Prevalence of *Chlamydia psittaci* in the feral pigeon population of Basel, Switzerland

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Feral pigeons (*Columba livia*) are commonly infected with *Chlamydia psittaci*, the agent of psittacosis in humans. To assess the risk of zoonosis posed by feral pigeons in the urban environment, we determined the prevalence of *Chlamydia psittaci* by detection of the outer-membrane protein A (*ompA*) gene of this pathogen in pharyngeal and cloacal samples of 202 feral pigeons present in a loft in Basel, Switzerland. Additionally, we examined 620 fresh faecal droppings of feral pigeons at six public sites in Basel. The *ompA* gene of *C. psittaci* could be detected in only 17 (8.4 %) of the 202 feral pigeons in the loft. *C. psittaci* DNA was present in nine (2.0 %) of 447 of the pharyngeal swabs and 11 (3.2 %) of the 348 cloacal swabs. Genotyping of the *ompA* gene revealed genotype B in seven of the birds. In one bird, a mixed infection was detected with the genotypes A, B and E/B, which, to our knowledge is the first time such an infection has been reported. Some of these birds immigrated into the loft as adults. To our knowledge, this is the first study to document how the interconnectedness between feral pigeon subpopulations favours the spread of *C. psittaci*. *C. psittaci* DNA was not detected in any of the faecal droppings collected at the six public areas. In spite of the low levels of *C. psittaci* shedding by feral pigeons in Basel, close contact to feral pigeons bears the risk of zoonotic transmission of *C. psittaci*. Feral pigeon management programmes and public education should be implemented to reduce the risk of a pigeon-to-human transmission of such pathogenic agents.

INTRODUCTION

Feral pigeons are descendants of the domesticated form of the wild rock dove (*Columba livia*, first described by Gmelin in 1789) and thrive in almost every city in the world. Due to their high numbers and close proximity humans, they pose a potential threat for public health, since they are carriers of at least 110 zoonotic pathogens (Haag-Wackernagel & Moch, 2004; Haag-Wackernagel, 2006a, b; Haag-Wackernagel & Bircher, 2010). The most significant pathogen that can be transmitted from feral pigeons to humans is *Chlamydia psittaci*, formerly also named *Chlamydothila psittaci* (Everett et al., 1999; Kuo & Stephens, 2011). Since Meyer (1941) first described two cases of psittacosis caused by contact with feral pigeons, a total of 113 cases of presumed or proved transmission of *C. psittaci* from feral pigeons to humans have been reported (Haag-Wackernagel, 2006a, b). *C. psittaci* is an obligate intracellular Gram-negative bacterium, which causes respiratory disease in birds and psittacosis/ornithosis in humans. Human *C. psittaci* infections are acquired by inhalation of aerosolized faecal dust, feather particles or dried respiratory tract secretions from infected birds (Andersen & Vanrompay, 2003). Humans come into close contact with feral pigeons and their excreta in public areas, at breeding or roosting sites on buildings and during occupational duties, e.g. cleaning activities or pigeon-control measures (Haag-Wackernagel, 2006a, b).

To assess the current risk of zoonosis in Basel, Switzerland, we examined chlamydial shedding in 202 free-ranging feral pigeons living in a pigeon loft. This loft provided a unique opportunity to study a feral pigeon subpopulation under natural conditions and it enabled us to study individual resident birds repeatedly. Additionally, we examined 620 feral pigeon faeces samples collected at six public sites in Basel taking in to account that streets and squares in the city centre are thoroughly cleaned on a regular basis and large accumulations of pigeon faeces are rarely seen. Also, feral pigeon droppings in the open urban environment are exposed to numerous physical environmental influences.

METHODS

Background. In 1988 the 'Pigeon Action of Basel' was founded as an interdisciplinary project of the University of Basel, the Government of
the Canton Basel-Stadt and the Society for the Protection of Animals of Basel (Haag-Wackernagel, 1993, 1995). At this time, many heavily diseased birds could be found in streets and other places, predominantly in the city centre (Haag, 1984). The aim of this project was to establish a small but healthy population of feral pigeons. A reduction of the population size could only be achieved by reducing the food supply provided by humans (Haag, 1984). Therefore, by means of large-scale information campaigns, the ‘Pigeon Action of Basel’ intended to encourage pigeon enthusiasts to stop or limit their feeding activities. The intention was to reverse the attitude towards pigeon feeding and convince the public that feeding is counterproductive and ultimately harms the feral pigeons since it leads to overpopulation and high-density, poor-quality living conditions. Concurrently, feral pigeons were trapped and killed (10–20 % of the population per year) to adapt the population size to the lowered food supply. Thus, it was possible to lower the feral pigeon population from ≥20000 to 8000 birds. Following this project, overtly diseased feral pigeons could rarely be seen in Basel. At the same time, nine supervised pigeon lofts were built in public buildings in Basel, where feral pigeons could be housed and cared for (Haag-Wackernagel, 1993, 1995).

