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1 ***Chlamydophila psittaci* infections in birds: a review with emphasis**  
2 **on zoonotic consequences**

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26 **Abstract**

27 The first part of the present review gives an overview on the history of infectious agents of  
28 the order *Chlamydiales* and the general infection biology of *Chlamydophila (C.) psittaci*, the  
29 causative agent of psittacosis. In the second part, the classification of *C. psittaci* strains, as  
30 well as issues of epidemiology of avian chlamydiosis., disease transmission routes, clinical  
31 disease, public health significance, present legislation and recommendations for prevention  
32 and control are reviewed.

33

34

35 *Keywords:* *Chlamydophila psittaci*, review, infection biology, epidemiology, diagnosis,  
36 zoonosis, prevention.

37

## 37 1. History

38 In 1879, psittacosis or parrot fever was documented for the first time when Ritter  
39 (1880) described an epidemic of unusual pneumonia associated with exposure to tropical pet  
40 birds in seven individuals in Switzerland. The term “psittacosis”, however, dates back to  
41 1893, when transmission from parrots to humans of an infectious agent causing flu-like  
42 symptoms was observed (Morange, 1895).

43 In 1907, Halberstaedter and von Prowazek were the first to make drawings of  
44 chlamydia-infected conjunctival cells of trachoma (*Chlamydia trachomatis*) patients. They  
45 took the observed vacuoles for "mantled" protozoa and consequently designated them  
46 *Chlamydozoa* after the Greek word "*chlamys*" for mantle (Halberstaedter and von Prowazek,  
47 1907). In the following years, similar infective agents causing inclusion conjunctivitis of the  
48 newborn or infection of the adult genital tract were described. The inability to grow these  
49 germs, in fact *Chlamydia. trachomatis* organisms, on artificial media made scientists assume  
50 that they were viruses. The relatedness to the agent of psittacosis as discovered by Ritter  
51 (1880) had not yet been realised.

52 During the winter of 1929-1930, a pandemic of human psittacosis occurred in the  
53 United States and Europe. The disease was attributed to the importation of Green Amazon  
54 parrots from Argentina. Shortly afterwards, 174 cases of human psittacosis were reported  
55 from the Faroe Islands in the period from 1930 to 1938. The human death rate was 20% and  
56 was especially high (80%) in pregnant women (Rasmussen-Ejde, 1938). Humans contracted  
57 the infection while capturing juvenile fulmars (*Fulmarus glacialis*) and preparing them for  
58 cooking. Herrmann et al., (2006), presented phylogenetic data on the outer membrane protein  
59 A (*ompA*) gene supporting the speculation that fulmars on the Faroes acquired psittacosis  
60 from infected and dead parrots thrown overboard during shipment from Argentina to Europe  
61 in 1930.

62           The agent of psittacosis was finally identified in humans by Bedson et al. (1930) and  
63 was classified as a virus of the psittacosis-LGV group, alongside the simultaneously  
64 identified causative agent of lymphogranuloma venereum (LGV). Bedson et al., (1930)  
65 defined the unique biphasic developmental cycle of these "viruses", and the organisms were  
66 referred to as *Bedsoniae* in the following years. Hereafter, Thygeson noted the resemblance  
67 between the developmental cycles of the agents of trachoma and psittacosis. He suggested  
68 that lymphogranuloma venereum (LGV) strains and the mouse pneumonitis strain also  
69 belonged to the same unique group of viruses (Thygeson, 1963). In the next years, the  
70 "viruses" were propagated in chorio-allantoic chick embryo membranes, monkey brains and  
71 mice. In 1965, with the development of electron microscopy, scientists could actually see that  
72 the chlamydial agent was not a virus, but a bacterium. Page (1966) proposed to combine all  
73 organisms of the psittacosis-LGV-trachoma group in the single genus *Chlamydia*.  
74 Nevertheless, chlamydiae were considered to belong to *Rickettsiae* for some time, but later on  
75 were found to be different because of the absence of an electron transport system, lack of  
76 cytochromes and the apparent inability to synthesise ATP and GTP in the same way as  
77 *Rickettsiae*. Finally, chlamydiae were classified as Gram-negative intracellular bacteria  
78 because they were found to divide by binary fission, to have cell walls comparable in  
79 structure to those of Gram-negative bacteria, to possess DNA containing nucleoids without  
80 membranes between the nucleoid and the cytoplasm, and to possess ribosomes with antibiotic  
81 susceptibilities characteristic for prokaryotic ribosomes. Shortly afterwards, the genus  
82 *Chlamydia* was placed in the family *Chlamydiaceae* of the order *Chlamydiales*. In the late  
83 1960s, two species were distinguished within the genus *Chlamydia*: *Chlamydia trachomatis*  
84 (Capponi and Haider, 1969) and *Chlamydia psittaci* (Tamura et al., 1971), to which the  
85 species *Chlamydia pneumoniae* (Grayston et al., 1989) and *Chlamydia pecorum* (Fukushi and  
86 Hirai, 1992) were added in the 1990s.

