



**HAL**  
open science

## **infection in the mouse: A useful model of the ovine disease**

M.R. Caro, A.J. Buendía, L. del Rio, N. Ortega, M.C. Gallego, F. Cuello, J.A. Navarro, J. Sanchez, J. Salinas

### ► **To cite this version:**

M.R. Caro, A.J. Buendía, L. del Rio, N. Ortega, M.C. Gallego, et al.. infection in the mouse: A useful model of the ovine disease. *Veterinary Microbiology*, 2009, 135 (1-2), pp.103. <10.1016/j.vetmic.2008.09.029>. <hal-00532486>

**HAL Id: hal-00532486**

**<https://hal.science/hal-00532486v1>**

Submitted on 4 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

## Accepted Manuscript

Title: *Chlamydophila abortus* infection in the mouse: A useful model of the ovine disease

Authors: M.R. Caro, A.J. Buendía, L. Del Rio, N. Ortega, M.C. Gallego, F. Cuello, J.A. Navarro, J. Sanchez, J. Salinas



PII: S0378-1135(08)00396-9  
DOI: doi:10.1016/j.vetmic.2008.09.029  
Reference: VETMIC 4181

To appear in: *VETMIC*

Please cite this article as: Caro, M.R., Buendía, A.J., Del Rio, L., Ortega, N., Gallego, M.C., Cuello, F., Navarro, J.A., Sanchez, J., Salinas, J., *Chlamydophila abortus* infection in the mouse: a useful model of the ovine disease, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2008.09.029

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



24 **Abstract**

25 *Chlamydophila (C.) abortus* is an obligate intracellular bacterium able to colonize the  
26 placenta of several species of mammals, which may induce abortion in the last third of  
27 pregnancy. The infection affects mainly small ruminants resulting in major economic  
28 losses in farming industries worldwide. Furthermore, its zoonotic risk has been reported  
29 in pregnant farmers or abattoir workers. Mouse models have been widely used to study  
30 both the pathology of the disease and the role of immune cells in controlling infection.  
31 Moreover, this animal experimental model has been considered a useful tool to evaluate  
32 new vaccine candidates and adjuvants that could prevent abortion and reduce fetal death.  
33 Future studies using these models will provide and reveal information about the precise  
34 mechanisms in the immune response against *C. abortus* and will increase the knowledge  
35 about poorly understood issues such as chlamydial persistence.

36

## 36 **1. Introduction**

37 Following reclassification of the Order *Chlamydiales* in 1999 (Everett et al., 1999), the  
38 genus *Chlamydophila* (*C.*) contains the species *C. abortus*, which is the most common  
39 infectious cause of abortion in sheep (ovine enzootic abortion, or OEA) and goats in  
40 Europe and results in significant losses to the agricultural industry worldwide. It is also  
41 recognized as a cause of reproductive failure in cattle, horses and pigs, although the  
42 economic impact of such infections is unknown because the lack of epidemiological data.  
43 *C. abortus* is also a zoonotic pathogen implicated in spontaneous miscarriages or  
44 stillbirths in farm women after contact with infected sheep or lambs. Abortion in woman  
45 had been suspected long ago, but it was documented for the first time by Roberts et al.  
46 (1967). Human abortion occurs weeks or months after animal contact, generally late in  
47 pregnancy and following a febrile flu-like syndrome, with or without disseminated  
48 intravascular coagulation or impaired liver and renal function (Hyde and Benirschke,  
49 1997; Longbottom and Coulter, 2003; Baud et al., 2008). As in small ruminants, *C.*  
50 *abortus* replicates in the human host within the trophoblast epithelium leading to a  
51 dysfunction of the placenta and foetal death (Pospischil et al, 2002). Pregnant women,  
52 especially those who live in rural areas, should generally be made aware of the risk of this  
53 zoonotic disease.

54 As regards OEA, adult ewes become infected as a result of contact with lambing pens or  
55 pasture contaminated by infected fetal membranes and discharges. No clinical signs are  
56 observed in non-pregnant animals and it is not until a subsequent pregnancy that they  
57 may abort, normally in the last weeks of gestation (Entrican et al., 2001).

58 Because of useful similarities between the experimental disease in mice and the natural  
59 disease in small ruminants, mouse models have been widely used to study *C. abortus*  
60 pathogenesis and the immune response to infection and also to evaluate the efficacy of  
61 commercial and experimental vaccines. This review focuses on the progress made in the  
62 field, summarizes our current understanding of the immune response and pathological  
63 basis of *C. abortus* infection, and presents data from studies with new vaccines against  
64 OEA using the mouse model as a useful tool of research.

65

## 66 **2. The intracellular development cycle of chlamydiae**

67 The key to understanding the pathophysiology caused by chlamydiae is the biphasic  
68 development cycle of these organisms (Figure 1). The bacteria exist in two development  
69 forms: the infectious extracellular elementary bodies (EBs) and non-infectious  
70 metabolically active, reticulate bodies (RBs), the former attach to the host-cell and are  
71 internalized in an entry vacuole that avoids fusion with host-cell lysosomes. Within 8-10  
72 h, the small EB (0.2-0.3  $\mu\text{m}$  in diameter) differentiate into RBs, the larger (0.5-1.6  $\mu\text{m}$ )  
73 form, which proliferate within the same membrane-bound vacuole. After several  
74 divisions by binary fission, the RBs differentiate back into EBs towards the end of the  
75 cycle (48-72 h, depending on the species) and are released from the infected cell either by  
76 lysis or exocytosis to begin a new cycle of infection (Hackstadt, 1999). The EB, in  
77 contrast to the RB, is structurally rigid, as a result of extensive disulphide linkages  
78 between various cysteine-rich proteins in, or associated with, the outer membrane. This  
79 rigidity results in EBs being resistant to both chemical and physical factors, so that they  
80 are adapted for prolonged extracellular survival, which is an important factor in terms of

81 chlamydial pathogenesis and the treatment of chlamydial infections. The host-cell death  
82 observed at the end of the infection cycle could thus be involved in the release of EBs  
83 from the host cell and could partially contribute to the inflammatory response of the host,  
84 since macrophages undergoing apoptosis secrete inflammatory cytokines and cells  
85 becoming necrotic stimulate inflammation (Hackstadt, 1999).

86         Apart from this typical developmental cycle, a third persistent form exists *in vitro*  
87 (in cell culture systems), where enlarged aberrant RBs have been experimentally induced  
88 by a variety of stimuli, including IFN- $\gamma$ , antibiotics and nutrient deprivation (Hogan et al.,  
89 2004) (Figure 1). These stimuli, particularly IFN- $\gamma$ , can alter chlamydial growth and  
90 facilitate a persistent or chronic infection. *C. trachomatis* has also been reported to cause  
91 chronic infections detectable by nucleic acid amplification tests, suggesting that this  
92 persistent form may also occur *in vivo* (Dean et al., 2000). Being intracellular pathogens,  
93 all chlamydial species have the potential to cause both acute and chronic infection. In  
94 addition, there is increasing evidence that these bacteria are capable of establishing a  
95 persistent infection that may become reactivated, thus causing recurrent infections  
96 (Stephens, 2003; Hogan et al., 2004; Goellner et al., 2006). While induction of persistent  
97 chlamydial bodies is an important area of research, extensive discussion on this topic is  
98 beyond the scope of this review.

