ON THE BIOLOGY AND EPIDEMIOLOGY OF THE FERAL PIGEON (*COLUMBA LIVIA*)

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Ila Geigenfeind

aus

Basel (BS), Schweiz

Basel, 2013

Namensnennung-Keine kommerzielle Nutzung-Keine Bearbeitung 2.5 Schweiz

---

Sie dürfen:

das Werk vervielfältigen, verbreiten und öffentlich zugänglich machen

Zu den folgenden Bedingungen:

Namensnennung. Sie müssen den Namen des Autors/Rechteinhabers in der von ihm festgelegten Weise nennen (wodurch aber nicht der Eindruck entstehen darf, Sie oder die Nutzung des Werkes durch Sie würden entlohnt).

Keine kommerzielle Nutzung. Dieses Werk darf nicht für kommerzielle Zwecke verwendet werden.

Keine Bearbeitung. Dieses Werk darf nicht bearbeitet oder in anderer Weise verändert werden.

- Im Falle einer Verbreitung müssen Sie anderen die Lizenzbedingungen, unter welche dieses Werk fällt, mitteilen. Am Einfachsten ist es, einen Link auf diese Seite einzubinden.
- Jede der vorgenannten Bedingungen kann aufgehoben werden, sofern Sie die Einwilligung des Rechteinhabers dazu erhalten.
- Diese Lizenz lässt die Urheberpersönlichkeitsrechte unberührt.

Quelle: http://creativecommons.org/licenses/by-nc-nd/2.5/ch/ Datum: 3.4.2009

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag von

Prof. Dr. Marcel Tanner

Prof. Dr. Daniel Haag-Wackernagel

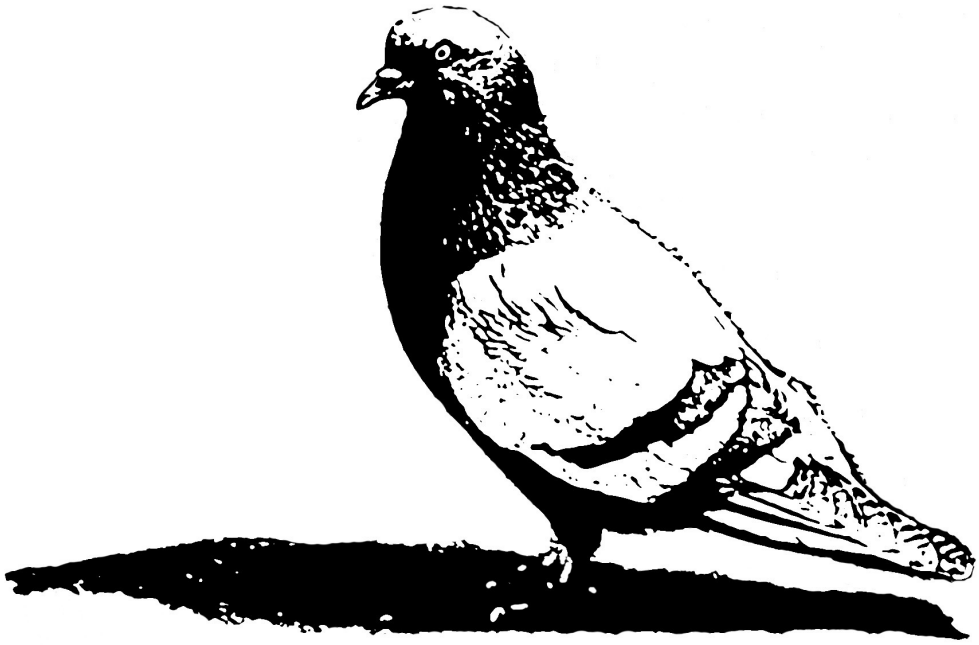
PD Dr. Peter Odermatt

Basel, den 21.Juni 2011

_____

Prof. Dr. Martin Spiess

Dekan



“To see a world in a grain of sand,
And a heaven in a wild flower,
Hold infinity in the palm of your hand,
And eternity in an hour.”

– William Blake (1757–1827), Auguries of Innocence –

Acknowledgements

I am deeply grateful to everyone who substantially contributed in many different ways to this PhD thesis over the past few years.

First of all, I would like to thank Prof. Dr. Daniel Haag-Wackernagel for giving me the opportunity to conduct my research for the present thesis at the Institute of Anatomy. I was very privileged to work in my own laboratory. I always enjoyed the excellent, inspiring and positive working atmosphere in our research group. I am also very grateful to have had the opportunity to engage in so many teaching activities.

I wish to thank PD Dr. Peter Odermatt for joining my PhD committee as a co-referee and Prof. Dr. Marcel Tanner for representing the faculty. Thanks go to Prof. Dr. Jürg Meier for acting as a chairman in the examination.

I am very grateful to Andreas Ochsenbein for his encouragement and for solving all technical problems. Furthermore, I thank my colleague and friend Adrian Schlageter for his help, encouragement and all the good times we had over the past years.

I am deeply grateful to Prof. Dr. Daisy Vanrompay and her research group at Ghent University for inviting me to learn the nested PCR technique, in particular Dr. Caroline Van Droogenbroeck and Dr. Delphine Beeckman for their valuable technical advice. Prof. Dr. Andreas Pospischil, PD Dr. Nicole Borel and Roseline Weilenmann are acknowledged for the PCR-tutorial at the Vetsuisse Faculty in Zürich. I would like to thank all my colleagues from the Institute of Anatomy and other Institutes for their help in so many different ways, particularly Dr. Simone Probst, Dr. Constanze Baranek, Dr. Lionel Nobs, Dr. Manuela Dittrich and Alexander Rufle. Rebecca Schmid is acknowledged for proofreading the manuscript.

I am very grateful to Prof. Dr. Magdalena Müller-Gerbl for supporting my work with a grant from the “Ressort Chancengleichheit”.

Finally, I thank my dear family and friends who always supported and encouraged me.

Abbreviations

A	Adenine
bp	Base Pair
C	Cytosine
°C	Centigrade
CFT	Complement Fixation Test
cm	Centimetre
DNA	Desoxyribonucleic Acid
EB	Elementary Body
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
G	Guanine
g	Gramme
g	Standard Gravity
HCl	Hydrogen Chloride
H_2O	Hydrogen Oxide (Water)
hsp	Heat Shock Protein
IB	Intermediate Body
IFU	Inclusion Forming Unit
kb	Kilobase
KCl	Potassium Chloride
LPS	Lipopolysaccharide
M	Molarity
m^2	Square Metre
mg	Milligramme
$MgCl_2$	Magnesium Chloride
ml	Millilitre
µl	Microlitre
ng	Nanogramme
nm	Nanometre
OD	Optical Density
ompA	Major Outer-Membrane Protein A Gene
PCR	Polymerase Chain Reaction

PPE	Personal Protective Equipment
RB	Reticulate Body
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
rpm	Revolutions per Minute
T	Thymine
TBE	TRIS/Borate/EDTA
TRIS	Tris(hydroxymethyl)aminomethane
UV	Ultraviolet

Table of Contents

Acknowledgements **I**
Abbreviations **III**
Table of Contents **V**

Summary **1**
Zusammenfassung **4**

CHAPTER 1
General Introduction **9**
1.1. Aim of this Thesis 14
1.2. Materials and Methods 16
1.3. References 20

CHAPTER 2
Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications **25**

CHAPTER 3
Detection of *Chlamydophila psittaci* from feral pigeons in environmental samples: problems with currently available techniques **41**
3.1. Abstract 43
3.2. Introduction 44
3.3. Materials and Methods 48
3.4. Results and Discussion 50
3.5. Acknowledgements 52
3.6. References 52

CHAPTER 4
Prevalence of *Chlamydia psittaci* in the feral pigeon population of Basel, Switzerland **57**
4.1. Abstract 59
4.2. Introduction 60
4.3. Methods 61
4.4. Results and Discussion 63
4.5. Conclusions 69

4.6. Acknowledgements 69
4.7. References 69

CHAPTER 5
Protecting Buildings against Feral Pigeons 75
5.1. Abstract 77
5.2. Introduction 78
5.3. Materials and Methods 80
5.4. Results 83
5.6. Discussion 89
5.7. Acknowledgements 92
5.8. References 92

CHAPTER 6
General Discussion and Conclusion 95
6.1. General Discussion 97
6.2. Conclusion 105
6.3. References 106

Curriculum vitae 111

Summary

Feral pigeons (*Columba livia,* Gmelin 1789) are among the most abundant vertebrates in the urban environment of almost every larger city in the world. Feral pigeons are well adapted to life in our cities, where they often come into close contact to humans. Such contacts bear the risk of transmission of zoonotic pathogens and parasites. The most important zoopathogen beeing transmitted from feral pigeons to humans is the bacterium *Chlamydia psittaci,* the agent of avian chlamydiosis and human psittacosis/ornithosis. Many of these transmissions reported in the medical literature were attributed to brief and transient contacts to feral pigeons in the urban environment. The aim of the present thesis is to investigate the prevalence of *C. psittaci* in the feral pigeon population of Basel, to identify possible routes of transmission in the city and to propose measures for the prevention of zoonotic *C. psittaci* transmissions. This research contributes to a better understanding of the health risk posed by feral pigeons.

