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Review of data and focus on public health implications

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1 **Chlamydial infections in feral pigeons in Europe: review of data and focus on public health**
2 **implications**

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39 **Abstract**

40

41 Feral pigeons (*Columba livia domestica*), which thrive in most European towns and cities, are
42 commonly infected with the zoonotic bacterium *Chlamydophila psittaci*, the agent of psittacosis
43 (also known as ornithosis) in humans. A number of surveys carried out over the last thirty years
44 across Europe have detected high seropositivity values and high percentages of infection in feral
45 pigeon populations. Overall, when considering data from 11 European countries, seropositivity
46 values to *C. psittaci* in the sampled populations ranged from 19.4 to 95.6 %. In most surveys, the
47 complement fixation test was used, and antibodies were detected in 19.4 to 66.3 % of the samples,
48 with a median of 46.1 %. Indirect immunofluorescence and ELISA tests were employed less
49 frequently, but led to detection of higher percentages of seropositivity (23.7 – 67.7 % and 35.9 –
50 95.6 %, respectively). Attempts to grow *C. psittaci* in cell culture or embryonated chicken eggs
51 were successful in 2 – 42.3 % and 0 – 57.1 % of samples, respectively, antigen detection methods
52 were positive in 2.3 – 40% of samples, while conventional PCR and real-time PCR using different

53 genomic targets detected the organism in 3.4 – 50 % of samples. Twenty-five *C. psittaci* isolates
54 from pigeons were typed as *ompA* genotype B (n=14), E (n=10) and E/B (n=1).

55 The huge increase of feral pigeon populations in Europe is a major cause of concern for the
56 detrimental effect of pigeon droppings on environmental hygiene, in addition to the extensive
57 damage due to the fouling of buildings and monuments. The most important pathogenic organism
58 transmissible from feral pigeons to humans is *C. psittaci*, with 101 cases of disease reported in the
59 literature. Exposure to *C. psittaci*-contaminated dust, direct contact with pigeons through handling
60 and, to a lesser extent, through pigeon feeding have been identified as hazardous exposures in more
61 than half of the human cases, while loose or transient contacts with feral pigeons have been
62 mentioned in about 40 % of the cases.

63 Education initiatives as to the communication of a health risk resulting from contact with pigeons
64 and pigeon excreta should primarily be targeted at individuals who may be exposed to *C. psittaci*-
65 contaminated dust, such as demolition/construction workers. Recommendations to this category of
66 workers include wearing protective clothes with hoods, boots, gloves and air filter face masks when
67 removing pigeon faeces from roofs, garrets and buildings, especially if working indoors.

68 Monitoring for *C. psittaci* infections in these workers over time should also be considered. Children
69 should be warned not to handle sick or dead pigeons, and immunocompromised individuals should
70 be advised to carefully limit their contact to feral pigeons.

71 Culling of pigeons by shooting or poisoning is both unethical and ineffective as the place of the
72 killed birds in the population is quickly filled by new juveniles or immigrating birds from
73 neighbouring areas. Pigeon-detering systems, such as nets and plastic or metal spikes applied to
74 buildings and monuments will prevent their fouling, and the administration of contraceptive drugs
75 may allow size regulation of the pigeon populations. Nevertheless, the measure that will ultimately
76 lead to permanent reduction and will establish healthy sustainable populations is the restriction of
77 indiscriminate feeding by pigeon lovers. The erection of dovecotes and artificial breeding facilities

78 should be considered for providing shelter and a balanced diet to the birds, as well as a chance of
79 interaction for pigeon lovers in a hygienically-controlled environment.

80

81 Keywords: *Chlamydophila psittaci*, diagnosis, epidemiology, feral pigeons, health hazard, zoonosis

82

83 1. Introduction

84

85 Feral pigeons (*Columba livia domestica*), also known as “urban”, “street” or “city” pigeons, are
86 descendants of the domesticated form of the free-living Rock Dove, or Rock Pigeon (*Columba livia*
87 Gmelin, 1789). During their domestication of more than five thousand years, hundreds of pigeon
88 breeds were produced according to the desires and wishes of man (Haag-Wackernagel, 1998; 1999).
89 Domestication in pigeons is characterized amongst others by a high annual reproduction success,
90 tameness and selection against aggressiveness in males. These features may partly be responsible
91 for the enormous thriving success of the feral pigeon in our cities around the world. In regions
92 where no rock pigeons live, feral pigeons are derived from escaped domestic pigeons, such as the
93 semi-domesticated dovecote pigeon, and from lost homing and fancy pigeons (Haag-Wackernagel,
94 2003).

95 After World War II, feral pigeon populations hugely increased worldwide in most larger cities
96 (Simms, 1979) to a level of concern for city administrators and communal health officers. Besides
97 being responsible for the massive fouling of buildings and monuments, feral pigeons were in fact
98 often shown to be naturally infected with a number of viruses, bacteria, fungi and protozoa that are
99 pathogenic to humans (Haag-Wackernagel and Moch, 2004). *Chlamydophila psittaci* (Everett et al.,
100 1999; Garrity et al., 2004), an obligate intracellular bacterium which is the agent of avian
101 chlamydiosis in birds and psittacosis in humans, is the most prevalent organism in feral pigeons
102 worldwide. As a consequence, feral pigeon populations have been repeatedly blamed as vectors for
103 the transmission of *C. psittaci* infections to humans.

104 COST Action 855 (<http://www.vetpathology.unizh.ch/forschung/CostAction855.html>), a Europe-
105 wide research network on animal chlamydioses and their zoonotic implications, has recently
106 provided a forum for researchers from several countries to discuss the public health risks associated
107 with chlamydiosis in feral pigeons.

108 The purpose of this communication is to (i) review the ecology of feral pigeons and the measures
109 that can be adopted to obtain healthy sustainable feral pigeon populations, (ii) review the methods
110 for detection of chlamydiae and chlamydial antibodies in feral pigeons and the present data on the
111 prevalence of chlamydiosis in avian populations established in several European countries, and (iii)
112 discuss the zoonotic relevance of chlamydial infections in feral pigeons.

113

114 **2. The ecology of feral pigeons in the urban environment**

115

116 Feral pigeons are a valuable enrichment of the urban environment and are one of the few animal
117 species able to survive in our noisy and hectic cities. They also represent a tourist attraction and
118 may have a cleaning up function by eating discarded food. In addition, the feeding and care of feral
119 pigeons are rewarding spare-time activities for many people who enjoy the company of animals,
120 and bring pleasure to a fraction of dedicated citizens, especially to children. In addition, feral
121 pigeons are an interesting study subject with a high scientific and educational value for hobby
122 ornithologists, as well as for biology scientists.

123 Today, the feeding of pigeons by “pigeon lovers” is mainly responsible for the establishment of
124 large pigeon populations in our cities and a supplemental input for their increase is provided by
125 rubbish and seasonally occurring natural food, such as grass and tree seeds in parks and gardens
126 (Haag, 1984). The extensive food supply indeed provides the ecological basis for the large
127 populations that occur in most cities of the world (Haag-Wackernagel, 1993; 1995; 2002; Kösters et
128 al., 1994). Pigeons in fact do not need to commute on risky flights to look for more natural food
129 supplies in the countryside and are minimally threatened by predatory birds, whose populations

130 have been drastically decimated over the years by hunting and by deliberate or accidental
131 poisoning. Regular feeding of pigeons by their feeders throughout the year allows pigeons extra
132 time for breeding, so that some individuals are able to breed throughout the year. Furthermore,
133 several behavioural changes have increased the chances of survival of feral pigeons in urban
134 environments. These birds are in fact extremely adaptable, which also enables them to accept
135 breeding places that are unnatural to them, e.g. on trees or over running ventilation systems.
136 As the density of nesting and roosting pigeons increases, the quality of life in the feral pigeon
137 population deteriorates. In fact, excessive population density activates and stimulates regulation
138 mechanisms that decimate nestlings and juvenile pigeons with infectious and parasitic diseases.
139 Crowded breeding places make pigeons behave more aggressively, which again mostly affects
140 nestlings and juveniles that are the weakest members of the population, leading to a progressive
141 spoiling of their physical condition.

142

143 **3. The impact of oversized feral pigeon populations in the urban environment**

144

145 Feral pigeons are gregarious birds that gather in swarms in streets, squares and parks, and along
146 rivers and lakes. Being often huge, these swarms are of increasing concern for owners of buildings,
147 city administrators and communal health officers (Haag-Wackernagel and Moch, 2004). Concerns
148 have been raised for the detrimental effect of pigeon droppings on environmental hygiene and for
149 the fouling of buildings and monuments. In fact, breeding sites for feral pigeons are usually man-
150 made structures such as holes in the façades of private and community buildings, churches and city
151 towers and structures under bridges (Kösters et al., 1991). Pigeon faeces are thus continuously shed
152 over monuments, statues, roofs, streets and sidewalks, leading to extensive fouling and progressive
153 damage due to the corrosive nature of the acidic contents. A pigeon produces around 12 kg of
154 faeces per year that are mainly deposited at the roosting, breeding and feeding sites (Haag, 1984;
155 Kösters et al., 1991). The progressive damage to marble and limestone is mainly due to the action of

156 organic acids other than uric acid, which does not seem to be able by itself to deteriorate calcareous
157 stone (Del Monte and Sabbioni, 1986; Dell’Omo, 1996). Pigeon droppings have also proved to be
158 an excellent substrate for the growth of microorganisms such as fungi and bacteria. In particular, the
159 mycelial growth of some fungi (e.g., *Aspergillus* spp.) may by itself cause alteration in marble
160 surfaces through the mechanical action exerted by the fungal hyphae. In addition, some fungal
161 species that grow on pigeon excrement secrete acidic products (especially low-molecular weight
162 organic acids) that contribute to the chemical erosion of calcareous material such as marble. Feral
163 pigeons can be a real problem for historical monuments, and in the case of the cathedral of Milan
164 they have probably contributed to the deterioration of many statues and pinnacles (Bassi and
165 Chiatante, 1976; Mendez-Tovar et al., 1995).

166 On the other hand, the issues of the contamination of the urban environment caused by feral pigeons
167 and the resultant health risks for humans have been known for a long time given the frequent
168 opportunities of direct and indirect contact with these birds. Close contact with humans commonly
169 occurs in squares, public gardens, parks, markets, and railway stations. In addition, the behavioural
170 habit of pigeons in assembling and resting on roofs, balconies, window sills and shutters brings
171 them even closer to humans. It should be noted that contact is sometimes also actively promoted by
172 enthusiastic pigeon feeders who directly provide the birds with food at their daily gathering.