99

### 100 **3. Pathogenesis and immune response in the natural host**

101 In ewes, *C. abortus* infection is characterized by abortions during the last 2 or 3 weeks of  
102 gestation or the birth of stillborn or weak lambs that die in the first days of life. It has also  
103 been reported that some lambs can be born healthy and survive infection, although they

104 may go on to abort during their first pregnancy (Papp and Shewen, 1997; Rodolakis et al.,  
105 1998; Entrican et al., 2001; Philips and Clarkson, 2002). Except for reproductive failure,  
106 sheep rarely display any other clinical signs of *C. abortus* infection, other than vulval  
107 discharge 2 to 3 days prior to abortion. Infected goats, on the other hand, may abort at  
108 any time during pregnancy, although most do so within the 2-3 weeks before the expected  
109 time of giving birth (Mathews, 1999). In some caprine cases, other signs, such as  
110 respiratory tract disease, polyarthritis, conjunctivitis and retained placentas maybe seen  
111 along with abortion (Rodolakis et al., 1984). Mild febrile symptoms are observed in non-  
112 pregnant animals after an experimental infection (Amin and Wilsmore, 1995).

113 The route of transmission in the flock is usually oronasal, after exposure to infected  
114 placentas and uterine discharges during lambing time. It is been reported that ewes and  
115 goats can excrete *C. abortus* up to two weeks after abortion and that EBs may remain  
116 infectious for several weeks thereafter, thus increasing the likelihood of spreading.  
117 Therefore, a proportion of asymptomatic animals can become carriers of the disease,  
118 which are shedding the bacteria during the oestrus and lambing time (Papp et al., 1994) or  
119 even in faeces. It is pertinent that an association of infection by enteric *C. abortus* strains  
120 with placental and foetal pathology has been reported (Rodolakis and Souriau, 1989;  
121 Tsakos et al., 2001). Some reports that demonstrate the susceptibility of the vaginal  
122 epithelium to experimental infection with *C. abortus* suggest that venereal transmission is  
123 also possible (Papp and Shewen, 1996), but the establishment of the role of this  
124 transmission route in the epidemiology of *C. abortus* infection requires further  
125 investigation.

126 After abortion due to *C. abortus*, ewes can conceive and give birth to normal lambs, but  
127 they may shed *C. abortus* for up to 2 or 3 years, during ovulation, suggesting that the host  
128 mounts a response that is sufficient to restrict but not eradicate the chlamydiae (Papp et  
129 al., 1994). If the primary infection is at lambing time the sheep probably do not  
130 seroconvert to the established tests, and it is not until abortion that titres develop and  
131 antibody levels persist for several months (Wilsmore et al., 1984) however, antibody  
132 titres may not be indicative of protective immunity since seropositive sheep may still  
133 abort (Dawson et al., 1986). Thus, humoral immunity is not sufficient on its own to  
134 induce effective protection following infection with *C. abortus* (McCafferty, 1990).

135 An interesting aspect of *C. abortus* infection is the lack of any pathological changes until  
136 after 90 days of gestation (normal gestation in sheep is approximately 147 days),  
137 irrespective of the timing of infection (Buxton et al., 1990). Following initial infection, it  
138 is thought that the organisms reside first in the tonsils from where it is disseminated by  
139 blood and lymph to other organs (Jones and Anderson, 1993). The data point to the  
140 existence of a latent stage of *C. abortus*, the localization of which remains unclear  
141 although lymphoid tissue is a candidate for such a site (Papp et al., 1993; Buxton et al.,  
142 1996).

143 Studies of placentitis in experimentally infected pregnant ewes demonstrated that  
144 chlamydial inclusions first appear in the trophoblast lining the chorioallantoic villi at the  
145 hilus of placentomes (Buxton et al., 1990). Subsequently, loss of the chorionic surface  
146 and inflammatory changes in the underlying chorioallantois developed and, as the disease  
147 progressed, chlamydial inclusions could develop in the endometrial epithelium at the  
148 edge of placentomes and associated with a focal endometritis (Buxton et al., 1990).

149 Recent reports described the immunopathological events that occur in the placenta of  
150 experimentally infected pregnant ewes, but little is known about the immune response  
151 and functional significance of the cells involved. The initial loss of trophoblastic  
152 epithelium is accompanied by severe inflammatory infiltration, composed mainly of  
153 neutrophils. Macrophages are scarce in the infiltrate, while lymphocytes are observed  
154 only occasionally in the deeper chorioallantois. Although low in numbers, CD8<sup>+</sup> T cells  
155 are more represented in the lesions than CD4<sup>+</sup> T cells, and number of the B cells is  
156 always very low (Buxton et al., 2002; Sammin et al., 2006). As with other bacterial  
157 placental pathogens, there may be chorionic arteriolitis with fibrinoid degeneration  
158 (Navarro et al., 2004; Sammin et al., 2006); some neutrophilic infiltration and numerous  
159 macrophages could be observed in the wall of those arterioles (Buxton et al., 2002). The  
160 inflammatory infiltrate in the endometrium has been shown to consist of a significant  
161 number of macrophages and lymphocytes where CD4<sup>+</sup> and CD8<sup>+</sup> T may also be found  
162 (Navarro et al., 2004; Sammin et al., 2006). The differences found in the immune  
163 response in the uterus and the placenta point up the difference in the immune response to  
164 *C. abortus* in these two tissues.

165 It has been reported that colonization of the placenta by *C. abortus* stimulates a strong  
166 inflammatory response, which is necessary to control infection, but which could also lead  
167 to the pathology associated with the infection (Brown et al., 2001; Entrican, 2002). This  
168 work also showed that the inflammatory cytokines, IFN- $\gamma$  and TNF- $\alpha$ , which are  
169 produced in response to the infection, can contribute to the pathology and threaten the  
170 maintenance of pregnancy. Therefore, the TNF- $\alpha$  released in response to chlamydial  
171 infection (possibly to the LPS component) may be contributing to the placental damage

172 that leads to abortion (Buxton et al., 2002). IFN- $\gamma$  is crucially involved in controlling  
173 chlamydial infections (Brown and Entrican, 1996). Although there is little evidence of  
174 IFN- $\gamma$  expression in response to infection within the placenta (Buxton et al., 2002),  
175 where it would probably be detrimental to the viability of pregnancy, IFN- $\gamma$  and other  
176 cytokines have been associated with the establishment of a persistent or latent infection in  
177 the host, and some authors suggested that the latent phase was mediated by host cytokine  
178 production in non-pregnant ewes (Brown and Entrican, 1996; Entrican et al., 2001).

179

#### 180 **4. Pathogenesis in the mouse model**

181 Mice are not a natural host for *C. abortus* infection. It is rather the former pneumonitis  
182 biotype of *Chlamydia trachomatis*, currently classified as *Chlamydia muridarum*, which  
183 is naturally encountered in these animals. However, mice can be experimentally infected  
184 with *C. abortus* through a variety of routes. Parenteral routes (i.e. intraperitoneal or  
185 endovenous) have been widely used in chlamydia research (Kerr et al., 2005), but also the  
186 intranasal infection was demonstrated to be a useful model (Huang et al., 1999; Buendia  
187 et al., 2007). More recently, the intragastrical route has been used to study some aspects  
188 of the pathogenesis of *C. abortus* infection (unpublished data from the laboratory of the  
189 authors). Depending on the chosen route, clinical findings can be different. For instance,  
190 parenteral or intragastrical inoculation lead to mild to severe systemic infection  
191 characterized by lethargia, roughed fur and huddling. This infection is controlled by the  
192 host in 4-8 days except in pregnant mice, where the final outcome of the infection is  
193 colonization of the placenta with subsequent abortion (Rodolakis, 1976). When the  
194 intranasal route is used, mice develop severe pneumonia. The initial peribronchial

195 interstitial lesion leads in a few days to a lobar pneumonia that can be lethal depending on  
196 the doses. Similarity of the main clinical findings in mice (abortion and pneumonia) with  
197 those observed in small ruminants suggested that mouse infection could be a useful  
198 model for studies on the pathogenesis of the diseases. Indeed, a number of analogies  
199 concerning pathogenesis and immune response have been reported. They are summarized  
200 in Table 1 and will be described in detail in the next paragraphs.