In a first study, 47 faecal samples were collected from nest boxes in a feral pigeon loft of the “Pigeon Action of Basel” in the St. Matthäus Church in Basel, Switzerland. In addition 34 samples were collected from the feather dust film on the water surface of public fountains, where feral pigeons regularly bathe. All 81 samples were tested for the presence of chlamydial antigen by use of an antigen-ELISA assay. Samples were tested using the IDEIA™ *PCE* Chlamydia Test (Dako Cytomation). Positive results were verified with IDEIA™ Chlamydia Blocking Reagents (Dako Cytomation). Initially the ELISA assay yielded a high proportion of positive results: 8 out of 47 (23.5 %) faecal samples and 26 out of 34 (76.5 %) of the water film samples were tested positive. However, the IDEIA™ Chlamydia Blocking test revealed only one faecal sample was a true positive and could be confirmed by microarray. This ELISA assay was not used for further studies.

In the main study, the prevalence of *C. psittaci* shedding in free ranging feral pigeons living in the loft in the St. Matthäus Church was investigated. A total of 202 individual birds were tested on four different time points between 2007 and 2009 by analysis of pharyngeal and cloacal swabs. Some of the birds could be tested repeatedly. Thus,

intermittent chlamydial shedding could be investigated. All samples were analyzed by use of a species-specific nested PCR assay targeting the *ompA* gene of *C. psittaci*. It could be documented, that the tested feral pigeons were shedding *C. psittaci* by respiratory secretions, since 9 out of 447 (2.0 %) of the pharyngeal swabs were tested positive. Furthermore, 11 out of 348 (3.2 %) of the cloacal swabs were tested positive, indicating that these birds are shedding *C. psittaci* in their faecal droppings. In total, *C. psittaci* was documented in 17 of 202 individual birds (8.4 %). The majority of the positive birds were shedding the pathogen intermittently. At present, this is the first study to test individual free-living feral pigeons repeatedly and therefore the first to prove intermittent shedding of *C. psittaci* in these birds. Genotyping of the positive samples by real-time PCR revealed *C. psittaci* genotype B in 7 of the birds, as well as a mixed infection with the genotypes A, B and E/B in one bird. A mixed infection with three different chlamydial genotypes could be documented for the first time. Seven of the birds that tested positive immigrated into the pigeon loft as adults, including the bird with the mixed infection. Thus, it could be proven how the interconnectedness of feral pigeon subpopulations favours the spread of pathogens. Additionally, 620 faecal samples from public sites in the city were analyzed. However, *C. psittaci* could not be detected in these faecal samples from the urban environment. We suspect either there were no shedding birds present at the time of sampling at these locations or this result may have been caused by previous destruction of the chlamydiae due to environmental influences.

Feral pigeons on building facades, on balconies and window ledges, or in open attics pose a health risk. Thus, keeping feral pigeons off buildings can contribute to the prevention of parasite infestations and the transmission of zoonotic agents. Avoiding attractive building structures already during building design is the best and most cost-effective way to prevent problems with feral pigeons in the future. In our study, we identified the structural parameters required to proof a building against feral pigeons. All experiments were performed with free ranging feral pigeons in the St. Matthäus Church pigeon loft. It could be demonstrated that feral pigeons are not able to pass through an outlet width of 4 cm, the respective outlet height is 5 cm, and a pigeon-safe square opening size is not exceeding 6 × 6 cm. A feral pigeon is not able to sit on a ledge if it’s width is 4 cm or smaller. The pigeon-safe angle of inclination for smooth construction materials (tinplate, glass, plastics) is 25°, for medium rough

materials (wood, plane concrete) 35°, and for rough materials (sandstone, rough concrete) at least 50°. With our study we provide the essential data required to proof a building against feral pigeons. Thus, we contribute to the prevention of disease transmissions and parasite infestations due to feral pigeons.

The low proportion of our feral pigeons in Basel, which are shedding *C. psittaci,* could be documented. However, *C. psittaci* could not be detected in faecal samples from public sites in the urban environment. At these unprotected sites pathogens in pigeon droppings are exposed to numerous physical environmental influences, such as sunlight, precipitation, and repeated freezing and thawing in winter. The regular cleaning of streets and squares in Basel additionally contributes to public health, since potentially infectious feral pigeon faeces are removed quickly. Considering the numerous opportunities of close contacts to feral pigeons in the urban environment, a transmission of the pathogen from feral pigeons to humans can never be ruled out. Therefore, feral pigeons present a significant potential health risk.

Zusammenfassung

Strassentauben (*Columba livia*, Gmelin 1789) gehören zu den häufigsten Wirbeltieren im städtischen Lebensraum und kommen in fast jeder grösseren Stadt der Welt vor. Strassentauben sind gut an das Leben in unseren Städten angepasst und es kommt oft zu engen Kontakten zu Menschen. Solche Kontakte bergen das Risiko einer zoonotischen Übertragung von Krankheitserregern und Parasiten. Der bedeutendste Krankheitserreger, der von Strassentauben auf den Menschen übertragen worden ist, ist *Chlamydia psittaci*, der Erreger der Aviären Chlamydiose und der Psittakose/Ornithose beim Menschen. Viele dieser Übertragungen wurden kurzen und vorübergehenden Kontakten zu Strassentauben im städtischen Lebensraum zugeschrieben. Ziel der vorliegenden Dissertation ist die Erfassung der Prävalenz von *C. psittaci* in der Basler Strassentaubenpopulation, die Identifikation von Übertragungswegen des Erregers in der Stadt sowie die Erarbeitung von Massnahmen zur Prävention von zoonotischen Übertragungen von *C. psittaci*. Diese Studien tragen zu einem besseren Verständnis des zoonotischen Gesundheitsrisikos bei, welches von Strassentauben ausgeht.

In einer ersten Studie wurden 47 Kotproben aus Nestboxen in einem Strassentaubenschlag der „Basler Taubenaktion“ in der St. Matthäus Kirche in Basel, Schweiz, gesammelt. Zusätzlich wurden 34 Proben vom Federpuderfilm auf der Wasseroberfläche von öffentlichen Brunnen entnommen wo Strassentauben regelmässig baden. Alle 81 Proben in dieser ersten Studie wurden mittels eines Antigen-ELISA Tests auf die Präsenz von chlamydialem Antigen untersucht. Hierzu wurde der IDEIA™ *PCE* Chlamydia Test (Dako Cytomation) verwendet. Positive Resultate wurden mit dem IDEIA™ Chlamydia Blocking Reagents (Dako Cytomation) verifiziert. Der erste Test lieferte eine hohe Anzahl an positiven Resultaten: 8 von 47 (23.5 %) Kotproben und 26 von 34 (76.5 %) Wasserfilmproben wurden positiv getestet. Der IDEIA™ Chlamydia Blocking Test hingegen konnte lediglich eine Kotprobe als positiv bestätigen. Dies konnte mittels Microarray bestätigt werden. Dieser ELISA-Test wurde für die weiteren Studien nicht mehr verwendet.

In der Hauptstudie wurde die Prävalenz der Ausscheidung von *C. psittaci* in frei lebenden Strassentauben im Taubenschlag der St. Matthäus Kirche untersucht. Insgesamt wurden 202 Individuen zu vier verschiedenen Zeitpunkten von 2007 bis 2009 mittels Rachen- und Kloakenabstrichen getestet. Einige der Tauben konnten mehrfach getestet werden. Auf diese Weise gelang es, die intermittierende Ausschüttung von Chlamydien zu untersuchen. Alle Proben wurden mit einer spezies-spezifischen Nested PCR Methode auf das *ompA* Gen von *C. psittaci* getestet. Es konnte nachgewiesen werden, dass die getesteten Tauben *C. psittaci* über die Atemwege ausscheiden, da 9 von 447 (2.0 %) Rachenabstriche positiv getestet wurden. Ausserdem wurden 11 von 348 (3.2 %) Kloakenabstriche positiv getestet, was darauf hinweist, dass diese Tauben *C. psittaci* über den Kot ausscheiden. Insgesamt konnte bei 17 von 202 Tauben *C. psittaci* nachgewiesen werden (8.4 %). Die Mehrzahl dieser Tiere zeigte intermittierende Erregerausschüttung. Dies ist zurzeit die erste Studie in der frei lebende Strassentauben wiederholt getestet wurden und somit auch die erste die intermittierende Ausschüttung von *C. psittaci* bei diesen Vögeln dokumentiert. Die Genotypisierung der positiven Proben mittels Real-time PCR ergab *C. psittaci* Genotyp B bei 7 Tauben, sowie eine Mischinfektion mit den Genotypen A, B und E/B bei einer Taube. Eine Mischinfektion mit diesen drei Genotypen konnte zum ersten Mal nachgewiesen werden. Sieben der positiv getesteten Tauben sind als Erwachsene Tiere in den Taubenschlag eingewandert, unter anderem auch die Taube mit der Mischinfektion. Hiermit wurde nachgewiesen, dass die Vernetzung zwischen den Subpopulationen die Verbreitung von Krankheitserregern begünstigt. Zusätzlich wurden 620 Taubenkotproben von öffentlichen Plätzen in der Stadt untersucht. In diesen Proben aus dem städtischen Lebensraum konnte *C. psittaci* nicht nachgewiesen werden. Wir vermuten, dass entweder zum jeweiligen Testzeitpunkt keine ausschüttenden Vögel an diesen Orten vorhanden waren oder dass dieses Resultat auf eine vorhergehende Zerstörung der Chlamydien durch Umwelteinflüsse zurückzuführen ist.