173 174 **4. Overview of management strategies of feral pigeon populations**

175
176 Various attempts have been made in towns and cities worldwide to reduce the size of the feral
177 pigeon populations. A detailed presentation and discussion of all methods applied through the years
178 can be found in several publications, including the ones by Barbieri et al. (1997) and Haag-
179 Wackernagel (1998).

180 At the beginning of the 20th century, the reduction of large populations was attempted in
181 Washington, London and Dresden by hunting and shooting (Haag-Wackernagel, 1998). Nowadays,

182 control programmes in some towns and cities still aim to reduce the number of feral pigeons by
183 killing as many individuals as possible, e.g. by trapping, shooting or poisoning. However, it should
184 be noted that several scientific studies have demonstrated that killing alone does not have an effect
185 on the population size because the place of the killed birds is quickly filled by new juveniles, or by
186 birds immigrating from neighbouring areas. Due to the high reproduction rate of feral pigeons of up
187 to 12 fledglings per pair per year, coupled with a low adult mortality rate of 10 % (Haag, 1984), a
188 lasting reduction of their populations simply cannot be achieved by killing.

189 Attempts for decreasing the birth rate of feral pigeons have been also made through the years in
190 several European towns and cities, with the aim of reducing the size of the populations. Physical
191 measures such as the destruction of pigeon eggs by eggshell puncture or by replacement of fertile
192 eggs with plastic ones, and pharmacological treatments with several drugs have been applied.

193 Cytostatic agents that inhibit the gametogenesis (e.g. busulfan), as well as drugs that interfere with
194 the birds' metabolic activities (azacholesterol, nicarbazine), and natural or synthetic progestinic and
195 estrogenic drugs (progesterone, mestranol, levonorgestrel, ethinylestradiol) have been administered
196 on different occasions (for references, see Ballarini et al., 1989; Bursi et al., 2001). It should be
197 noted that control measures for feral pigeons based on the administration of any such drugs are very
198 controversial. Some results in terms of a reduction of the population size and improvement in the
199 health status of the birds have been reported in the past, and recently also in the city of Ljubljana,
200 Slovenia (Dovč et al., 2003; Dovč et al., pers. comm. 2006). However, in order to remain effective
201 this measure should be supported by other actions such as a feeding ban (Dobeic, 2003). Overall,
202 there is in fact no clear evidence of a significant long-term effect of the administration of drugs on
203 the reduction of the size of feral pigeon populations.

204 Other measures that have been applied in several European towns and cities for protecting buildings
205 from fouling include pigeon deterring systems such as net-like barriers in front of possible nesting
206 places on the façades of buildings, metal or plastic spikes on preferred resting sites, and
207 electrorepulsive systems. Such measures have been also applied, among others, also in the cities of

208 Zagreb and Paris (Prukner-Radovčić et al., 2005; Laroucau et al., 2005). Although these devices, if
209 properly installed, can effectively prevent most damages to buildings and monuments, they just
210 displace the problem of fouling to other urban areas and have little or no effect at all on the size of
211 the pigeon populations.

212 All experiences up to now have led ultimately to the conclusion that a permanent reduction of feral
213 pigeon populations can only be achieved by reducing their food supply, and the most effective way
214 of achieving this, is through education of the community to not feeding pigeons (Haag-
215 Wackernagel, 1993). Since food supply and availability are in fact the main ecological factors that
216 influence the population size of feral pigeons, the single most important control measure is the
217 enforcement of a feeding ban, which opposes the zoophilic behaviour of pigeon feeders.

218 In the city of Basel, Switzerland, the importance of the restriction of the feeding of feral pigeons has
219 been brought to the attention of the community through an interdisciplinary project of the
220 University of Basel, the government and the Society for the Protection of Animals of Basel (Haag-
221 Wackernagel, 1995). Large information and education campaigns with leaflets, posters and
222 advertisements in media through radio, television and newspapers conveyed the message that
223 feeding pigeons was in fact harmful to the health of the population, as it leads to over-population
224 and ultimately to poor living conditions for many birds. As a special education initiative, supervised
225 pigeon dovecotes were set up, where birds could stay healthy and find shelter, and could be visited
226 and fed by citizens and pigeon fanciers. This intervention proved crucial for illustrating the
227 beneficial effects of the resizing of the population on the health of the individual pigeons.

228

229 **5. Microorganisms harboured by feral pigeons, with an emphasis on chlamydiae**

230

231 Communal health officers, operators in hospitals, schools, railway stations and even prisons, see in
232 feral pigeons a significant hazard for human health and well being (Kösters et al., 1991). A wealth
233 of publications provides firm evidence that feral pigeons are indeed the source of a large number of

234 zoonotic agents. Epidemiological studies in feral pigeon populations detected at least 110 organisms
235 that are pathogenic to humans (supplemented data according to Haag-Wackernagel and Moch,
236 2004). Eight of them were viruses, 41 bacteria, 55 fungi and 6 protozoa. However, of these human
237 pathogens harboured by feral pigeons, only seven (namely *Salmonella enterica* serovar *Kiambu*,
238 *Chlamydophila psittaci*, *Aspergillus* spp., *Candida parapsilosis*, *Cryptococcus neoformans*,
239 *Histoplasma capsulatum* and *Toxoplasma gondii*) caused a total of 230 human infections, of which
240 13 had a fatal outcome (supplemented data according to Haag-Wackernagel, 2006a; 2006b).

241 Natural infections by *C. psittaci* widely occur in many avian species. In a recent review, Kaleta and
242 Taday (2003) have listed 467 species belonging to 30 orders of birds where *C. psittaci* has been
243 identified. The associated disease may cause significant morbidity and mortality in companion birds
244 and in poultry, and the infection can be transmitted to humans, where clinical signs may also be
245 severe.

246 *C. psittaci* commonly infects feral pigeons worldwide. Almost all investigations carried out
247 worldwide in a representative sample size of feral pigeon populations identified some birds
248 seropositive to *C. psittaci*. In 51 investigations of feral pigeon populations carried out from 1966 to
249 2006, a mean seroprevalence rate of 42.3 % was found with a minimum detection rate of 10 % and
250 a maximum of 95.6 %. Chlamydiae-excreting feral pigeons are often detected as well. In 14
251 investigations, detection of chlamydial antigen was successful in 13.2 % of feral pigeon specimens
252 with a range of values from 0 to 33.3 % (supplemented data according to Haag-Wackernagel,
253 2005).

254 Most infected feral pigeons are asymptomatic and latent carriers of *C. psittaci*. Shedding of the
255 organism occurs in faeces as well as in respiratory and conjunctival secretions, often intermittently
256 and without clinical signs, which makes it difficult to assess the risk of transmission of *C. psittaci* to
257 other animals, including humans. Increased shedding of chlamydiae may be triggered by stress
258 factors such as other concurrent infections or infestations, lack of food, breeding and overcrowding
259 (Andersen and Vanrompay, 2003; NASPHV, 2006). The elementary body (EB), which is the

260 infectious form of *C. psittaci* shed from the birds, can retain its infectivity for months under suitable
261 environmental conditions (Albrecht et al., 2003) and may travel long distances, carried by the wind
262 (Kukowka et al., 1960). Overt disease with clinical signs of depression, serous conjunctivitis,
263 blepharitis, rhinitis and diarrhea has been reported in pigeons (Andersen and Vanrompay, 2003).
264 Lesions observed at necropsy may include conjunctivitis, hyperemia and enlargement of spleen,
265 hyperemia and degeneration of liver, enteritis and airsacculitis (Pavlak et al., 2000).

266

267 **6. Direct diagnosis of chlamydial infections in feral pigeons**

268

269 Chlamydiae can be mainly detected in pigeon faeces, cloacal swabs or smears from the surface of
270 viscera such as liver, spleen and lung by simple laboratory methods, i.e. by staining with several
271 techniques including Giménez, Machiavello, or modified Ziehl-Neelsen (Machiavello, 1937; Stamp
272 et al., 1950; Giménez, 1964; Quinn et al., 1994) or with a direct immunofluorescence assay (DIF).
273 The isolation of *C. psittaci* in tissue culture or in embryonated chicken eggs is still referred to as the
274 gold standard for the direct diagnosis and is the preferred diagnostic method according to OIE
275 (Andersen, 2004), but it requires specialized laboratories and expertise and is time-consuming and
276 expensive. In addition, the test requires that the viability of chlamydiae has been preserved with a
277 suitable transport media when samples are collected and forwarded to the laboratory (Andersen,
278 1998). The use of the Buffalo Green Monkey (BGM) cell line is recommended for the isolation of
279 avian chlamydial strains (Vanrompay et al., 1992). Chlamydial inclusions in the cytoplasm of the
280 infected cells are visualized by specific staining procedures such as Giemsa (Giemsa, 1902; 1904)
281 or Giménez (1964), or by immunofluorescence. Immunochromatographic (ICT) and ELISA tests
282 for the detection of chlamydial antigens can be used as well and are quick and easy to perform
283 (Fudge, 1991). These two methods can be used also for the detection of non-viable chlamydiae.
284 However, most of the commercially available kits based on these methods were originally
285 developed for detecting chlamydial species other than *C. psittaci* in human samples. Thus, their

286 reliability for testing animal samples (and especially faeces) is generally lower than culture-based
287 and molecular methods. In particular, false-positive results may occur due to the cross-reactivity
288 with the lipopolysaccharide (LPS) antigen of other Gram-negative bacteria (Vanrompay et al.,
289 1994; Andersen, 2004).

290 As an alternative to the cultivation of chlamydiae, molecular methods have been adopted in many
291 laboratories in the last few years. In particular, several PCR protocols have been made available.
292 The genomic targets for the PCR assays include the single *ompA* and *ompB* genes (Hewinson et al.,
293 1997; Kaltenböck et al., 1997; Yoshida et al., 1998; Hartley et al., 2001; Sachse and Hotzel, 2003),
294 the 16S rRNA gene (Ossewaarde and Meijer, 1999) or the *pmp* gene family (Laroucau et al., 2001;
295 2007). Recently, real-time PCR protocols have been also recommended, targeting either conserved
296 *Chlamydiaceae* genomic sequence (Ehrlich et al., 2006) or a species-specific and genotype-specific
297 *C. psittaci* genomic sequences (Geens et al., 2005b; Heddema et al., 2006a; Pantchev et al., 2008).
298 These tests are very sensitive and their detection limits has been found to be equivalent to a few
299 genomic copies of chlamydiae (Geens et al., 2005b; Ehrlich et al., 2006; Heddema et al., 2006a).
300 Very recently, two DNA microarrays for the detection of chlamydiae were developed. One targets
301 the 23S rRNA gene of the *Chlamydiaceae* family and allows species identification (Sachse et al.,
302 2005), while the other has been specifically developed for *ompA*-based *C. psittaci* genotyping
303 (Sachse et al., 2008).

