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2 High prevalence of antibodies against *Chlamydiaceae* and *Chlamydomphila*
3 *abortus* in wild ungulates using two “in house” blocking-ELISA tests4 J. Salinas^{a,*}, M.R. Caro^a, J. Vicente^b, F. Cuello^a, A.R. Reyes-García^b, A.J. Buendía^a,
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ABSTRACT

Few data are available on the prevalence and relevance of chlamydiae in wild mammals, and even fewer studies have been conducted to determine the prevalence of *Chlamydomphila abortus* in wildlife hosts, most probably due to the absence of suitable species-specific serological assays for testing sera from wild animals. In light of this, we have developed two in-house blocking-ELISA tests for detection of antibodies against *Chlamydiaceae* and *C. abortus* in wild ungulates, and analyzed the relationship between geographical and biological factors and the prevalence of antibodies against *Chlamydiaceae* and *C. abortus* in 434 wild ungulates from Spain, including sera from European wild boar, Red deer, Fallow deer, Roe deer, Mouflon, Barbary sheep, Southern chamois, and Iberian ibex. Serology revealed that $41.7 \pm 4\%$ of the sera were positive for the b-ELISA-LPS (*Chlamydiaceae*-specific) and $18.9 \pm 3\%$ for the b-ELISA-rPOMP (*C. abortus*-specific). Antibodies against *Chlamydiaceae* lipopolysaccharide (LPS) were detected in sera from all eight ungulate species, the prevalence ranging from 23 to 60%. Iberian ibex was the only wild ungulate not showing seropositivity to the *C. abortus* specific polymorphic outer membrane protein (POMP). The prevalence of anti-POMP antibodies in the other seven wild ungulate species ranged from 7 to 40%. While significant seroprevalence differences were detected among species and among sampling regions, no effect of age and sex was observed. The high prevalence levels found should be considered with regards to livestock and human health, and warrant further research.

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10 1. Introduction

11 The link between wildlife, environment, pathogens and
12 human activities creates a dynamic where new pathogens
13 or new hosts frequently emerge; changes in population
14 density or host behaviour affect disease prevalence, and
15 disease agents can suddenly boost their virulence and
16 widen their host range (Gortázar et al., 2007). There is

growing evidence that chlamydial infections may result in
adverse pregnancy outcomes in humans and domestic
animals (Pospischil et al., 2002; Baud et al., 2008), and it is
well known that chlamydiosis is a multi-host disease
affecting a huge variety of vertebrates (Everett et al., 1999).

Chlamydomphila, which includes six species, is a bacterial
genus that, together with the genus *Chlamydia*, both
belongs to the family *Chlamydiaceae*. Ovine enzootic
abortion (OEA) caused by *Chlamydomphila abortus* is the
most common cause of reproductive failure in sheep- and
goat-breeding countries in Europe, and where it has a
substantial economic impact (Aitken, 1993). Infected
pregnant ewes and goats abort late in gestation or give

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birth to weak lambs as a result of affected placenta. Bacteria excreted at abortion with the uterine discharge are the main source of infection for susceptible animals through ingestion or inhalation. After the onset of abortion, most animals acquire immunity and rebreed successfully (Rodolakis et al., 1998). Data from the few existing reports on chlamydial seroprevalence in domestic ruminants show geographical differences, ranging from 11 to 51% in sheep and goat flocks (Cuello et al., 1992; Markey et al., 1993; Mainar-Jaime et al., 1998; Borel et al., 2004) and from 24 to 45% in cattle (Cavirani et al., 2001; Petit et al., 2008). Data related to the prevalence and relevance of chlamydial infections in wild mammals are even more scarce (Hotzel et al., 2004).

