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Khalil Yousef Mohamad, Abdessalem Rekiki, Mustapha Berri, Annie Rodolakis. Recombinant 35-kDa inclusion membrane protein IncA as a candidate antigen for serodiagnosis of. *Veterinary Microbiology*, 2010, 143 (2-4), pp.424. 10.1016/j.vetmic.2009.11.017. hal-00594812

HAL Id: hal-00594812

<https://hal.science/hal-00594812>

Submitted on 21 May 2011

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Accepted Manuscript

Title: Recombinant 35-kDa inclusion membrane protein IncA as a candidate antigen for serodiagnosis of *Chlamydophila pecorum*

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PII: S0378-1135(09)00566-5
DOI: doi:10.1016/j.vetmic.2009.11.017
Reference: VETMIC 4672

To appear in: *VETMIC*

Received date: 18-8-2009
Revised date: 9-11-2009
Accepted date: 12-11-2009

Please cite this article as: Mohamad, K.Y., Rekiki, A., Berri, M., Rodolakis, A., Recombinant 35-kDa inclusion membrane protein IncA as a candidate antigen for serodiagnosis of *Chlamydophila pecorum*, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.11.017

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**Recombinant 35-kDa inclusion membrane protein InCA as a candidate
antigen for serodiagnosis of *Chlamydophila pecorum***

Khalil Yousef Mohamad, Abdessalem Rekiki, Mustapha Berri and Annie Rodolakis*

Institut National de la Recherche Agronomique (INRA), UR1282, Infectiologie Animale et
Santé Publique, F-37380, Nouzilly (Tours), France

*Corresponding author: Tel.: +33 2 47 42 76 34; fax: +33 2 47 42 77 79

E- Mail address: rodolaki@tours.inra.fr

1 Abstract

2 *Chlamydophila pecorum* strains are commonly found in the intestine and vaginal mucus of
3 asymptomatic ruminants and may therefore induce a positive serological response when the
4 animals are tested for *C. abortus*. They have also been associated with different pathological
5 diseases in ruminants, swine and koala. The aim of this study was to identify specific *C.*
6 *pecorum* immunodominant antigens which could be used in ELISA tests allowing to
7 distinguish between animals infected with *C. pecorum* and those infected with other
8 chlamydial species. A gene encoding 35-kDa inclusion membrane protein *incA* of *C. pecorum*
9 was isolated by immunoscreening of the *C. pecorum* DNA library using ovine anti-*C.*
10 *pecorum* antibodies. The recombinant IncA protein did not react with a murine serum directed
11 against *C. abortus* but did react with a specific monoclonal antibody of *C. pecorum* and
12 toward several ovine serum samples obtained after experimental infection with different *C.*
13 *pecorum* strains. This protein could be a good candidate for specific diagnosis of *C. pecorum*
14 infection.

15
16 **Keywords:** *Chlamydophila pecorum*, serodiagnosis, IncA

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26 **Introduction**

27 *Chlamydomphila pecorum* strains have been isolated from small and large ruminants, koalas
28 and swine. It is commonly found in the intestine and vaginal mucus of ruminants without any
29 clinical sign. However, some *C. pecorum* strains cause diseases in ruminants and swine,
30 including pneumonia, polyarthritis, conjunctivitis, enteritis, encephalomyelitis and abortion
31 (Kaltenboeck and Storz, 1992; Rodolakis et al., 1998). In koalas, *C. pecorum* is known to
32 cause reproductive disease, infertility and urinary tract disease (Jackson et al., 1999). *C.*
33 *pecorum* strains can also cause infections in wild animals, for example, conjunctivitis in the
34 western barred bandicoot and abortion in water buffalo (Greco et al., 2008; Warren et al.,
35 2005).