304 Among all chlamydial species, only *C. psittaci* has been detected in pigeons so far. Isolates of this
305 species have been grouped into serovars by a microimmunofluorescence assay employing serovar-
306 specific monoclonal antibodies (MAbs) directed against the major outer membrane protein
307 (MOMP). Six avian serovars (A to F) are currently recognized (Andersen, 1991), and at least three
308 of them infect pigeons. Serovar B is considered to be host specific and the most prevalent pigeon-
309 associated serovar worldwide (Vanrompay et al., 1993; Hoop et al., 2002; Andersen, 2005;
310 Laroucau et al., 2007). Serovar E also commonly infects pigeons. Initially, it was detected less
311 frequently compared to serovar B both in the US (Andersen, 2005) and in Europe (Vanrompay et

312 al., 1993; 1997; Duan et al., 1999). Serovar A, which is commonly associated with psittacine birds,
313 has also been detected in feral pigeons (Vanrompay et al., 1993).

314 Besides serotyping, a genotyping procedure consisting of restriction enzyme analysis of the PCR-
315 amplified MOMP gene (*ompA*) of chlamydiae (restriction fragment length polymorphism or RFLP
316 analysis) has been introduced for typing of avian *C. psittaci* strains (Sayada et al., 1995). This
317 technique is highly reproducible and can be directly applied to clinical samples without the need for
318 culturing the organism. However, a major drawback is its limited discriminatory ability compared
319 to other genotyping methods. In fact, PCR-RFLP lacks high sensitivity since the DNA content of
320 the sample may not be high enough to generate large amounts of amplified product and
321 unambiguous restriction cleavage patterns. In addition, this technique fails to recognise the new
322 genotype E/B or any of the atypical *C. psittaci* strains.

323 Seven genotypes in total, from A to F and an additional E/B recognized by nucleotide sequencing,
324 have been identified in birds. Genotypes A, B, C, D, E and E/B have all been detected in pigeons,
325 and mixed infections with different genotypes have been documented as well (Geens et al., 2005a).
326 Serovars and genotypes are closely related (Table 1). All *C. psittaci* genotypes can be transmitted to
327 humans (Andersen and Vanrompay, 2003; Geens et al., 2005b; Heddema et al., 2006c; Gaede et al.,
328 2008), including the recently described genotype E/B, whose zoonotic transmission from parrots
329 and from turkeys has been just reported (Harkinezhad et al., 2007; Verminnen et al., 2008).

330 Besides the current *C. psittaci* classification based on the *ompA* gene sequence, a novel approach
331 based on the identification of tandem repeats in DNA (multilocus variable number of tandem
332 repeats analysis or MLVA) has been recently applied to *C. psittaci*. This method targets eight
333 distinct genomic areas dispersed throughout the genome, none of them being localised within the
334 *ompA* gene (Laroucau et al., 2008). So far, isolates from pigeons have yielded four distinct MLVA
335 patterns (number 1, 7, 12 and 19).

336 For more detailed reading on diagnostic issues, the reader is referred to the review "Recent
337 developments in the laboratory diagnosis of chlamydial infections" in this volume.

338

339 7. Serological diagnosis of chlamydial infections in feral pigeons

340

341 The most widely used serological test for detection of antibodies to *C. psittaci* in pigeons is the
342 complement fixation test (CFT), which is the standard test for chlamydial antibodies in birds
343 according to the OIE (Andersen, 2004). The test was originally described by Bedson (1935), and it
344 can be used for analysing sera from pigeons, as recommended by Page (1975). The CFT has been
345 generally considered suitable for the analysis of sera from pigeons since it often detects both high
346 percentages of seropositivity in the sampled population and high antibody titers in individual
347 pigeons. However, this test has some important limitations. It only detects antibodies capable of
348 fixing the complement and directed to a group-specific chlamydial antigen, while other serological
349 methods can detect all IgG capable of binding the antigen, as well as species- and type-specific
350 antibodies (Salinas et al., 1993b). As an alternative to the CFT, an indirect immunofluorescence test
351 (IIF) and a microimmunofluorescence test (MIF) have been also employed (Salinas et al., 1993b;
352 Dovč, 1995; Donati et al., 2006). Moreover, the use of an ELISA based on the chlamydial LPS,
353 which is more sensitive than the CFT, easier to standardize and more suitable for large-scale
354 epidemiological studies, has been also recommended (Schmeer, 1983; Fudge, 1991).

355 For more detailed reading on serological methods, the reader is referred to the review "Recent
356 developments in the laboratory diagnosis of chlamydial infections" in this volume.

357

358 8. Data on chlamydial infections in feral pigeon populations across Europe

359

360 Tables 2 and 3 illustrate laboratory data from surveys on *C. psittaci* infections carried out in the
361 feral pigeon populations of 11 European countries over the last 30 years.

362 Overall, when considering data from all countries, seropositivity values to *C. psittaci* in the sampled
363 populations ranged from 19.4 % to 95.6 %. Four different methods were used for the detection of

364 antibodies, with CFT being the most frequently employed (6 countries) followed by ELISA and IIF
365 (2 countries each) and MIF (one country). In one survey, a pool of sera was examined with three
366 serological methods (CFT, MIF and ELISA) for comparison. With CFT, seropositivities ranged
367 from 19.4 % to 66.3 % with a median of 46.1 %, while similar values were obtained in surveys
368 employing the indirect immunofluorescence test (23.7 – 67.7 %). The highest seropositivities,
369 ranging from 56 % to 95.6 %, were reported with the LPS-based ELISA.

370 In most surveys, *C. psittaci* was also detected using direct methods in a percentage of up to 50 % of
371 the examined samples. Direct detection was carried out either from cloacal or combined
372 conjunctival/choanal/cloacal swabs of live birds, or from the intestinal content or viscera collected
373 at necropsy. Pigeon droppings were examined in one survey only. The isolation of *C. psittaci* in cell
374 culture or embryonated chicken eggs was attempted in 8 countries, and yielded positive results in 2
375 - 42.3 % and 0 - 57.1 % of samples, respectively. Non-culturable antigen detection methods were
376 employed in 4 countries and were positive in 2.3 – 40 % of samples. As to molecular methods,
377 several PCR protocols were applied, with genomic targets as diverse as the *ompA*, the 16S rRNA
378 and the 23S rRNA genes, which allowed the detection of *C. psittaci* in 3.4 - 50 % of the samples.
379 Genotyping was carried out on 25 *C. psittaci* strains detected in pigeons sampled in 3 countries.
380 Fourteen of them were assigned to genotype B, 10 to genotype E and one to genotype E/B.

381

382 **9. Comments on the different methods employed for diagnosing chlamydial infections in feral** 383 **pigeons**

384

385 Overall, chlamydial infections are widespread in the feral pigeon populations of several European
386 towns and cities. Indeed, in most of them, moderate to high percentages of seropositivity to *C.*
387 *psittaci* have been detected for several years. The results of the serological investigations in some
388 countries may not be directly comparable due, among others, to the different cut-offs used in the
389 analysis, yet they clearly indicate that European feral pigeons are frequently exposed to *C. psittaci*.

390 This finding is not unexpected, since all investigations performed worldwide in a representative
391 number of birds have demonstrated the detection of chlamydial antibodies in feral pigeons. In most
392 surveys mentioned in this communication, serological investigations have been carried out using
393 CFT, which has for a long time proven to be a suitable method, since pigeon sera are able to fix
394 guinea pig complement, unlike sera from other birds such as turkeys and some parrot species.
395 ELISA, IFI and MIF have been also used, albeit less frequently. Limited data have been published
396 concerning the comparison of the sensitivity and specificity of serological methods for the detection
397 of anti-*C. psittaci* antibodies in pigeon sera. Milon et al. (1983) and Trap et al. (1986) compared IIF
398 and CFT, and concluded that IIF was more sensitive than CFT for the analysis of pigeon sera, but
399 also suggested that the cut-off of 1:40 for the IIF was too high. Ceglie et al. (2007) recently reported
400 good agreement between the CFT and the MIF and again detected more positive samples with the
401 latter test. Eidebenz (1990) tested a blocking antibody ELISA against the CFT and found that the
402 former was specific and more sensitive than CFT. Salinas et al. (1993b) compared the performance
403 of five serological methods (CFT, indirect CFT, IIF, MIF and ELISA) for the detection of
404 chlamydial antibodies in pigeon sera. Taking the IIF as a reference method, they found that the
405 ELISA and MIF were more sensitive and allowed the detection of more positive samples than the
406 CFT, but the ELISA was found to be less specific than the other methods. Overall, serological
407 methods other than the CFT have shown a higher sensitivity, but still needs to be fully evaluated as
408 to their specificity (Andersen, 2004).

409 In the surveys considered in this communication, infection with *C. psittaci* has been frequently
410 demonstrated directly with both non-cultural and molecular methods. In addition, the carriage of
411 viable organisms by apparently healthy birds has been ascertained in some cases by isolation of *C.*
412 *psittaci* in cell culture or embryonated eggs. The cultural method is highly sensitive provided that
413 the viability of chlamydiae in the sample has been preserved, while the use of non-culturable
414 antigen detection methods has been questioned in the last few years due to the possible occurrence
415 of false-positive results arising from cross-reactivity with other bacterial antigens. On the other

416 hand, some of the recently developed molecular methods, i.e. PCR-RFLP, real-time PCR and DNA
417 microarrays, look particularly attractive for their specificity, sensitivity and flexibility, since they
418 allow more rapid detection and typing of *C. psittaci* without the need for culturing the organism,
419 which also makes them safer. There is no consensus at the moment for recommending a single PCR
420 assay for the diagnosis of avian (including pigeon) chlamydiosis. However, in a comparison of
421 different conventional PCR protocols, the PCR assay targeting the *pmp* gene family has been
422 recently found highly specific and more sensitive, up to 10 times, than assays targeting other
423 chlamydial genes (Laroucau et al., 2007). Real-time PCR protocols are characterized by high
424 sensitivity and also allow quantification of genome copy numbers in the samples. A validation
425 study has recently compared conventional PCR, real-time PCR, immunohistochemistry, cell culture
426 and a DNA microarray assay. Sensitivities of microarray testing and real-time PCR have been found
427 to be equivalent (Borel et al., 2008).

428

429 **10. The zoonotic relevance of chlamydiae acquired from feral pigeons**

430

431 The most important pathogenic organism transmissible from feral pigeons to humans is *C. psittaci*,
432 the agent of avian chlamydiosis in birds and psittacosis (also known as ornithosis) in humans. The
433 clinical presentations of the disease in humans range from a mild influenza-like illness to a severe
434 atypical pneumonia and systemic disease with extra-pulmonary involvement. Humans get infected
435 by inhalation of aerosols contaminated with faecal dust, feather particles or dried excreta from
436 infected birds (Leopold, 1965).