Early diagnosis of chlamydial infections is important to prevent and limit the spread of the microorganism. For diagnosis purposes, serological assays are more suitable for screening large numbers of samples. The complement fixation test (CFT) is the most widely used test and is still recommended by the Organisation Internationale des Epizooties (OIE) (<http://www.oie.int>). However, it lacks specificity due to its antigen, which consists mainly of the heat-resistant lipopolysaccharide (LPS), common to all *Chlamydiaceae* species (Brade et al., 1987), particularly in ruminants, which are widely infected by *Chlamydophila pecorum* (Rodolakis et al., 1998). Moreover, CFT requires a prime quality serum which, in wildlife studies, is not always available (Mason and Fleming, 1999). Several ELISA methods have been developed in order to improve chlamydial serology. These include ELISA tests using purified whole elementary bodies (EB), LPS or more specific assays based on the *C. abortus* major outer membrane protein (MOMP) (Kaltenboeck et al., 1997; Salti-Montesanto et al., 1997; Hoelzle et al., 2004; Livingstone et al., 2005). Comparative serological studies have revealed that a new commercially available ELISA test based on a polymorphic outer membrane protein (POMP) could serve as an improved alternative to CFT or to classical indirect ELISAs for the serological diagnosis of OEA Q1 (Buendia et al., 2001; Vretou et al., 2007).

Although there are numerous indications that *Chlamydiaceae* are present in a wide range of wildlife host species, few studies have been conducted to determine the prevalence of *C. abortus*, most probably due to the absence of suitable species-specific serological tools and reagents to test sera from wild animals. While the role of *Chlamydiaceae* as pathogens in European wild boar (*Sus scrofa*) has yet to be established, the detection by PCR of a spectrum of *Chlamydiaceae* species identical to those in domestic pigs (including *C. abortus*) in a small sample of hunter-harvested wild boar from Germany, revealed a possible wildlife reservoir for these bacteria. The possibility that these animals acquired chlamydiae through contact with domestic pigs appeared very unlikely (Hotzel et al., 2004).

As regards wild ruminants, the literature on abortions caused by *C. abortus* includes a case report on a springbok (*Antidorcas marsupialis*) in Paris zoo (Berri et al., 2004). However, most studies have been made by serological analysis using CFT. In Italy, Giovannini et al. (1988) showed a prevalence of 79% in fallow deer (*Dama dama*), while Giacometti et al. (1995) showed a prevalence of 31% in

alpine ibex (*Capra ibex*). Similarly, a CFT-based study in populations of wild ruminants including fallow deer, red deer (*Cervus elaphus*), mouflon (*Ovis aries musimon*) and Iberian ibex (*Capra pyrenaica*) in southern Spain indicated that *Chlamydiaceae* infections could be present in wild ruminant species (Cubero-Pablo et al., 2000). Antibody seroprevalence ranged from 24 to 37% and was higher in those areas where contact with domestic sheep and goats was likely. Salwa et al. (2007) found low titers of a serological reaction to *Chlamydiaceae* in 45% of European bison (*Bison bonasus*) selected between 1991 and 2001 for poor body condition, cachexia, lameness and balanoposthitis in Bialowieza (Poland), suggesting that the transmission of pathogens can occur between domestic and wild ruminants.

In the present study our objectives were (1) to develop two “in-house” blocking-ELISA tests to detect antibodies against *Chlamydiaceae* and *C. abortus* in sera from wild ungulates. These methods would make it possible to analyze simultaneously sera from phylogenetically non-related species, including birds; (2) to establish the relationship between geographical and biological factors such as animal species, age and sex and prevalence of antibodies against *Chlamydiaceae* and *C. abortus* in wild ungulates from Spain.

2. Materials and methods

2.1. Wild animal sera and geographical location

The sample included 434 field sera from eight wild ungulate species including one suid species (European wild boar), three cervids (red deer; fallow deer; and roe deer, *Capreolus capreolus*), and four bovids (mouflon; Barbary sheep, *Ammotragus lervia*; Southern chamois, *Rupicapra pyrenaica*; and Iberian ibex). Samples were obtained opportunistically during the hunting seasons between 2005 and 2007 from different geographical regions in Spain. Sampling sites are representative of the most biogeographically relevant landscape types of mainland Spain, with a bias towards the Mediterranean shrub-lands of the central and southern regions, where hunting activities are traditionally important. Thus, sampling sites were divided into four geographic regions, named Cantabrian Mountains, Northern Plateau, South–Central, and South Coast.