201 The first murine models of *C. abortus* infection were developed in order to establish the  
202 clinical signs and pathogenesis of the disease. In non-pregnant animals, the infection is  
203 self-limiting, and in mice it resolves within two weeks without anti-microbial  
204 chemotherapy (Buzoni-Gatel and Rodolakis, 1983). In pregnant mice, inoculation at mid-  
205 pregnancy is followed by colonization of the placenta and subsequent abortion in the last  
206 seven days of gestation (Rodolakis, 1976). Other studies (Buendia et al., 1998) showed  
207 that abortion occurs in the late stages of gestation, independently of whether the mice are  
208 infected early in pregnancy (day 7) or mid-pregnancy (day 11). The bacteria rapidly  
209 colonize the liver and spleen and chlamydial antigen can be detected in the placenta 5  
210 days post-infection, mainly in the metrial gland and decidua basalis (Sanchez et al.,  
211 1996). The placenta is significantly colonized by *C. abortus* during the following days to  
212 reach a maximum at day 17-18 and resulting in abortion. Uterine NK cells (formerly  
213 Granulated metrial gland cells) are the most abundant lymphoid cells in rodent placentas  
214 and they may become infected with *C. abortus* just before abortion occurs. The  
215 degeneration due to bacterial multiplication, in addition to neutrophil infiltration and the  
216 endotoxin environment, might be responsible for initiating abortion at this stage.

217 An important aspect of study into the disease included the comparison of the virulence of  
218 different strains of *C. abortus* performed in mouse models, which has permitted graded  
219 differentiation between virulent and non-virulent strains (Buzoni-Gatel and Rodolakis,  
220 1983; Anderson, 1986; Rodolakis et al.1989; Buendía et al., 1999b). Such studies have  
221 shown that, in propitious circumstances, intestinal strains, now included in the *C.*  
222 *pecorum* species (Everett et al., 1999), could reach and colonize the placenta and disrupt  
223 pregnancy as shown by Rodolakis et al. (1989). These authors concluded that the  
224 difference in virulence between intestinal strains and abortion-causing strains is linked to  
225 their ability to invade cells. This triggered further investigations into genetic and  
226 serological differences between strains. Subsequently, the genetic control of mouse  
227 susceptibility to *C. abortus* infection was determined by Buzoni-Gatel et al. (1994) who  
228 observed differences in the innate capacity of various inbred lines of mice to control  
229 bacterial load in infected organs following a challenge by the microorganism. Later  
230 studies comparing the immune response of *C. abortus* in two inbred strains of mice,  
231 namely CBA and C57BL/6 mice (Del Rio et al., 2000) suggested that the difference in  
232 the clearance rate of the microorganisms during primary infection was attributable to the  
233 establishment of an earlier innate response and a more pronounced CD8 T-cell presence  
234 in the more resistant C57BL/6 mice. These findings established the basis for subsequent  
235 research to design experiments with different mice strains and mouse models that allowed  
236 a deeper understanding of the immunological mechanisms presented in response to the  
237 infection, as well as the validation of new vaccines, adjuvants and routes of inoculation.  
238 These will be discussed below.  
239

## 240 **5. Immunity in the mouse model against *C. abortus* infection**

241 The important role of innate immunity, especially in relation to neutrophils, has  
242 been shown in the mouse abortion model (Buendía et al., 1999a) in the early stages of a  
243 primary infection, when it contributes to establishing specific immunity through the  
244 secretion of different cytokines. It may also control bacterial multiplication prior to the  
245 establishment of a specific immune response. The authors reported that in mice in which  
246 the neutrophils have been depleted, mortality was increased with early abortion in  
247 pregnant mice. The role of neutrophils in the recruitment of T cells to inflammatory foci  
248 has been demonstrated in the response to a primary infection with *C. abortus* in mice  
249 (Montes de Oca et al., 2000a). The reduced recruitment mainly affects the CD8<sup>+</sup> T cells, a  
250 key subpopulation for the clearance of *C. abortus* infection (Buzoni-Gatel et al., 1992;  
251 Del Rio et al., 2000; Martinez et al., 2006). However, neutrophils have been shown to be  
252 of limited importance in a secondary infection (Montes de Oca et al., 2000b).  
253 Subsequently, natural killer (NK) cells, another component of innate immunity, were  
254 shown to have a role. Buendía and colleagues (2004) demonstrated the relationship  
255 between NK cells and early IFN- $\gamma$  production in the control of infection of *C. abortus*, as  
256 well as the complex and close relationship with neutrophils, which could produce  
257 cytokines that are chemotactic and activators of NK cells.

258 Despite the fact that innate immunity plays an important role in the response to  
259 chlamydial infection, protection against *C. abortus* requires cell-mediated immunity  
260 (Buzoni-Gatel et al., 1987). The relative importance of the T-cell subsets in this response  
261 depends on the species of the family *Chlamydiaceae*, but cellular immunity seems to be  
262 mediated by IFN- $\gamma$  in all species (Perry et al., 1997). Some of these previous findings

263 were confirmed by adoptive cell transfer and re-infection in mouse experiments (Buzoni-  
264 Gatel et al., 1992). The authors concluded that CD8<sup>+</sup> T cells played a major role in host  
265 resistance against *C. abortus*, although the precise mechanism by which this  
266 subpopulation developed its protective role was not elucidated. The findings of studies  
267 performed with neutrophil-depleted and infected mice (Montes de Oca et al., 2000a)  
268 showed that recruitment of CD8<sup>+</sup> T cells to inflammatory foci in affected tissues is  
269 important for the clearance of *C. abortus* (Martinez et al., 2006). Their crucial role in  
270 primary infection has been confirmed, and it has been suggested that they may have a  
271 regulatory role whereby they prevent an exacerbated Th1 response from inducing severe  
272 injury to the mouse, as seen in *C. pneumoniae* infection (Penttila et al., 1999). In contrast  
273 to the immune response to *C. trachomatis*, in which CD4<sup>+</sup> T cells are essential for host  
274 resistance to chlamydial genital tract infection (Su and Caldwell, 1995), *C. abortus*  
275 infection is mainly controlled by a specific Th1 immune response, which is, at least  
276 partly, IL-12-independent and characterized by the early production of high  
277 concentrations of IFN- $\gamma$  (Del Rio et al., 2001), as well as the presence of T cells,  
278 particularly CD8<sup>+</sup>, which have been shown to be crucial for the clearance of infection  
279 (Martinez et al., 2006)

280 The specific cell-mediated immune response to *C. abortus* is comprised of a  
281 complex balance of mechanisms requiring elucidation. Some reports have shown that in  
282 mice an exacerbated production of cytokines in response to *C. abortus* infection can  
283 induce pathological changes, and abortion has been associated with the detrimental effect  
284 of inflammatory cytokines (IFN- $\gamma$ , TNF- $\alpha$ ) induced by the infection in the placenta  
285 (Bouakane et al., 2003). The role of IFN- $\gamma$  in the host response against *C. abortus* was