36 *C. pecorum* could be responsible for the positive serological response sometimes seen in
37 flocks, tested for *C. abortus*. Serodiagnostic tests, such as the complement fixation test (CFT)
38 and enzyme-linked immunosorbent assays (ELISA), are the simplest methods for detecting
39 chlamydial infections. The CFT was generally used until the development of ELISA tests
40 because of the false positive response obtained with anti-complementary serum samples in the
41 CFT. Thus, neither the CFT nor ELISA tests using whole bacteria or protein extracts are able
42 to differentiate between antibodies directed against *C. abortus* and *C. pecorum* (Brade et al.,
43 1987; Markey et al., 1993). Several experimental (Hoelzle et al., 2004; Kaltenboeck et al.,
44 1997; Longbottom et al., 2001) or commercial ELISAs developed by INRA-Nouzilly
45 (Buendia et al., 2001; Vretou et al., 2007) have been proposed to detect species-specific anti-
46 *C. abortus* antibodies in ovine and bovine sera.

47 The present study focuses on the search for a specific antigen to identify animals infected
48 with *C. pecorum*. This would allow to estimate the prevalence of *C. pecorum* in
49 epidemiological studies and to improve the diagnosis of *C. abortus* by removing the
50 serological cross-reactivity between *C. pecorum* and *C. abortus*.

51

52 **Materials and Methods**

53 **Chlamydial strains**

54 Five chlamydial strains were used: 4 strains of *C. pecorum* and 1 strain of *C. abortus* (Table
55 1). All strains were propagated in the yolk sac of chicken embryos and stored at -70 °C as
56 previously described (Rodolakis, 1976).

57 **Serum samples**

58 Serum samples were obtained after experimental infection of sheep and mice with various *C.*
59 *pecorum* isolates. Maintenance and care of experimental animals were in accordance with
60 National Decree, N° 2001-464, May 2001, concerning animal testing in France. Samples were
61 collected from 10 ewes: 3 inoculated with *C. pecorum* M14, 3 with *C. pecorum* AB10, 2 with
62 *C. pecorum* LW679, and 2 with *C. pecorum* iB5. Approximately 10^6 plaque-forming units
63 (PFU)/Sheep of each strain were inoculated subcutaneously 4 times at 3-week intervals. Two
64 serum samples obtained from mice inoculated with *C. pecorum* M14 or *C. pecorum* AB10
65 were previously used in our laboratory (Rekiki et al., 2004). One serum was pooled from 10
66 mice infected experimentally with *C. abortus* AB7. Approximately 10^4 PFU/mouse were
67 inoculated subcutaneously 3 times at 2-week intervals.

68 The specific monoclonal anti-*C. pecorum* antibody PD3 was produced at INRA- Nouzilly as
69 previously described (Salinas et al., 1996).

70 In addition, 8 bovine serum samples were collected from a herd with no clinical signs.

71 All these serum samples were examined using microimmunofluorescence (MIF) as previously
72 described (Salinas et al., 1996).

73 **Construction and immunoscreening of *C. pecorum* genomic DNA expression library**

74 Genomic DNA was prepared from purified *C. pecorum* M14 strain as previously described
75 (Boumedine and Rodolakis, 1998). The genomic library was constructed using Lambda ZAP

76 Express vector following the manufacturer's protocol (Stratagene, Lyon, France). The
77 recombinant phages were selected using the ovine anti-*C. pecorum* M14 serum. Several phage
78 clones were plaque-purified and converted to the recombinant pBK-CMV plasmid in *E. coli*
79 XLOR following the manufacturer's instructions (Stratagene).

80 **Immunoblotting**

81 Total proteins expressed by cloned *C. pecorum* M14 DNA in *E. coli* XLOR were analyzed
82 by immunoblotting. *E. coli* XLOR containing the recombinant plasmid was cultured in
83 Luria-Bertani Media (LB) at 37°C overnight. The cells were pelleted by centrifugation
84 (5000g/ 10 min), resuspended in Laemmli sample buffer containing 5% of 2-mercaptoethanol
85 (Biorad, Marnes-la-Coquette, France), and boiled at 95°C for 10 min. The proteins were
86 separated by SDS-PAGE electrophoresis (Laemmli, 1970) and transferred onto nitrocellulose
87 membranes in Tris-glycine buffer (containing 0.1 M Tris base, 0.192 M glycine, and 10%
88 methanol) at 65 mA for 60 min (Towbin et al., 1979). Immunoreactive proteins of *C. pecorum*
89 were detected by Western blot analysis using an ovine serum sample as the first antibody,
90 then reacted with anti-sheep alkaline phosphatase conjugate (Sigma-Aldrich, Lyon, France) as
91 the secondary antibody, and visualized by BCIP/NBT color substrate (Promega,
92 Charbonnières-Les-Bains, France).