Strassentauben an Hausfassaden, auf Balkonen, auf Fenstersimsen oder in offenen Dachstöcken stellen ein Gesundheitsrisiko dar. Strassentauben von Gebäuden fern zu halten, trägt somit zur Prävention von Parasitenbefall und der Übertragungen von Krankheitserregern bei. Das Vermeiden von attraktiven Strukturen, bereits während

der Planungsphase eines Gebäudes, ist die beste und kostengünstigste Methode um zukünftige Probleme mit Strassentauben zu vermeiden. In unserer Studie haben wir die wichtigsten strukturellen Parameter identifiziert, die es ermöglichen, Tauben von Gebäuden fern zu halten. Alle Experimente wurden mit frei lebenden Strassentauben im Taubenschlag in der St. Matthäus Kirche durchgeführt. Wir konnten zeigen, dass Tauben nicht dazu in der Lage sind, eine 4 cm breite Öffnung zu durchqueren, die entsprechende Höhe beträgt 5 cm und eine taubensichere quadratische Öffnung darf nicht grösser sein als 6 x 6 cm. Eine Strassentaube ist nicht in der Lage, auf einem Sims von 4 cm Breite oder weniger zu sitzen. Der taubensichere Neigungswinkel für geneigte Oberflächen beträgt für glattes Baumaterial 25° (Kupferblech, Glas, Kunststoff), für mittelraues Baumaterial 35° (Holz, glatter Beton) und für raues Baumaterial mindestens 50° (Sandstein, rauer Beton). Mit unserer Studie liefern wir die essentiellen Daten, die notwendig sind um ein Gebäude gegen Strassentauben abzusichern. Auf diese Weise tragen wir zur Prävention von Krankheits- und Parasitenübertragung durch Strassentauben bei.

Es konnte dokumentiert werden, dass ein vergleichsweise geringer Prozentsatz unserer Strassentauben in Basel *C. psittaci* ausscheidet. In Kotproben von öffentlichen Plätzen in der Stadt konnten wir *C. psittaci* jedoch nicht nachweisen. An solch ungeschützten Orten sind die Krankheitserreger im Taubenkot zahlreichen physikalischen Einflüssen ausgesetzt wie z.B. Sonnenlicht, Regen und wiederholtes Gefrieren und Auftauen im Winter. Die regelmässige Reinigung der Strassen und Plätze in Basel trägt zusätzlich zur öffentlichen Gesundheit bei. Potenziell infektiöser Strassentaubenkot wird schnell entfernt. In Anbetracht der zahlreichen Möglichkeiten für enge Kontakte zu Strassentauben im städtischen Lebensraum kann eine Übertragung des Krankheitserregers von Strassentauben auf den Menschen nie völlig ausgeschlossen werden. Deshalb stellen Strassentauben ein erhebliches, potenzielles Gesundheitsrisiko dar.

Chapter 1

General Introduction

1. General Introduction

The feral pigeon (*Columba livia*) is part of the townscape of almost every larger city in the world. More than any other animal species living in our cities, the feral pigeon has become a symbol of wildlife in the urban environment. At present, between 170–340 million feral pigeons are estimated to live in cities around the world (Haag-Wackernagel, 2010). Feral pigeons are descendants of the domesticated form of the wild living rock dove (*Columba livia*, Gmelin 1789). Rock doves prefer crevices and caves on rocky cliffs for breeding (Haag-Wackernagel, 1998). In the urban habitat, window ledges, balconies, and facade ornaments on buildings and monuments represent structures analogue to the natural habitat of the rock dove and provide places for roosting and breeding (Haag-Wackernagel, 1998). Feral pigeons are well adapted to life in our cities, where they often come into close contact with humans (Johnston & Janiga, 1995; Haag-Wackernagel, 1998). The size of feral pigeon populations predominantly depends on the food supply provided by humans and to a lesser extent on seasonally occurring natural food (Haag, 1984).

Figure 1: Feral pigeons feeding on discarded bread at the Marketplace in Basel.

Feral pigeons can cause numerous problems. At roosting and breeding sites, large amounts of faecal droppings, nesting material, and decaying corpses of dead nestlings can accumulate. Feral pigeon droppings cause fouling and biodeterioration of buildings and monuments and thus generate high costs for building owners and communities. Furthermore, feral pigeons are carriers of numerous zoopathogens and parasites that can be transmitted to humans. To date, a total of 110 microorganisms, which can potentially infect humans, have been detected in feral pigeons (supplemented data according to Haag-Wackernagel, 2006a,b). However, the mere presence of a pathogenic organism in a feral pigeon population does not allow any statement about the actual zoonotic threat (Haag-Wackernagel & Moch, 2004). Additional important factors need to be taken into account. Such factors are the virulence of the respective pathogen, the transmission route, the infectious dose, the immune status of the exposed person, and the opportunity of a close contact that favours a pathogen transmission (Haag-Wackernagel & Moch, 2004; Haag-Wackernagel, 2006a,b). This may explain why only seven of the 110 pathogens have evidentially been transmitted from feral pigeons to humans. In total, 242 cases of disease transmission have been reported in the medical literature, 13 of them took a fatal course (supplemented data according to Haag-Wackernagel, 2006a,b). The seven pathogens transmitted from feral pigeons to humans were: *Chlamydia psittaci* (113), *Histoplasma capsulatum* (91), *Aspergillus* ssp. (13), *Candida* ssp. (12), *Cryptococcus neoformans* (11), *Salmonella enterica* serovar Kiambu (1) and *Toxoplasma gondii* (1) (number of cases in parentheses). All cases of aspergillosis and candidosis, 7 cases of cryptococcosis, and 6 cases of psittacosis affected persons with known immunosuppression. Despite these case reports documented in the medical literature, many animal protection activists still deny the possible threat to public health feral pigeons can pose. Reliable data about the actual zoonotic risk for humans, their pets and livestock are therefore needed.

The present thesis is focused on *C. psittaci*, which is the most important pathogen transmitted from feral pigeons to humans. *C. psittaci* accounts for 113 of proved or presumed disease transmissions from feral pigeons to humans, two of them with a fatal outcome. A detailed description of *C. psittaci* is given in Chapter 3. Until recently, the bacterium was termed *Chlamydophila psittaci*, as proposed by Everett *et al*. (1999). However, in the latest edition of “Bergey’s Manual of Systematic

Bacteriology", the original genus *Chlamydia* is retained, since the genus *Chlamydophila* has not equally been accepted throughout the scientific community (Kuo *et al*. 2011). In this thesis, the genus *Chlamydia* is used. In all parts published before 2011, the original published version is given.

C. psittaci is an obligate intracellular gram-negative bacterium, which causes avian chlamydiosis in birds and psittacosis in humans, also referred to as ornithosis or parrot fever (Andersen & Vanrompay, 2003). Human psittacosis infections cause a wide range of signs and symptoms that can range from mild, influenza-like symptoms to severe atypical pneumonia, diarrhoea, endocarditis, myocarditis, hepatitis, arthritis, keratoconjunctivitis, encephalitis, and probably also ocular adnexal lymphoma (NASPHV, 2010). Due to the wide range of unspecific symptoms, it is very likely that the disease often gets misdiagnosed and is therefore underreported. Unapparent infections have also been documented in humans (Harkinezhad *et al*., 2009; NASPHV, 2010).