286 initially described by McCafferty et al. (1994) in infected athymic mice. The authors  
287 investigated the effects of endogenous IFN- $\gamma$  on the resolution of *C. abortus* infection and  
288 showed that this cytokine is crucial and plays a major role in the early immune response  
289 against the microorganism. Subsequently, Huang et al. (1999) confirmed that exogenous  
290 IL-12 administered early in *C. abortus* lung infection in BALB/c mice eliminated  
291 mortality and reduced morbidity. Del Rio et al. (2001), in a study with IFN- $\gamma$ -depleted  
292 C57BL/6 and IL12p40-deficient (IL-12 $^{-/-}$ ) mice, reported that an interleukin 12-  
293 dependent overproduction of IFN- $\gamma$  may result in an increase in morbidity and pathology.  
294 The authors showed that residual IFN- $\gamma$  present in IL-12 $^{-/-}$  mice is sufficient to induce  
295 the host response and hence might contribute to the elimination of the bacteria. The low  
296 level of this cytokine detected in the absence of IL-12, but presumably produced by cells  
297 of the innate immune system, was essential for the survival of the animals.

298         The role played by humoral immunity against *C. abortus* has been poorly studied,  
299 although it has been demonstrated that the passive transfer of anti-MOMP monoclonal  
300 antibodies protected pregnant mice against chlamydial abortion (De Sa et al., 1995), in  
301 contrast to anti-LPS monoclonal antibody transfer (Salinas et al., 1994). Thus, B cells not  
302 only play a pivotal role in the production and amplification of humoral responses, but  
303 also act as antigen-presenting cells (APCs) in the generation of T cell-mediated immune  
304 response. With the development of B cell-deficient mice ( $\mu$ MT) through the disruption of  
305 the transmembrane portion of the  $\mu$  chain gene, the functions of B cells other than  
306 antibody production can readily be studied in infections in which the cellular immune  
307 response is important. In order to further our knowledge of the immune mechanisms  
308 involved in the clearance of *C. abortus* the role of B cells was studied during primary and

309 secondary infections with *C. abortus* (Buendia et al., 2000a). The data presented in this  
310 study demonstrated that  $\mu$ MT were highly susceptible to a primary infection with *C.*  
311 *abortus* based on the development of a rapid and exaggerated pro-inflammatory response,  
312 with higher levels of IFN- $\gamma$ , TNF- $\alpha$  and IL-6 than their wild-type mouse counterparts. In  
313 contrast,  $\mu$ MT mice developed an effective response to a secondary infection after a low-  
314 level primary infection, thus demonstrating that when the increase of production of pro-  
315 inflammatory cytokines is limited, T cells can be primed in the absence of B cells. The  
316 study suggested that B cells are not essential for controlling the multiplication of *C.*  
317 *abortus* in a secondary infection, but could play a role in controlling the exacerbated  
318 inflammatory response induced by *C. abortus* primary infection. An interesting  
319 hypothesis is that B cells might have an immunoregulatory function in mouse infections  
320 with *C. abortus*, while their absence could lead to an uncontrolled response (Buendia et  
321 al., 2002a).

322

## 323 **6. The mouse model in the development of vaccines against *C. abortus***

324 The observation that the immune response is directly or indirectly involved in the  
325 pathogenesis of disease caused by *C. abortus*, further complicated the vaccine  
326 development process. The successful design and delivery of an effective vaccine against  
327 *C. abortus* infection will depend on a better understanding of this phenomenon.  
328 Inactivated vaccines prepared from egg-grown or cell cultures have formed the basis for  
329 the prevention of the infection since the early 1950s (McEwen et al., 1951). However,  
330 efficiency varies, since outbreaks of the disease have been reported in vaccinated flocks  
331 (Rodolakis and Souriau, 1979; Aitken et al., 1990). Killed vaccines can reduce the

332 incidence of abortion and also the shedding of *C. abortus* at lambing, although they may  
333 not stop shedding completely in all cases, which leads to endemic cycles of infection that  
334 have serious consequences regarding the epidemiology of OEA. An alternative approach  
335 to solve this problem has been to develop a live temperature-sensitive attenuated vaccine  
336 (Rodolakis, 1983), which is commercially available and which has been shown to offer  
337 good protection against *C. abortus*-induced abortion (Rodolakis and Souriau, 1983;  
338 Chalmers et al., 1997) including atypical strains of *C. abortus* (Bouakane et al., 2005).  
339 However, the potential dangers of this kind of vaccine need to be understood, particularly  
340 since *C. abortus* can also cause abortion and severe illness in pregnant women  
341 (Longbottom and Coulter, 2003).

342 To date, efforts directed at obtaining a suitable subcellular vaccine against OEA  
343 have largely focused on the MOMP, which reportedly induces protection if used in its  
344 native oligomer form (De Sa et al., 1995). In contrast, the efficacy of a MOMP-based  
345 vaccine against *C. trachomatis* has proved to be limited (Caro et al., 2005b). However,  
346 since cell culture yields of *C. abortus* are poor, the purification of the MOMP oligomer  
347 from the bacterium is very difficult and prohibitively expensive for an ovine vaccine.  
348 Furthermore, vaccine studies performed in ewes to examine the efficacy of different  
349 forms of recombinant MOMP against experimental infection have been disappointing  
350 (Herring et al., 1998). Finally, vaccination attempts in mice with different DNA  
351 preparations, including the genes of Dnak (Hsp70) and MOMP have failed to induce  
352 protection (Hechard and Grapinet, 2004).

353 The experimental mouse model is a useful tool for validating commercial or  
354 experimental vaccines against *C. abortus* (Rodolakis et al., 1981; De Sa et al., 1995; Caro

355 et al., 2001; 2003; Rekiki et al., 2004; Hechard and Grapinet, 2004; Bouakane et al.,  
356 2005). Mice have been used in this way to test different vaccine production procedures in  
357 order to design new inactivated vaccines against *C. abortus* (Caro et al., 2003). The results  
358 showed that several experimental vaccines induced good protection in pregnant mice and  
359 elicited an adequate degree of cellular immunity required to clear infection and prevent  
360 abortion as well as the shedding of *C. abortus* at the time of delivery. Subsequently,  
361 García de la Fuente et al. (2004) showed that these new inactivated vaccines also  
362 increased protection in the natural host and minimized *C. abortus* shedding at delivery,  
363 thus also limiting the spread of infection in the flock.

364 One important aspect of vaccination against OEA is the choice of specific  
365 adjuvants that help to activate the appropriate effector cells and cytokines, and so favour a  
366 Th1 type of immune response. Of relevance is a study in C57BL/6 mice, in which aTh2  
367 type immune response was induced by *Nippostrongylus brasiliensis* in a co-infection  
368 model with *C. abortus* (Buendia et al., 2002b). Using this mouse co-infection model  
369 either after or before vaccination, Caro et al. (2005a) showed that the efficiency offered  
370 by a vaccine tested, adjuvanted with aluminium hydroxide (which is commonly included  
371 in vaccines for veterinary medicine) was reduced when the Th2 response induced by *N.*  
372 *brasiliensis* was established just prior to infection with *C. abortus*. In field conditions, it  
373 is usually not possible to prevent parasitic infections before *C. abortus* infection, so care  
374 should be taken to select the most appropriate adjuvant or type of vaccine so as to avoid  
375 any deleterious immune effects of a high parasitic burden.