93 **DNA sequence analysis**

94 Plasmid DNAs were purified by a QIAprep Miniprep kit (Qiagen, Courtaboeuf, France) from
95 cultures of *E. coli* XLOR clones containing different *C. pecorum* M14 DNA inserts. The
96 cloned DNAs identified by Western blotting were sequenced at Genome Express (Cogenic,
97 Meylan, France) using T7 forward and reverse primers. The cloned genes were identified
98 using BLASTn and BLASTx in NCBI against the complete genome sequence of *Chlamydia*.

99 **Cloning of *inca* gene**

100 The DNA of the ovine *C. pecorum* AB10 strain was extracted using the Dneasy kit following
101 the manufacturer's instructions (Qiagen). The *incA* gene was amplified by polymerase chain
102 reaction (PCR) with two primers, containing *Bam*HI and *Kpn*I restriction sites respectively
103 (restriction sites are underlined), as follows: In-E58-F (5'-
104 CTCGGATCCACAGTGAATCCCCTACGAA -3') and In-E58-R (5'-
105 CTCGGTACCTTTTGAAGCGCTGTTTCAT -3'). PCR was performed using the GoTaq
106 Flexi DNA Polymerase protocol (Promega) in an automated DNA thermal cycler (Biometra,
107 Goettingen, Germany). After an initial denaturation period of 5 min at 94 °C, reactions were
108 subjected to 30 cycles of 30 sec at 94 °C, 45 sec at an annealing temperature of 63 °C, then 72
109 °C for 1.5 min with a final extension step at 72 °C for 10 min. The PCR product was purified
110 using the QIAquick PCR kit (Qiagen) and ligated into the pQE30-HisTag expression vector
111 (Qiagen). The recombinant construct (pQE30/*incA*) was used to transform *E. coli* TG1 cells.
112 The recombinant IncA protein was purified under native conditions using a Nickel affinity
113 chromatography column following the manufacturer's instructions (Qiagen).

114

115 **Results**

116 Immunoscreening of the *C. pecorum* genomic DNA library identified 16 immunoreactive
117 clones with varying signal intensities (Table 2). The molecular masses of four (B3, A10, B15
118 and B11) of the sixteen clones were identified by Western blotting (Figure 1). Three clones
119 (B3, A10 and B15) with a molecular mass of 32-33 kDa reacted with the same MAbs PD3
120 (Fig. 2). Two immunoreactive proteins showing a molecular mass of 18 and 30 kDa were
121 identified in the fourth clone (B11) (Figure 1).

122 Sequence analysis and BLAST search of the complete chlamydial genome sequence showed
123 that the 18, 30 and 32-33 kDa proteins corresponded to the rRNA methylase family spoU

124 protein (459 bp), peptidyl-prolyl cis-trans isomerase Mip precursor (744 bp), and inclusion
125 membrane protein IncA (981 bp), respectively.

126 The full-length *incA* gene of *C. pecorum* was cloned in pQE-30 vector and a recombinant 35
127 kDa protein was expressed. Immunoblotting analysis showed that the purified rIncA reacted
128 with the MAb PD3 (Figure 3) and with all murine and ovine serum samples obtained after
129 experimental infection with different *C. pecorum* strains. However, no reaction was seen with
130 the murine serum directed against *C. abortus*. Moreover, the ability of the recombinant IncA
131 to detect anti-*C. pecorum* antibodies in healthy ruminant herds was tested using 8 bovine
132 serum samples: 6 of these samples were found positive (Figure 4).

133

134 **Discussion**

135 To diagnose *C. pecorum*, several DNA-based techniques have been proposed using
136 conventional PCR (Everett et al., 1999; Kaltenboeck et al., 1992) or real-time PCR with
137 primers derived from 23S rRNA or *ompA* genes (DeGraves et al., 2003; Yang et al., 2006). A
138 multiplex PCR has recently been developed to detect *C. abortus*, *C. pecorum* and *Coxiella*
139 *burnetii* (Berri et al., 2009). However, at present, no serodiagnostic system can specifically
140 detect *C. pecorum* infection.