437 In 1941, Meyer described the first case of transmission of *C. psittaci* from feral pigeons to humans.
438 A mother and her daughter had picked up a sick feral pigeon in the street in New York City. The
439 pigeon died after 4 days and, two weeks later, both mother and daughter developed ornithosis with
440 fever and pneumonia. Two thirds of the feral pigeons examined in their environment were positive
441 for *C. psittaci* (Meyer, 1941). Since the first description, a number of case reports have

442 demonstrated the successful transmission of chlamydiae from pigeons to humans (for references,
443 see Süß et al., 1996). A recent extensive search of the literature identified 101 case reports of
444 ornithosis in humans where the route of transmission could be traced to a contact with feral pigeons
445 (Haag-Wackernagel and Moch, 2004).

446 In most cases (95 %), detailed information has been provided as to the circumstances of interaction
447 between humans and pigeons (supplemented data according to Haag-Wackernagel, 2006a; 2006b).

448 Overall, 53 % of all reported cases of disease in humans could be referred to a close contact with
449 feral pigeons or their excreta as detailed hereafter. About one fourth (27 %) of cases was related to
450 occupational exposure to contaminated dust, while fewer cases (15 %) followed the handling of sick
451 or dead pigeons. Fatalities – one case each - following both exposures were reported. Few cases (11
452 %) were linked to the habit of feeding pigeons. On the other hand, loose or transient contact with
453 feral pigeons were mentioned in 43 cases (42 %) of human disease, of which 11 were children and 6
454 were immunosuppressed patients. In this case, the activities leading to infection included, e.g.,
455 eating lunch in a park frequented by pigeons, walking through a pigeon flock, and living in a
456 neighbourhood frequented by pigeons. Only in a limited percentage of cases (5 %) no information
457 was provided as to the nature and circumstances of the contact with pigeons.

458 Demolition/construction labourers may get professionally exposed to *C. psittaci*-contaminated dust
459 when they work in parts of buildings such as over roofs, in garrets and close to gutters where pigeon
460 faeces have accumulated. Inside such buildings, the beating of wings of pigeons may further
461 contribute to build up and spread contaminated aerosols that are especially hazardous. Children may
462 be particularly exposed to the risk of infection when they handle sick birds, as they may be prone to
463 give them some shelter and assistance. Feeding pigeons may lead to exposure to chlamydiae when
464 birds congregate and spread contaminated dust with the beating of their wings, or when pigeon
465 feeders indulge in intimate contacts with the birds.

466 Loose or transient contact with feral pigeons leading to disease in humans is difficult to identify and
467 document, but may indeed be relevant, as shown by the analysis of the occurrence of the disease in

468 people only temporarily exposed to infected birds or contaminated aerosols, e.g. customs officers
469 transiently exposed to imported parrots (De Schrijver, 1995) and veterinarians visiting a duck
470 processing plant on a single occasion (Palmer et al., 1981; Kaleta, pers. comm. 2008).

471 Although the prevalence of chlamydial infections in feral pigeons is consistently high across
472 Europe, the actual risk for humans of acquiring psittacosis from these birds is difficult to quantify.
473 In general, the relevance of feral pigeons as a source of zoonotic chlamydiae is poorly understood.
474 It is somehow puzzling to note that in spite of the exceptionally wide distribution of *C. psittaci* in
475 feral pigeon populations and the variety of possible contacts with humans, only very few cases of
476 transmission of *C. psittaci* from feral pigeons to humans have been reported worldwide. One
477 possible underlying reason is that many pigeon-derived *C. psittaci* strains may not be highly
478 pathogenic in humans, or at least not as pathogenic as the strains commonly encountered in other
479 birds, e.g. parrots, ducks and turkeys. In this scenario, pigeon-borne psittacosis in humans would
480 often be undetected or misdiagnosed, due to the associated poor clinical or non-specific influenza-
481 like signs. Actually, feral pigeons are known to harbour a variety of genotypes of *C. psittaci*. In the
482 surveys of feral pigeons whose results are summarized in this communication, only genotypes B, E,
483 and E/B have been detected. Other genotypes that may occur in pigeons, namely A, C and D, which
484 are often present in parrots, ducks and turkeys, respectively, and have been associated with more
485 severe disease in humans, have not been identified. The recently described occurrence of a mild
486 form of psittacosis in humans infected with *C. psittaci* genotype E/B provides evidence that mild
487 disease induced by *C. psittaci* in humans may be overlooked (Harkinezhad et al., 2007). The
488 genotyping of additional strains of *C. psittaci* recovered from feral pigeons is needed in order to
489 assess the relative prevalence of each genotype in these avian populations and ultimately to trace
490 human cases of psittacosis to infections in this animal reservoir.

491 On the other hand, it may be difficult to unequivocally trace a human case of ornithosis to contact
492 with feral pigeons, since contact with other *C. psittaci*-infected free-living birds that dwell close to
493 humans may have simultaneously occurred. For example, free-living tits (*Parus major* and other

494 *Paridae*) are frequently infected with chlamydiae (Holzinger-Umlauf et al., 1997) and they too
495 might be a source of infection for humans.

496 Assessing the risk of acquiring psittacosis from feral pigeons is also difficult because there is a lack
497 of information and understanding about the mechanism of infection of humans through loose and
498 transient contact with these birds. Research is ongoing to clarify this issue, as well as investigations
499 on indirect ways of transmission of *C. psittaci* from feral pigeons. For example, the relevance of
500 additional transmission routes of *C. psittaci* to humans in the urban environment, such as the
501 inhalation of contaminated water droplets from public fountains where feral pigeons regularly
502 bathe, is currently being investigated at the University of Basel (Geigenfeind and Haag-
503 Wackernagel, pers. comm. 2007).

504

505 **11. Recommendations aimed at preventing pigeon-related psittacosis in humans**

506

507 The degree of exposure to feral pigeons and their excreta, as well as the susceptibility to *C. psittaci*
508 is not homogeneous in the human population. Thus, specific measures for the prevention of feral
509 pigeon-related cases of psittacosis in humans should be adopted at different levels.

510 Education initiatives to communicate the health risks and recommendations for minimizing this risk
511 should be primarily directed at occupationally exposed groups, such as demolition/construction
512 labourers that are exposed to dust contaminated with pigeon excreta. Preventive measures for these
513 categories include wearing protective clothes with hoods, boots, gloves and P2 or P3 air filter face
514 masks when removing pigeon faeces from roofs, garrets and buildings, especially if working
515 indoors. Keeping the pigeon droppings damp while removing them is a simple hygienic measure
516 that helps reduce the risk of inhaling *C. psittaci*-contaminated dust. After work, all clothing should
517 be disposed of, or disinfected in case of future intended use. In Switzerland and Germany, clear
518 guidelines have been published for the prevention of psittacosis when working in areas frequented
519 by feral pigeons and contaminated with their excreta (Tiefbau-Berufsgenossenschaft, 2006).

520 Monitoring for *C. psittaci* infections over time, by direct detection of the organism and/or by
521 specific antibody testing, should also be considered for this category of workers.

522 It may be also speculated that other workers in the urban environment, such as street sweepers and
523 traffic wardens might be particularly exposed to *C. psittaci* through inhalation of dust contaminated
524 with pigeon excreta. However, no information is available as to an increased risk of infection in this
525 group compared to the general population. Targeted studies might be helpful to clarify this issue.

526 Recommendations should also be directed to vulnerable sections of the population that may develop
527 severe clinical manifestations after exposure to *C. psittaci*. Accordingly, children should be warned
528 not to handle sick or dead pigeons and immunocompromised individuals should be educated to
529 carefully limit their contact with feral pigeons and enforce strict hygienic procedures when dealing
530 with the birds.

531 In many European towns and cities, a reduced and healthier population of feral pigeons should be
532 included among the aims of administrators and health officers, as a general intervention for
533 preserving urban hygiene. The management of feral pigeon populations in the urban environment is
534 a complex issue that requires careful planning. Before any intervention, an evaluation of the local
535 situation as to the number of birds and their aggregation sites is mandatory. Fencing of buildings
536 with pigeon deterring systems such as net-like structures and other mechanical devices represents a
537 first-line intervention measure for preventing fouling. Administration of contraceptive drugs may be
538 useful for reducing the bird population, but this measure is unlikely to lead to a permanent solution
539 and should be coupled with others, in particular with a feeding ban. Pigeon feeders should be
540 encouraged to stop or limit their activity by at least enforcing a feeding ban in defined urban areas
541 that are close to hospitals, railway stations, kindergardens and prisons, where avoidance of pigeon
542 aggregation is considered as a priority. Building dovecotes and artificial breeding facilities may be
543 also considered for providing a balanced diet to the pigeons and a chance of interaction between
544 pigeon lovers and the birds in a hygienically controlled environment. The personnel attending
545 dovecotes should be adequately informed about the health risks arising from contact with pigeons

546 and be regularly monitored for *C. psittaci* infections by DNA or antigen detection methods and/or
547 by antibody testing. For the sake of animal protection, overtly sick birds should be captured and
548 taken into veterinary care. In case chlamydiosis is confirmed, the birds should be appropriately
549 treated with effective drugs such as tetracyclines (chlortetracycline, doxycycline), quinolones
550 (enrofloxacin, difloxacin) or macrolides (clarithromycin) (Theis, 2007; Kinndle, 2007). In the case
551 of very poor conditions, the birds should be euthanized in order to adapt the population to the
552 reduced food supply resulting from public restriction of feeding. Education initiatives directed to
553 the general public are strongly encouraged to illustrate the relationship between feeding,
554 overcrowding, and the deterioration of living conditions of pigeons. In this context, reliable and
555 unbiased information concerning the health hazards arising from the uncontrolled increase of feral
556 pigeon populations should also be provided to the citizens through a variety of media. Regular
557 interaction with the associations involved in the protection of animal welfare and health, such as the
558 Society for the Protection of Animals, is recommended in order to illustrate the implementation of
559 regulatory measures which need to be adopted. Education and information are fundamental, since
560 the imposition of feeding bans usually does not prove successful given the solidarity that pigeon
561 feeders tend to get from the general population. In this scenario, the usefulness of sanctions for
562 those who defy the ban is questionable, since they might actually prove ineffective as to their
563 intended scope of controlling the bird populations.

