251 trapping and investigation on asymptomatic or latent and chronic carriers are needed too.

252 The importance of vertical transmission is unknown too, and sampling calves before ingestion
253 of colostral antibodies could help to specify the importance of this phenomenon.

254 The tools we developed can contribute to target these objectives.

255

256 **Conflict of interest statement**

257 None of the authors of this paper has a financial or personal relationship with other people or
258 organizations that could inappropriately influence or bias the content of the paper

259 **Authorship**

260 LX. Nouvel and MC. Hygonenq carried out DNA extraction and PCR design and analysis. E.
261 Martinelli and G. Catays carried out part of DNA extraction and PCR analysis. Ph. Lepage
262 and E. Collin carried out animal sampling and case story collection. H.Inokuma provided
263 positive controls and PCR protocols. C. Citti, LX. Nouvel and R. Maillard drafted the
264 manuscript. LX. Nouvel, F. Schelcher, C. Citti and R. Maillard conceived and participated in
265 the design of the study, which was coordinated by R. Maillard. All authors read and approved
266 the final manuscript.

267

268 **Declarations of interest:**

269 None

270

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274

275

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360

361 **Figure captions**

362

Figure 1: *M. wenyonii* PCR assays

363 Princeps cases detection targeting 16S rRNA (A) or polC (C) genes, and assays specificity
364 testing (B). T+: MW for 16S rRNA, *rnpB* and MW-*polC*; MO for MO-*polC*; M: Smart ladder
365 Eurogentec; 1: *Mycoplasma fermentans*; 2: *Achleplasma laidlawi*; 3: *M. capricolum* subsp.
366 *capricolum*; 4: *M. leachi*; 5: *M. putrefaciens*; 6: *M. ovipneumoniae*; 7: *M. mycoides* subsp.
367 *capri*; 8: *M. bovis genitalium*; 9: *M. conjunctivae*; 10: *M. arginini*; 11: *M. canis*; 12:
368 *U. diversum* 310[#]; 13: *U. diversum* 246^{#£}; 14: *M. bovis*; 15: *M. agalactiae**; 16: *M. wenyonii**;
369 (*: DNA extract of blood from positive animal, [#]: culture after PK lysis, [£]: no culture growth)

370 **Figure 1C:** 16S and *polC* sequences from isolates 14-1145 and 14-1146

371

372

Figure 2: Sensitivity testing of *M. wenyonii* PCR assays

373 (A) 16S rRNA, *rnpB*, MW-*polC* and MO-*polC* PCR with specific plasmid in serial dilution
374 corresponding to 10 to 10¹⁰ gene copy as template (B) Quantification of gene copy in positive
375 sample 14-1145: 16S rRNA, *rnpB*, MW-*polC* PCR with serial dilution of DNA extract as
376 template.

377

Figure 3: Global survey results

378 Detection results in all animals or when selecting animal on clinical signs

379 **Figure 4:** *polC* gel *Mycoplasma wenyonii* specific

380

381 **Figure 5:** *polC* phylogenetic tree

382

383 **Tables**

384

Table 1: Primers and PCR conditions

Targeted gene	Primers	Ta*	Expected amplicon size		References
			<i>M. wenyonii</i>	<i>M. ovis</i>	
16S_rRNA	16S-F2 (5'-GGCCCATATTCCTRCGGGAAG-3') 16S-R2 (5'-ACRGGATTACTAGTGATTCCA-3')	62°C	193 bp	176 and 193 bp	Tagawa <i>et al.</i> [2008]
<i>rnpB</i>	<i>rnpB</i> -F (5'-AGTCTGAGATGACTRTAGTG-3') <i>rnpB</i> -R (5'-TRCTTGMGGGTTTGCCTCG-3')	58°C	189 bp	187 bp	Sasaoka <i>et al.</i> [2015]
<i>MW_polC</i>	MW-PolCF1 (5'-GTAGTACCACCACCAGAGCAG-3') MW-PolCR1 (5'-ACTACCTCGTGCCCAAGTG-3')	58°C	530 bp	-	This study
<i>MO_polC</i>	MO-PolCF1 (5'-GGACAGTGAGCGGAGATGG-3') MO-PolCR1 (5'-AAGAGTGGGGCTTGAATGGG-3')	58°C	-	545 bp	This study