87

88 **2. Infection biology**89 *2.1. Morphology*

90 Morphologically distinct forms of *Chlamydophila* are termed elementary body (EB),  
91 reticulate body (RB), and intermediate body (IB). The EB is a small, electron dense, spherical  
92 body, of about 0.2-0.3  $\mu\text{m}$  in diameter (Costerton et al., 1976; Carter et al., 1991; Miyashita  
93 et al., 1993). The EB is the infectious form of the organism, which attaches to the target cell  
94 and finally enters it. The EBs are characterized by a highly electron-dense nucleoid, located  
95 at the periphery and clearly separated from an electron-dense cytoplasm. The chromatin is  
96 maintained condensed by the histon H1-like protein, Hc1. Following entry into the host cell,  
97 the EB expands in size to form the RB, which is the intracellular metabolically active form.  
98 Differentiation of EBs into RBs is accompanied by chromatin dispersal as chlamydiae  
99 become transcriptionally active. The RB is larger, measuring approximately 0.5-2.0  $\mu\text{m}$  in  
100 diameter. The inner and outer bacterial membranes of RBs are relatively closely apposed.  
101 The RB divides by binary fission and thereafter matures into new EBs. During this  
102 maturation, morphologically intermediate forms (IB) measuring about 0.3-1.0  $\mu\text{m}$  in  
103 diameter can be observed inside the host cell. The IB has a central electron-dense core with  
104 radially arranged individual nucleoid fibers surrounding the core. Cytoplasmic granules are  
105 tightly packed at the periphery of the IB and are separated from the core by a translucent  
106 zone. At the end of the replication cycle, less and highly condensed EBs can be observed  
107 (Litwin et al., 1961; Eb et al., 1976; Costerton et al., 1976; Louis et al., 1980; Soloff et al.,  
108 1982; Matsumoto 1982a; Matsumoto 1988; Vanrompay et al., 1996; Rockey and Matsumoto  
109 2000). Immature, less condensed EBs have few fibrous elements transigrating the granular  
110 cytoplasm towards the condensed electron-dense nucleoid. The latter lies adjacent to the  
111 inner bacterial membrane. Mature, more condensed EBs possess a homogeneous ovoid,

112 elongated or irregular electron-dense nucleoid separated from cytoplasmic elements by a very  
113 distinct electron-transparent space.

114 Electron microscopy and freeze-fracture structures clearly indicate the existence of  
115 curious surface projections on both EBs (Fig 1) and RBs (Fig 2) (Matsumoto et al., 1976;  
116 Matsumoto 1982b). These projections extend from the chlamydial surface into the inclusion  
117 membrane. Matsumoto also observed images of arrayed-rosettes in negatively stained EB  
118 preparations. It is tempting to speculate that these dome-like and needle-like surface  
119 projections correspond to Type III Secretion pores used to deliver virulence proteins into the  
120 host cell (Bavoil and Hsia 1998).

121

## 122 2.2. Developmental cycle

123 *C. psittaci* is an obligate intracellular bacterium replicating within a non-acidified  
124 vacuole, termed an inclusion. Within the inclusion, *C. psittaci* undergoes a unique biphasic  
125 developmental cycle alternating between the EB, which guarantees extracellular survival and  
126 infection of host cells and RB, which is responsible for intracellular replication and  
127 generation of infectious progenitor bacteria.

128 Infection is initiated by attachment and subsequent parasite-mediated endocytosis of  
129 the infectious EB. The EBs are sometimes seen in association with clathrin-coated pits. In  
130 vitro, EBs rely on the actin cytoskeleton to facilitate their entry into host cells. Once  
131 internalized, EBs are redistributed from the periphery of the cell and aggregate at the Golgi  
132 region of the cell that corresponds to the Microtubule Organizing Centre (MTOC). While  
133 internalized EBs occupy small vesicles or inclusions they avoid fusion with lysosomes and  
134 other endocytic organelles. Thus, the nascent inclusion is derived from host plasma  
135 membrane proteins and lipids. Within the first few hours after internalisation, EBs begin to  
136 differentiate into RBs, which is characterised by a loss in infectivity, nucleoid decondensation

137 and enlargement of the organism. By 8 hours post infection, RBs start to replicate by binary  
138 fission and remain in close association with the inclusion membrane. Numerous mitochondria  
139 surround the inclusion (Vanrompay et al., 1996). Host kinesin possibly plays a role in  
140 mitochondrial recruitment to the inclusion. *C. psittaci* may not be as capable of generating its  
141 own ATP as other chlamydial species, and, therefore, a close association of the inclusion with  
142 mitochondria might provide an alternative mechanism to obtain ATP from the host.

143 Unlike bacteria that replicate in the host cell cytosol and have free access to cytosolic  
144 nutrients, *C. psittaci* must import host nutrients across the inclusion membrane. Chlamydial  
145 organisms indeed require amino acids and nucleotides from host pools, but the mechanisms  
146 that facilitate delivery of these molecules into the inclusion have not been clearly defined.  
147 Because the inclusion is disconnected from the endosomal/lysosomal pathway, delivery of  
148 nutrients by fusion of endocytic vesicles is unlikely. Furthermore, although exocytic vesicles  
149 containing sphingomyelin are known to fuse with the inclusion, and may be a source of  
150 nutrients and lipids, the luminal contents of these vesicles have not been identified. Recent  
151 data demonstrate that the inclusion membrane is freely permeable to small molecules with a  
152 molecular weight of 100-520 Da (Hackstadt, 1999; Grieshaber et al., 2002). After delivery  
153 into the lumen by passive diffusion, specific membrane transporters located in the bacterial  
154 membrane would facilitate uptake into the organisms themselves. In addition, specific  
155 membrane transporters would still be needed to transport molecules larger than 520 Da across  
156 the inclusion membrane. However, no such membrane transporters have been found to be  
157 localised to the inclusion membrane yet.