564

565 **12. Conflict of interest statement**

566

567 None of the authors (Magnino, Haag-Wackernagel, Geigenfeind, Helmecke, Dovč, Prukner-
568 Radovčić, Residbegović, Ilieski, Laroucau, Donati, Martinov, Kaleta) has a financial or personal
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570 entitled “Chlamydial infections in feral pigeons in Europe: review of data and focus on public
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572

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579

580 **References**

581

582 Albrecht, A., Schies, U., Kämpfer, P., Scholbeck, R., 2003. Gesundheitsgefährdung durch
583 Taubenkot. Sonderdruck aus TIEFBAU, Tiefbau-Berufsgenossenschaft, München; Heft 5/2001 und
584 3/2002, 1–14.

585 Andersen, A.A., 1991. Serotyping of *Chlamydia psittaci* isolates using serovar-specific monoclonal
586 antibodies with the microimmunofluorescence test. J. Clin. Microbiol. 29 (4), 707-711.

587 Andersen, A.A., 1997. Two new serovars of *Chlamydia psittaci* from North American birds. J. Vet.
588 Diagn. Invest. 9, 159-164.

589 Andersen, A.A., 1998. Chlamydiosis. In: Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson,
590 J.E. and Reed W.M. (eds.). A laboratory manual for the isolation and identification of avian
591 pathogens, 4th edition. American Association of Avian Pathologists, University of Pennsylvania,
592 New Bolton Center, Kennett Square, PA, p. 81-88.

593 Andersen, A.A., 2004. Avian chlamydiosis. In: Manual of Diagnostic Tests and Vaccines for
594 Terrestrial Animals (Terrestrial Manual), 5th Edition, Office International des Epizooties, Paris,
595 France, p. 856-867.

- 596 Andersen, A.A., 2005. Serotyping of US isolates of *Chlamydophila psittaci* from domestic and wild
597 birds. J. Vet. Diagn. Invest. 17, 479-482.
- 598 Andersen, A.A., Vanrompay, D., 2003. Avian chlamydiosis (psittacosis, ornithosis). In: Saif, Y.M.
599 (ed.), Diseases of Poultry, 11th edition, Iowa State University Press, Iowa, USA, p. 863-879.
- 600 Ballarini, G., Baldaccini, N.E., Pezza, F., 1989. Colombi in città, Aspetti biologici, sanitari e
601 giuridici. Metodologie di controllo. Istituto Nazionale di Biologia della Selvaggina, Documenti
602 Tecnici 6, 1-58.
- 603 Barbieri, F., Caldonazzi, M., Zanghellini, S., Zorzi, S., 1997. The street pigeon (*Columba livia*
604 forma *domestica*) in the municipality of Trento. Centro di Ecologia Alpina. Report n. 7. p. 1-62.
- 605 Bassi, M., Chiatante, D., 1976. The role of pigeon excrement in stone biodeterioration. Int.
606 Biodeterior. Bull. 12 (3), 73-79.
- 607 Bedson, S.P., 1935. The use of complement-fixation reaction in the diagnosis of human psittacosis.
608 Lancet 2, 1277-1280.
- 609 Borel, N., Kempf, E., Hotzel, H., Schubert, E., Torgerson, P., Slickers, P., Ehricht, R., Tasara, T.,
610 Pospischil, A., Sachse, K., 2008. Direct identification of chlamydiae from clinical samples using a
611 DNA microarray assay – a validation study. Mol. Cell. Probes 22, 55-64.
- 612 Bursi, E., Gelati, A., Ferraresi, M., Zannetti, G., 2001. Impiego della nicarbazina nel controllo della
613 riproduzione del colombo randagio di città. Ann. Fac. Med. Vet. Univ. Parma 21, 97-115.
- 614 Ceglie, L., Lafisca, S., Guadagno, C., Dalla Pozza, G., Capello, K., Bano, L., Vicari, N., Donati, M.,
615 Mion, M., Giurisato, I., Lombardo, D., Pozzato, N., Cevenini, R., Natale, A., 2007. Serological
616 surveillance in north-eastern Italy for the presence of *Chlamydophila* spp. from birds and molecular
617 characterisation of PCR isolates within the area of Venice. In: Proceedings of the 5th Annual
618 Workshop of COST Action 855 “Animal chlamydioses and its zoonotic implications”, Pulawy
619 (Poland), September 10-11, 2007, p. 62-67.

- 620 Cerri, D., Andreani, E., Salvi, G., Perelli G., 1989. The pigeons as carriers of some pathogenic
621 agents for man and animals. In: Proceedings of the International Symposium “Environmental
622 pollution and animal populations”. Pisa, Italy, October 3-4, 1989, p. 195-202.
- 623 Del Monte, M., Sabbioni, C., 1986. Chemical and biological weathering of an historical building:
624 Reggio Emilia Cathedral. *Sci. Total Environ.* 50, 165–182.
- 625 Dell’Omo, A., 1996. Il ruolo degli escrementi dei piccioni nella bioalterazione delle rocce. Tesina
626 di Università degli Studi di Roma “La Sapienza”, Facoltà di Scienze Matematiche, Fisiche e
627 Naturali, Dipartimento di Biologia Animale e dell’Uomo: 18 pages.
- 628 De Schrijver, K., 1995. A psittacosis outbreak in Belgian customs officers. *Euro Surveill.* 0 (0), 3.
629 Available online: <http://www.eurosurveillance.org/em/v00n00/0000-222.asp>
- 630 Dobeic, M., 2003. Regulation of population size of street pigeons in Ljubljana, Slovenia. *Acta Vet.*
631 (Beograd) 53 (2-3), 171-182
- 632 Donati, M., Renzi, M., Magnino, S., Di Francesco, A., Rinieri, G., Pignanelli, S., Cevenini, R.,
633 2006. In vitro detection of neutralizing antibodies to *Chlamydophila psittaci* in pigeon sera with a
634 microneutralization test. In: Proceedings of the 4th Annual Workshop of COST Action 855 “Animal
635 chlamydioses and its zoonotic implications”, Edinburgh, Scotland (UK), September 3-5, 2006, p.
636 113-114.
- 637 Dovč, A., 1995. Introduction of immunofluorescence method in diagnosing chlamydiosis
638 (*Chlamydia psittaci*) and the epidemiology of the disease in parrots and pigeons. *Slov. Vet. Res.* 32
639 (2), 257-276 (In Slovenian with English abstract and summary).
- 640 Dovč, A., Račnik, J., Pišot, J., Cestnik, V., Čebulj-Kadunc, N., Dobeic, M., 2003. Progesterone
641 concentration in the sera of pigeon treated with Ornisteril. In: 7th European A.A.V. Conference. 5th
642 ECAMS Scientific Meeting in cooperation with Loro Parque & Loro Parque Fundación. Tenerife:
643 European Committee of the Association of the Avian Veterinarians, p. 73-74.

- 644 Dovč, A., Zorman-Rojs, O., Vergles Rataj, A., Bole-Hribovsek, V., Krapez, U., Dobeic, M., 2004.
645 Health status of free-living pigeons (*Columba livia domestica*) in the city of Ljubljana. Acta Vet.
646 Hung. 52 (2), 219-226.
- 647 Duan, Y.J., Souriau, A., Mahé, A.M., Trap, D., Andersen, A.A., Rodolakis, A., 1999. Serotyping of
648 chlamydial clinical isolates from birds with monoclonal antibodies. Avian Dis. 43, 22-28, 1999.
- 649 Ehricht, R., Slickers, P., Goellner, S., Hotzel, H., Sachse, K., 2006. Optimized DNA microarray
650 assay allows detection and genotyping of single PCR-amplifiable target copies. Mol. Cell. Probes
651 20, 60-63.
- 652 Eidebenz, C., 1990. Comparative studies on the demonstration of antibodies against *Chlamydia*
653 *psittaci* using CF and ELISA. In: Proceedings, VII Tagung über Vogelkrankheiten. Munich, Federal
654 Republic of Germany. p. 290-294.
- 655 Everett, K.D., Bush, R.M., Andersen, A.A., 1999. Emended description of the order *Chlamydiales*,
656 proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one
657 monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five
658 new species, and standards for the identification of organisms. Int. J. Sys. Bacteriol. 49 Pt 2: 415-
659 440.
- 660 Fudge, A.M., 1991. ELISA testing for avian chlamydiosis. Vet. Clin. North Am. Small Anim. Pract.
661 21 (6), 1181-1187.
- 662 Gaede, W., Reckling, K.-F., Dresenkamp, B., Kenklies, S., Schubert, E., Noack, U., Irmischer, H.-
663 M., Ludwig, C., Hotzel, H., Sachse, K., 2008. *Chlamydophila psittaci* infections in humans during
664 an outbreak of psittacosis from poultry in Germany. Zoonoses Public Health 55 (4), 184-188.
- 665 Gaffuri, A., Garbarino, C., Consoli, T., Carrara, M., Broussard, D., Pietra, V., Magnino, S.,
666 Paterlini, F., 2000. Study on the health status of pigeons in Bergamo province. Sel. Vet. 8-9/2000,
667 827-830.

- 668 Garrity, G.M., Bell, J.A., Lilburn, T.G., 2004. Taxonomic Outline of the Prokaryotes. *Bergey's*
669 *Manual of Systematic Bacteriology*, Second Edition, Release 5.0, Springer-Verlag, New York.
670 p.302. DOI: 10.1007/bergeysoutline200405
- 671 Geens, T., Desplanques, A., Van Loock, M., Bönner, B.M., Kaleta, E.F., Magnino, S., Andersen,
672 A.A., Everett, K.D.E., Vanrompay, D., 2005a. Sequencing of the *Chlamydophila psittaci ompA*
673 gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. *J.*
674 *Clin. Microbiol.* 43 (5), 2456-2461.
- 675 Geens, T., Dewitte, A., Boon, N., Vanrompay, D., 2005b. Development of a *Chlamydophila psittaci*
676 species-specific and genotype-specific real-time PCR. *Vet. Res.* 36, 787-797.
- 677 Giemsa, G., 1902. Färbemethoden für Malariaparasiten. *Centralblatt für Bakteriologie,*
678 *Parasitenkunde und Infektionskrankheiten I. Abteilung, Originale* 32, 307-313.
- 679 Giemsa, G., 1904. Eine Vereinfachung und Vervollkommnung meiner Methylenazur-
680 Methylenblau-Eosin-Färbemethode zur Erzielung der Romanowsky-Nacht'schen Chromatin-
681 Färbung. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten I. Abteilung,*
682 *Originale* 37, 308-311.
- 683 Giménez, D. F., 1964. Staining Rickettsiae in yolk-sac cultures. *Stain Technol.* 39, 135-140.
- 684 Haag, D., 1984. Ein Beitrag zur Ökologie der Stadttaube. Dissertation, Phil.Nat. Fakultät der
685 Universität Basel, Verlag Medizinische Biologie.
- 686 Haag-Wackernagel, D., 1993. Street pigeons in Basel. *Nature* 361, 200.
- 687 Haag-Wackernagel, D., 1995. Regulation of the street pigeon in Basel. *Wildlife Soc. Bull.* 23(2),
688 256–260.
- 689 Haag-Wackernagel, D., 1998. Die Taube. Vom heiligen Vogel der Liebesgöttin zur Strassentaube.
690 Verlag Schwabe & Co. AG, Basel.