385 *: temperature of annealing

Table 2: Specificity of the PCR assays targeting the detection of MW

Species and reference of the strain or isolate	Host	PCR assay			
		16S rRNA	<i>rnpB</i>	MW- <i>polC</i>	MO- <i>polC</i>
<i>M. wenyonii</i> Isolate 14-1146 this study	Ruminants	+	+	+	-*
<i>M. ovis</i> isolate 10-373, this study	Ruminants	+*	+	-*	+
<i>M. fermentans</i> type strain PG 12	Human	+	-	-	-
<i>M. hominis</i> type strain PG21	Human	+	-	-	-
<i>M. orale</i> type strain CH 19299	Human	+	-	-*	-*
<i>A. laidlawii</i> type strain PG8	Free	+	-	-	-*
<i>M. capricolum</i> subsp. <i>Capricolum</i> type strain California kid	Ruminants	+	-	-	-
<i>M. mycoides</i> subsp. <i>Capri</i> type strain Y goat	Ruminants	+	-	-	-
<i>M. leachii</i> type strain PG50	Ruminants	+	-	-	-
<i>M. putrefaciens</i> type strain KST	Ruminants	+	-	-	-

<i>M. ovipneumoniae</i> type strain Y98	Ruminants	+	-	-	-
<i>M. bovigenitalium</i> type strain PG 11	Ruminants	+	-	-	-
<i>M. conjunctivae</i> type strain HRC581	Ruminants	+	-	-	-
<i>M. arginini</i> type strain G230	Ruminants	+	-	-	-
<i>M. gallisepticum</i> type strain PG31	Poultry	+	+f	-	-
<i>M. canis</i> type strain PG14	Ruminants	-	-	-	-
<i>U. diversum</i> type strain A417	Ruminants	-	-	-	-
<i>M. bovis</i> type strain PG45	Ruminants	+	-	-	-
<i>M. agalactiae</i> type strain PG2	Ruminants	+	-	-	-

387 +: amplicon at expected size; -: no amplification detected; -*: weak signal at a different
388 size than expected; +f: positive weak signal at the expected size

389

390 Table 3: Sensitivity of the PCR assays

	Plasmid insert target	Detection threshold (genome/ μ L blood)
16S rRNA	MW	30
<i>rnpB</i>	MW	200
	MO	90
MW- <i>polC</i>	MW	1700
MO- <i>polC</i>	MO	240

391

392

MO: *Mycoplasma ovnis*; MW: *Mycoplasma wenyonii*

A

16S

M

14-1145

14-1146

H2O

T+

PG45



>Seq1 [Mycoplasma wenyonii] M.wenyonii[14_1145]partial16S

ACGAAAGTCTGATGGAGCAATACCACGTGAACGATGAAGGTCTTCTGATTGTAAAGTTCTTTTATTTAGGAAA
AAAAGCGTGCTAGGAAATGAGCGCGCCTTGATGGTACTAATTGAATAAGTGACAGCTAACTATGTGCCAGCAG
CTGCGGTAAAACATAGGTCACGAGCATTATCCGGATTTATTGGGCGT

>Seq2 [Mycoplasma wenyonii] M.wenyonii[14_1146]partial16S

ACGAAAGTCTGATGGAGCAATACCACGTGAACGATGAAGGTCTTCTGATTGTAAAGTTCTTTTATTTAGGAAA
AAAAGCGTGCTAGGAAATGAGCGCGCCTTGATGGTACTAATTGAATAAGTGACAGCTAACTATGTGCCAGCAG
CTGCGGTAAAACATAGGTCACGAGCATTATCCGGATTTATTGGGCGT