141 In this study, we proposed *C. pecorum* inclusion membrane protein *incA* as a diagnostic
142 antigen. Our results showed that the *incA* was obtained by immunoscreening the *C. pecorum*
143 genomic library with the ovine anti-*C. pecorum* serum sample. Three out of sixteen clones
144 had the *incA* gene, and a strong band reacting with ovine serum directed against different *C.*
145 *pecorum* strains was detected, suggesting that this protein could be a common and
146 immunodominant antigen for *C. pecorum*. The recombinant IncA protein reacted with the
147 specific anti-*C. pecorum* MAb PD3, but not with the murine serum directed against *C.*
148 *abortus*, suggesting that IncA could be a specific *C. pecorum* antigen. Although the

149 biochemical properties of IncA proteins, including their multimeric structure, were conserved
150 in all the chlamydial species, the *incA* sequences showed little similarity and antibodies
151 against IncA in the different chlamydial species did not cross-react (Delevoeye et al., 2004).
152 This gene has therefore been proposed to detect *C. psittaci* and *C. trachomatis* using
153 quantitative PCR and immunodiagnostic tests respectively (Menard et al., 2006; Tsai et al.,
154 2007). Furthermore, a recent *in silico* study proposed IncA as a major antigen for *C.*
155 *pneumoniae* diagnosis (Park et al., 2009).

156 A coding tandem repeats (CTR) variant was identified along the *incA* gene sequence of *C.*
157 *pecorum* allowing 19 *C. pecorum* strains isolated from ruminants to be divided into 3 groups
158 based on the different CTR motifs (Yousef Mohamad et al., 2008a). Seven out of eight
159 pathogenic strains of different clinical and geographical origins were found in one group in
160 which the CTR of *incA* contained only alanine-proline (APA) motif. In addition, a multi-
161 virulence locus sequence typing (MVLST) technique was used to divide *C. pecorum* strains
162 into 4 groups, one containing only 6 out of 8 pathogenic strains (Yousef Mohamad et al.,
163 2008b). That study suggested that *ompA*, *incA* and ORF663 genes could be used as molecular
164 markers in epidemiological studies of *C. pecorum*.

165 Although the CTR in the *incA* sequence varied among *C. pecorum* strains, they did not
166 hamper the reaction of recombinant IncA protein with serum samples from sheep immunized
167 with *C. pecorum*, including pathogenic or intestinal strains. This suggests that the IncA
168 protein has common epitopes recognized by different *C. pecorum* strains. It would be
169 interesting to examine the antigenicity of these motifs and to produce synthetic peptide
170 antigens based on them which could be useful for distinguishing between animals infected by
171 pathogenic and non-pathogenic *C. pecorum* strains.

172 The rIncA also reacted with 6 of the 8 serum samples collected from healthy bovine herds.
173 This suggests that *C. pecorum* persists in the intestine and vaginal mucus of healthy ruminants

174 as previously demonstrated (Longbottom, 2004). More than 51% of female calves acquired
175 natural infection with *C. pecorum* in the first 2 months after birth (Kaltenboeck et al., 2005).
176 In addition, an investigative study showed that *C. pecorum* was more widespread in cattle
177 than *C. abortus*, and the bacteria were frequently detected in vaginal swabs and fecal samples
178 (Jee et al., 2004). It is therefore important to develop an approach able to detect clinically
179 unapparent intestinal infection caused by *C. pecorum* which has been reported to be prevalent
180 in both abortion-affected and -unaffected ruminant flocks (Reinhold et al., 2008).
181 In conclusion, this is the first report about the utilization of a recombinant InCA as a potential
182 specific antigen for *C. pecorum* diagnosis. Further analysis is needed to evaluate the use of
183 this antigen in ELISAs.