- 691 Haag-Wackernagel, D., 1999. The Pigeon. From the Holy Bird of the Goddess of Love to the Feral
692 Pigeon. An English supplementum translated by Derek Goodwin. Verlag Schwabe & Co. AG,
693 Basel, Switzerland.
- 694 Haag-Wackernagel, D., 2002. Feral pigeons: management experiences in Europe. In: Dinetti M.
695 (ed.) Specie ornamentiche problematiche: Biologia e gestione nelle città e nel territorio. Atti 2°
696 Convegno Nazionale sulla Fauna Urbana, Firenze, 10 Giugno 2000, ARSIA e LIPU. Regione
697 Toscana, Firenze, 25–37.
- 698 Haag-Wackernagel, D., 2003. Die Strassentaube: Geschichte – Probleme – Lösungen. Der
699 Ornithologische Beobachter 100, 33–57.
- 700 Haag-Wackernagel, D., 2005. Feral pigeons (*Columba livia*) as a potential source of human
701 ornithosis. In: Proceedings of the 3rd Annual Workshop of COST Action 855 “Animal
702 chlamydioses and its zoonotic implications”, Siena, Italy, September 22-23, 2005, p. 15-17.
- 703 Haag-Wackernagel, D., 2006a. Human diseases caused by feral pigeons. Advances in Vertebrate
704 Pest Management 4, 31-58, 2006. Chris J. Feare & David P. Cowan (eds.), Filander Verlag, Fürth.
- 705 Haag-Wackernagel, D., 2006b. Gesundheitsgefährdung durch die Strassentaube *Columba livia*.
706 Amtstierärztlicher Dienst und Lebensmittelkontrolle 4, 262–272.
- 707 Haag-Wackernagel, D., Moch, H., 2004. Health hazards posed by feral pigeons. J. Infect. 48 (4),
708 307–313.
- 709 Harkinezhad, T., Verminnen, K., Van Droogenbroeck, C., Vanrompay, D., 2007. *Chlamydothyla*
710 *psittaci* genotype E/B transmission from African grey parrots to humans. J. Med. Microbiol. 56,
711 1097-1100.
- 712 Hartley, J.C., Kaye, S., Stevenson, S., Bennett, J., Ridgway, G., 2001. PCR detection and molecular
713 identification of *Chlamydiaceae* species. J. Clin. Microbiol. 39 (9), 3072-3079.

- 714 Heddemma, E.R., Beld, M.G.H.M., de Wever, B., Langerak, A.A.J., Pannekoek, Y., Duim, B., 2006a.
715 Development of an internally controlled real-time PCR assay for detection of *Chlamydomphila*
716 *psittaci* in the LightCycler 2.0 system. Clin. Microbiol. Infect. 12 (6), 571-575.
- 717 Heddemma, E.R., ter Sluis, S., Buys, J.A., Vandenbroucke-Grauls, C.M.J.E., van Wijnen, J.H.,
718 Visser, C.E., 2006b. Prevalence of *Chlamydomphila psittaci* in fecal droppings from feral pigeons in
719 Amsterdam, the Netherlands. Appl. Environ. Microbiol. 72 (6), 4423-4425.
- 720 Heddemma, E.R., Van Hannen, E.J., Duim, B., Vandenbroucke-Grauls, C.M., Pannekoek, Y., 2006c.
721 Genotyping of *Chlamydomphila psittaci* in human samples. Emerg. Infect. Dis. 12 (12), 1989–1990.
- 722 Helmecke, S., 2007. Retrospektive Analyse diagnostizierter Untersuchungsbefunde zu Tauben
723 (*Columba livia* forma domestica) aus dem Zeitraum 1979 bis 2004. Veterinary Dissertation,
724 University of Giessen, 156 pages.
- 725 Hewinson, G.L., Griffiths, P.C., Bevan, B.J., Kirwan, S.E.S., Field, M.E., Woodward, M.J.,
726 Dawson, M., 1997. Detection of *Chlamydia psittaci* DNA in avian clinical samples by polymerase
727 chain reaction. Vet. Microbiol. 54, 155-156.
- 728 Holzinger-Umlauf, H. A.-M., Marschnag R.E., Gravendyck, M., Kaleta, E., 1997. Investigation of
729 *Chlamydia* sp. infections in tits (*Paridae*). Avian Pathol. 26, 779-789.
- 730 Hoop, R.K., Schiller, I., Fraefel, D., 2002. Serotyping of avian *Chlamydomphila psittaci* isolates. Vet.
731 Rec. 150, 315-316.
- 732 Ilieski, V., Naletoski, I., Pendovski, L., Sachse, K., Laroucau, K., Dovč, A., 2007. Detection of
733 *Chlamydiaceae* spp. in clinical samples from pigeons by real time PCR in Macedonia. In:
734 Proceedings of the 5th Annual Workshop of COST Action 855 “Animal chlamydioses and its
735 zoonotic implications”, Pulawy (Poland), September 10-11, 2007, p. 48.
- 736 Kaleta E.F., Hönicke A., 2004. Review of the literature on avian influenza A viruses in pigeons and
737 experimental studies on the susceptibility of domestic pigeons to influenza A viruses of the
738 haemagglutinin subtype H7. Dtsch Tierarztl Wochenschr. 111 (12), 467-472.

- 739 Kaleta, E.F., Taday, E.M.A., 2003. Avian host range of *Chlamydophila* spp. based on isolation,
740 antigen detection and serology. Avian Pathol. 32, 435-462.
- 741 Kaltenböck, B., Schmeer, N., Schneider, R., 1997. Evidence for numerous alleles of porcine
742 *Chlamydia trachomatis* and novel *Chlamydia* species obtained by PCR. J. Clin. Microbiol. 35,
743 1835-1841.
- 744 Kinndle, M.B.P., 2007. Untersuchung der Sensitivität von Chlamydien-Isolaten unter Verwendung
745 verschiedener antibakterieller Wirkstoffe (chlortetracycline, doxycycline, enrofloxacin, diflo-
746 xacin, clarithromycin, erythromycin). Veterinary Dissertation, University of Giessen. 135 pages.
- 747 Kösters, J., Döring, N., Grimm, F., 1994. Bemühungen zur Reduzierung freilebender
748 Stadttaubenpopulationen Verh. Ber. Erkr. Zootiere 36, 352-357.
- 749 Kösters, J., Kaleta, E.F., Monreal, G., Siegmann, O., 1991. Das Problem der Stadttauben. Deutsches
750 Tierärzteblatt 39 (4), 272-276.
- 751 Kukowka, A., Stephan, H., Krebs, W., 1960. Entenfarm als Ausgangspunkt von
752 Ornithoseerkrankungen beim Menschen. Dtsch Gesundheitsw. 15, 2477-2484.
- 753 Laroucau, K., Mahé, H., Bouillin, C., Deville, M., Gandouin, C., Touati, F., Guillot, J., Boulouis,
754 H.J., 2005. Health status of free-living pigeons in Paris. In: Proceedings of the 3rd Annual
755 Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Siena, Italy,
756 September 22-23, 2005, p. 17-18.
- 757 Laroucau, K., Souriau, A., Rodolakis, A., 2001. Improved sensitivity of PCR for *Chlamydophila*
758 using *pmp* genes. Vet. Microbiol. 82, 155-164.
- 759 Laroucau, K., Thierry, S., Vorimore, F., Aaziz, R., Blanco, K., Kaleta, E.F., Hoop, R., Magnino, S.,
760 Vanrompay, D., Sachse, K., Myers, G.S.A., Bavoil, P., Vergnaud, G., Pourcel, C., 2008. High
761 resolution typing of *Chlamydophila psittaci* by Multilocus VNTR Analysis (MLVA). Infect. Genet.
762 Evol. 8 (2), 171-181.

- 763 Laroucau, K., Trichereau, A., Vorimore, F., Mahé, A.M., 2007. A *pmp* genes-based PCR as a
764 valuable tool for the diagnosis of avian chlamydiosis. *Vet. Microbiol.* 121 (1-2), 150-157.
- 765 Leopold, P.G., 1965. Ornithose als Berufskrankheit. *Monatsh. Veterinarmed.* 20 (23), 964–969.
- 766 Machiavello, A. M., 1937. Estudios sobre tifos exantimatico. II. Un nuevo método para tenir
767 *Rickettsia*. *Rev. Chil. Hig. Med. Prev.* 1, 101-106.
- 768 Manfredi, M.T., Fabbi, M., Magnino, S., Rizzoli, A., Genchi, C., 1997. Survey of health status of
769 the street pigeons (*Columba livia* forma *domestica*) in Trento. Centro di Ecologia Alpina. Report n.
770 7. p. 63-68.
- 771 Martinov, S.P., 2006. Avian chlamydiosis in Bulgaria and zoonotic implications. In: Proceedings of
772 the 4th Annual Workshop of COST Action 855 “Animal chlamydioses and its zoonotic
773 implications”, Edinburgh, Scotland (UK), September 3-5, 2006, 159-169.
- 774 Martinov, S.P., Kazachka, D., Shoilev, H., Runevska, P., 1997. Studies on the *Chlamydia psittaci*
775 infection in birds and humans. *Biotechnol. Equip.* 11(1), 74-80.
- 776 Mendez-Tovar, L.-J., Mainou, L.-M., Pizarro, S.-A., Fortoul-Vandergoes, T., Lopez-Martinez, R.,
777 1995. Fungal biodeterioration of colonial facades in Mexico City. *Rev. Mex. Micol.* 11 (0), 133–
778 144.
- 779 Meyer, K.F., 1941. Pigeons and barn yard fowls as possible sources of human psittacosis or
780 ornithosis. *Schweiz. Med. Wochenschr.* 44, 1377–1379.
- 781 Milon, A., Geral, M.F., Pellerin, J.L., Thises, I., Lautie, R., 1983. Enquête sur le portage et
782 l'excrétion de *Chlamydia psittaci* par les pigeons semi-domestiques (*Columba livia*) de
783 l'agglomération toulousaine. *Rev. Med. Vet.* 134 (10), 559-565.
- 784 Mitevski, D., Pendovski, L., Naletoski, I., Ilieski, V., 2005. Surveillance for the presence of
785 *Chlamydomphila psittaci* in pigeons and doves from several towns in Macedonia. In: Proceedings of