Feral pigeons are commonly infected with *C. psittaci* and in most cases no signs of illness can be observed (Andersen & Vanrompay, 2003; Harkinezhad *et al*., 2009). Feral pigeons can become asymptomatic, latent carriers of the disease. In clinically healthy, asymptomatic birds, chlamydial shedding by faeces or ocular- and nasal secretions can occur intermittently (Harkinezhad *et al*., 2009). Chlamydial shedding is mainly triggered by stress factors, such as overcrowding, breeding, chilling, malnutrition, other diseases and parasites (Andersen & Vanrompay, 2003; Harkinezhad, *et al*., 2009). The replicating intracellular form of *C. psittaci* is the reticulate body (RB). RBs can differentiate into the infectious, yet metabolically inactive elementary body (EB). These EBs are released from the host cell where they infect other cells or they are shed into the environment by ocular- or respiratory secretions, or by faeces (Andersen & Vanrompay, 2003). Cryptic persistent forms of RBs that remain inside the host cell can also be found. These forms are not reproducing or transforming into EBs, but they remain metabolically active. These aberrant RBs can quickly retransform into normal RBs and start to differentiate into infectious EBs again (Harkinezhad *et al*., 2009). These cryptic, persistent forms are responsible for chronic chlamydial infections. The mechanisms of persistence are poorly understood and are currently investigated (Belland *et al*., 2003; Harkinezhad

et al., 2009). Intermittent shedding of *C. psittaci* is typical of chronic infections in birds, which are known to occur in numerous bird species, including pigeons and doves (Andersen & Vanrompay, 2003). Shedding of infectious EBs into the environment contributes to the spread of the disease in the feral pigeon population and represents a zoonotic risk for humans. Since shedding occurs intermittently, the zoonotic potential of feral pigeons with subclinical, persistent *C. psittaci* infections is difficult to assess. Repeated examination of the birds can therefore improve prevalence estimates.

Humans acquire *C. psittaci* infections by inhaling contaminated faecal dust or dried ocular- and respiratory secretions from infected birds (Andersen & Vanrompay, 2003). Haag-Wackernagel (2006a,b) proposes four major risk factors for acquiring *C. psittaci* from feral pigeons:

- Occupational dust exposure
- Handling of sick or dead feral pigeons
- Feral pigeon feeding
- Loose and transient contacts to feral pigeons in the urban environment

Occupational dust exposure

Feral pigeon faeces can accumulate on window ledges, balconies, open attics, or other architectural structures on buildings and monuments. Accumulations of faecal droppings provide optimal conditions for the survival of pathogenic microorganisms that can be transmitted to humans, including elementary bodies of *C. psittaci* (Albrecht *et al*., 2003). In closed rooms, the removal of large amounts of faecal material can lead to heavy dust formation. Accumulations of pigeon droppings pose a zoonotic risk for construction workers and pest control workers in contaminated areas (Albrecht *et al*., 2003; Haag-Wackernagel 2006a,b). Thus, the use of appropriate personal protective equipment (PPE) is crucial for the prevention of infections.

Handling of sick or dead feral pigeons

Compassionate animal lovers or other caring persons, especially children, may take sick feral pigeons home in order to care for them. Feral pigeons showing overt signs of illness may be shedding large amounts of infectious elementary bodies (Andersen

& Vanrompay, 2003). Therefore, handling of sick or dead feral pigeons can pose a severe risk of infection. Children should be educated not to touch feral pigeons or other wild living animals.

Feral pigeon feeding

Feral pigeon feeding has additionally been identified as a risk factor for psittacosis. However, it is difficult to assess the closeness of contact to the birds in these cases.

Loose and transient contacts

Haag-Wackernagel (supplemented data, 2006a,b) documented in his review a total of 53 cases in which loose and transient contacts to feral pigeons have been identified as the possible source of infection. However, *C. psittaci* infections due to loose and transient contact to feral pigeons are difficult to prove and thus they are often based on speculation. Such contacts were e.g. feral pigeons staying on window ledges, feral pigeons breeding at house facades, feral pigeons in the neighbourhood, or the mere presence of feral pigeons in the city environment (Babudieri, 1956, 1964; Jansson, 1960; Süss *et al*., 1996). Loose and transient contacts to feral pigeons in the urban environment are often unintended and therefore difficult to avoid (Fig.2). This stands in contrast to intended contacts, where infection can be prevented by the use of appropriate PPE.

To assess the actual zoonotic risk posed by feral pigeons, it is important to know the prevalence of *C. psittaci* infections in the feral pigeon population. According to Haag & Gurdan (1990), 62 % of the feral pigeons in Basel are seropositive for *C. psittaci*. However, serology alone does not provide sufficient information about the current state of the disease in a bird. Single point positive sera indicate merely a present or past *C. psittaci* infection that may be reactivated at any point in the future, if cryptic persistent chlamydiae are present in organ tissues (Harkinezhad, *et al*. 2009). In contrast, ill birds shedding chlamydiae can be negative by serology (Babudieri, 1964). Hence, serology alone does not allow a conclusion about whether a bird is currently shedding the pathogen or not.

Figure 2: Feral pigeons assemble daily at the Centralbahnplatz in front of the Basel SBB railway station, causing considerable accumulations of faecal droppings.

1.1. AIM OF THIS THESIS

The aim of this thesis is to investigate the epidemiology of *C. psittaci* in the feral pigeon population of Basel, Switzerland, to contribute to a better understanding of the zoonotic risk posed by feral pigeons in the urban environment. The prevalence of *C. psittaci* in our feral pigeon population is assessed and possible transmission routes in the city are identified.

The following objectives are pursued:

- to review the literature concerning *C. psittaci* infections in feral pigeons.
- to find suitable diagnostic methods for the identification of *C. psittaci* in different kinds of samples.

- to assess the prevalence of *C. psittaci* in the local feral pigeon population.
- to describe practically applicable methods for feral pigeon management and disease prevention.

The following research questions will be addressed:

- What is the prevalence of *C. psittaci* in the feral pigeon population of Basel?
- Does intermittent shedding of *C. psittaci* occur in individual birds?
- Is *C. psittaci* present in the urban environment of Basel?
- Which routes of *C. psittaci* transmission can be identified in the city?
- What preventive measures can we take to minimize the risk of human *C. psittaci* infections caused by feral pigeons?
- How can buildings be protected against feral pigeons by the use of architectural measures?
- What is the dimension of the zoonotic risk presented by feral pigeons in Basel?

The present thesis consists of a general introduction (Chapter 1), followed by a review article, to which I contributed (Chapter 2) and three original papers (Chapters 3, 4 and 5). In Chapter 6 the main results are discussed and the main conclusions are provided. In Chapter 2, 3, and 4, the problem of zoonotic transmission of *C. psittaci* from feral pigeons to humans is addressed. In Chapter 5, the essential parameters, which are important to proof buildings against feral pigeon, are described.

The following chapters are manuscripts published independent of each other.

Chapter 2 is a review by Magnino *et al*. (2009) that has been published in a special issue of “Veterinary Microbiology”. Numerous studies have been conducted in different European countries over the past years to assess the prevalence of *C. psittaci* in feral pigeons. The results of these studies and their implications for public health are summarized and discussed. This review has been elaborated as a collaboration of scientists participating in the COST Action 855 “Animal Chlamydiosis and the Zoonotic Implications”. I contributed to this review by providing parts of the

introduction, the biology of the feral pigeon, feral pigeon management, epidemiology of the feral pigeon, and different parts of the discussion.

In **Chapter 3** a preliminary study conducted to address the issue of the risk of zoonotic transmission of *C. psittaci* in the urban environment is described. A commercial Antigen-ELISA-Kit was used to detect chlamydial antigen in environmental samples, such as feral pigeon faeces and water-film samples from public fountains. Different diagnostic methods for the detection of *C. psittaci* and their suitability for the present study are discussed.

Chapter 4 provides the results of the main study conducted from 2007 to 2009 with feral pigeons of the pigeon loft in the St. Matthäus Church in Basel, Switzerland. All the birds resident in the loft were tested at four time points for the presence of *C. psittaci* in swab samples taken from the pharynx and the cloaca by use of a nested PCR assay. Additionally, faecal samples taken from different sites in the public environment of Basel were analyzed.

Chapter 5 provides the results of a study conducted in the St. Matthäus Church loft to assess the architectural parameters, which are required to keep feral pigeons away from buildings.

In **Chapter 6** the main findings are discussed and the conclusions are provided.

1.2. MATERIALS AND METHODS

All materials and methods are described in detail in the Chapters 3–5. In this introduction, the feral pigeons investigated and the methods used for the identification of *C. psittaci* are described.

Feral pigeons studied

All experiments were performed with the feral pigeons resident in a pigeon loft in the St. Matthäus Church in Basel, Switzerland (hereafter referred to as the St. Matthäus loft). This loft is part of the “Pigeon Action of Basel” (“Basler Taubenaktion”). This feral pigeon control programme was implemented between 1988 and 1990 and

involved the construction of nine feral pigeon lofts in public buildings in Basel (Haag-Wackernagel, 1993, 1995). More details on this programme are given in Chapter 4. The loft is located above the nave of the St. Matthäus Church and is constantly inhabited by about 120 birds. The loft is cleaned of droppings, nesting material and carcasses every 14 days. The birds use either the 39 breeding boxes on the wall or the loft floor for breeding. All birds hatching in the loft, as well as individuals that immigrated from other subpopulations are individually marked with a metal foot ring and registered in a database. Thus, the life history of each bird is documented. The pigeons are not fed in the loft and are forced to search for food and water themselves. They use the loft for roosting and breeding and are free to enter or leave the loft at will. Juveniles and adults can stay in their home colony or establish in other breeding flocks. Thus, feral pigeon subpopulations are interconnected by emigration and immigration, which offers opportunities for the transmission of diseases and parasites. Rose *et al*. (2006) showed that feral pigeon subpopulations of Basel overlap at important feeding sites where disease and parasite transmission can occur. Therefore, the birds of the St. Matthäus Loft loft are representative for the whole feral pigeon population of Basel. The direct access to free ranging feral pigeons in this loft offers a unique opportunity to perform epidemiological studies representing the conditions of an uninfluenced, urban feral pigeon population.