184

185 **References**

- 186 Berri, M., Rekiki, A., Sidi Boumedine, K., and Rodolakis, A., 2009. Simultaneous differential
187 detection of *Chlamydomphila abortus*, *Chlamydomphila pecorum* and *Coxiella burnetii*
188 from aborted ruminant's clinical samples using multiplex PCR. BMC Microbiol. 9,
189 130.
- 190 Boumedine, K. S., and Rodolakis, A., 1998. AFLP allows the identification of genomic
191 markers of ruminant *Chlamydia psittaci* strains useful for typing and epidemiological
192 studies. Res. Microbiol. 149, 735-44.
- 193 Brade, H., Brade, L., and Nano, F. E., 1987. Chemical and serological investigations on the
194 genus-specific lipopolysaccharide epitope of *Chlamydia*. Proc. Natl. Acad. Sci. U S A.
195 84, 2508-12.
- 196 Buendia, A. J., Cuello, F., Del Rio, L., Gallego, M. C., Caro, M. R., and Salinas, J., 2001.
197 Field evaluation of a new commercially available ELISA based on a recombinant

- 198 antigen for diagnosing *Chlamydophila abortus* (*Chlamydia psittaci* serotype 1)
199 infection. Vet. Microbiol. 78, 229-39.
- 200 DeGraves, F. J., Gao, D., Hehnen, H. R., Schlapp, T., and Kaltenboeck, B., 2003. Quantitative
201 detection of *Chlamydia psittaci* and *C. pecorum* by high-sensitivity real-time PCR
202 reveals high prevalence of vaginal infection in cattle. J. Clin. Microbiol. 41, 1726-9.
- 203 Delevoeye, C., Nilges, M., Dautry-Varsat, A., and Subtil, A., 2004. Conservation of the
204 biochemical properties of IncA from *Chlamydia trachomatis* and *Chlamydia caviae*:
205 oligomerization of IncA mediates interaction between facing membranes. J. Biol.
206 Chem. 279, 46896-906.
- 207 Everett, K. D., Hornung, L. J., and Andersen, A. A., 1999. Rapid detection of the
208 *Chlamydiaceae* and other families in the order *Chlamydiales*: three PCR tests. J. Clin.
209 Microbiol. 37, 575-80.
- 210 Faye, P., Charton, L., Mage, C., Bernard, C., and Le Layec, C., 1972. Propriétés
211 hémagglutinantes du virus de l'avortement enzootique des petits ruminants (Souches
212 de *Rakeia* d'origine ovine et caprine). Bul. Acad. Vet. Fr. 45, 169-173.
- 213 Greco, G., Corrente, M., Buonavoglia, D., Campanile, G., Di Palo, R., Martella, V.,
214 Bellacicco, A. L., D'Abramo, M., and Buonavoglia, C., 2008. Epizootic abortion
215 related to infections by *Chlamydophila abortus* and *Chlamydophila pecorum* in water
216 buffalo (*Bubalus bubalis*). Theriogenology. 69, 1061-9.
- 217 Hoelzle, L. E., Hoelzle, K., and Wittenbrink, M. M., 2004. Recombinant major outer
218 membrane protein (MOMP) of *Chlamydophila abortus*, *Chlamydophila pecorum*, and
219 *Chlamydia suis* as antigens to distinguish chlamydial species-specific antibodies in
220 animal sera. Vet. Microbiol. 103, 85-90.
- 221 Jackson, M., White, N., Giffard, P., and Timms, P., 1999. Epizootiology of *Chlamydia*
222 infections in two free-range koala populations. Vet. Microbiol. 65, 255-64.