The Atlantic climate is represented by the samples from the Cantabrian Mountains, a mountainous coastal region in the north of Spain. Here, no feed is provided to wildlife because of conflict regarding wildlife damage to croplands. Contact between wild ungulates and livestock, mostly cattle, is frequent. Mediterranean shrub-lands are dominant in the South–Central and South Coast sampling sites. These areas are characterized by high densities of wild ungulates due to intensive game management. Fencing and feeding are common and contact with livestock (mostly goats and cattle) is limited within fenced estates. Finally, only Iberian ibex was tested in the Northern Plateau. Most samples came from a fenced estate with ibex, wild boar and roe deer but no domestic ungulates, and two samples were obtained from an open hunting reserve with diverse wild and domestic ungulates.

149 Except for the Iberian ibex samples, obtained by the
150 venipuncture of live animals during a capture and
151 translocation operation, all blood samples were obtained
152 post-mortem from the heart and thoracic cavity of legally
153 hunted animals. Serum was obtained after centrifugation
154 and stored at -20°C until use.

155 2.2. Ovine control sera

156 Seven sera from aborted ewes experimentally infected
157 with the AB7 strain of *C. abortus* (García de la Fuente et al.,
158 2004), two sera from ewes experimentally infected with
159 the iB1 strain of *C. pecorum* (Rodolakis and Souriau, 1989)
160 and three sera from SPF lambs were used as ovine control
161 sera. Additionally, 10 field ovine sera from a flock with a
162 known OEA clinical history (Buendia et al., 2001) were
163 used in this work.

164 2.3. Chlamydial antigen

165 McCoy cell monolayer in tissue culture flasks of 75 cm^2
166 (Sterilin Ltd, Hounslow, UK) were infected with the AB7
167 strain of *C. abortus* and elementary bodies (EB) were
168 purified using a renografin method as previously described
169 (Buendía et al., 1997). Infected cells were sonicated for
170 rupture and then centrifuged at $500 \times g$ for 10 min to
171 remove gross cell debris. The resulting supernatant portion
172 was placed on a 40% Renografin 76 solution (Schering, Lys-
173 Lez-Lannoy, France) buffered in PBS (0.1 M, pH 7.2) and
174 centrifuged at $38,000 \times g$ for 1 h at 4°C . The pellet was
175 collected in PBS, placed in a 60% Renografin solution and
176 centrifuged as above. The pellet representing the purified
177 chlamydiae was collected in PBS-sucrose (0.1 M) and was
178 used to coat ELISA plates. Protein concentration was
179 determined using the bicinchoninic acid protein assay
180 with bovine serum albumin as a standard (Sigma, Madrid,
181 Spain).

182 2.4. Serological tests

183 2.4.1. Reference techniques

184 Three serological assays were carried out as reference
185 techniques using control and field ovine sera to select the
186 cut-off for the "in house" blocking-ELISAs: (i) the comple-
187 ment fixation test (CFT), (ii) a classical indirect ELISA using
188 purified *C. abortus* EB as antigen (ELISA-EB), and (iii) the
189 ELISA kit commercialized by Pourquier Laboratories
190 (Institut Pourquier, Montpellier, France, now IDEXX
191 Laboratories) that used an *C. abortus* recombinant frag-
192 ment of the 80–90 kDa protein not defined by the
193 manufacturer (ELISA-Pourquier).

194 The CFT and ELISA-EB were performed essentially as
195 described previously by Buendía et al. (2001) and a titre of
196 $\geq 1/40$ was considered positive for CFT. In the ELISA-EB, a
197 cut-off value was calculated using a group of 10 sera tested
198 negative by CFT and ELISA-Pourquier in a previous study
199 (Buendía et al., 2001). Sera with an OD ≥ 0.207 (mean
200 optical density for the 10 serum specimens + 3 standard
201 deviations) were considered positive. The ELISA-Pourquier
202 test was performed according to the instructions of the
203 manufacturer. The final values were expressed as %

Sample/Positive control (% S/P). Sera with % S/P equal to
or lower than 50% were considered negative for *C. abortus*
infection, sera with a % S/P between 50 and 60% were
considered doubtful and sera with a % S/P equal to or
higher than 60% were considered positive.