Feral pigeon population studies. During the first part of the research project (2007–2009), a pigeon loft in the St Matthäus Church was under study. In this loft, the long-term population dynamics were observed and no control measures were applied. Thus, the population of the loft represents the real urban scenario. All birds that hatched in the loft were marked with individual foot rings and registered in a database. Therefore, their exact age was known. Adult birds of unknown age and origin that immigrated into the loft were estimated to be at least 7 months old (Johnston & Janiga, 1995). The pigeons present at the start of the experiment, as well as all new immigrating birds, represented an observed population of 202 feral pigeons over the 2 years. The loft had a floor space of 31 m² and was cleaned of droppings, nesting material and carcasses every 14 days. The pigeons were not fed and had to search for food and water themselves. They used the loft as a roosting and breeding site and were free to enter or leave the loft at any time. Juveniles and adults were free to stay in the loft or establish themselves in other breeding flocks throughout the city. During the second part of this study (Nov 2008–Nov 2009) 520 faecal droppings were examined, which were collected from the Marketplace, one of the most popular feeding sites of local feral pigeons situated in the city centre of Basel. Ringed feral pigeons from pigeon lofts and unmarked pigeons breeding in the city were observed daily. Feral pigeon subpopulations can overlap at important feeding sites. These sites are where transmission of human infection sources. DNA was extracted from the samples, as well as from a positive control (C. psittaci strain 92/1293), prepared as described previously and tested by using C. psittaci-specific nested PCR (Van Loock et al., 2005). Briefly, the PCR targeted a 472 bp fragment of the ompA gene of C. psittaci, as well as a 703 bp fragment of an internal control plasmid, which served as an inhibition control to rule out false negative results. PCR-products were analysed by gel electrophoresis, stained with ethidium bromide and visualized using UV-illumination. ompA-positive samples were further characterized using a genotyping real-time PCR for detecting the C. psittaci ompA genotypes A–F and E/B (Geens et al., 2005a).

RESULTS AND DISCUSSION

The seroprevalence of C. psittaci in feral pigeons has been investigated in 38 studies from 1966 to 2005. These studies revealed rates of seropositivity ranging from 12.5 to 95.6 % (Haag-Wackernagel, 2005; Laroucau et al., 2005; Mitevski et al., 2005; Prukner-Radovčić et al., 2005; Tanaka et al., 2005). However, all these studies used serological assays based on detecting antibodies against chlamydial whole organisms or chlamydial LPS. These assays are prone to yielding false positive results due to serological cross-reaction with heat-shock proteins and/or LPS of other bacteria (Yuan et al., 1992). Culture methods and nucleic acid amplification tests for studying the epidemiology of C. psittaci infections in birds are more accurate and the latter allows molecular characterization and even tracing of human infection sources in case of psittacosis (Heddema et al., 2006a).

In 14 studies conducted in European cities from 1979 to 2007, cultures of C. psittaci revealed positive results in 1.2–57 % of investigated feral pigeons (Magnino et al., 2009). The highest percentage of positive cultures was found in feral pigeons in Paris (Trap et al., 1986). In 11 studies conducted from 2003 to 2007, the presence of C. psittaci DNA could be proved in 3.4–52.6 % of the examined feral pigeons by the use of nucleic acid amplification assays (Magnino et al., 2009; Vázquez et al., 2010). Interestingly, during the 1990s, C. psittaci prevalence rates in studied populations of ≥20 feral pigeons were much higher than during the 2000s with mean prevalence rates of 22 and 10 %, respectively (Magnino et al., 2009). This could be due to use of more specific diagnostic techniques like nucleic acid amplification tests. Research on optimal strategies for the management of feral pigeons (Magnino et al., 1993, 1995).
et al., 2009) and increased implementation of such strategies in cities may also have played a role. However, at present, successful management programmes resulting in a scientifically proven sustainable reduction of the feral pigeon population have only been documented in Basel, Lucerne and Lausanne, Switzerland (Cuendet & Beaud, 2009; Haag-Wackernagel, 1993, 1995; Keller, 2007).