- 786 the 3rd Annual Workshop of COST Action 855 “Animal chlamydioses and its zoonotic
787 implications”, Siena, Italy, September 22-23, 2005, p. 141-145.
- 788 National Association of State Public Health Veterinarians (NASPHV), 2006. Compendium of
789 measures to control *Chlamydophila psittaci* infection among humans (psittacosis) and pet birds
790 (avian chlamydiosis), 2006. Available at <http://www.nasphv.org/83416/index.html>
- 791 Ossewaarde, J.M., Meijer, A., 1999. Molecular evidence for the existence of additional members of
792 the order *Chlamydiales*. *Microbiology* 145, 411-417.
- 793 Page, L.A., 1975. Chlamydiosis (ornithosis-psittacosis). In: Hitchner, S.B., Domermuth, C.H.,
794 Purchase, H.G., Williams, J.E. (eds.), *Isolation and identification of avian pathogens*. Arnold
795 Printing Corporation, Ithaca, New York, p. 118-131.
- 796 Palmer, S.R., Andrews, B.E., Major, R., 1981. A common-source outbreak of ornithosis in
797 veterinary surgeons. *Lancet* ii (8250), 798-799.
- 798 Pantchev, A., Sting, R., Bauerfeind, R., Tyczka, J., Sachse, K., 2008. New real-time PCR tests for
799 species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue
800 samples. *Vet. J.* (in press) doi:10.1016/j.tvjl.2008.02.025
- 801 Pavlak, M., Vlahović, K., Gregurić, J., Župančić, Ž., Jerčić, J., Božikov, J., 2000. An epidemiologic
802 study of *Chlamydia* sp. in feral pigeons (*Columba livia domestica*). *Z. Jagdwiss.* 46, 84-95.
- 803 Prukner-Radovčić, E., Horvatek, D., Gottstein, Z., Ciglar Grozdanić, I., Mazija, H., 2005.
804 Epidemiological investigation on *Chlamydophila psittaci* in pigeons and free living birds in Croatia.
805 *Vet. Res. Commun.* 29 (Suppl 1), 17-21.
- 806 Quinn, P.J., Carter, M.E., Markey, B.K., Carter, G.R., 1994. *Clinical Veterinary Microbiology*.
807 Mosby-Year Book Europe Limited, London, UK.

- 808 Rampin, T., Grilli, G., Sironi, G., Volorio, A., Schmitz, G., Magnino, S., Vigo, P.G., Fabbi, M.,
809 Schiavini, N., 1998. Observations on the sanitary conditions of feral pigeons in Milan. *Sel. Vet.* 8-
810 9/1998, 667-684.
- 811 Renzi, M., Magnino, S., 1998. Isolamento di *Chlamydia psittaci* da piccioni urbanizzati (*Columba*
812 *livia*) della provincia di Bologna. Final report of a research funded by the Italian Ministry of Health.
- 813 Rešidbegović, E., Kavazović, A., Abduzaimović-Maglajlić, J., Dizdarević, Z., Kustura, A., Gagić,
814 A., Dovč, A., 2006. Some bacterial and viral pathogens in free-living pigeons from the city of
815 Sarajevo. In: Proceedings of the 4th Annual Workshop of COST Action 855 “Animal chlamydioses
816 and its zoonotic implications”, Edinburgh, Scotland, September 3-5, 2006, p. 9.
- 817 Rešidbegović, E., Kavazović, A., Satrović, E., Alibegović-Zečić, F., Keše, D., Dovč, A., 2007.
818 Detection of antibodies and isolation of *Chlamydia psittaci* in free-living pigeons. In:
819 Proceedings of the 5th Annual Workshop of COST Action 855 “Animal chlamydioses and its
820 zoonotic implications”, Pulawy, Poland, September 10-11, 2007, p. 81-85.
- 821 Sachse, K., Hotzel, H., 2003. Detection and differentiation of chlamydiae by nested PCR. *Meth.*
822 *Molec. Biol.* 216, 123-136.
- 823 Sachse, K., Hotzel, H., Slickers, P., Ellinger, T., Ehricht, R., 2005. DNA microarray-based
824 detection and identification of *Chlamydia* and *Chlamydophila* spp. *Mol. Cell. Probes* 19, 41-50.
- 825 Sachse, K., Laroucau, K., Hotzel, H., Schubert, E., Ehricht, R., and Slickers, P. 2008. Genotyping
826 of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of *ompA*
827 genes. *BMC Microbiology* 8, 63 (17Apr2008)
- 828
- 829 Salinas, J., Caro, M.R., Cuello, F., 1993a. Antibody prevalence and isolation of *Chlamydia psittaci*
830 from pigeons (*Columba livia*). *Avian Dis.* 37 (2), 523-527.
- 831 Salinas, J., Caro, M.R., Cuello, F., 1993b. Comparison of different serological methods for the
832 determination of antibodies to *Chlamydia psittaci* in pigeon sera. *J. Vet. Med. B* 40, 239-244.

- 833 Sayada, C.H., Andersen, A.A., Storey, C.H., Milon, A., Eb, F., Hashimoto, N., Hirai, K., Elion, J.,
834 Denamur, E., 1995. Usefulness of *omp1* restriction mapping for avian *Chlamydia psittaci* isolate
835 differentiation. Res. Microbiol. 146, 155-165.
- 836 Schmeer, N., 1983. Enzyme immunoassay for detection of IgG and IgM antibodies against
837 *Chlamydia psittaci* in the pigeons. Zentralbl. Veterinarmed. B 30, 356-370.
- 838 Simms, E., 1979. The public life of the Street Pigeon. Hutchinson & Co. LTD., London: 144 pages.
- 839 Stamp, J.T., McEwen, A.D., Watt, J.A., Nisbet, A.D.I., 1950. Enzootic abortion in ewes. I.
840 Transmission of the disease. Vet. Rec. 62, 251-264.
- 841 Süss, A., Reetz, J., Schulze, P., Kretzschmar, M., Schirrmeistr, W., Süss, J., 1996. Schwerer Verlauf
842 einer Ornithose und ihre intensivmedizinische und diagnostische Problematik – eine Kasuistik.
843 Anaesthesiol. Reanim. 21, 97-102.
- 844 Theis, H.-P., 2007. *In-vitro*-Prüfung der Empfindlichkeit von aviären Chlamydien gegen Difloxazin
845 im Vergleich zu den als “wirksame Mittel” eingestuftem Arzneimitteln Chlortetrazyklin, Doxizylin
846 und Enrofloxazin. Veterinary Dissertation, University of Giessen, 124 pages.
- 847 Tiefbau-Berufsgenossenschaft, 2006. Gesundheitsgefährdungen durch Taubenkot.
848 Handlungsanleitung zur Gefährdungsbeurteilung nach Biostoffverordnung (BioStoffV). Tiefbau-
849 Berufsgenossenschaft, Technischer Aufsichtsdienst. Available online:
850 http://www.bgbau.de/d/pages/koop/forschung/Handlungsanleitung_Tk.pdf
- 851 Trap, D., Louzis, C., Gourreau, J.M., Gaumont, R., 1986. Chlamydiose aviaire chez les pigeons de
852 Paris. Rev. Med. Vet. 137 (11), 789-796.
- 853 Vanrompay, D., Andersen, A.A., Ducatelle, R., Haesebrouck, F., 1993. Serotyping of European
854 isolates of *Chlamydia psittaci* from poultry and other birds. J. Clin. Microbiol. 31 (1), 134-137.

- 855 Vanrompay, D., Butaye, P., Sayada, C., Ducatelle, R., Haesebrouck, F., 1997. Characterization of
856 avian *Chlamydia psittaci* strains using *omp1* restriction mapping and serovar-specific monoclonal
857 antibodies. Res. Microbiol. 148, 327-333.
- 858 Vanrompay, D., Ducatelle, R., Haesebrouck, F., 1992. Diagnosis of avian chlamydiosis: specificity
859 of the modified Giménez staining on smears and comparison of the sensitivity of isolation in eggs
860 and three different cell cultures. J. Vet. Med. B 39, 105-112.
- 861 Vanrompay, D., Van Nerom, A., Ducatelle, R., Haesebrouck, F., 1994. Evaluation of five
862 immunoassays for detection of *Chlamydia psittaci* in cloacal and conjunctival specimens from
863 turkeys. J. Clin. Microbiol. 32 (6), 1470-1474.
- 864 Verminnen, K., Duquenne, B., De Keukeleire, D., Duim, B., Pannekoek, Y., Braeckman, L.,
865 Vanrompay, D., 2008. Evaluation of a *Chlamydophila psittaci* diagnostic platform for zoonotic risk
866 assessment. J. Clin. Microbiol. 46 (1), 281-285.
- 867 Vlahović, K., Župančić, Ž., Gregurić, J., Pavlak, M., 1998. Zur Zuverlässigkeit diagnostischer
868 Verfahren beim Nachweis von Infektionen mit *Chlamydia* sp. bei Vögeln. Z. J. Jagdwiss. 44 (3),
869 133-139.
- 870 Vlahović, K., Matica, B., Bata, I., Pavlak, M., Pavičić, Ž., Popović, M., Nejedli, S., Dovč, A., 2004.
871 Campylobacter, salmonella and chlamydia in free-living birds of Croatia. Eur. J. Wildl. Res. 50,
872 127-132.
- 873 Yoshida, H., Kishi Y., Shiga S., Hagiwara T., 1998. Differentiation of *Chlamydia* species by
874 combined use of polymerase chain reaction and restriction endonuclease analysis. Microbiol.
875 Immunol. 42, 411-414.