2.4.2. Monoclonal antibodies

Two monoclonal antibodies (mAbs) were selected from
a panel in preliminary assays (data not shown) using the
ELISA-EB or the ELISA-Pourquier tests: the *Chlamydiaceae*
LPS-specific HA5C5 mAb (Salinas et al., 1994) and the *C.*
abortus POMP91B-specific JA6C7 mAb (Souriau et al., 1993; Q2
Vretou et al., 2003), respectively. These mAbs were
produced from ascitic fluid in Balb/c mice. An optimal
working dilution of the mAbs was determined in
preliminary experiments, using a checker-board design
(data not shown). Both mAbs were titrated to determine
the saturation point (OD between 1.6 and 1.8), and
working dilutions of 1/100 for HA5C5 and 1/500 for
JA6C7 were selected for use in the blocking-ELISA-LPS and
blocking-ELISA-rPOMP, respectively, with an OD below the
saturation point.

2.4.3. "In house" blocking-ELISA Chlamydiaceae lipopolysaccharide-specific (b-ELISA-LPS)

The 96-well ELISA plates (Maxisorp, Nunc, Roskilde,
Denmark) were coated overnight at 4°C with purified EB at
a concentration of $0.2\ \mu\text{g}$ of protein/well in $100\ \mu\text{l}$ of
0.05 M sodium carbonate/bicarbonate buffer (pH 9.6).
Non-specific binding was blocked with 5% non-fat dried
milk in PBS (PBS-M) for 30 min at room temperature. After
three washes with PBS containing 0.05% Tween-20 (PBS-T),
serum samples, including pooled sera from SPF lambs as
negative control, were applied in duplicate to the plate at
1/20 dilution in PBS-M, incubated at room temperature for
1 h and washed three times as before. The LPS-HA5C5 mAb
was added to the plates at the above defined 1/100 dilution
and incubated at room temperature for 30 min. After
another washing step, goat peroxidase-conjugated anti-
mouse immunoglobulins (Sigma), diluted at 1/1000 in
PBS-M, was added and incubated at room temperature for
1 h. Bound peroxidase was developed by adding a
commercially available substrate containing the chromo-
gen 3,3',5,5' tetramethylbenzidine (TMB). The reaction was
stopped after 10 min by addition of 1 M H_2SO_4 . Optical
densities were read in a microtitre plate reader at 450 nm
wavelength. The results were expressed as percentage of
inhibition:

$$\% \text{ of inhibition} = 100$$

$$- \left[\frac{\text{mean net OD of sample serum}}{\text{mean net OD of control negative serum}} \right] \\ \times 100.$$

2.4.4. "In house" blocking-ELISA recombinant *C. abortus* POMP-specific (b-ELISA-rPOMP)

The b-ELISA-rPOMP was performed using the ELISA kit
commercialized by Pourquier Laboratories according to
the instructions of the manufacturer with some modifica-
tions. The plates were coated with a *C. abortus*-specific

Table 1

Q4 Serological results for ovine control sera and for field sera from sheep with known clinical OEA history, estimated by all the ELISA tests.

Control sera from ewes	ELISA-EB (OD) ^a	b-ELISA-LPS (% Inhibition) ^b	ELISA-Pourquier (% S/P) ^c	b-ELISA-rPOMP (% Inhibition) ^b
Sera from <i>C. abortus</i> infected ewes				
Serum number 1	0.610 (+)	41	70 (+)	39
Serum number 2	1.275 (+)	72	82 (+)	52
Serum number 3	1.032 (+)	38	76 (+)	46
Serum number 4	0.512 (+)	44	61 (+)	32
Serum number 5	1.719 (+)	69	113 (+)	71
Serum number 6	0.826 (+)	36	92 (+)	48
Serum number 7	0.410 (+)	43	48 (–)	29
Sera from <i>C. pecorum</i> infected ewes				
Serum number 1	0.221 (+)	38	33 (–)	21
Serum number 2	0.276 (+)	35	27 (–)	16
Sera from SPF lambs				
Serum number 1	0.097 (–)	21	24 (–)	0
Serum number 2	0.139 (–)	18	12 (–)	11
Serum number 3	0.116 (–)	12	31 (–)	7
Field sera from ewes				
Serum number 1	0.196 (–)	34	21 (–)	25
Serum number 2	0.143 (–)	25	32 (–)	22
Serum number 3	0.118 (–)	16	17 (–)	9
Serum number 4	0.465 (+)	35	66 (+)^d	27
Serum number 5	1.541 (+)	49	103 (+)	61
Serum number 6	0.620 (+)	39	72 (+)	30
Serum number 7	0.963 (+)	57	66 (+)	39
Serum number 8	1.640 (+)	72	112 (+)	84
Serum number 9	0.306 (+)^d	33	11 (–)	18
Serum number 10	0.820 (+)	49	63 (+)	33