158 As the developmental cycle progresses, the inclusion enlarges to accommodate the  
159 increasing numbers of bacteria. The growing inclusion intercepts biosynthetic membrane  
160 trafficking pathways and acquires the ability to fuse with a subset of Golgi-derived vesicles,  
161 thereby increasing its membrane surface (Hackstadt, 1999). When more and more RBs are



162 actively replicating the following 20 hours (each RB can be responsible of a progeny of up to  
163 1000 new RBs), the inner side of the inclusion membrane becomes overcrowded, leading to  
164 RB detachment from the inclusion membrane. This could be the signal for RBs to convert  
165 into intermediate bodies and new infectious EBs. At the end of the cycle, approximately 50  
166 hours post infection, the host cell and its inclusion(s) either lyse or the EBs and some non-  
167 differentiated RBs are released via reverse endocytosis.

168 In some cases, the developmental cycle can be altered in favour of persistence.  
169 Persistent chlamydiae fail to complete their development from RBs into infectious EBs, but  
170 retain metabolic activity. Persistent RBs appear morphologically aberrant from oval shaped to  
171 strongly enlarged and form small inclusions. While they accumulate chromosomes because of  
172 continuing DNA replication, they do not divide. Persistent growth forms have been  
173 associated with chronic infections. These cryptic persistent forms can rapidly retransform into  
174 normal RBs and infectious EBs. How chlamydiae modulate between normal and persistent  
175 growth is not yet understood (Belland et al., 2003; Hogan et al., 2004; Gieffers et al., 2004).

176

### 177 **3. Avian chlamydiosis**

#### 178 *3.1. Classification and typing of strains*

179 The family *Chlamydiaceae* was recently reclassified into two genera and nine species  
180 (Everett et al., 1999). The new genera *Chlamydia* and *Chlamydophila* correlate with the  
181 former species *Chlamydia trachomatis* and *Chlamydia psittaci*. The genus *Chlamydia* now  
182 includes the species of *Chlamydia trachomatis* (main host: humans), *Chlamydia suis* (swine),  
183 and *Chlamydia muridarum* (mouse, hamster). The genus *Chlamydophila* includes *C. psittaci*  
184 (birds), *C. felis* (cat), *C. abortus* (sheep, goat, cattle), *C. caviae* (guinea pig), and the  
185 previously defined species of *C. pneumoniae* (human) and *C. pecorum* (ruminants). All  
186 known avian strains belong to the species *C. psittaci*, which comprises six avian serovars, i.e.

187 A to F, and two mammalian isolates, i.e. WC and M56 (Andersen, 1991; Vanrompay et al.,  
188 1993; Vanrompay et al., 1997). The isolates WC and M56 originated from epizootics in cattle  
189 and muskrats, respectively. The avian serovars are relatively host-specific. Serovars A and B  
190 are usually associated with psittacine birds and pigeons, respectively. The natural hosts of the  
191 other serovars are more uncertain. Serovar C has primarily been isolated from ducks and  
192 geese, and serovar D mainly from turkeys. Serovar F was isolated from a psittacine bird and  
193 from turkeys. The host range of serovar E is the most diverse among these types, as it was  
194 isolated from about 20% of pigeons, from many cases of fatal chlamydiosis in ratites, from  
195 outbreaks in ducks and turkeys, and occasionally from humans. All serovars should be  
196 considered to be readily transmissible to humans.

197 Genetic diversity based on 16S rRNA gene sequences is less than 0.8 % among *C.*  
198 *psittaci* strains. Several strains were found to have an extrachromosomal plasmid, and many  
199 strains are susceptible to bacteriophage Chp1 (Storey et al., 1989a; 1989b). At present,  
200 analysis of the gene encoding outer membrane protein A (*ompA*) is often used to classify  
201 avian *C. psittaci* strains into genotypes A to F and E/B (Geens et al., 2005). So far, genotype  
202 E/B has been isolated mainly from ducks.

203

204

### 205 3.2. Epidemiology

206 The psittacosis outbreaks of 1929-1930 and 1930-1938 were attributed to psittacine birds.  
207 However, in the following years it became clear that *C. psittaci* infections were not limited to  
208 psittacine birds, but could also affect other avian species. In 1939, the agent was isolated  
209 from two South African racing pigeons, and soon additional pigeon isolates were obtained,  
210 this time from Californian racing pigeons. At that time, cases of psittacosis in two New York  
211 citizens could be attributed to contact with infected feral pigeons. In 1942, serological  
212 evidence showed ducks and turkeys to be frequently infected, and, within the next 3 years,

213 human infections due to contact with infected ducks were reported in California and New  
214 York. In the early 1950s, *C. psittaci* could be isolated from turkeys and humans in contact  
215 with infected turkeys during severe respiratory outbreaks in the US turkey industry (Meyer,  
216 1967). The incidence of severe *C. psittaci* epidemics in US poultry declined during the 1960s.  
217 However, avian chlamydiosis remained a continuing threat to both birds and humans. In the  
218 1980s, chlamydiosis in turkeys was again reported in the US (Grimes and Wyrick 1991), and  
219 in the 1990s also in Europe (Ryll et al., 1994; Vanrompay et al., 1997; Sting et al., 2006).  
220 Nowadays, *C. psittaci* is nearly endemic in the Belgian turkey industry (Van Loock et al.,  
221 2005; Verminnen et al., 2006), and there is evidence to assume that it is also endemic in other  
222 European countries (Hafez and Sting, 1997; Van Loock et al., 2005). However, in contrast to  
223 the devastating explosive outbreaks in the first half of the 20th century, the present outbreaks  
224 are characterised by respiratory signs and low mortality. More recently, an increase in the  
225 number of outbreaks in ducks affecting contact persons was reported (Laroucau, 2007,  
226 personal communication; Laroucau et al., 2008; Andersen and Vanrompay, 2008). Human  
227 infections associated with outbreaks in ducks had already been described in past two decades  
228 (Arzey and Arzey, 1990; Arzey et al., 1990; Martinov and Popov, 1992; Newman et al.,  
229 1992; Hinton et al., 1993; Goupil et al., 1998; Lederer and Muller, 1999; Bennedsen and  
230 Filskov, 2000).