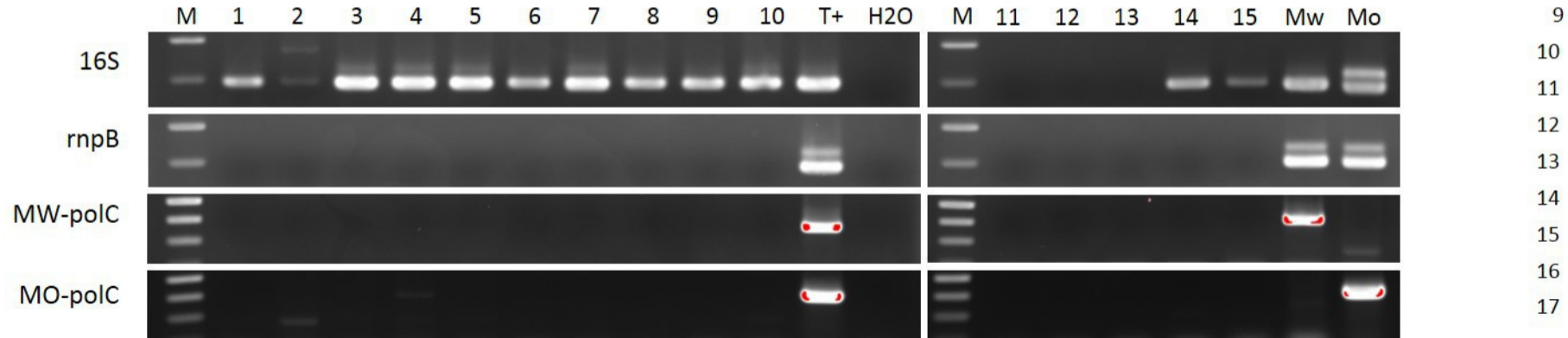
> Seq3 [Mycoplasma wenyonii] M.wenyonii[14_1145]polC

GTAGTACCACcACCAGAGCAGATACTAGAGGAAGATGTAAGTGTAAAGGAAGCCCCTTTAATAGCAAAAAGAGA
CAGAAGTATTGGGAGCAATACAACTATTGACTCTAAGGTAAAAGGTATTTTCAAGCTCTATCTCCTAGAAGAG
ATAGATAGCTACAGTTACAGAGGTCAGAGTATGTATAAGGTAGCTTTTACTAACTTCTCTGATCATTACATATTA
CTACTAGGTAGATTAATTACTCCAgAAAAGAAAGCAGAATATCTAAAGATACTAAAAGTTGAAGAGTGATTTG
AGATAGAAATTCAACTAGATGTAAAGAGTTCTACTAGTTCAAAACTCTATCTAACTATGATGGTTGACTAATT
AGTTGTGCTTCAGTTGATTATCCAAAGGGATTAAGTctAGAAGATAACTCACCAAAGAAAGCCTTTTCCTTAAAT
GTATATACCAAATACTCTAGCTTTGATGGGTTATTTAACTCAGATCAATGAGCAGAAAAGCTAAAGCACTTGG
GCACGAGGTAGT

>Seq4 [Mycoplasma wenyonii] M.wenyonii[14-1146]polC

GTAGTACCACCACCAGAGCAGATACTAGAGGAAGATGTAAGTGTAAAGGAAGCCCCTTTAATAGCAAAAAGAG
ACAGAAGTATTGGGAGCAATACAACTATTGACTCTAAGGTAAAAGGTATTTTCAAGCTCTATCTCCTAGAAGA
GATAGATAGCTACAGTTACAGAGGTCAGAGTATGTATAAGGTAGCTTTTACTAACTTCTCTGATCATTACATAT
TACTACTAGGTAGATTAATTACTCCAGAAAAGAAAGCAGAATATCTAAAGATACTAAAAGTTGAAGAGTGATT
TGAGATAGAAATTCAACTAGATGTAAAGAGTTCTACTAGTTCAAAACTCTATCTAACTATGATGGTTGACTAA
TTAGTTGTGCTTCAGTTGATTATCCAAAGGGATTAAGTCTAGAAGATAACTCACCAAAGAAAGCCTTTTCCTTA
AATGTATATACCAAATACTCTAGCTTTGATGGGTTATTTAACTCAGATCAATGAGCAGAAAAGCTAAAGCACT
TGGGCACGAGGTAGT