Serum with % S/P ≤50: negative result; serum with % S/P 50–60: doubtful result; serum with % S/P ≥60: positive result.

^a OD ≥0.207: Positive serum (average for OD of 10 CFT and ELISA-Pourquier negative sera + 3 S.D.).

^b % Inhibition = 100 – [(OD of test serum/OD of control negative serum) × 100].

^c % serum/positive control = (Corrected OD of test serum/Corrected OD of control positive serum) × 100.

^d Discordant results between b-ELISAs and their reference techniques.

258 recombinant protein (unknown fragment of the POMP).
 259 Serum samples, including pooled sera from SPF lambs as
 260 negative control, were applied in duplicate to the plate at
 261 1/20 dilution in the buffer provided by the commercial kit,
 262 incubated at room temperature for 1 h and washed three
 263 times with the washing buffer. The *C. abortus* POMP91B-
 264 specific JA6C7 mAb was added to the plates at the
 265 previously defined 1/500 working dilution and incubated
 266 at room temperature for 30 min. The remaining steps were
 267 carried out and the results expressed as in the b-ELISA-LPS
 268 methodology described above.

269 2.5. Statistical analysis

270 Chi square tests or, where appropriate, Fisher's exact
 271 tests were used to compare prevalence data. 95% confidence
 272 intervals for prevalence were estimated by the expression
 273 $S.E. 95\%C.I. = 1.96[p(1 - p)]/n^{1/2}$ (Martin et al., 1987). Sex
 274 was known in 282 individuals and the age category was
 275 known in 127 hosts. For statistical purposes, animals were
 276 classified into age classes following Vicente et al. (2006).

277 3. Results

278 3.1. Determination of the cut-off for the b-ELISAs

279 Control sera from seven *C. abortus* and two *C. pecorum*-
 280 infected ewes, three SPF lambs and ten ewes from a flock

with known OEA clinical history were tested by all the
 ELISA tests (Table 1) to establish the cut-off for both b-
 ELISA tests. The results were expressed as percentage of
 inhibition. The selected cut off was calculated considering
 the lowest inhibition percentage that showed a sensitivity
 higher than 90%, while maintaining the maximum
 specificity in relation to the reference tests (ELISA-EB for
 b-ELISA-LPS and ELISA-Pourquier for b-ELISA-rPOMP).
 Thus, we selected a cut off of 35% in the case of b-ELISA-
 LPS and 30% for b-ELISA-rPOMP, showing a sensitivity of
 93.7 and 91.6%, respectively, with a specificity of 100% in
 both cases.

3.2. Analysis of the wild ungulate sera

The serology analyses revealed that 41.7 ± 4 of the
 sera were positive for the b-ELISA-LPS and $18.9 \pm 3\%$ for
 the b-ELISA-rPOMP (Table 2). Antibodies to *Chlamydiaceae*
 LPS were detected in sera from all eight ungulate species
 with a prevalence ranging from 23 to 60%. Iberian ibex was
 the only wild ungulate without seropositivity to the *C.*
abortus specific POMP. The prevalence of anti-POMP
 antibodies in the other seven wild ungulate species ranged
 from 7 to 40%. The inter-specific differences found in
 antibody seroprevalence (Fig. 1) were significant both for
 anti-LPS antibodies (Chi-square statistic = 22.3, 7 d.f.,
 $p < 0.01$) and for anti-POMP antibodies (Chi-square sta-
 tistic = 29.9, 7 d.f., $p < 0.001$).