231 The list of avian species in which *C. psittaci* infections occur increased rapidly. Today, *C.*  
232 *psittaci* has been demonstrated in about 465 bird species comprising 30 different bird orders  
233 (Kaleta and Taday, 2003). The highest infection rates are found in psittacine birds  
234 (*Psittacidae*) and pigeons (*Columbiformes*). The prevalence in psittacine birds ranges  
235 between 16 and 81 %, and a mortality rate of 50 % or even higher is not unusual (Raso et al.,  
236 2002; Dovc et al., 2005; Dovc et al., 2007). *Psittacidae* are major reservoirs of chlamydiae,  
237 especially under captive conditions, but also among those kept as pets (Chahota et al., 2006;

238 Vanrompay et al., 2007).

239 Data on the seropositivity of racing pigeons range from 35.9 to 60 %. Free-living pigeons  
240 are present in urban and rural areas all over the world and get in close contact with people in  
241 public places. Thirty-eight studies on the seroprevalence of *C. psittaci* in feral pigeons  
242 conducted from 1966 to 2005 revealed a seropositivity rate ranging from 12.5 to 95.6 %  
243 (Tanaka et al., 2005; Haag-Wackernagel 2005; Laroucau et al., 2005; Mitevski et al., 2005;  
244 Prukner-Radovic et al., 2005). More recent studies performed in feral pigeons in Italy,  
245 Bosnia and Herzegovina, and Macedonia showed a seropositivity of 48.5%, 26.5% and  
246 19.2%, respectively (Ilieski et al., 2006; Residbegovic et al., 2007; Ceglie et al., 2007).

247 Birds living on sea shores and other waters, such as gees, ducks, gulls and penguins are  
248 more frequently infected than hens, pheasants and quails (Kaleta and Taday, 2003). Common  
249 reservoirs of *C. psittaci* include wild and feral birds, such as sea gulls, ducks, herons, egrets,  
250 pigeons, blackbirds, grackles, house sparrows, and killdeer, all of which freely intermingle  
251 with domestic birds. Highly virulent strains of *C. psittaci* can be carried and extensively  
252 excreted by sea gulls and egrets without any apparent effect on the hosts themselves.

253

### 254 3.3. *Transmission between birds*

255 Transmission of *C. psittaci* primarily occurs from one infected bird to another susceptible  
256 bird in close proximity. The agent is excreted in faeces and nasal discharges. Faecal shedding  
257 occurs intermittently and can be activated through stress caused by nutritional deficiencies,  
258 prolonged transport, overcrowding, chilling, breeding, egg-laying, treatment or handling.  
259 Bacterial excretion periods during natural infection can vary depending on virulence of the  
260 strain, infection dose and host immune status. However, shedding may occur for several  
261 months. Transmission of chlamydiae occurs mainly through inhalation of contaminated  
262 material and, sometimes, ingestion. Large numbers of *C. psittaci* cells can be found in

263 respiratory tract exudate and faecal material of infected birds. The importance of the  
264 respiratory exudate has become more apparent. In turkeys, the lateral nasal glands become  
265 infected early and remain infected for more than 60 days. Choanal/oropharyngeal swabs are  
266 more consistent for isolation of the agent than faecal swabs, especially during early stages of  
267 infection. Direct aerosol transmission through aerosolisation of respiratory exudate must be  
268 considered as the primary method of transmission.

269 Avian species, including domestic poultry sharing aquatic or moist soil habitats with wild  
270 infected aquatic birds may become infected via contaminated water. Granivorous birds like  
271 pigeons, doves, pheasants and house sparrows may become infected by dust inhalation in  
272 faeces contaminated barnyards and grain storage sites. The consumption of infected carcasses  
273 may transmit *C. psittaci* to host species that are predators or scavengers of other birds.

274 Transmission of *C. psittaci* in the nest is possible. In many species, such as  
275 *Columbiformes*, cormorants, egrets, and herons, transmission from parent to young may  
276 occur through feeding, by regurgitation, while contamination of the nesting site with infective  
277 exudates or faeces may be important in other species, such as snow geese, gulls and  
278 shorebirds. Furthermore, *C. psittaci* can be transmitted from bird to bird by blood-sucking  
279 ectoparasites such as lice, mites and flies or, less commonly, through bites or wounds  
280 (Longbottom and Coulter, 2003). Transmission of *C. psittaci* by arthropod vectors would be  
281 facilitated in the nest environment.

282 Vertical transmission has been demonstrated in turkeys, chickens, ducks, parakeets,  
283 seagulls and snow geese, although the frequency appears to be fairly low (Wittenbrink et al.,  
284 1993). However, it could serve as a route to introduce chlamydiae into a poultry flock.