376 Little is known about the role of the innate response in *C. abortus* infection of  
377 vaccinated animals. It is possible that an innate response could modulate the development

378 of adaptative immunity through the secretion of different cytokines, and so help to  
379 establish a suitable immunological environment and favour antigen presentation by the  
380 APC. Related to this, some studies have attempted to determine how the cells involved in  
381 innate immunity may influence protection against *C. abortus* induced by vaccination.  
382 Ortega et al. (2007), using the live attenuated 1B vaccine and two inactivated  
383 experimental vaccines in mice, depleted of PMN and NK cells, and which were  
384 subsequently infected with *C. abortus*, confirmed that both cell types play a secondary  
385 role in the response induced by vaccination.

386 Most mouse models used to date for testing vaccines have involved parenteral  
387 routes of inoculation of *C. abortus*. Such routes induce a systemic infection that allows  
388 the study of the multiplication of *C. abortus* in different organs but these experiments  
389 have not provided information on infection via mucosal surfaces. Since oronasal entry  
390 has been established as the most likely route of entry of *C. abortus* in the natural host  
391 (Jones and Anderson, 1988; Amin and Wilsmore, 1995), a model of intranasal  
392 inoculation that mimics the features of early infection in the natural host was developed  
393 (Buendía et al., 2007). The protection conferred by two previously designed inactivated  
394 vaccines (Caro et al., 2003; 2005a) and the live vaccine 1B, in C57BL/6 mice was tested.  
395 This mouse model offered two advantages: the development of a clear endpoint in non-  
396 immunized mice, which permits the rapid and simple evaluation of the effectiveness of  
397 vaccines, and the use of an infection route that better reflects what happens in the natural  
398 host. In addition, the route of inoculation used permits the response to mucosal surface  
399 infection of *C. abortus* to be investigated in immunized mice. This last aspect is  
400 important since a strong local immune response could avoid systemic dissemination and

401 the subsequent multiplication of the bacterium in the placenta, but whether this avoids  
402 persistent infection needs to be established.

403

## 404 **7. Concluding remarks**

405 There are certain significant similarities between features of *C. abortus* infection in sheep  
406 and mice, which emphasize the value of the mouse model in research (Table 1). In recent  
407 years, this research has provided new insights into the pathogenesis and control of  
408 infection with *C. abortus*. However, many questions remain unanswered, including the  
409 identification of the specific mechanisms of acquired immunity that will assist in the  
410 identification of vaccine candidate antigens. Current research aims at improving the  
411 presentation of the chosen antigens and the protective efficacy of vaccines through  
412 careful selection of adjuvants and routes of immunisation and infection. *C. abortus*  
413 vaccine research will continue to focus on the role of pro-inflammatory cytokines in  
414 influencing Th1/Th2 responses and the identification of additional antigens that induce  
415 protective T cell responses. The latter has become more promising now that the complete  
416 genome sequence of *C. abortus* is available (Thomson et al., 2005). Future studies along  
417 these lines will undoubtedly provide valuable insights and reveal the precise mechanisms  
418 of immunity that will permit the development of novel control strategies in sheep.

419

420 ***Conflict of Interest Statements:*** none declared.

421

422

423

424 **Acknowledgements**

425 Some investigations reviewed in this work were financed by the following grants:  
426 AGF97-0459, 1FD97-1242-CO2-01, AGL2001-0627 and AGL2004-06571, all from  
427 MEC, MCyT and FEDER.

428

429 **References**

- 430 Aitken, I.D., Clarkson, M.J., Linklater, K., 1990. Enzootic abortion in ewes. *Vet. Rec.*  
431 126, 136-138.
- 432 Amin, J.D., Wilsmore, A.J., 1995. Studies on the early phase of the pathogenesis of ovine  
433 enzootic abortion in the non-pregnant ewe. *Br. Vet. J.* 151, 141-155.
- 434 Anderson, I.E., 1986. Comparison of the virulence in mice of some ovine isolates of  
435 *Chlamydia psittaci*. *Vet. Microbiol.* 12, 213-220.
- 436 Baud, D., Regan, L., Greub, G., 2008. Emerging role of *Chlamydia* and *Chlamydia*-like  
437 organisms in adverse pregnancy outcomes. *Curr. Op. Infect. Dis.* 21, 70-76.
- 438 Bouakane, A., Benchaïeb, I., Rodolakis, A., 2003. Abortive potency of *Chlamydomphila*  
439 *abortus* in pregnant mice is not directly correlated with placental and fetal  
440 colonization levels. *Infect. Immun.* 71, 7219-7222.
- 441 Bouakane, A., Rekiki, A., Rodolakis, A., 2005. Protection of pregnant mice against  
442 placental and splenic infection by three strains of *Chlamydomphila abortus* with a live  
443 1B vaccine. *Vet. Rec.* 157, 771-774.
- 444 Brown, J., Entrican, G., 1996. Interferon-gamma mediates long-term persistent  
445 *Chlamydia psittaci* infection in vitro. *J. Comp. Pathol.* 115, 373-383.

- 446 Brown, J., Howie, S.E., Entrican, G., 2001. A role for tryptophan in immune control of  
447 chlamydial abortion in sheep. *Vet. Immunol. Immunopathol.* 82,107-119.
- 448 Buendía, A.J., Sánchez, J., Martínez, C.M., Cámara, P., Navarro, J.A., Rodolakis, A.,  
449 Salinas, J., 1998. Kinetics of infection and effects on placental cell population in a  
450 murine model of *Chlamydia psittaci*-induced abortion. *Infect. Immun.* 66, 2128-2134.
- 451 Buendía, A.J., Montes de Oca, R., Navarro, J.A., Sánchez, J., Cuello, F., Salinas, J., 1999a.  
452 Role of polymorphonuclear neutrophils in a murine model of *Chlamydia psittaci*-  
453 induced abortion. *Infect. Immun.* 67, 2110-2116.
- 454 Buendía, A.J., Sánchez, J., Del Río, L., Garcés, B., Gallego, M.C., Caro, M.R., Bernabé,  
455 A., Salinas, J., 1999b. Differences in the immune response against ruminant chlamydial  
456 strains in a murine model. *Vet. Res.* 30, 495-507.
- 457 Buendía, A.J., Del Río, L., Ortega, N., Sánchez, J., Gallego, M.C., Caro, M.R., Navarro,  
458 J.A., Cuello, F., Salinas J., 2002a. B-cell-deficient mice show an exacerbated  
459 inflammatory response in a model of *Chlamydomydia abortus* infection. *Infect.*  
460 *Immun.* 70, 6911-6918.
- 461 Buendía, A.J., Fallon, P.G., Del Río, L., Ortega, N., Caro, M.R., Gallego, M.C., Salinas,  
462 J., 2002b. Previous Infection with the nematode *Nippostrongylus brasiliensis* alters  
463 the immune specific response against *Chlamydomydia abortus* infection. *Microb.*  
464 *Pathog.* 33, 7-15.
- 465 Buendía, A.J., Martínez, C.M., Ortega, N., Del Río, L., Caro, M.R., Gallego, M.C.,  
466 Sánchez, J., Navarro, J.A., Cuello, F., Salinas, J., 2004. Natural Killer (NK) cells play a  
467 critical role in the early innate immune response to *Chlamydomydia abortus* infection in  
468 mice. *J. Comp. Pathol.* 130, 48-57.