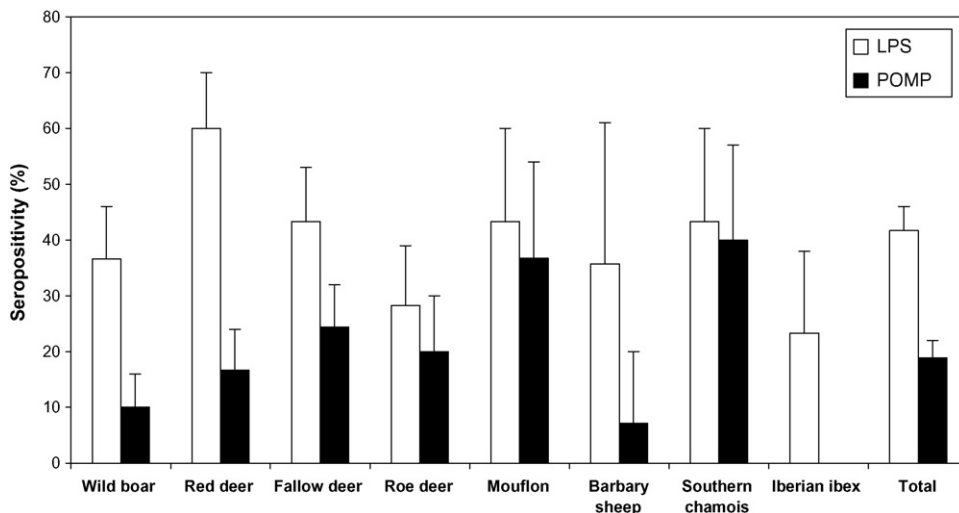


Fig. 1. Mean serum antibody prevalence (%) against the *Chlamydiaceae* LPS and the *Chlamydophila abortus* POMP, estimated by “in house” blocking-ELISA tests in eight wild ungulates from Spain. Error bars indicate 95% confidence intervals.

prevalence in wild animals is related to the absence of species-specific serological assays for this microorganism, unlike the cases for domestic ruminant species (Salti-Montesanto et al., 1997; Kaltenboeck et al., 1997; Buendía et al., 2001; Hoelzle et al., 2004; Livingstone et al., 2005; Vretou et al., 2007). However, using a commercial kit that offers good sensitivity and specificity (Buendía et al., 2001; Vretou et al., 2007), we developed a *C. abortus*-specific “in house” blocking-ELISA test. Similarly, we developed another “in house” blocking-ELISA test to detect anti-*Chlamydiaceae* LPS antibodies, adapted from a previously evaluated indirect ELISA (Buendia et al., 2001).

The high LPS/POMP antibody ratios detected in wild boar, red deer and Barbary sheep suggest frequent contact of these hosts with *Chlamydiaceae* other than *C. abortus*, such as *C. pecorum*, *Chlamydia suis* and *Chlamydophila*

psittaci, the last, found by PCR as the predominant species in wild boar from Germany (Hotzel et al., 2004). Alternatively, we might suggest that the particular POMP-fragment used as antigen varies in the *C. abortus*-subtypes present in the field, as occurs with the MOMP in atypical *C. abortus* strains (Vretou et al., 2001); these variants would raise less-reactive sera from the infected wild ungulates analyzed.

The surprising finding of 4.3% positive sera in wild ungulates to r-POMP but negative to LPS may be explained by the much earlier presence of anti-POMP antibodies detected in naturally (Buendía et al., 2001) and experimentally (Livingstone et al., 2005) *C. abortus*-infected ewes. Thus, these last authors showed that r-POMP ELISA is considerably more sensitive than other serological tests as CFT, where the LPS is used as antigen. In view of these findings, the use of r-POMP would allow to adopt appropriate control measures to limit contamination and the spread of infection, since it enables a much earlier detection of *C. abortus* antibodies.