285 *C. psittaci* can be introduced into susceptible pet birds and poultry through the wild bird  
286 population. Contaminated feed or equipment can also be a source of infection, and feed  
287 should therefore be protected from wild birds. Careful cleaning of equipment is extremely

288 important as *C. psittaci* can survive in faeces and bedding for up to thirty days. Cleaning and  
289 disinfection with most detergents and disinfectants will inactivate *C. psittaci*, as this Gram-  
290 negative bacterium has a high lipid content.

291

### 292 3.4. Clinical disease

293 Depending on the chlamydial strain and the avian host, chlamydiae cause pericarditis, air  
294 sacculitis, pneumonia, lateral nasal adenitis, peritonitis, hepatitis, and splenitis. Generalised  
295 infections result in fever, anorexia, lethargy, diarrhoea, and occasionally shock and death.  
296 Chlamydiosis is a very common chronic infection of psittacine birds. Infections cause  
297 conjunctivitis, enteritis, air sacculitis, pneumonitis, and hepatosplenomegaly. Droppings are  
298 often green to yellow-green. Many of the birds become chronically infected, but show no  
299 clinical signs until stressed. These birds often shed chlamydiae intermittently and serve as a  
300 source of infection of humans and other birds.

301 Chlamydiosis is also a common chronic infection of pigeons. Clinical signs include  
302 conjunctivitis, blepharitis, and rhinitis (Andersen 1996). Survivors can become asymptomatic  
303 carriers.

304 Infected turkeys show vasculitis, pericarditis, pneumonitis, air sacculitis, and  
305 hepatosplenomegaly, and lateral nasal adenitis at necropsy. Mortality rates of 5–40 % may  
306 occur unless early antibiotic treatment is introduced (Grimes et al., 1970; Andersen 1996).  
307 Experimentally, virulent turkey strains cause little, if any, disease in chickens, pigeons, and  
308 sparrows; however, cockatiels and parakeets succumb rapidly to infection by these agents.  
309 Outbreaks in turkeys with low mortality and little or no human involvement usually are  
310 caused by the "pigeon serovar". The mortality rate is usually less than 5%.

311 Chlamydiosis in ducks is rare in the United States (Andersen 1996), but is a serious  
312 economic and occupational health problem in Europe (Laroucau et al., 2008). Trembling,

313 conjunctivitis, rhinitis, and diarrhea are observed in infected ducks. Mortality ranges to up to  
314 30 %.

315

### 316 3.5. Public health significance

317 *C. psittaci* can infect humans and should be handled carefully under conditions of bio-  
318 containment. Humans most often become infected by inhaling the organism when urine,  
319 respiratory secretions or dried faeces of infected birds are dispersed in the air as very fine  
320 droplets or dust particles. Other sources of exposure include mouth-to-beak contact, a bite  
321 from an infected bird or handling the plumage and tissues of infected birds. Therefore, post-  
322 mortem examinations of infected birds and handling of cultures should be carried out in  
323 laminar flow hoods or with proper protective equipment. Human infection can result from  
324 transient exposures. The disease is of significance for public health because of the popularity  
325 of psittacine birds as pets and the increased placement of these birds in child care facilities  
326 and in homes for the elderly. The risk of catching psittacosis is not only associated with direct  
327 contact to birds, but is also relevant in rural environments and gardening activities, such as  
328 lawn mowing and trimming without a grass catcher, where individuals can be exposed to the  
329 infectious agent (Telfer et al., 2005; Fenga et al., 2007). Person-to-person transmission of  
330 psittacosis is possible, but is believed to be rare (Hughes et al., 1997; Ito et al., 2002).

331 The incubation period is usually 5–14 days, but longer incubation periods are known.  
332 Human infections vary from inapparent to severe systemic disease with interstitial pneumonia  
333 and occasionally encephalitis. The disease is rarely fatal in properly treated patients;  
334 therefore, awareness of the danger and early diagnosis are important. Infected humans  
335 typically develop headache, chills, malaise and myalgia, with or without signs of respiratory  
336 involvement. While pulmonary involvement is common, auscultatory findings may appear to  
337 be normal. A number of severe cases of human psittacosis, i.e. atypical pneumonia, were

338 reported recently (Chorazy et al., 2006; Haas et al., 2006; Pandeli and Ernest, 2006; Strambu  
339 et al., 2006). Besides, *C. psittaci* has been associated with ocular lymphoma, although this  
340 still is a matter of debate as conflicting results have been found (Ferreri et al., 2004; Chanudet  
341 et al., 2006; Rosado et al., 2006; Zhang et al., 2007). However, severe cases of psittacosis are  
342 probably only the tip of the iceberg. Apart from these, there seems to be a larger number of  
343 less severe, clinically unnoticed infections, which are misdiagnosed due to symptoms  
344 suggesting involvement of other respiratory pathogens, as well as asymptomatic infections  
345 (Harkinezhad et al., 2007; Vanrompay et al., 2007). The impact of these types of *C. psittaci*  
346 infections on human health is difficult to determine. One can try to extrapolate present  
347 knowledge on avian infections to human psittacosis. As in birds, the carrier status might be  
348 common, as well as pathogenic synergies with other respiratory pathogens (Van Loock et al.,  
349 2006).