- 469 Buendía, A.J., Nicolás, L., Ortega, N., Gallego, M.C., Martínez, C.M., Sánchez, J., Caro,  
470 M.R., Navarro, J.A., Salinas, J., 2007. Characterization of a murine model of  
471 intranasal infection suitable for testing vaccines against *Chlamydomphila abortus*. *Vet.*  
472 *Immunol. Immunopathol.* 115, 76-86.
- 473 Buxton, D., Barlow, R.M., Finlayson, J., Anderson, I.E., Mackellar, A., 1990.  
474 Observations on the pathogenesis of *Chlamydia psittaci* infection of pregnant sheep.  
475 *J. Comp. Pathol.* 102, 221-237.
- 476 Buxton, D., Rae, A.G., Maley, S.W., Thomson, K.M., Livingstone, M., Jones, G.E.,  
477 Herring, A.J., 1996. Pathogenesis of *Chlamydia psittaci* infection in sheep: detection  
478 of the organism in a serial study of the lymph node. *J. Comp. Pathol.* 114, 221-230.
- 479 Buxton, D., Anderson, I.E., Longbottom, D., Livingstone, M., Wattedgedera, S., Entrican,  
480 G., 2002. Ovine chlamydial abortion: characterization of the inflammatory immune  
481 response in placental tissues. *J. Comp. Pathol.* 127, 133-141.
- 482 Buzoni-Gatel, D., Bernard, F., Pla, M., Rodolakis, A., Lantier, F., 1994. Role of H-2 and  
483 non-H-2-related genes in mouse susceptibility to *Chlamydia psittaci*. *Microb. Pathog.*  
484 16, 229-233.
- 485 Buzoni-Gatel, D., Guilloteau, L., Bernard, F., Chardes, T., Rocca, A., 1992. Protection  
486 against *Chlamydia psittaci* in mice conferred by Lyt-2+ cells. *Immunology* 77, 284-  
487 288.
- 488 Buzoni-Gatel, D., Rodolakis, A., 1983. A mouse model to compare virulence of abortive  
489 and intestinal ovine strains of *Chlamydia psittaci*: influence of the route of  
490 inoculation. *Ann. Microbiol. (Paris)* 134, 91-99.

- 491 Buzoni-Gatel, D., Rodolakis, A., Plommet, M., 1987. T cell mediated and humoral  
492 immunity in a mouse *Chlamydia psittaci* systemic infection. Res. Vet. Sci. 43, 59-63.
- 493 Caro, M.R., Ortega, N., Buendía, A.J., Gallego, M.C., Del Río, L., Cuello, F., Salinas, J.,  
494 2001. Protection conferred by commercially available vaccines against  
495 *Chlamydomphila abortus* in a mouse model. Vet. Rec. 149, 492-493.
- 496 Caro, M.R., Ortega, N., Buendía, A.J., Gallego, M.C., Del Río, L., Cuello, F., Salinas, J.,  
497 2003. Relationship between the immune response and protection conferred by new  
498 designed inactivated vaccines against the enzootic abortion of ewes in a mouse  
499 model. Vaccine 21, 3126-3136.
- 500 Caro, M.R., Buendía, A.J., Ortega, N., Gallego, M.C., Martínez, C.M., Cuello, F., Ruiz-  
501 Ybañez, M.R., Erb, K.J., Salinas, J., 2005a. Influence of the Th2 immune response  
502 established by *Nippostrongylus brasiliensis* infection on the protection offered by  
503 different vaccines against *Chlamydomphila abortus* infection. Vet. Res. Commun. 29,  
504 51-59.
- 505 Caro, M.R., Buendía, A.J., Del Río, L., Cuello, F., Ortega, N., Gallego, M.C., Salinas, J.,  
506 2005b. *Chlamydia trachomatis* genital infection: Immunity and prospects for vaccine  
507 development. Inmunología 24, 298-312.
- 508 Chalmers, W.S., Simpson, J., Lee, S.J., Baxendale, W., 1997. Use of live chlamydial  
509 vaccine to prevent ovine enzootic abortion. Vet. Rec. 141, 63-67.
- 510 Dawson, M., Zaghoul, A., Wilsmore, A.J., 1986. Ovine enzootic abortion: experimental  
511 studies of immune responses. Res. Vet. Sci. 40, 59-64.

- 512 De Sa, C., Souriau, A., Bernard, F., Salinas, J., Rodolakis, A., 1995. An oligomer of the  
513 major outer membrane protein of *Chlamydia psittaci* is recognized by monoclonal  
514 antibodies which protect mice from abortion. *Infect. Immun.* 63, 4912-4916.
- 515 Dean, D., Suchland, R.J., Stamm, W.E., 2000. Evidence for long-term cervical  
516 persistence of *Chlamydia trachomatis* by omp1 genotyping. *J. Infect. Dis.* 182, 909-  
517 916.
- 518 Del Río, L., Buendía, A.J., Sánchez, J., Garcés, B., Caro, M.R., Gallego, M.C., Bernabé,  
519 A., Cuello, F., Salinas, J., 2000. *Chlamydophila abortus* (*Chlamydia psittaci* serotype  
520 1) clearance is associated with the early recruitment of neutrophils and CD8<sup>+</sup> T cell in  
521 a mouse model. *J. Comp. Pathol.* 132, 171-181.
- 522 Del Río, L., Buendía, A.J., Sánchez, J., Gallego, M.C., Caro, M.R., Ortega, N., Seva, J.,  
523 Pallarés, F.J., Cuello, F., Salinas, J., 2001. Endogenous IL-12 is not required for  
524 resolution of *Chlamydophila abortus* (*Chlamydia psittaci* serotype 1) infection in  
525 mice. *Infect. Immun.* 69, 4808-4815.
- 526 Entrican, G., Buxton, D., Longbottom, D., 2001. Chlamydial infection in sheep: immune  
527 control versus fetal pathology. *J. R. Soc. Med.* 94, 273-277.
- 528 Entrican, G., 2002. Immune regulation during pregnancy and host-pathogen interactions  
529 in infectious abortion. *J. Comp. Pathol.* 126, 79-94.
- 530 Everett, K., Bush, R., Andersen, A., 1999. Emended description of the order  
531 *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam.  
532 nov., each containing one monotypic genus, revised taxonomy of the family  
533 *Chlamydiaceae*, including a new genus and five new species, and standards for the  
534 identification of organisms. *Int. J. Syst. Bacteriol.* 49, 415-440.

- 535 García de la Fuente, J.N., Gutiérrez-Martín, C.B., Ortega, N., Rodríguez Ferri, E.F., del  
536 Río, M.L., González, O.R., Salinas, J., 2004. Efficacy of different commercial and new  
537 inactivated vaccines against ovine enzootic abortion. *Vet. Microbiol.* 100, 65-76.
- 538 Goellner, S., Schubert, E., Liebler-Tenorio, E., Hotzel, H., Saluz, H.P., Sachse, K., 2006.  
539 Transcriptional response patterns of *Chlamydophila psittaci* in different in vitro  
540 models of persistent infection. *Infect. Immun.* 74, 4801-4808.
- 541 Hackstadt, T., 1999. The *Chlamydia trachomatis* IncA protein is required for homotypic  
542 vesicle fusion. *Cell. Microbiol.* 1, 119-130.
- 543 Héchard, C., Grepinet, O., 2004. DNA vaccination against *Chlamydiaceae*: current status  
544 and perspectives. *Vet. Res.* 35, 149-161.
- 545 Herring, A.J., Jones, G.E., Dunbar, S.M., 1998. Recombinant vaccines against *Chlamydia*  
546 *psittaci* an overview of results using expression and a new approach using a plan  
547 virus “overcoat” system. In: Stephens, R.S., Byrne, G.I., Christiansen, G. (Eds.),  
548 Disease of sheep, Società Editrice Esculapio, Bologna, pp. 434-437.
- 549 Hogan, R.J., Mathews, S.A., Mukhopadhyay, S., Summersgill, J.T., Timms, P., 2004.  
550 Chlamydial persistence: beyond the biphasic paradigm. *Infect. Immun.* 72, 1843-  
551 1855.
- 552 Huang, J., Wang, M.D., Lenz, S., Gao, D., Kaltenboeck, B., 1999. IL-12 administered  
553 during *Chlamydia psittaci* lung infection in mice confers immediate and long-term  
554 protection and reduces macrophage inflammatory protein-2 level and neutrophil  
555 infiltration in lung tissue. *J. Immunol.* 162, 2217-2226.
- 556 Hyde, S.R., Benirschke, K., 1997. Gestational psittacosis: case report and literature  
557 review. *Mod. Pathol.* 10, 602-607.