Detection of *Chlamydia psittaci*

Antigen-ELISA

Fast and cost-effective screening methods like commercial antigen-ELISA tests can be used to detect chlamydiae. These tests were originally intended for the diagnosis of *Chlamydia trachomatis* in swab samples from humans. However, these tests are not officially licensed for the use in veterinary medicine (OIE, 2009). Some authors stated that these tests yielded a high number of false positive results, while others use these methods for routine diagnostics (Gaede *et al*. 2005; Vanrompay *et al*., 1994). In the study described in Chapter 3, one of these commercial ELISA-assays has been used. All methods are described in detail in Chapter 3.

Nested PCR

Swab samples taken from feral pigeons and faecal samples from public sites in the city were tested for the presence of *C. psittaci* DNA. Nucleic acid amplification

techniques, such as the polymerase chain reaction (PCR), are very sensitive and specific. Moreover, these methods offer the opportunity to determine the chlamydial genotype of a positive sample. Thus it is possible to trace back human infections to specific avian hosts (Harkinezhad *et al*., 2009; Heddema *et al*., 2006). This study is described in Chapter 4. The nested PCR assay described by Van Loock *et al*. (2005) appeared suitable. This nested PCR targets a conserved region of the Major Outer Membrane Protein A gene (*ompA*) of *C. psittaci*. This assay is highly sensitive and species specific for *C. psittaci*. According to Van Loock *et al*. (2005), the sensitivity is established at 10^{-2} inclusion forming units (IFU) and the specificity is 100 %. The assay is well established and is routinely used in studies conducted by the research group of Prof. Dr. Daisy Vanrompay at Ghent University (Belgium). I had the opportunity to collaborate with this research group for my study. The sensitivity of nested PCR procedures can equal the sensitivity of the more expensive real-time PCR technique. However, nested PCR procedures are known to be particularly prone to carry-over contamination. Thus, special precautions must be taken. Each step of the nested PCR procedure was conducted with a dedicated set of pipettes using aerosol barrier tips (Vaudaux-Eppendorf, Switzerland). Setup of reagents, DNA extraction and post-PCR analysis by gel electrophoresis were performed in separate rooms.

DNA extraction

Swab samples were taken using sterile rayon-tipped aluminium-shafted swabs (Copan, Italy). Samples were transported on ice. DNA from swab samples and faecal samples was extracted using the STD-method, as previously described by Van Loock *et al*. (2005). Swabs were thawed and shaken for one hour at 300 rpm at room temperature. Specimens were briefly vortexed and the swabs were discarded. Remaining suspensions were centrifuged at 2'700 × *g* for 10 minutes at room temperature. Supernatants were transferred to fresh 1.5 ml microcentrifuge tubes and pelleted at 14'000 × *g* for one hour at room temperature. Pellets were resuspended in 198 µl STD buffer (0.01 M Tris-HCl [pH 8.3], 0.05 M KCl, 0.0025 M $MgCl_2$* $6H_2O$, 0.5 % Tween 20) and 2 µl of Proteïnase K (20 mg/ml stock solution, Applied Biosystems). Specimens were incubated for one hour at 56° C, subsequently heated at 100° C for 10 minutes and stored at -20° C until testing.

ompA nested PCR

The nested PCR for detection of the *C. psittaci* major outer-membrane protein A gene (*ompA*) was performed as previously described (Van Loock *et al*., 2005). The method targets a 472-bp fragment of the *ompA* gene of *C. psittaci*, as well as a 703-bp fragment of an internal control plasmid, which serves as an inhibition control to rule out false negative results due to PCR inhibition. All PCR reactions were prepared using a PCR-cooler (Vaudeaux-Eppendorf, Switzerland). The following primers were used (Microsynth, Switzerland):

- sense outer *ompA* (5´-CCT GTA GGG AAC CCA GCT GAA-3´)
- anti-sense outer *ompA* (5´-GGC TGA GCA ATG CGG ATA GTG T-3´)
- sense inner *ompA* (5´-GCA GGA TAC TAC GGA GA-3´)
- anti-sense inner *ompA* (5´-GGA ACT CGG CTC CTA AAG-3´)

Both nested PCR rounds were performed in a buffer consisting of 50 mM KCl, 20 mM Tris-HCl (pH 8.3), 2 mM MgCl2, 0.1% Tween 20, 200 µM each dNTP (Qiagen, Switzerland), 0.625 µM each outer primer (round 1), 1 µl Super-Taq buffer and 0.1 U Super-Taq polymerase (5 U/µl) (Endotell, Switzerland). To 45 µl of this reaction mixture, 5 µl of DNA extract were added resulting in a final reaction volume of 50 µl. Initial denaturation in both rounds occurred at 95° C for 5 minutes. In the first round, 20 cycles of one minute at 95° C, two minutes at 59° C and three minutes at 72° C, were performed (Unocycler, VWR International). The final elongation was performed at 72° C for 5 minutes in both rounds. For the second round, 10 µM of each inner primer were used. The annealing temperature was lowered to (47° C) and the number of cycles was changed to 25. All samples were tested in parallel, once including 10 ng of inhibition control plasmid to detect possible inhibition of PCR. After the second nested PCR round, the *ompA*- specific band (472 bp) could clearly be distinguished from the band of the control plasmid (703 bp). In each run, a positive control was included to test the performance of the PCR (0.2 ng/ml of genomic DNA of *C. psittaci* strain 92/1293). A negative control containing MilliQ water instead of sample DNA was also included in every test. PCR-products were analyzed by gel electrophoresis in a 1.2 % agarose gel in 0.5 × TBE buffer (Invitrogen AG, Switzerland), stained with ethidium bromide and visualized using UV-illumination.

The size of the bands was determined using the BenchTop 1kb DNA Ladder (Promega AG, Switzerland).

Real-time PCR for genotyping of the positive samples was performed at Ghent University as previously described (Geens *et al*. 2005). The inhibition control plasmid and the positive control DNA were kindly provided by Prof. Dr. Daisy Vanrompay (Ghent University, Belgium).

1.3. REFERENCES

Albrecht A, Schies U, Kämpfer P, Scholbeck R. (2003). Gesundheitsgefährdung durch Taubenkot [Health hazards posed by pigeon droppings]. *Sonderdruck aus TIEFBAU, Tiefbau-Berufsgenossenschaft, München*; Heft 5/2001 und 3/2002, 1–14.

Andersen AA, Vanrompay D. (2003). Avian Chlamydiosis (psittacosis, ornithosis). In: Saif YM, ed. *Diseases of Poultry*, 11th edition, Iowa State Press, Iowa, USA, pp. 863–879.

Babudieri B. (1956). L'ornitosi: sua presenza e frequenza in Italia. *Terapia*. 317, 3–19.

Babudieri B. (1964). Epidemiologie und Virologie der Ornithose beim Menschen. *Archiv Exper Vet Med*. 18, 5–18.

Belland RJ, Nelson DE, Virok D, Crane DD, Hogan D, Sturdevant D, Beatty WL, Caldwell HD. (2003). Transcriposome analysis of chlamydial growth during IFN-gamma-mediated persistence and reactivation. *Proc Natl Acad Sci U S A*. Dec 23, 100(26), 15971–15976. Epub 2003 Dec 12.

Everett KD, Bush RM, Andersen AA. (1999). Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. *Int J Syst Bacteriol*. 49 (Pt 2.), 415–440.

Gaede W, Kenklies S, Dresenkamp B. (2005). Effectiveness of PCR, antigen-ELISA and cell cultivation for the diagnosis of chlamydia-infections in different animal species. *Proceedings of the third annual workshop of the COST Action 855, Diagnosis and Pathogenesis of Avian Chlamydiosis*, Bononia University Press, Bologna, Italy, pp. 95–105.

Geens T, Dewitte A, Boon N, Vanrompay D. (2005) Development of a *Chlamydophila psittaci* species-specific and genotype-specific real-time PCR. *Vet Res*. 36(5-6), 787–97.

Haag D. 1984. Ein Beitrag zur Oekologie der Stadttaube. *Dissertation*. Phil.Nat. Fakultät der Universität Basel, Verlag Medizinische Biologie.

Haag-Wackernagel, D. (1993). Street Pigeons in Basel. *Nature*. 361, 200.

Haag-Wackernagel D. (1995). Regulation of the street pigeon in Basel. *Wildl Soc Bull*. 23 (2), 256–260.