350         Diagnosis of *C. psittaci* infection can indeed be difficult and is usually accomplished  
351 through testing paired sera, or only one serum sample, using the micro-immunofluorescence  
352 (MIF) test, which is more sensitive and specific than the complement fixation test, which was  
353 frequently used in the past. However, the MIF test shows cross-reactivity with other  
354 chlamydial species. Highly sensitive nucleic acid amplification assays can be used to  
355 specifically detect *C. psittaci*. Culture is also possible, but technically rather difficult for  
356 requiring biosafety level 3 laboratories. Like in birds, tetracyclines are the drugs of choice for  
357 treating human psittacosis. Doxycycline or tetracycline is usually administered, unless  
358 contraindicated, like in the case of pregnant women and children under 9 years, where  
359 erythromycin can be used. The length of treatment will vary with the drug, but should be  
360 continued for at least 14 days for tetracycline.



361 Psittacosis is a notifiable disease in Australia, the US and most European countries,  
362 e.g. Belgium, France, Germany, Italy, and Switzerland. An overview of reported psittacosis  
363 cases in several countries is presented in Table 1.

364

### 365 *3.6. Legislation and recommendations for prevention and control*

366 Although research on MOMP-based DNA vaccination in SPF turkeys was very  
367 promising (Vanrompay et al., 1999a; Vanrompay et al., 1999b), there are no vaccines  
368 currently available. DNA vaccination significantly diminished clinical signs, lesions and  
369 excretion of chlamydiae, but did not provide complete protection. However, the latter is  
370 perhaps impossible. In this situation, we have to rely on risk reduction strategies and  
371 available treatment. In a worldwide context, recommendations for prevention and control of  
372 psittacosis, as well as legislation are focusing on a reduction of human morbidity.

373 Until recently, EU legislation for the importation of birds other than poultry from  
374 third countries was encompassed in Decisions 2000/666/EC (CEC, 2000) and 2005/760/EG  
375 of the European Commission, which were primarily concerned with restricting the  
376 introduction of Newcastle disease or avian influenza. Essentially, these decisions stipulate  
377 that 1) the birds must come from a flock in the country of origin where they were kept for at  
378 least 21 days prior to export, 2) the birds are transported in cages or crates that contain only  
379 one species of bird or one species per compartment, if compartmentalised and, 3) the birds  
380 are moved to designated licensed quarantine premises, where they are held for 30 days and  
381 subjected to at least two veterinary inspections, usually beginning and end, before release.  
382 There was only a single mention of the agent *C. psittaci*, i.e. “if during quarantine it is  
383 suspected or confirmed that *Psittaciformes* are infected with *C. psittaci*, all birds of the  
384 consignment must be treated by a method approved by the competent authority and the  
385 quarantine must be prolonged for at least two months following the last recorded case”. Thus,

386 these requirements did not apply to any other bird species, nor were there any rules that  
387 applied to the management of psittacosis within the EU. Therefore, decisions 2000/666/EC  
388 and 2005/760/EC were repealed and a new regulation, i.e. EC no. 318/2007, came into force  
389 on July 1 in 2007, after being published in the Official Journal of the EU. This regulation lays  
390 down the animal health conditions for imports of certain birds from third countries and parts  
391 thereof into the EU. Thus it is not applicable to: a) poultry, b) racing pigeons, c) birds  
392 imported from Andorra, Liechtenstein, Monaco, Norway, San Marino, Switzerland and the  
393 Vatican City, and not to, d) third countries which can use an animal health certificate referred  
394 to in Annex I of the regulation. The regulation also lays down the quarantine conditions. For  
395 instance: 1) approved quarantine facilities and centres, 2) direct transport of birds to  
396 quarantine stations, 3) attestation by the importers or their agents, 4) quarantine for at least 30  
397 days, 5) examination, sampling and testing to be carried out by an official veterinarian, 6)  
398 actions in case of disease suspicion, which are in case of chlamydiosis: treatment of all birds  
399 and prolonged quarantine for at least two months following the date of the last recorded case.  
400 Importantly, the regulation only allows imports of birds from approved breeding  
401 establishments, thus for birds other than poultry, only birds bred in captivity carrying an  
402 individual identification number and accompanied by an animal health certificate are  
403 allowed.

404 In the US, State regulatory agencies may impose quarantine on intrastate movement  
405 of diseased poultry flocks and may require antibiotic treatment of the flock prior to slaughter.  
406 Since regulations may vary from state to state, the appropriate public health and/or animal  
407 health agencies should be consulted as necessary. According to USDA regulations,  
408 movement of poultry, carcasses, or offal from any premise is prohibited where the existence  
409 of chlamydiosis has been proved by isolation of a chlamydial agent. The Animal and Plant  
410 Health Inspection Service of the USDA and the US Department of Health and Human

411 Services forbid interstate movement of birds from infected flocks, but there is no restriction  
412 on movement of eggs from such flocks.

413 In 2006, the US National Association of State Public Health Veterinarians (NASPHV;  
414 [www.nasphv.org](http://www.nasphv.org)) published a compendium of measures to control *C. psittaci* infections  
415 among humans and pet birds, including prevention and control recommendations such as: 1)  
416 protect persons at risk, 2) maintain accurate records of all bird-related transactions for at least  
417 one year to aid in identifying sources of infected birds and potentially exposed persons, 3)  
418 avoid purchasing or selling birds that have signs consistent with avian chlamydiosis, 4)  
419 isolate newly acquired, ill, or exposed birds, 5) test birds before they are to be boarded or sold  
420 on consignment, 6) screen groups of birds with frequent public contact routinely for anti-  
421 chlamydial antibodies, 7) practice preventive husbandry, 8) control the spread of the infection  
422 and, finally, 9) use disinfection measures.