- 223 Jee, J., Degraives, F. J., Kim, T., and Kaltenboeck, B., 2004. High prevalence of natural
224 *Chlamydophila* species infection in calves. J. Clin. Microbiol. 42, 5664-72.
- 225 Kaltenboeck, B., Heard, D., DeGraves, F. J., and Schmeer, N., 1997. Use of synthetic
226 antigens improves detection by enzyme-linked immunosorbent assay of antibodies
227 against abortigenic *Chlamydia psittaci* in ruminants. J. Clin. Microbiol. 35, 2293-8.
- 228 Kaltenboeck, B., Hehnen, H. R., and Vaglenov, A., 2005. Bovine *Chlamydophila* spp.
229 infection: do we underestimate the impact on fertility? Vet. Res. Commun. 29 Suppl 1,
230 1-15.
- 231 Kaltenboeck, B., Kousoulas, K. G., and Storz, J., 1992. Two-step polymerase chain reactions
232 and restriction endonuclease analyses detect and differentiate *ompA* DNA of
233 *Chlamydia* spp. J. Clin. Microbiol. 30, 1098-104.
- 234 Kaltenboeck, B., and Storz, J., 1992. Biological properties and genetic analysis of the *ompA*
235 locus in *chlamydiae* isolated from swine. Am. J. Vet. Res. 53, 1482-7.
- 236 Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of
237 bacteriophage T4. Nature. 227, 680-5.
- 238 Longbottom, D., 2004. Chlamydial infections of domestic ruminants and swine: new
239 nomenclature and new knowledge. Vet. J. 168, 9-11.
- 240 Longbottom, D., Psarrou, E., Livingstone, M., and Vretou, E., 2001. Diagnosis of ovine
241 enzootic abortion using an indirect ELISA (rOMP91B iELISA) based on a
242 recombinant protein fragment of the polymorphic outer membrane protein POMP91B
243 of *Chlamydophila abortus*. FEMS Microbiol. Lett. 195, 157-61.
- 244 Markey, B. K., McNulty, M. S., and Todd, D., 1993. Comparison of serological tests for the
245 diagnosis of *Chlamydia psittaci* infection of sheep. Vet. Microbiol. 36, 233-52.

- 246 Menard, A., Clerc, M., Subtil, A., Megraud, F., Bebear, C., and de Barbeyrac, B., 2006.
247 Development of a real-time PCR for the detection of *Chlamydia psittaci*. J. Med.
248 Microbiol. 55, 471-3.
- 249 Norton, W. L., and Storz, J., 1967. Observations on sheep with polyarthritis produced by an
250 agent of the psittacosis-lymphogranuloma venereum-trachoma group. Arthritis
251 Rheum. 10, 1-12.
- 252 Park, S. H., Kwon, S. J., Lee, S. J., Kim, Y. C., Hwang, K. Y., Kang, Y. H., and Lee, K. J.,
253 2009. Identification of immunogenic antigen candidate for *Chlamydophila*
254 *pneumoniae* diagnosis. J. Proteome Res. 8, 2933-2943.
- 255 Reinhold, P., Jaeger, J., Liebler-Tenorio, E., Berndt, A., Bachmann, R., Schubert, E., Melzer,
256 F., Elschner, M., and Sachse, K., 2008. Impact of latent infections with
257 *Chlamydophila* species in young cattle. Vet. J. 175, 202-11.
- 258 Rekiki, A., Bouakane, A., Hammami, S., El Idrissi, A. H., Bernard, F., and Rodolakis, A.,
259 2004. Efficacy of live *Chlamydophila abortus* vaccine 1B in protecting mice placentas
260 and fetuses against strains of *Chlamydophila pecorum* isolated from cases of
261 abortion. Vet. Microbiol. 99, 295-9.
- 262 Rodolakis, A., 1976. Abortive infection of mice inoculated intraperitoneally with *Chlamydia*
263 *ovis*. Ann. Rech. Vet. 7, 195-205.
- 264 Rodolakis, A., Bernard, F., and Lantier, F., 1989. Mouse models for evaluation of virulence of
265 *Chlamydia psittaci* isolated from ruminants. Res. Vet. Sci. 46, 34-9.
- 266 Rodolakis, A., Salinas, J., and Papp, J., 1998. Recent advances on ovine chlamydial abortion.
267 Vet. Res. 29, 275-88.
- 268 Salinas, J., Souriau, A., De Sa, C., Andersen, A. A., and Rodolakis, A., 1996. Serotype 2-
269 specific antigens from ruminant strains of *Chlamydia pecorum* detected by
270 monoclonal antibodies. Comp. Immunol. Microbiol. Infect. Dis. 19, 155-61.