- 558 Jones, G.E., Anderson, I., 1988. *Chlamydia psittaci*: is tonsillar tissue the portal of entry  
559 in ovine enzootic abortion?. Res. Vet. Sci. 44, 260-261.
- 560 Kerr, K., Entrican, G., McKeever, D., Longbottom D., 2005. Immunopathology of  
561 *Chlamydomphila abortus* infection in sheep and mice. Res. Vet. Sci. 78, 1-7.
- 562 Longbottom, D., Coulter, L.J., 2003. Animal chlamydioses and zoonotic implications. J.  
563 Comp. Pathol. 128, 217-244.
- 564 Martínez, C.M., Buendía, A.J., Sánchez, J., Ortega, N., Caro, M.R., Gallego, M.C.,  
565 Navarro, J.A., Cuello, F., Salinas, J., 2006. Relative importance of CD4+ and CD8+ T  
566 cells in the resolution of *Chlamydomphila abortus* primary infection in mice. J. Comp.  
567 Pathol. 134, 297-307.
- 568 Matthews, J., 1999. Abortion. Diseases of the Goat, Blackwell Science, Oxford, pp. 22-  
569 36.
- 570 McCafferty, M.C., 1990. Immunity to *Chlamydia psittaci* with particular reference to  
571 sheep. Vet. Microbiol. 25, 87-89.
- 572 McCafferty, M.C., Maley, S.W., Entrican, G., Buxton, D., 1994. The importance of  
573 interferon-gamma in an early infection of *Chlamydia psittaci* in mice. Immunology  
574 81, 631-636.
- 575 McEwen, A.D., Stamp, J.T., Littlejohn, A.I., 1951. Enzootic abortion in ewes. II.  
576 Immunization and infection experiments. Vet. Rec. 63, 197-201.
- 577 Montes de Oca, R., Buendía, A.J., Del Río, L., Sánchez, J., Salinas, J., Navarro, J.A.,  
578 2000a. Polymorphonuclear neutrophils are necessary for the recruitment of CD8(+) T  
579 cells in the liver in a pregnant mouse model of *Chlamydomphila abortus* (*Chlamydia*  
580 *psittaci* serotype 1) infection. Infect. Immun. 68, 1746-1751.

- 581 Montes de Oca, R., Buendía, A.J., Sánchez, J., Del Río, L., Seva, J., Navarro, J.A.,  
582 Salinas, J., 2000b. Limited role of polymorphonuclear neutrophils in a pregnant  
583 mouse model of secondary infection by *Chlamydophila abortus* (*Chlamydia psittaci*  
584 serotype 1). *Microb. Pathog.* 29, 319-327.
- 585 Navarro, J.A., García de la Fuente, J.N., Sánchez, J., Martínez, C.M., Buendía, A.J.,  
586 Gutiérrez-Martín, C.B., Rodríguez-Ferri, E.F., Ortega, N., Salinas, J., 2004. Kinetics  
587 of infection and effects on the placenta of *Chlamydophila abortus* in experimentally  
588 infected pregnant ewes. *Vet. Pathol.* 41, 498-505.
- 589 Ortega, N., Caro, M.R., Buendía, A.J., Gallego, M.C., Del Río, L., Martínez, C.M.,  
590 Nicolás, L., Cuello, F., Salinas, J., 2007. Role of polymorphonuclear neutrophil  
591 (PMN) and NK cells in the protection conferred by different vaccines against  
592 *Chlamydophila abortus* infection. *Res. Vet. Sci.* 82, 314-322.
- 593 Papp, J.R., Shewen, P.E., Gartley, C.J., 1993. *Chlamydia psittaci* infection and associated  
594 infertility in sheep. *Can. J. Vet. Res.* 57, 185-189.
- 595 Papp, J.R., Shewen, P.E., Gartley, C.J., 1994. Abortion and subsequent excretion of  
596 *chlamydiae* from the reproductive tract of sheep during estrus. *Infect. Immun.* 62,  
597 3786-3792.
- 598 Papp, J.R., Shewen, P.E., 1996. Localization of chronic *Chlamydia psittaci* infection in  
599 the reproductive tract of sheep. *J. Infect. Dis.* 174, 1296-1302.
- 600 Papp, J.R., Shewen, P.E., 1997. *Chlamydia psittaci* infection in sheep: a paradigm for  
601 human reproductive tract infection. *J. Reprod. Immunol.* 34, 185-202.
- 602 Penttilä, J.M., Anttila, M., Varkila, K., Puolakkainen, M., Sarvas, M., Mäkelä, P.H.,  
603 Rautonen, N., 1999. Depletion of CD8<sup>+</sup> cells abolishes memory in acquired

- 604 immunity against *Chlamydia pneumoniae* in BALB/c mice. Immunology 97, 490-  
605 496.
- 606 Perry, L.L., Feilzer, K., Caldwell, H.D., 1997. Immunity to *Chlamydia trachomatis* is  
607 mediated by T helper 1 cells through IFN-gamma-dependent and -independent  
608 pathways. J. Immunol. 158, 3344-3352.
- 609 Philips, H.L., Clarkson, M.J., 2002. Investigation of Pre-natal *Chlamydophila abortus*  
610 (*Chlamydia psittaci*) exposure of female lambs and the outcome of their first  
611 pregnancy. Vet. J. 163, 329-330.
- 612 Pospischil, A., Thoma, R., Hilbe, M., Grest, P., Gebbers, J.O., 2002. Abortion in woman  
613 caused by caprine *Chlamydophila abortus* (*Chlamydia psittaci* serovar 1). Swiss. Med.  
614 Wkly., 132, 64-66.
- 615 Rekiki, A., Bouakane, A., Rodolakis, A., 2004. Combined vaccination of live 1B  
616 *Chlamydophila abortus* and killed phase I *Coxiella burnetii* vaccine does not destroy  
617 protection against chlamydiosis in a mouse model. Can. J. Vet. Res. 68, 226-228.
- 618 Roberts, W., Grist, N.R., Giroud, P., 1967. Human abortion associated with infection by  
619 ovine abortion agent. Br. Med. J., 4,37.
- 620 Rodolakis, A., 1976. Infection abortive de la souris inoculée par voie intrapéritonéale  
621 avec *Chlamydia ovis*. Ann. Rech. Vet. 7, 195-205.
- 622 Rodolakis, A., Souriau, A., 1979. Clinical evaluation of a commercial vaccine against  
623 chlamydial abortion of ewes. Ann. Rech. Vet. 10, 41-48.
- 624 Rodolakis, A., Gestin, L., Bertin, A., 1981. Méthode de contrôle des vaccines contre la  
625 chlamydie abortive ovine utilisant la souris gestante. Ann. Rech. Vet. 12, 371-377.