From 2007 to 2009, the \textit{ompA} gene of \textit{C. psittaci} could be detected in only 17 (8.4 %) of 202 feral pigeons sampled in the loft. \textit{C. psittaci} DNA was present in 9 (2.0 %) of the 447 pharyngeal and 11 (3.2 %) of 348 cloacal swabs (Table 1) but was only found once in a pigeon’s pharynx and cloaca simultaneously. Thus, in feral pigeons, sampling both sites is advisable. In the pigeon loft, we had the unique opportunity to test some of the birds repeatedly. By retesting individual birds, we were able to document intermittent shedding of \textit{C. psittaci} in free-ranging feral pigeons. This is in accordance with findings in domestic pigeons and other bird species (Andersen & Vanrompay, 2003; Harkinezhad et al., 2009; Kaleta & Taday, 2003). Shedding of \textit{C. psittaci} could be demonstrated in feral pigeons of all ages and was not limited to young birds present in the loft (Supplementary Table 1, available in JMM Online).

Outer-membrane protein \textit{A} (\textit{ompA}) genotyping using real-time PCR was successful in 8 (47.1 %) of the 17 nested-PCR-positive pigeons, revealing genotype \textit{B} in seven pigeons and a mixed infection with genotypes \textit{A}, \textit{B} and \textit{E/B} in one pigeon (Supplementary Table 1). To date, seven genotypes (\textit{A}, \textit{B}, \textit{C}, \textit{D}, \textit{E}, \textit{F}, and \textit{E/B}) of the \textit{ompA} gene of \textit{C. psittaci} have been described, all of which can be transmitted to humans (Geens et al., 2005b; Harkinezhad et al., 2009; Heddema et al., 2006a). Genotype \textit{B} is commonly found in feral pigeons but infection with genotypes \textit{A}, \textit{C}, \textit{D}, \textit{E} and \textit{E/B} as well as mixed infections with two genotypes have been documented as well (Geens et al., 2005b). To our knowledge, we are the first to document a mixed infection with three different genotypes in an individual feral pigeon. This is of special interest as the bird was infected with genotype \textit{A}, which is associated with a more severe disease in humans than that caused by genotypes \textit{B} and \textit{E/B} (Magnino et al., 2009).

Seven of the birds positive for \textit{C. psittaci} were adults of unknown age. They had most likely immigrated into the loft from other subpopulations in the city. Three of the samples from these birds were successfully genotyped (Supplementary Table 1). These data contribute to the understanding of the epidemiology of \textit{C. psittaci} in the feral pigeon. We were unable to genotype the other nested-PCR-positive samples. This was probably due to the presence of only small amounts of DNA, since the nested PCR is more sensitive than the genotyping real-time PCR. However, this could also be due to the presence of unknown \textit{ompA} genotypes.

The results of the present study are in accordance with those of other studies. In a recent study in Ghent, Belgium, only one out of 61 (1.6 %) feral pigeons was found to be positive for \textit{C. psittaci} by analysis of cloacal swabs (Dickx et al., 2010). The chlamydial genotype could not be determined in this study. In another study, conducted in Switzerland, Zweifel et al. (2009) demonstrated that two out of 60 (3.3 %) feral pigeons in the city of Lucerne were positive for \textit{C. psittaci}. Interestingly, in the same study, the prevalence of \textit{C. psittaci} in feral pigeons in Zurich was found to be significantly higher than in Lucerne. In Zurich, 10 out of 24 (41.7 %) clinically healthy feral pigeons tested positive by analysis of cloacal swabs. Genotyping revealed genotype \textit{B} in one sample from Lucerne and five of the samples from Zurich. Genotype \textit{E} was detected in one sample from Zurich. The authors found no explanation for the remarkably differing prevalence of \textit{C. psittaci} in these two Swiss cities. However, in Lucerne, a feral pigeon management project similar to the ‘Pigeon Action of Basel’ has been successfully implemented (Keller, 2007). These findings suggest that the sustainable reduction of the feral pigeon population has had a beneficial effect on the health status of the birds. Further investigations are needed to detect the underlying reasons behind the prevalence of different \textit{C. psittaci} genotypes in different feral pigeon populations.