Haag-Wackernagel D. (1998). *Die Taube. Vom heiligen Vogel der Liebesgöttin zur Strassentaube*. Verlag Schwabe & Co. AG, Basel.

Haag-Wackernagel D. (2006a). Human diseases caused by feral pigeons. In: Chris J. Feare, David P. Cowan, (Eds.), *Advances in Vertebrate Pest Management,* 4. Filander Verlag, Fürth, pp. 31–58.

Haag-Wackernagel D. (2006b). Gesundheitsgefährdungen durch die Strassentaube *Columba livia*: Krankheiten. *Amtstierärztlicher Dienst und Lebensmittelkontrolle.* 4, 262–272.

Haag-Wackernagel D. (2010). Gesundheitsgefährdungen durch Strassentauben. *Praxis der Naturwissenschaften*. 7(59), 26–30.

Haag D, Gurdan P. (1990). Über den hygienischen Zustand der Strassentauben in Basel. *Swiss Vet* – Schweizerische Zeitschrift für Veterinärmedizin. 7/6, 19–22.

Haag-Wackernagel D, Moch H. (2004). Health hazards posed by feral pigeons. *The Journal of Infection*. 48(4), 307–313.

Harkinezhad T, Geens T, Vanrompay D. (2009). *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. *Vet Microbiol*. 135 (1–2), 68–77.

Heddema ER, Van Hannen EJ, Duim B, Vandenbroucke-Grauls CM, Pannekoek Y (2006b). Genotyping of *Chlamydophila psittaci* in human samples. *Emerg Infect Dis.* 12(12), 1989–1990.

Jansson E. (1960). Ornithosis in Helsinki and some other localities in Finland. *Annales medicinae experimentalis et biologiae Fenniae* 38, suppl 4, 6–110.

Johnston RF, Janiga M. (1995). *Feral Pigeons*. Oxford University Press, New York, Oxford, 320 S.

Kuo C, Stephens R. (2011). Family I. Chlamydiacaea. in *Bergey's Manual of Systematic Bacteriology. Second Edition.* pp. 845. Edited by William B. Whitman. Springer Science+Business Media, New York, USA.

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovč A, Prukner-Radovčić E., Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF. (2009). Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Vet Microbiol*. 135, 54–67.

National Associaton of State Public Health Veterinarians (NASPHV). (2010). Compendium of Measures to control *Chlamydophila psittaci* infection among humans (psittacosis) and birds (avian chlamydiosis). Available at: *www.nasphv.org/documentsCompendiaPsittacosis.html*

OIE (World Organisation for Animal Health). (2009). *Manual for Diagnostic Tests and Vaccines for Terrestrial Animals*. pp. 431–442.

Rose E, Nagel P, Haag-Wackernagel D. (2006). Spatio-temporal use of the urban habitat by feral pigeons (*Columba* livia). *Behav Ecol Sociobiol*. 60, 242–254.

Süss A, Reetz J, Schulze P, Kretzschmar M, Schirrmeister W, Süss J. (1996). [Severe course of ornithosis and its intensive care and diagnostic problems – a case report]. *Anaesthesiol Reanim*. 21(4), 97-102.

Van Loock, M, Verminnen, K, Messmer, TO, Volckaert, G, Goddeeris, BM, Vanrompay D (2005). Use of a nested PCR-enzyme immonoassay with an internal control to detect *Chlamydophila psittaci* in turkeys. *BMC Infectious Diseases* 26, 5, 76.

Vanrompay D, Van Nerom, A Ducatelle R, Haesebrouck F (1994). Evaluation of five immunoassays for detection of *Chlamydia psittaci* in cloacal and conjunctival specimens from turkeys. *J Clin Microbiol*. 32, 1470–14

Chapter 2

Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications

S. Magnino, D. Haag-Wackernagel, I. Geigenfeind, S. Helmecke, A. Dovč, E. Prukner-Radovčić, E. Residbegović , V. Ilieski, K. Laroucau, M. Donati, S. Martinov, E.F. Kaleta

---

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovč A, Prukner-Radovčić E, Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF. (2009). Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Veterinary Microbiology.* 135, 54–67

Veterinary Microbiology 135 (2009) 54–67

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications

S. Magnino [a,*], D. Haag-Wackernagel [b], I. Geigenfeind [b], S. Helmecke [c], A. Dovč [d], E. Prukner-Radovčić [e], E. Residbegović [f], V. Ilieski [g], K. Laroucau [h], M. Donati [i], S. Martinov [j], E.F. Kaleta [c]

[a] *National Reference Laboratory for Animal Chlamydioses, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Sezione Diagnostica di Pavia, Strada Campeggi 61, 27100 Pavia, Italy*
[b] *Institute of Anatomy, Department of Biomedicine, University of Basel, Pestalozzistrasse 20, CH-4056 Basel, Switzerland*
[c] *Klinik für Vögel, Reptilien, Amphibien und Fische, Justus-Liebig-Universität Giessen, Frankfurter Strasse 91-93, D-35392 Giessen, Germany*
[d] *Institute for Health Care of Poultry, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia*
[e] *Department of Avian Diseases, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia*
[f] *Faculty of Veterinary Medicine, University of Sarajevo, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina*
[g] *Faculty of Veterinary Medicine, University Sts Cyril and Methodius, Lazar Pop-Trajkov 5/7, 1000 Skopje, The FYR of Macedonia*
[h] *French Food Safety Agency, Bacterial Zoonosis Unit, LERPAZ, 23 Avenue du Général de Gaulle, 94706 Maisons-Alfort, France*
[i] *DMCSS, University of Bologna, St Orsola Hospital, Via Massarenti 9, 40138 Bologna, Italy*
[j] *National Diagnostic and Research Veterinary Medical Institute, 15, P. Slaveykov blvd., Sofia 1606, Bulgaria*

ARTICLE INFO

Keywords:
Chlamydophila psittaci
Diagnosis
Epidemiology
Feral pigeons
Health hazard
Zoonosis

ABSTRACT

Feral pigeons (*Columba livia domestica*), which thrive in most European towns and cities, are commonly infected with the zoonotic bacterium *Chlamydophila psittaci*, the agent of psittacosis (also known as ornithosis) in humans. A number of surveys carried out over the last thirty years across Europe have detected high seropositivity values and high percentages of infection in feral pigeon populations. Overall, when considering data from 11 European countries, seropositivity values to *C. psittaci* in the sampled populations ranged from 19.4% to 95.6%. In most surveys, the complement fixation test was used, and antibodies were detected in 19.4–66.3% of the samples, with a median of 46.1%. Indirect immunofluorescence and ELISA tests were employed less frequently, but led to the detection of higher percentages of seropositivity (23.7–67.7% and 35.9–95.6%, respectively). Attempts to grow *C. psittaci* in cell culture or embryonated chicken eggs were successful in 2–42.3% and 0–57.1% of samples, respectively, antigen detection methods were positive in 2.3–40% of samples, while conventional PCR and real-time PCR using different genomic targets detected the organism in 3.4–50% of samples. Twenty-five *C. psittaci* isolates from pigeons were typed as *ompA* genotype B ($n = 14$), E ($n = 10$) and E/B ($n = 1$).

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

* Corresponding author. Tel.: +39 0382 422006; fax: +39 0382 422066.
E-mail address: simone.magnino@bs.izs.it (S. Magnino).

0378-1135/$ – see front matter
doi:10.1016/j.vetmic.2008.09.045

Education initiatives as to the communication of a health risk resulting from contact with pigeons and pigeon excreta should primarily be targeted at individuals who may be exposed to *C. psittaci*-contaminated dust, such as demolition/construction workers. Recommendations to this category of workers include wearing protective clothes with hoods, boots, gloves and air filter face masks when removing pigeon faeces from roofs, garrets and buildings, especially if working indoors. Monitoring for *C. psittaci* infections in these workers over time should also be considered. Children should be warned not to handle sick or dead pigeons, and immunocompromised individuals should be advised to carefully limit their contact to feral pigeons.

Culling of pigeons by shooting or poisoning is both unethical and ineffective as the place of the killed birds in the population is quickly filled by new juveniles or immigrating birds from neighbouring areas. Pigeon-deterring systems, such as nets and plastic or metal spikes applied to buildings and monuments will prevent their fouling, and the administration of contraceptive drugs may allow size regulation of the pigeon populations. Nevertheless, the measure that will ultimately lead to permanent reduction and will establish healthy sustainable populations is the restriction of indiscriminate feeding by pigeon lovers. The erection of dovecotes and artificial breeding facilities should be considered for providing shelter and a balanced diet to the birds, as well as a chance of interaction for pigeon lovers in a hygienically controlled environment.