423 Large-scale commercial import of psittacine birds ended in the US in 1993 with the  
424 implementation of the Wild Bird Conservation Act. The Veterinary Services of the Animal  
425 and Plant Health Inspection Service of the USDA regulate the legal importation of pet birds  
426 set forth in the Code of Federal Regulations, (Title 9, Chapter 1). The Department of the  
427 Interior (DOI) monitors and regulates wildlife shipments at US ports of entry and conducts  
428 investigations on smuggling and interstate trafficking of endangered and protected species  
429 including birds, which is extremely important as these birds are a potential source of new *C.*  
430 *psittaci* infections in domestic birds.

431

#### 432 ***Conflict of Interest Statements***

433 None of the authors (Taher Harkinezhad, Tom Geens and Daisy Vanrompay) has a  
434 financial or personal relationship with other people or organizations that could

435 inappropriately influence or bias the paper entitled “*Chlamydophila psittaci* infections in  
436 birds: a review with emphasis on zoonotic consequences”.

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438 **References**

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693 **Figure captions**

694 Fig. 1. Electron micrographs of elementary bodies (EBs) of *C. psittaci* and *Chlamydia*  
695 *trachomatis*.

696 a) Transmission electron microscopic image of an EB of *C. psittaci* strain Cal 10. The  
697 sample was treated with ruthenium red to enhance the electron opacity of the surface  
698 projections (arrows). The most obvious feature is the eccentric, electron dense (black) DNA  
699 core (n), which is tightly packed onto chlamydial histone protein. Histones are basic DNA-  
700 binding proteins commonly found in higher plants and animal cells, but unusual in bacteria.  
701 Note that the cytoplasm of EBs (c) has a granular appearance due to the presence of 70S  
702 ribosomes. Surrounding the cytoplasm is a lipid cytoplasmic membrane and a rigid outer  
703 envelope (both ~8 nm) containing a regularly packed hexagonal structure of 16.7 nm. This  
704 structure, together with extensive -S-S- bridging of the sulphur amino-acid rich outer  
705 envelope proteins, probably accounts for much of the outer envelope's rigidity (bar 0.15  $\mu\text{m}$ ).  
706 Adapted from Matsumoto (1988). b) Rotational symmetry of EBs from *C. psittaci* strain Cal  
707 10. A rosette comprising 9 subunits is presented (bar 30 nm). Adapted from Matsumoto  
708 (1979). c) Negatively stained envelope fragment of EBs from *C. psittaci* strain Cal 10. Note  
709 the dark centred rosettes (arrows; bar 0.1  $\mu\text{m}$ ). Adapted from Matsumoto (1979). d) Deep  
710 freeze-etching replica of EBs from *Chlamydia trachomatis* strain D-12N within a McCoy cell  
711 inclusion at 44 hours p.i. Note the projections radiating from the surface of each EB (bar 0.1  
712  $\mu\text{m}$ ). Adapted from Matsumoto ([http://www.chlamydiae.com/docs/biology\\_biol\\_EB.asp](http://www.chlamydiae.com/docs/biology_biol_EB.asp)). e)  
713 Rosettes on the surface of EBs from *Chlamydia trachomatis* strain D9-3 exposed by deep  
714 freeze-etching. In contrast to *C. psittaci*, this rosette seems to be composed of 8 subunits (bar  
715 0.1  $\mu\text{m}$ ). Adapted from Matsumoto *et al.* (1999).

716

717 Fig. 2. Electron micrographs of reticulate bodies (RBs) of *C. psittaci* and *Chlamydia*  
718 *trachomatis*. a) Transmission electron micrograph of RBs from *C. psittaci* strain Cal 10. Note  
719 the surface projections penetrating the inclusion membrane (IcM), the latter being treated  
720 with tannic acid to reveal the projections (arrows; bar, 0.1  $\mu\text{m}$ ). Adapted from Matsumoto  
721 (1988). b) Transmission electron micrograph of an immature inclusion of *Chlamydia*  
722 *trachomatis* LGV 404. The micrograph shows a mixture of small electron dense EBs (E) and  
723 much larger and less dense RBs (R). Note the homogenous granular cytoplasm of the RBs,  
724 due to the presence of many ribosomes and the typical hourglass profile of a dividing  
725 reticulate body (DR) (bar 1.0  $\mu\text{m}$ ). Adapted from Ward (1988). c) Carbon replica of a freeze-  
726 fractured face of a *C. psittaci* Cal 10 inclusion at 18 hours p.i. (bar 0.1  $\mu\text{m}$ ). Note the clusters  
727 of projections studding the inclusion membrane. Adapted from Rockey *et al.* (2000). d)  
728 Negatively stained outer membrane of a RB from *C. psittaci* strain Cal 10, showing the  
729 rosettes (bar 0.1  $\mu\text{m}$ ). Adapted from an electron micrograph of Matsumoto.  
730 ([http://www.chlamydiae.com/docs/biology/biol\\_RB.asp](http://www.chlamydiae.com/docs/biology/biol_RB.asp)).