Figure 4 : 16S and *PolC* sequences of isolates 14-1145 et 14-1146

B

C

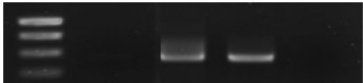
MW-polC

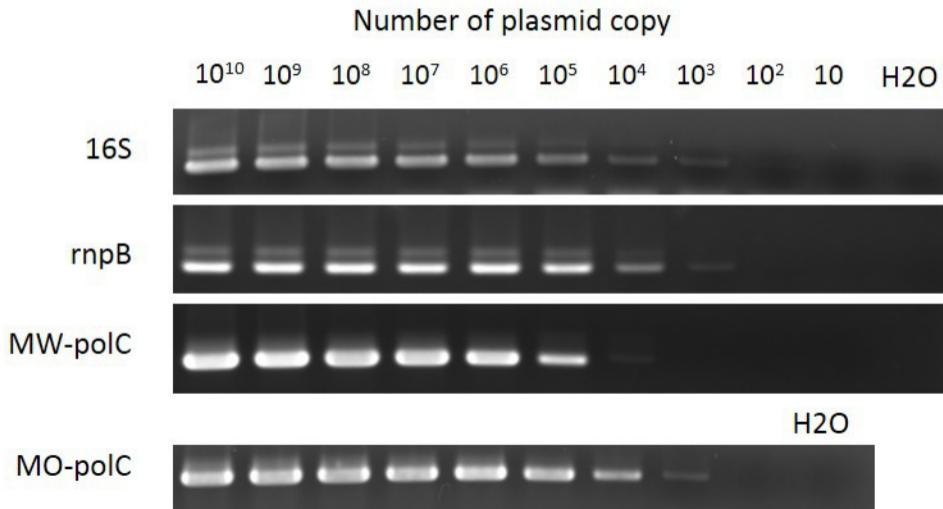
M

14-1145

14-1146

H₂O



A

B