Table 1 – Geographical distribution of serotypes and genotypes of *C. psittaci* detected in feral pigeons

Country	Serovars	Genotypes	References
United States	B, E	B, E	Andersen, 1997; Vanrompay <i>et al.</i> , 1997; Geens <i>et al.</i> , 2005a
Belgium	A, B	A, B, D	Vanrompay <i>et al.</i> , 1993; 1997
England	n.d.	A, B	Sayada <i>et al.</i> , 1995
The FYR of Macedonia	n.d.	B, E	Ilieski <i>et al.</i> , 2007
France	B	B, E	Duan <i>et al.</i> , 1999; Laroucau <i>et al.</i> , 2008
Italy	A, B, E	A, B, D, E, E/B	Geens <i>et al.</i> , 2005a; Laroucau <i>et al.</i> , 2008
Japan	n.d.	C	Sayada <i>et al.</i> , 1995
The Netherlands	n.d.	B	Heddema <i>et al.</i> , 2006b
Switzerland	n.d.	B	Hoop <i>et al.</i> , 2002

n.d. = not determined

Table 2 - A summary of surveys for detecting antibodies to *C. psittaci* in feral pigeon populations in some European towns and cities

Country	Town or city	Year of sampling	Laboratory test	Results positive/total (% positive)	Notes	Reference
Bosnia and Herzegovina	Sarajevo	2005	IIF	53/176 (30.1 %)	sera from captured pigeons with no clinical signs	Rešidbegović <i>et al.</i> , 2006
		2006	IIF	62/234 (26.5 %)	sera from captured pigeons with no clinical signs	Rešidbegović <i>et al.</i> , 2007
Bulgaria	Pleven	1993-1994	CFT	7/20 (35%)	cut-off: 1 :8	Martinov <i>et al.</i> , 1997
Croatia	Zagreb	1992-1997	CFT	18/44 (40.9 %)	cut-off: 1:8	Vlahović <i>et al.</i> , 1998
		1988-1993	CFT	410/834 (49.2 %)	higher antibody titers were detected in pigeons with lesions at necropsy cut-off: 1:8	Pavlak <i>et al.</i> , 2000
		2000-2003	Ab-ELISA (LPS)	174/182 (95.6 %)	high antibody titers were detected in 57/182 sera (32.8 %)	Prukner-Radovčić <i>et al.</i> , 2005
France	Toulouse	1980-1982	CFT, IIF	186/501 (37.1 %)	CFT cut-off: 1:8 IIF cut-off: 1:40	Milon <i>et al.</i> , 1983
			CFT, IIF	46/101 (45.5 %)		
	Paris	1984	CFT, IIF	315/475 (66.3 %)	CFT cut-off: 1:8 IIF cut-off: 1:40	Trap <i>et al.</i> , 1986
		1990	CFT	176/415 (42 %)	cut-off: 1:8	Laroucau, pers. comm. 2007
		1999	CFT	316/658 (48 %)	pigeons sampled in the 20 districts, in the Bois de Boulogne and in the Bois de Vincennes	Laroucau <i>et al.</i> , 2005

					cut-off: 1:8	
		2003 (March)	CFT	38/75 (51 %)	cut-off: 1:8	Laroucau, pers. comm. 2007
		2003 (December)	CFT	21/43 (49 %)	cut-off: 1:8	Laroucau, pers. comm. 2007
	Troyes	2007 (June)	CFT	7/29 (24 %)	cut-off: 1:8	Laroucau, pers. comm. 2007
Germany	Giessen	1979-2004	CFT	46/81 (56.8 %)	sera from diseased and necropsied feral pigeons	Kaleta and Hönicke, 2004; Kaleta, pers. comm. 2007; Helmecke, 2007
			CFT	286/1,474 (19.4 %)	sera from feral pigeons with no clinical history	
Italy	Pisa	1988	CFT	263/495 (53.1 %)	cut-off: 1 :16	Cerri <i>et al.</i> , 1989
	Bologna and Ferrara	1997	CFT	39/178 (21.8 %)	sera from feral pigeons with no clinical history	Renzi and Magnino, 1998
					cut-off: 1:16	
	Bolzano	2006	CFT	38/68 (55.9 %)	cut-off: 1:10	Ceglie <i>et al.</i> , 2007
	Venice	2006	CFT	111/267 (41.6 %)	cut-off: 1:10	Ceglie <i>et al.</i> , 2007
	Padua	2006	CFT	65/100 (65 %)	cut-off: 1:10	Ceglie <i>et al.</i> , 2007
	Verona	2006	CFT	78/167 (46.7 %)	cut-off: 1:10	Ceglie <i>et al.</i> , 2007
Spain	Murcia	1991	CFT	36/128 (28.1 %)	CFT cut-off: 1:43	Salinas <i>et al.</i> , 1993a

				(28.6 %)		
			MIF	44/128 (33.5 %)	MIF cut-off: 1:32	
			Ab-ELISA (EB)	45/128 (35.9 %)		
Slovenia	Ljubljana	1991-1992	IIF	10/15 (67.7 %)	cut-off: 1:40	Dovč, 1995
		2000	IIF	33/139 (23.7 %)	cut-off : 1:40	Dovč <i>et al.</i> , 2004
		2006	IIF	26/86 (30.2 %)	cut-off: 1:40	Dovč, pers. comm. 2006
Switzerland	Luzern	2001	Ab-ELISA (LPS)	33/59 (56 %)		Haag-Wackernagel, 2006a

Legenda

CFT = complement fixation test; Ab-ELISA (EB) = antibody-detection ELISA based on crude antigen from chlamydial elementary bodies;
 Ab-ELISA (LPS) = antibody-detection ELISA based on chlamydial lipopolysaccharide; IIF = indirect immunofluorescence assay;
 MIF = microimmunofluorescence assay

Table 3 - A summary of surveys for detecting *C. psittaci* in feral pigeon populations in some European towns and cities

Country	Town or city	Year of sampling	Laboratory test	Results positive/total (% positive)	Notes on the type of sample(s)	Reference
Bosnia and Herzegovina	Sarajevo	2006	PCR (<i>ompA</i>)	3/8 (37.5 %)	tissue samples from dead birds	Rešidbegović <i>et al.</i> , 2007
Bulgaria	Sofia	2006	EI	2/15 (13.3 %)	pool of spleen, liver and lung from necropsied birds	Martinov, 2006
Croatia	Zagreb	1992-1997	DIF	4/39 (10.2 %)	tissue samples from necropsied birds	Vlahović <i>et al.</i> , 1998
		2000-2003	ICT	3/107 (2.8 %)	cloacal swabs	Vlahović <i>et al.</i> , 2004
			EI	0/3 (0 %)		
		2000-2003	Ag-ELISA	44/278 (15.8 %)	cloacal swabs	Prukner-Radovčić <i>et al.</i> , 2005
		2006	Ag-ELISA	120/787 (15.3 %)	cloacal swabs	Prukner-Radovčić, pers. comm. 2007
France	Toulouse	1980-1982	EI	3/101 (3 %)	pool of spleen and lung	Milon <i>et al.</i> , 1983
			TC	3/150 (2 %)	cloacal, intestinal and pharyngeal swabs	
	Paris	1984	EI	4/7 (57.1 %)	tissue samples from necropsied birds	Trap <i>et al.</i> , 1986
		2003 (March)	Rt PCR (23S rRNA)	5/33 (15.2 %)	cloacal swabs	Laroucau, pers. comm. 2007
		2003 (December)	Rt PCR (23S rRNA)	4/20 (20 %)	cloacal swabs	Laroucau, pers. comm. 2007

		(December)	(23S rRNA)	(20 %)		2007
	Troyes	2007 (March)	Rt PCR (23S rRNA)	5/33 (15.2 %)	cloacal swabs	Laroucau, pers. comm. 2007
		2007 (June)	Rt PCR (23S rRNA)	1/29 (3.4 %)	cloacal swabs	Laroucau, pers. comm. 2007
Germany	Giessen	1979-2004	TC	10/77 (13 %)	tissue samples from necropsied birds	Kaleta and Hönicke, 2004; Kaleta, pers. comm 2007; Helmecke, 2007
Italy	Pisa	1988	EI	14/35 (40 %)	pool of viscera (lung, liver and spleen) from necropsied birds	Cerri <i>et al.</i> , 1989
	Trento	1995	TC	12/35 (34.3 %)	intestinal content from necropsied birds with no clinical history	Manfredi <i>et al.</i> , 1997
	Milan	1996-1997	TC	30 ¹ /163 (18.4 %)	intestinal content from necropsied birds with no clinical history	Rampin <i>et al.</i> , 1998
	Bologna and Ferrara	1997	TC	34 ² /178 (19.1 %)	intestinal content from necropsied birds with no clinical history	Renzi and Magnino, 1998
	Bergamo	1998-1999	TC	11/26 (42.3 %)	tissue samples from necropsied birds with no clinical history	Gaffuri <i>et al.</i> , 2000
	Venice	2006	PCR (16S rRNA)	7/50 (14 %)	liver and spleen collected from necropsied birds	Ceglie <i>et al.</i> , 2007
The FYR of Macedonia	Skopje	2004-2005	Ag-ELISA	10/25 (40 %)	conjunctival, choanal and cloacal swabs	Mitevski <i>et al.</i> , 2005
		2006	Rt PCR (23S rRNA)	2 ³ /36 (5.6 %)	cloacal swabs	Ilieski <i>et al.</i> , 2007
	Prilep	2004-2005	Ag-ELISA	4/16 (25 %)	conjunctival, choanal and cloacal swabs	Mitevski <i>et al.</i> , 2005

				(25 %)	swabs	
	Kumanovo	2004-2005	Ag-ELISA	2/10 (20 %)	conjunctival, choanal and cloacal swabs	Mitevski <i>et al.</i> , 2005
	Bogdanci	2004-2005	Ag-ELISA	2/12 (16.7 %)	conjunctival, choanal and cloacal swabs	Mitevski <i>et al.</i> , 2005
	Vinica	2006	Rt PCR (23S rRNA)	10 ⁴ /20 (50 %)	cloacal swabs	Ilieski <i>et al.</i> , 2007
	Stip	2006	Rt PCR (23S rRNA)	4 ⁵ /60 (6.7 %)	cloacal swabs	Ilieski <i>et al.</i> , 2007
The Netherlands	Amsterdam	2005	Rt PCR (<i>ompA</i>)	26 ⁶ /331 (7.9 %)	fresh faecal droppings	Heddema <i>et al.</i> , 2006b
Spain	Murcia	1991	TC	7/39 (18 %)	cloacal swabs	Salinas <i>et al.</i> , 1993a
			EI	5/39 (12.8 %)	cloacal swabs	
Slovenia	Ljubljana	2006	DIF	2/86 (2.3 %)	cloacal swabs	Dovč, pers. comm. 2006
			EI	1/86 (1.2 %)	cloacal swabs	
Switzerland	Luzern	2001	Ag-ELISA	2/60 (3.3 %)	cloacal swabs	Haag-Wackernagel, 2006a
			EI	1/60 (1.6 %)	cloacal swabs	

Notes

- (1) Three isolates were genotype B
- (2) Four isolates were genotype E and one was genotype E/B
- (3) One PCR product was genotype E
- (4) Four PCR products were genotype E
- (5) One PCR product was genotype B, and one was genotype E

(6) Ten PCR products were genotype B

Legenda

TC = tissue culture; EI = egg inoculation; Ag-ELISA = antigen-detection ELISA; DIF = direct immunofluorescence assay; ICT = immunochromatographic test

PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; Rt PCR (gene) = real-time PCR (targeted gene)

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