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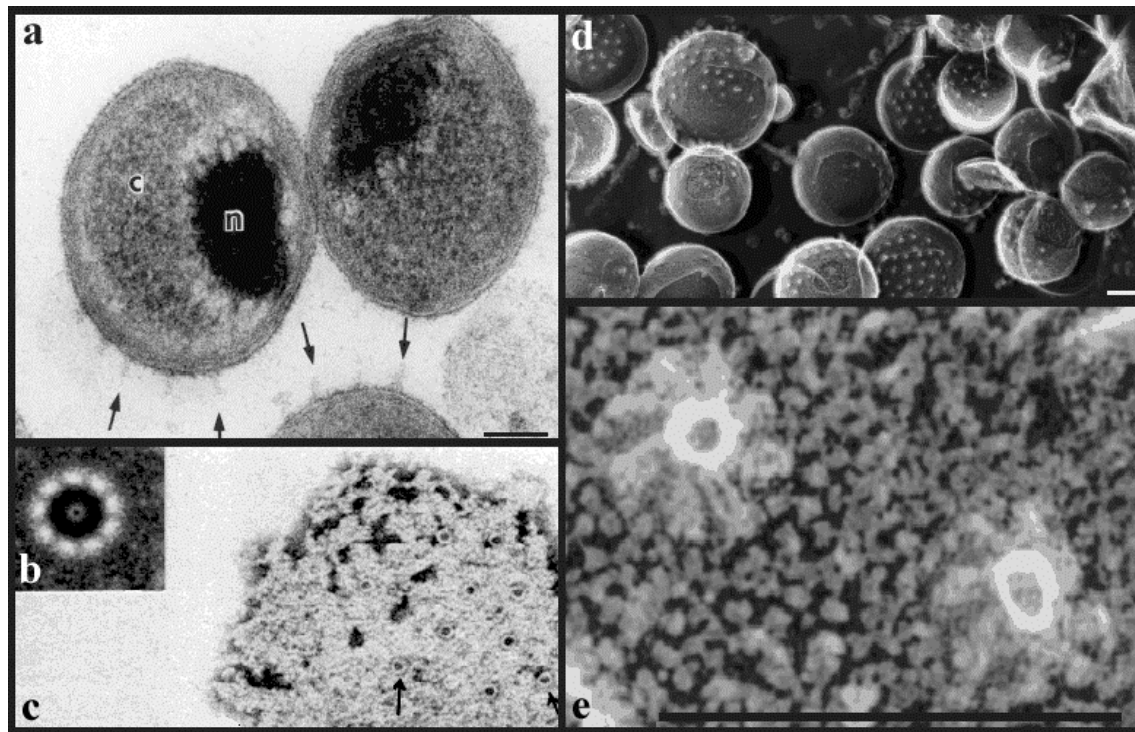


Fig. 1

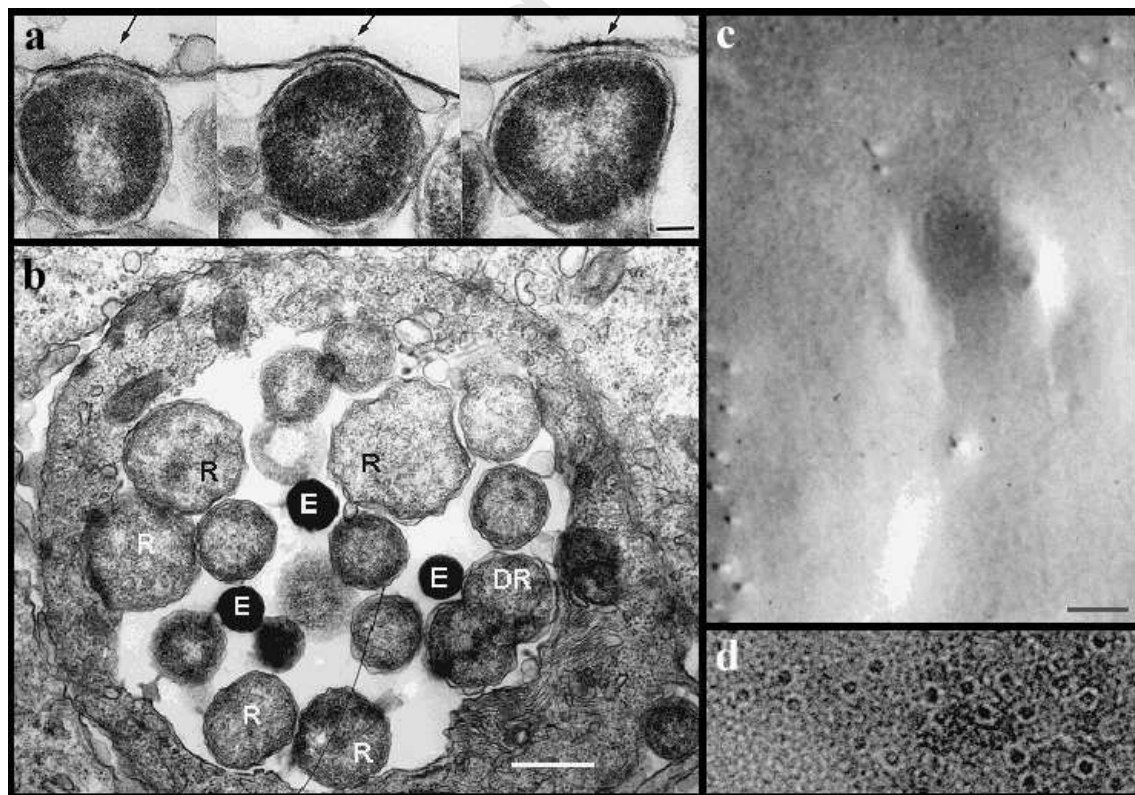


Fig. 2