\textit{C. psittaci} DNA was not detected in any of the faecal dropping samples collected despite the fact that a mean of 3.2 % of the birds tested were positive for \textit{C. psittaci} by analysis of cloacal swabs. In a similar setting in Amsterdam, Heddema et al. (2006b) detected the \textit{C. psittaci ompA} gene in 7.9 % of examined faecal droppings. According to Buijs & Van Wijnen (2001), there are \textasciitilde 30 000 feral pigeons in Amsterdam, most of which live in the city centre. Thus, the feral pigeon population in Amsterdam is much larger than in Basel. As a consequence of the ‘Pigeon Action of Basel’, the population of feral pigeons in Basel decreased to around two thirds its previous size and is currently stable at a level of up to 8000 birds, of which \textasciitilde 500 live in public pigeon lofts (Haag-Wackernagel, 1993, 1995). Lofts are regularly cleaned and birth control is performed in some of them by egg and nestling removal. In 2007, about 1265 kg of droppings and nesting material were removed from the lofts, which would otherwise have led to contamination and fouling in the public environment (Haag-Wackernagel, unpublished data). Thus, the use of pigeon lofts reduces

### Table 1. Proportion of pharyngeal and cloacal samples from feral pigeons in the St Matthäus-Loft in Basel that tested positive for \textit{C. psittaci} by nested PCR

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Number of positive samples/number of feral pigeons tested (% positives)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pharyngeal swab</td>
</tr>
<tr>
<td>1 (1.2.2007)</td>
<td>5/99 (5.1 %)</td>
</tr>
<tr>
<td>2 (7.2.2008)</td>
<td>1/104 (1.0 %)</td>
</tr>
<tr>
<td>3 (8.7.2008)</td>
<td>3/124 (2.4 %)</td>
</tr>
<tr>
<td>4 (6.5.2009)</td>
<td>0/120 (0 %)</td>
</tr>
<tr>
<td>Total</td>
<td>9/447 (2.0 %)</td>
</tr>
</tbody>
</table>
the amount of potentially infectious feral pigeon droppings in the public urban environment and, therefore, the risk to public health. By reducing the feral pigeon population of Basel, the ‘Pigeon Action of Basel’ may have contributed to an improved health status of the birds and may have reduced the number of chlamydial infections in feral pigeons. In 1990, an investigation of the health status of the feral pigeons in Basel showed that the birds were in a surprisingly good condition of health. However, 62% of the pigeons tested were seropositive for C. psittaci (Haag & Gurdan, 1990).

A small but healthy feral pigeon population also makes it less likely that pigeons and humans will come into close contact, thus lowering the potential risk of disease transmission. Accumulation of pigeon faeces is rarely seen in Basel, since the resident shop and restaurant owners, as well as the employees of the city cleaning department, quickly remove these faecal accumulations. The city centre of Basel is cleaned daily in the early morning hours throughout the year, mostly by dry brushing. In addition, to avoid dust formation, streets and squares are periodically cleaned with water. Thorough cleaning could play an important role in preventing pathogen survival and spread by contaminated dust. C. psittaci remains viable at low temperatures and is resistant to desiccation but the bacterium is highly susceptible to repeated freeze–thawing cycles and is destroyed within 3 min when exposed to UV-light (Fritzsche, 1961; Andersen & Vanrompay, 2003). Therefore, we suppose that C. psittaci cells in feral pigeon faeces are eliminated from the urban environment in winter. However, it is difficult to know how long C. psittaci cells can survive in the unprotected urban environment where they are exposed to numerous physical influences. Feral pigeon faeces in attics or other sites can present a health risk to construction and pest-control workers. Psittacosis due to dust exposure during pigeon culling in a loft has been reported previously (Haag-Wackernagel, 2006a). This highlights the importance of using personal protective clothing during the handling or removal of sick or dead feral pigeons and during occupational contact with feral pigeons and pigeon faeces. Moreover, Wreghitt (2003) reported six cases of psittacosis in immunocompromised patients in a transplant ward due to contaminated pigeon faeces on a window ledge. Since all zoonotic pathogens pose a severe risk for immunocompromised persons, feral pigeons should not be tolerated in the vicinity of hospitals.

Conclusions
Feral pigeons can become infected with C. psittaci and thus present a health risk to the public. Despite the low level of shedding detected in feral pigeons, the risk of disease transmission can never be ruled out, since there is an increased likelihood of close contact between feral pigeons and humans in city environments. Due to the problems pigeon faeces cause with respect to environmental hygiene as well the detrimental effect it has on buildings and historical monuments, strategies for the management of feral pigeon populations in the urban environment need to be implemented. This, however, is a complex issue that requires careful planning and should involve the community, the government and animal protection societies as well as scientists.

ACKNOWLEDGEMENTS
The authors are very grateful to Delphine Beeckman, Caroline van Droogenbroeck, Kristel Verminnen and Annelien Dumont (Department of Molecular Biotechnology, Ghent University) for their valuable technical advice. We thank Andreas Ochsnebin, Simone Probst and Alex Ruffe (University of Basel) for their assistance in the laboratory. Andreas Pospischil and Nicole Borel (University of Zurich) are acknowledged for their technical assistance. This study was financially supported by the Gottfried und Julia Bangerter-Rhyner-Stiftung.

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