In contrast, there was a high degree of agreement between both b-ELISAs and the prevalence of anti-*C. abortus* antibodies in Southern chamois and mouflon. In chamois, this may reflect contact with domestic livestock in the open pastures of the Cantabrian Mountains. The high prevalence found in mouflon is more striking, since these animals were sampled exclusively in fenced estates with no direct contact with domestic ruminants. In fact, the detection of anti-*C. abortus* antibodies in wildlife hosts from fenced estates where there is no contact with livestock suggests that wild animals alone are capable of maintaining the disease cycle, constituting a reservoir of infection, as shown for other shared diseases such as bovine tuberculosis (Gortazar et al., 2005).

This observational study did not allow conclusions to be drawn on the possible effect of *Chlamydiaceae* on Spanish wild ungulates. However, the high anti-POMP antibody prevalence recorded in some hosts and sampling regions,

Table 3

Number and percentage of positive ELISA reactions to the *Chlamydiaceae* LPS antigen and the *C. abortus* POMP antigen, estimated by “in house” blocking-ELISA tests in wild ungulates from Spain classed by age.

Age	LPS		POMP		n
	Positive	%	Positive	%	
1	24	40.7	11	18.6	59
2	21	38.9	15	27.7	54
3	7	36.8	2	10.5	19
4	59	48.0	18	14.1	127

Table 4

Number and percentage of positive ELISA reactions to the *Chlamydiaceae* LPS antigen and the *Chlamydophila abortus* POMP antigen, estimated by “in house” blocking-ELISA tests in wild ungulates from Spain classed by sex.

Sex	LPS		POMP		n
	Positive	%	Positive	%	
Male	46	44.2	20	19.2	104
Female	71	39.9	28	15.7	178

e.g. in Southern chamois and wild boar from the Cantabrian Mountains, mouflon and fallow deer from South-Central Spain, and red deer from the South Coast, suggests that adverse effects on host population dynamics may occur locally. In contrast with previous reports (Cubero-Pablo et al., 2000; Salwa et al., 2007), we found no sex- or age-related differences in seroprevalence. The apparent absence of an age-increasing pattern suggests that contact probability with *Chlamydiaceae* is not age-cumulative and, therefore, that limited immunity is acquired with age. However, larger species-specific sampling from more homogeneous study areas is needed to clarify this and to further investigate any possible effect on the reproductive success of wild hosts (Ruiz-Fons et al., 2006).

Nonetheless, the high prevalence reported herein should be taken into account as regards livestock and human health. Several studies have shown that strains of *C. abortus* and other *Chlamydiaceae* found in wildlife are similar to those found in domestic animals and in humans (Herrmann et al., 2000; Berri et al., 2004; Hotzel et al., 2004; Blumer et al., 2007). Hence, our data suggest that wildlife hosts, particularly wild ungulates, need to be considered in the control of diseases such as OEA. Moreover, hunters are thought to be at risk (Deutz et al., 2003), and so could other personnel handling carcasses and raw game meat (Hotzel et al., 2004).

In conclusion, contacts with *Chlamydiaceae*, including *C. abortus* and possibly *C. pecorum*, *C. psittaci* and *C. suis* are frequent among wild ungulates. While possible effects on the wildlife hosts remain unknown, a role of wildlife as a reservoir for *Chlamydiaceae* is clear and its possible importance for animal and public health warrants further studies, including the use of direct assays as PCR, immunohistochemistry or isolation of the pathogen. Also, in which host species, including birds and other wildlife animals, and in which conditions *C. abortus* acts just as a commensal or as a pathogen, should be clarified. In view of this, there is an urgent requirement for standardized serological tests (with greater sensitivity and specificity) for the diagnosis of OEA and other chlamydial infections in wildlife hosts in order to ensure comparability and harmonization among international veterinary laboratories. For that reason, the authors suggest the possibility to include new standardized serological assays, as *C. abortus*-specific ELISAs, in substitution of CFT (reference serological test listed in the OIE Manual) that would contribute to the improvement of OEA serological diagnosis.

Conflict of interest statement

None declared.

Uncited reference

Souriau et al. (1994).

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