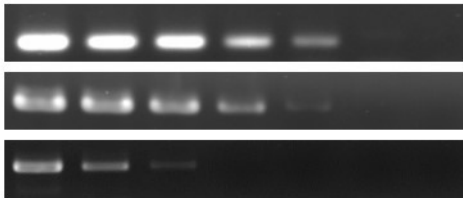
Extract 14-1145

 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}

16S

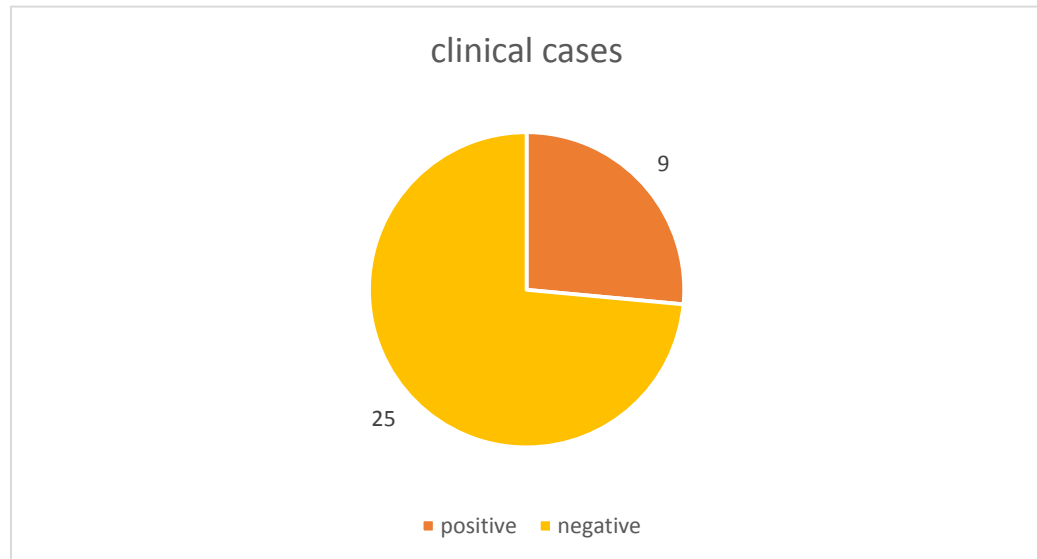
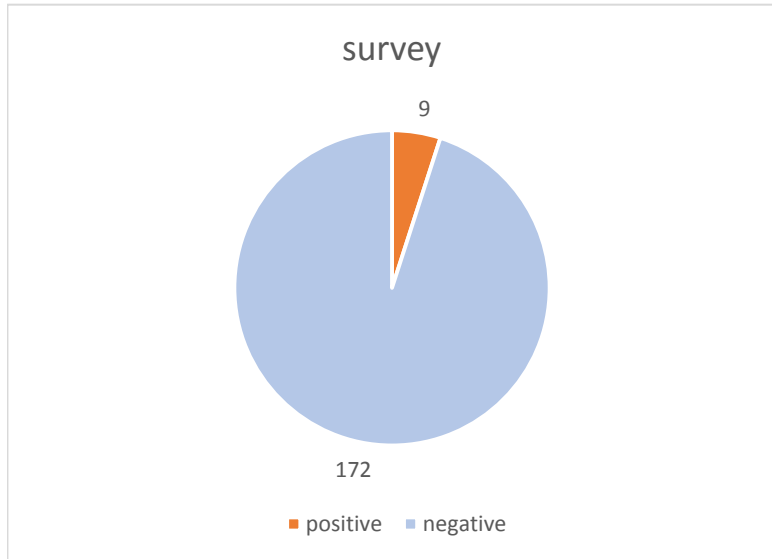
rnpB