1. Introduction

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

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

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

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

2. The ecology of feral pigeons in the urban environment

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

Today, the feeding of pigeons by "pigeon lovers" is mainly responsible for the establishment of large pigeon populations in our cities and a supplemental input for their increase is provided by rubbish and seasonally occurring natural food, such as grass and tree seeds in parks and gardens (Haag, 1984). The extensive food supply indeed provides the ecological basis for the large populations that occur in most cities of the world (Haag-Wackernagel, 1993, 1995, 2002; Kösters et al., 1994). Pigeons in fact do not need to commute on risky flights to look for more natural food supplies in the countryside and are minimally threatened by predatory birds, whose populations have been drastically decimated over the years by hunting and by deliberate or accidental poisoning. Regular feeding of pigeons by their feeders throughout the year allows pigeons extra time for breeding, so that some individuals

are able to breed throughout the year. Furthermore, several behavioural changes have increased the chances of survival of feral pigeons in urban environments. These birds are in fact extremely adaptable, which also enables them to accept breeding places that are unnatural to them, e.g. on trees or over running ventilation systems.

As the density of nesting and roosting pigeons increases, the quality of life in the feral pigeon population deteriorates. In fact, excessive population density activates and stimulates regulation mechanisms that decimate nestlings and juvenile pigeons with infectious and parasitic diseases. Crowded breeding places make pigeons behave more aggressively, which again mostly affects nestlings and juveniles that are the weakest members of the population, leading to a progressive spoiling of their physical condition.

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

Feral pigeons are gregarious birds that gather in swarms in streets, squares and parks, and along rivers and lakes. Being often huge, these swarms are of increasing concern for owners of buildings, city administrators and communal health officers (Haag-Wackernagel and Moch, 2004). Concerns have been raised for the detrimental effect of pigeon droppings on environmental hygiene and for the fouling of buildings and monuments. In fact, breeding sites for feral pigeons are usually man-made structures such as holes in the façades of private and community buildings, churches and city towers and structures under bridges (Kösters et al., 1991). Pigeon faeces are thus continuously shed over monuments, statues, roofs, streets and sidewalks, leading to extensive fouling and progressive damage due to the corrosive nature of the acidic contents. A pigeon produces around 12 kg of faeces per year that are mainly deposited at the roosting, breeding and feeding sites (Haag, 1984; Kösters et al., 1991). The progressive damage to marble and limestone is mainly due to the action of organic acids other than uric acid, which does not seem to be able by itself to deteriorate calcareous stone (Del Monte and Sabbioni, 1986; Dell'Omo, 1996). Pigeon droppings have also proved to be an excellent substrate for the growth of microorganisms such as fungi and bacteria. In particular, the mycelial growth of some fungi (e.g., *Aspergillus* spp.) may by itself cause alteration in marble surfaces through the mechanical action exerted by the fungal hyphae. In addition, some fungal species that grow on pigeon excrement secrete acidic products (especially low-molecular weight organic acids) that contribute to the chemical erosion of calcareous material such as marble. Feral pigeons can be a real problem for historical monuments, and in the case of the cathedral of Milan they have probably contributed to the deterioration of many statues and pinnacles (Bassi and Chiatante, 1976; Mendez-Tovar et al., 1995).

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

4. Overview of management strategies of feral pigeon populations

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

At the beginning of the 20th century, the reduction of large populations was attempted in Washington, London and Dresden by hunting and shooting (Haag-Wackernagel, 1998). Nowadays, control programmes in some towns and cities still aim to reduce the number of feral pigeons by killing as many individuals as possible, e.g. by trapping, shooting or poisoning. However, it should be noted that several scientific studies have demonstrated that killing alone does not have an effect on the population size because the place of the killed birds is quickly filled by new juveniles, or by birds immigrating from neighbouring areas. Due to the high reproduction rate of feral pigeons of up to 12 fledglings per pair per year, coupled with a low adult mortality rate of 10% (Haag, 1984), a lasting reduction of their populations simply cannot be achieved by killing.

Attempts for decreasing the birth rate of feral pigeons have been also made through the years in several European towns and cities, with the aim of reducing the size of the populations. Physical measures such as the destruction of pigeon eggs by eggshell puncture or by replacement of fertile eggs with plastic ones, and pharmacological treatments with several drugs have been applied. Cytostatic agents that inhibit the gametogenesis (e.g. busulfan), as well as drugs that interfere with the birds' metabolic activities (azacholesterol, nicarbazine), and natural or synthetic progestinic and estrogenic drugs (progesterone, mestranol, levonorgestrel, ethinylestradiol) have been administered on different occasions (for references, see Ballarini et al., 1989; Bursi et al., 2001). It should be noted that control measures for feral pigeons based on the administration of any such drugs are very controversial. Some results in terms of a reduction of the population size and improvement in the health status of the birds have been reported in the past, and recently also in the city of Ljubljana, Slovenia (Dovč et al., 2003; Dovč et al., 2006, personal communication). However, in order to remain effective this measure should be supported by other actions such as a feeding ban (Dobeic, 2003). Overall, there is in fact no clear evidence of a significant long-term effect of the administration of drugs on the reduction of the size of feral pigeon populations.

Other measures that have been applied in several European towns and cities for protecting buildings from

fouling include pigeon deterring systems such as net-like barriers in front of possible nesting places on the façades of buildings, metal or plastic spikes on preferred resting sites, and electrorepulsive systems. Such measures have been also applied, among others, also in the cities of Zagreb and Paris (Prukner-Radovčić et al., 2005; Laroucau et al., 2005). Although these devices, if properly installed, can effectively prevent most damages to buildings and monuments, they just displace the problem of fouling to other urban areas and have little or no effect at all on the size of the pigeon populations.

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

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

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

Communal health officers, operators in hospitals, schools, railway stations and even prisons, see in feral pigeons a significant hazard for human health and well being (Kösters et al., 1991). A wealth of publications provides firm evidence that feral pigeons are indeed the source of a large number of zoonotic agents. Epidemiological studies in feral pigeon populations detected at least 110 organisms that are pathogenic to humans (supplemented data according to Haag-Wackernagel and Moch, 2004). Eight of them were viruses, 41 bacteria, 55 fungi and 6 protozoa. However, of these human pathogens harboured by feral pigeons, only seven (namely *Salmonella enterica* serovar *Kiambu*, *Chlamydophila psittaci*, *Aspergillus* spp., *Candida parapsilosis*, *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Toxoplasma gondii*) caused a total of 230 human infections, of which 13 had a fatal outcome (supplemented data according to Haag-Wackernagel, 2006a,b).

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

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

Most infected feral pigeons are asymptomatic and latent carriers of *C. psittaci*. Shedding of the organism occurs in faeces as well as in respiratory and conjunctival secretions, often intermittently and without clinical signs, which makes it difficult to assess the risk of transmission of *C. psittaci* to other animals, including humans. Increased shedding of chlamydiae may be triggered by stress factors such as other concurrent infections or infestations, lack of food, breeding and overcrowding (Andersen and Vanrompay, 2003; NASPHV, 2006). The elementary body (EB), which is the infectious form of *C. psittaci* shed from the birds, can retain its infectivity for months under suitable environmental conditions (Albrecht et al., 2003) and may travel long distances, carried by the wind (Kukowka et al., 1960). Overt disease with clinical signs of depression, serous conjunctivitis, blepharitis, rhinitis and diarrhea has been reported in pigeons (Andersen and Vanrompay, 2003). Lesions observed at necropsy may include conjunctivitis, hyperemia and enlargement of spleen, hyperemia and degeneration of liver, enteritis and airsacculitis (Pavlak et al., 2000).

6. Direct diagnosis of chlamydial infections in feral pigeons

Chlamydiae can be mainly detected in pigeon faeces, cloacal swabs or smears from the surface of viscera such as liver, spleen and lung by simple laboratory methods, i.e. by staining with several techniques including Giménez, Machiavello, or modified Ziehl-Neelsen (Machiavello, 1937; Stamp et al., 1950; Giménez, 1964; Quinn et al., 1994) or with a direct immunofluorescence assay (DIF). The isolation of *C. psittaci* in tissue culture or in embryonated chicken eggs is still referred to as the gold standard for the direct diagnosis and is the preferred diagnostic method according to OIE (Andersen, 2004), but it requires specialized laboratories and expertise and is time-consuming and expensive. In addition, the test requires that the viability of chlamydiae has been preserved with a suitable transport media when samples are

collected and forwarded to the laboratory (Andersen, 1998). The use of the Buffalo Green Monkey (BGM) cell line is recommended for the isolation of avian chlamydial strains (Vanrompay et al., 1992). Chlamydial inclusions in the cytoplasm of the infected cells are visualized by specific staining procedures such as Giemsa (Giemsa, 1902, 1904) or Giménez (1964), or by immunofluorescence. Immunochromatographic (ICT) and ELISA tests for the detection of chlamydial antigens can be used as well and are quick and easy to perform (Fudge, 1991). These two methods can be used also for the detection of non-viable chlamydiae. However, most of the commercially available kits based on these methods were originally developed for detecting chlamydial species other than *C. psittaci* in human samples. Thus, their reliability for testing animal samples (and especially faeces) is generally lower than culture-based and molecular methods. In particular, false-positive results may occur due to the cross-reactivity with the lipopolysaccharide (LPS) antigen of other Gram-negative bacteria (Vanrompay et al., 1994; Andersen, 2004).