- 626 Rodolakis, A., 1983. In vitro and in vivo properties of chemically induced temperature-  
627 sensitive mutants of *Chlamydia psittaci* var. *ovis*: screening in a murine model. Infect.  
628 Immun. 42, 525-530.
- 629 Rodolakis, A., Souriau, A., 1983. Response of ewes to temperature-sensitive mutants of  
630 *Chlamydia psittaci* (var *ovis*) obtained by NTG mutagenesis. Ann. Rech. Vet. 14,  
631 155-161.
- 632 Rodolakis, A., Bouillet, C., Souriau, A., 1984. *Chlamydia psittaci* experimental abortion  
633 in goats. Am. J. Vet. Res. 45, 2086-2089.
- 634 Rodolakis, A., Salinas, J., Papp, J.R., 1998. Recent advances on ovine chlamydial  
635 abortion. Vet. Res. 29, 275-288.
- 636 Rodolakis, A., Bernard, F., Lantier, F., 1989. Mouse models for evaluation of virulence  
637 of *Chlamydia psittaci* isolated from ruminants. Res. Vet. Sci. 46, 34-39.
- 638 Rodolakis, A., Souriau, A., 1989. Variations in the virulence of strains of *Chlamydia*  
639 *psittaci* for pregnant ewes. Vet. Rec. 125, 87-90.
- 640 Salinas, J., Sánchez, J., Buendía, A.J., Souriau, A., Rodolakis, A., Bernabé, A., Cuello,  
641 F., 1994. The LPS localization might explain the lack of protection of LPS-specific  
642 antibodies in abortion-causing *Chlamydia psittaci* infections. Res. Microbiol. 145,  
643 611-620.
- 644 Sammin, D.J., Markey, B.K., Quinn, P.J., McElroy, M.C., Bassett, H.F., 2006.  
645 Comparison of fetal and maternal inflammatory responses in the ovine placenta after  
646 experimental infection with *Chlamydia abortus*. J. Comp. Pathol. 135, 183-192.

- 647 Sánchez J., Buendía, A.J., Salinas, J., Bernabé, A., Rodolakis, A., Stewart, I.J., 1996.  
648 Murine granulated metrial gland cells are susceptible to *Chlamydia psittaci* infection  
649 *in vivo*. Infect. Immun. 64, 3897-3900.
- 650 Stephens, R.S., 2003. The cellular paradigm of chlamydial pathogenesis. Trends  
651 Microbiol. 11, 44-51.
- 652 Su, H., Caldwell, H.D., 1995. CD4+ T cells play a significant role in adoptive immunity  
653 to *Chlamydia trachomatis* infection of the mouse genital tract. Infect. Immun. 63,  
654 3302-3308.
- 655 Thomson N.R., Yeats, C., Bell, K., Holden, M.T., Bentley, S.D., Livingstone, M.,  
656 Cerdeno-Tarraga, A.M., Harris, B., Doggett, J., Ormond, D., Mungall, K., Clarke, K.,  
657 Feltwell, T., Hance, Z., Sanders, M., Quail, M.A., Price, C., Barrell, B.G., Parkhill, J.,  
658 Longbottom, D., 2005. The *Chlamydophila abortus* genome sequence reveals an  
659 array of variable proteins that contribute to interspecies variation. Genome Res. 15,  
660 629-640.
- 661 Tsakos, P., Siarkou, V., Guscetti, F., Chowdhury, H., Papaioannou, N., Vretou, E.,  
662 Papadopoulos, O., 2001. Experimental infection of pregnant ewes with enteric and  
663 abortion-source *Chlamydophila abortus*. Vet. Microbiol. 82, 285-291.
- 664 Wilsmore, A.J., Parsons, V., Dawson, M., 1984. Experiments to demonstrate routes of  
665 transmission of ovine enzootic abortion. Br. Vet. J. 140, 380-391.
- 666

666 **Figure caption**

667 **Figure 1. The developmental cycle of *Chlamydiaceae*.** Chlamydial infection is initiated  
668 by attachment of the infectious EBb to the host cell, followed by EB entry into a  
669 membrane-bound vesicle, called inclusion. The EBs rapidly differentiate into a RBs  
670 that replicate by binary fission within the inclusion. Following several rounds of  
671 replication, the RBs reorganize to form infectious EBs, which are released from the  
672 cell. Under certain conditions, such as an inflammatory environment where IFN- $\gamma$  is  
673 produced, the intracellular development of chlamydial strains may be altered to adopt  
674 a non-infectious, non-replicating form (aberrant form) that retains viability  
675 (persistence). Aberrant forms can re-differentiate into infectious EBs upon removal of  
676 IFN- $\gamma$  and subsequent replenishing of intracellular bacterium.

677

678

679

Table1. Comparison of immunological features of *C. abortus* infection in the natural host and the mouse model

| THE NATURAL HOST   | THE MOUSE MODEL   |
|--|---|
| Experimental chlamydial infection induces abortion in the third term of gestation or stillbirth (Papp et al., 1994)                              | Chlamydial infection induces abortion in the last days of gestation (Buzoni-Gatel and Rodolakis, 1983)  |
| Time of abortion is independent of the time of infection (Buxton et al., 1990)   | Infection in early or mid-pregnancy induces pre-term abortion (Buendia et al., 1998)  |
| Differentiation between virulent and non-virulent strains is shown (Rodolakis and Souriau, 1989)   | Differentiation between virulent and non-virulent strains is shown (Rodolakis et al., 1989)   |
| Trophoblastic epithelium is the target cell of the infection (Buxton et al., 2002)   | Infection in the placenta starts between decidua and the giant cells layer (trophoblast) (Buendia et al., 1998)   |
| Neutrophilic infiltration is the most evident feature of chlamydial infection in the placenta (Navarro et al., 2004)                             | Neutrophils acts as the first line of defence and are the main population observed in decidua and labyrinth during chlamydial infection (Buendia et al., 1999a) |
| Immune response against chlamydial infection is mainly T-cell dependent (Entrican et al., 2001)  | Cellular immunity is responsible for protection against chlamydial infection (Buzoni-Gatel et al., 1987)  |
| CD8 T cells are the most cell represented lymphoid population both in the placenta and in the adjacent areas of the uterus (Sammin et al., 2006) | CD8 T cells have a crucial role in the control and clearance of chlamydial infection (Buzoni-Gatel et al., 1992; Martinez et al., 2006)                         |
| IFN- $\gamma$ is able to control <i>C. abortus</i> replication in ovine cells (Brown et al., 2001)   | IFN- $\gamma$ has an important role in the control of early <i>C. abortus</i> infection (McCafferty et al., 1994; Del Rio et al., 2001)                         |
| Live vaccines confer optimal protection against chlamydial infection (Chalmers et al., 1997)   | Immunization with live vaccine avoid both abortion and shedding of chlamydiae (Rodolakis, 1983)   |
| Selection of adequate adjuvants significantly increases the protection induced by killed vaccines (García de la Fuente et al., 2004)             | Killed vaccines in combination with some adjuvants (QS-21, Montanide 773) induce a level of protection similar to live vaccines (Caro et al., 2003)             |

Figure 1