MW-polC

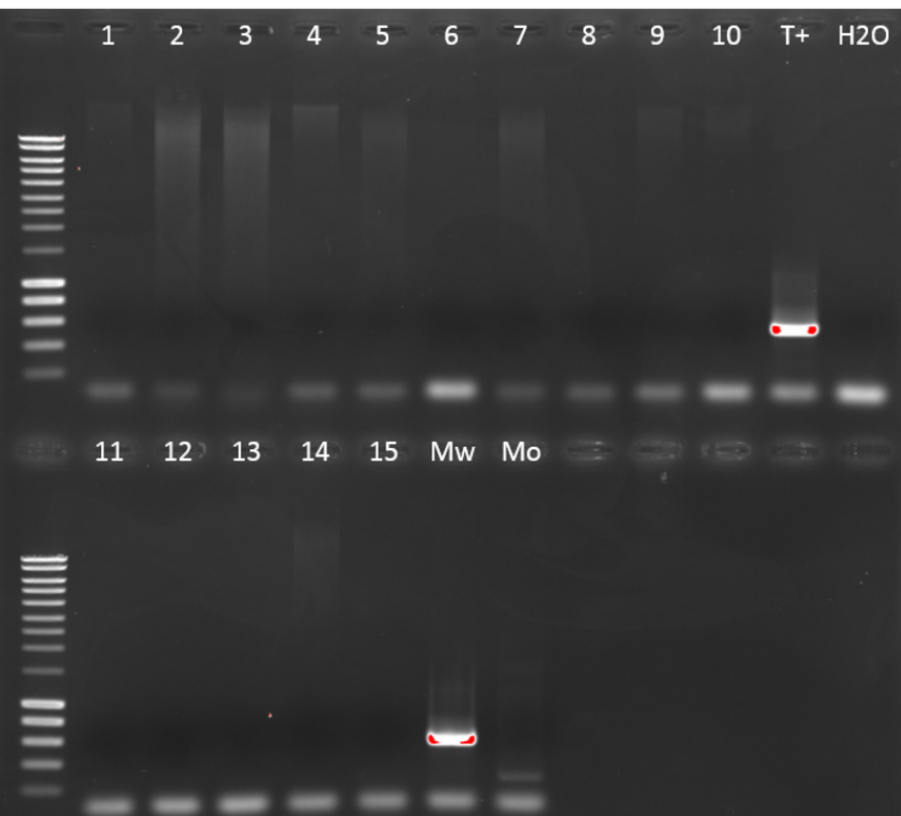
 10^7 cp

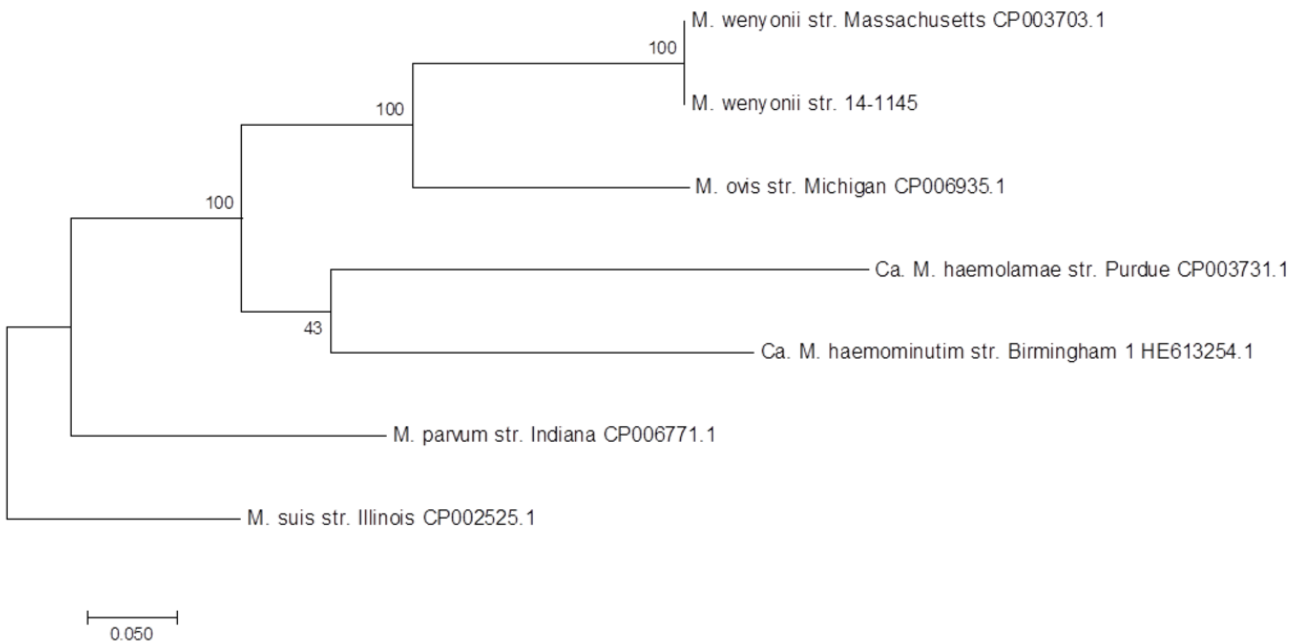
survey
positive 9
negative 172

clinical cases
positive 9
negative 25



polC Mwen (2016-09-29 11hr16min)





Phylogenetic tree based on *polC* gene sequence

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [1]. The tree with the highest log likelihood (-22940.9572) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 4202 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2].

1. Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10:512-526.
2. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.

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