- 271 Towbin, H., Staehelin, T., and Gordon, J., 1979. Electrophoretic transfer of proteins from
272 polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc.
273 Natl. Acad. Sci. U S A. 76, 4350-4.
- 274 Tsai, P. Y., Hsu, M. C., Huang, C. T., and Li, S. Y., 2007. Human antibody and antigen
275 response to IncA antibody of *Chlamydia trachomatis*. Int. J. Immunopathol.
276 Pharmacol. 20, 156-61.
- 277 Vretou, E., Radouani, F., Psarrou, E., Kritikos, I., Xylouri, E., and Mangana, O., 2007.
278 Evaluation of two commercial assays for the detection of *Chlamydophila abortus*
279 antibodies. Vet. Microbiol. 123, 153-61.
- 280 Warren, K., Swan, R., Bodetti, T., Friend, T., Hill, S., and Timms, P., 2005. Ocular
281 chlamydiales infections of western barred bandicoots (*Perameles bougainville*) in
282 Western Australia. J. Zoo. Wildl Med. 36, 100-2.
- 283 Yang, J. M., Liu, H. X., Hao, Y. X., He, C., and Zhao, D. M., 2006. Development of a rapid
284 real-time PCR assay for detection and quantification of four familiar species of
285 *Chlamydiaceae*. J. Clin. Virol. 36, 79-81.
- 286 Yousef Mohamad, K., Rekiki, A., Myers, G., Bavoil, P. M., and Rodolakis, A., 2008a.
287 Identification and characterisation of coding tandem repeat variants in *inca* gene of
288 *Chlamydophila pecorum*. Vet. Res. 39, 56.
- 289 Yousef Mohamad, K., Roche, S. M., Myers, G., Bavoil, P. M., Laroucau, K., Magnino, S.,
290 Laurent, S., Rasschaert, D., and Rodolakis, A., 2008b. Preliminary phylogenetic
291 identification of virulent *Chlamydophila pecorum* strains. Infect. Genet. Evol. 8, 764-
292 71.
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295

296 **Tables:**297 **Table 1:** Chlamydial strains used in this study

Strain	Geographic origin	Host	Pathology	Reference
<i>C. pecorum</i> M14	Morocco	Goat	Abortion	(Rekiki et al., 2004)
<i>C. pecorum</i> AB10	France	Sheep	Abortion	(Rodolakis et al., 1989)
<i>C. pecorum</i> LW679	USA	Sheep	Arthritis	(Norton and Storz, 1967)
<i>C. pecorum</i> iB5	France	Sheep	No clinical sign (Fecal carriage)	(Rodolakis et al., 1989)
<i>C. abortus</i> AB7	France	Sheep	Abortion	(Faye et al., 1972)

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308 **Table 2:** Signal intensity of the antigenic reaction of the 16 recombinants with the ovine anti-
309 *C. pecorum* M14 serum sample.

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Signal intensity	Clone
Strong (+++)	B1, B3, A10
Medium (++)	B2, A7, B14, B15, B17, A4
Weak (+)	A2, A1, B5, B7, B18, B19, B11

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329 **Figure captions:**

330 **Fig. 1:** Western Blot analysis of 5 clones using ovine anti-*C. pecorum* serum sample. Lane 1,
331 clone B3 (33 kDa); lane 2, negative control; lane 3, clone A10 (33 kDa); lane 4, clone B15
332 (32 kDa); lane 5, clone B11 (30 and 18 kDa).

333

334 **Fig. 2:** Western Blot analysis of 6 clones using MAb PD3. Lane 1, clone B3; lane 2, clone B7;
335 lane 3, clone B15; lane 4, clone B17; lane 5, clone B2; lane 6, clone A10. Three clones, B3,
336 B15 and A10, reacted with the Mab PD3.

337

338 **Fig. 3:** Western Blot analysis of rIncA using MAb PD3. Lane 1, *E. coli* TG1 sample not
339 induced by IPTG; lane 2, *E. coli* TG1 sample induced by IPTG (35 kDa); lane 3, clone B15
340 (33 kDa).

341

342 **Fig. 4:** Western Blot analysis of rIncA (35 kDa). Lane 1, ovine anti-*C. pecorum* M14 serum
343 sample (positive control); lane 2, 3, 4, 5 and 6, bovine serum samples collected from a healthy
344 herd.

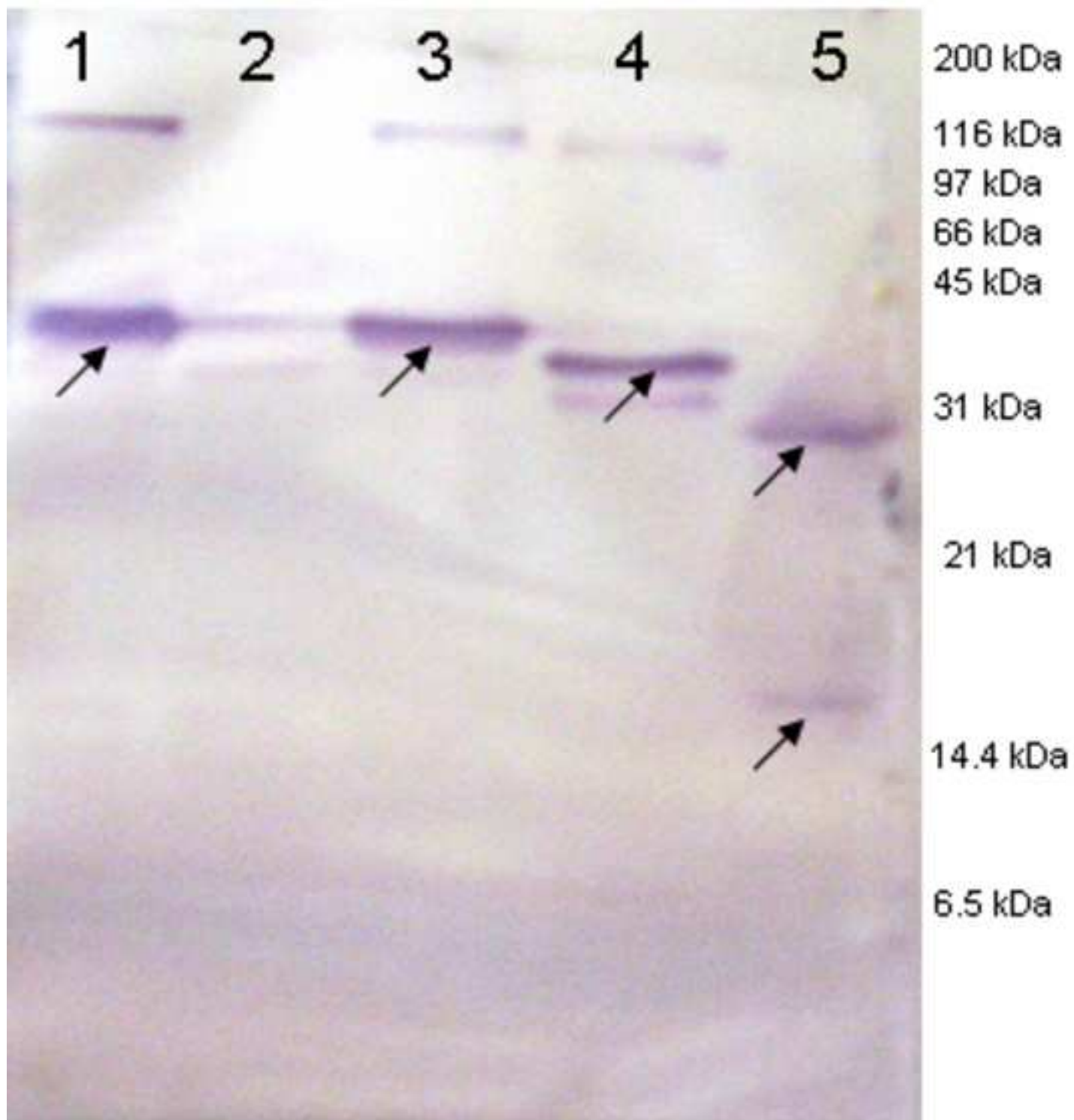


Figure 2