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

Among all chlamydial species, only *C. psittaci* has been detected in pigeons so far. Isolates of this species have been grouped into serovars by a microimmunofluorescence assay employing serovar-specific monoclonal antibodies (MAbs) directed against the major outer membrane protein (MOMP). Six avian serovars (A to F) are currently recognized (Andersen, 1991), and at least three of them infect pigeons. Serovar B is considered to be host specific and the most prevalent pigeon-associated serovar worldwide (Vanrompay et al., 1993; Hoop et al., 2002; Andersen, 2005; Laroucau et al., 2007). Serovar E also commonly infects pigeons. Initially, it was detected less frequently compared to serovar B both in the US (Andersen, 2005) and in Europe (Vanrompay et al., 1993, 1997; Duan et al., 1999). Serovar A, which is commonly associated with psittacine birds, has also been detected in feral pigeons (Vanrompay et al., 1993).

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

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

Besides the current *C. psittaci* classification based on the *ompA* gene sequence, a novel approach based on the identification of tandem repeats in DNA (multilocus variable number of tandem repeats analysis or MLVA) has been recently applied to *C. psittaci*. This method targets

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 et al. (1997); Geens et al. (2005a)
Belgium	A, B	A, B, D	Vanrompay et al. (1993, 1997)
England	n.d.	A, B	Sayada et al. (1995)
The FYR of Macedonia	n.d.	B, E	Ilieski et al. (2007)
France	B	B, E	Duan et al. (1999); Laroucau et al. (2008)
Italy	A, B, E	A, B, D, E, E/B	Geens et al. (2005a); Laroucau et al. (2008)
Japan	n.d.	C	Sayada et al. (1995)
The Netherlands	n.d.	B	Heddema et al. (2006b)
Switzerland	n.d.	B	Hoop et al. (2002)

n.d. = not determined.

eight distinct genomic areas dispersed throughout the genome, none of them being localized within the *ompA* gene (Laroucau et al., 2008). So far, isolates from pigeons have yielded four distinct MLVA patterns (number 1, 7, 12 and 19).

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

7. Serological diagnosis of chlamydial infections in feral pigeons

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

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

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

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

Overall, when considering data from all countries, seropositivity values to *C. psittaci* in the sampled populations ranged from 19.4% to 95.6%. Four different methods were used for the detection of antibodies, with CFT being the most frequently employed (6 countries) followed by ELISA and IIF (2 countries each) and MIF (one country). In one survey, a pool of sera was examined with three serological methods (CFT, MIF and ELISA) for comparison. With CFT, seropositivities ranged from 19.4% to 66.3% with a median of 46.1%, while similar values were obtained in surveys employing the indirect immunofluorescence test (23.7–67.7%). The highest seropositivities, ranging from 56% to 95.6%, were reported with the LPS-based ELISA.

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

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

Overall, chlamydial infections are widespread in the feral pigeon populations of several European towns and cities. Indeed, in most of them, moderate to high percentages of seropositivity to *C. psittaci* have been detected for several years. The results of the serological investigations in some countries may not be directly comparable due, among others, to the different cut-offs used in the analysis, yet they clearly indicate that European feral pigeons are frequently exposed to *C. psittaci*. This finding is not unexpected, since all investigations performed worldwide in a representative number of birds have demonstrated the detection of chlamydial antibodies in feral pigeons. In most surveys mentioned in this communication, serological investigations have been carried out using CFT, which has for a long time proven to be a suitable method, since pigeon sera are able to fix guinea pig complement, unlike sera from other birds such as turkeys and some parrot species. ELISA, IFI and MIF have been also used, albeit less frequently. Limited data have been published concerning the comparison of the sensitivity and specificity of serological methods for the detection of anti-*C. psittaci* antibodies in pigeon sera. Milon et al. (1983) and Trap et al. (1986) compared IIF and CFT, and concluded that IIF was more sensitive than CFT for the analysis of pigeon sera, but also suggested that the cut-off of 1:40 for the IIF was too high. Ceglie et al. (2007) recently reported good agreement between the CFT and the MIF and again detected more positive samples with the latter test. Eidebenz (1990) tested a blocking antibody ELISA against the CFT and found that the former was specific and more sensitive than CFT. Salinas et al. (1993b) compared the performance of five serological methods (CFT, indirect CFT, IIF, MIF and ELISA) for the detection of chlamydial antibodies in pigeon sera. Taking the IIF as a reference method, they found that the ELISA and MIF were more sensitive and allowed the detection of more positive

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 et al. (2006)
		2006	IIF	62/234 (26.5%)	Sera from captured pigeons with no clinical signs	Rešidbegović et al. (2007)
Bulgaria	Pleven	1993–1994	CFT	7/20 (35%)	Cut-off: 1:8	Martinov et al. (1997)
Croatia	Zagreb	1992–1997	CFT	18/44 (40.9%)	Cut-off: 1:8	Vlahović et al. (1998)
		1988–1993	CFT	410/834 (49.2%)	Higher antibody titers were detected in pigeons with lesions at necropsy cut-off: 1:8	Pavlak et al. (2000)
		2000–2003	Ab-ELISA (LPS)	174/182 (95.6%)	High antibody titers were detected in 57/182 sera (32.8%)	Prukner-Radovčić et al. (2005)
France	Toulouse	1980–1982	CFT, IIF	186/501 (37.1%)	CFT cut-off: 1:8 IIF cut-off: 1:40	Milon et al. (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 et al. (1986)
		1990	CFT	176/415 (42%)	Cut-off: 1:8	Laroucau (2007, personal communication)
		1999	CFT	316/658 (48%)	Pigeons sampled in the 20 districts, in the Bois de Boulogne and in the Bois de Vincennes cut-off: 1:8	Laroucau et al. (2005)
		2003 (March)	CFT	38/75 (51%)	Cut-off: 1:8	Laroucau (2007, personal communication)
		2003 (December)	CFT	21/43 (49%)	Cut-off: 1:8	Laroucau (2007, personal communication)
	Troyes	2007 (June)	CFT	7/29 (24%)	Cut-off: 1:8	Laroucau (2007, personal communication)
Germany	Giessen	1979–2004	CFT	46/81 (56.8%)	Sera from diseased and necropsied feral pigeons	Kaleta and Hönicke (2004); Kaleta (2007, personal communication); Helmecke (2007)
			CFT	286/1,474 (19.4%)	Sera from feral pigeons with no clinical history cut-off: 1:8	
Italy	Pisa	1988	CFT	263/495 (53.1%)	Cut-off: 1:16	Cerri et al. (1989)
	Bologna and Ferrara	1997	CFT	39/178 (21.8%)	Sera from feral pigeons with no clinical history cut-off: 1:16	Renzi and Magnino (1998)
	Bolzano	2006	CFT	38/68 (55.9%)	Cut-off: 1:10	Ceglie et al. (2007)
	Venice	2006	CFT	111/267 (41.6%)	Cut-off: 1:10	Ceglie et al. (2007)
	Padua	2006	CFT	65/100 (65%)	Cut-off: 1:10	Ceglie et al. (2007)
	Verona	2006	CFT	78/167 (46.7%)	Cut-off: 1:10	Ceglie et al. (2007)
Spain	Murcia	1991	CFT	36/128 (28.6%)	CFT cut-off: 1:10	Salinas et al. (1993a)
			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 et al. (2004)
		2006	IIF	26/86 (30.2%)	Cut-off: 1:40	Dovč (2006, personal communication)
Switzerland	Luzern	2001	Ab-ELISA (LPS)	33/59 (56%)		Haag-Wackernagel (2006a)

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.

samples than the CFT, but the ELISA was found to be less specific than the other methods. Overall, serological methods other than the CFT have shown a higher sensitivity, but still need to be fully evaluated as to their specificity (Andersen, 2004).

In the surveys considered in this communication, infection with *C. psittaci* has been frequently demonstrated directly with both non-cultural and molecular methods. In addition, the carriage of viable organisms by apparently healthy birds has been ascertained in some cases by

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 (*ompA*)	3/8 (37.5%)	Tissue samples from dead birds	Rešidbegović et al. (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 et al. (1998)
		2000–2003	ICT EI	3/107 (2.8%) 0/3 (0%)	Cloacal swabs	Vlahović et al. (2004)
		2000–2003	Ag-ELISA	44/278 (15.8%)	Cloacal swabs	Prukner-Radovčić et al. (2005)
		2006	Ag-ELISA	120/787 (15.3%)	Cloacal swabs	Prukner-Radovčić (2007, personal communication)
France	Toulouse	1980–1982	EI	3/101 (3%)	Pool of spleen and lung	Milon et al. (1983)
			TC	3/150 (2%)	Cloacal, intestinal and pharyngeal swabs	
	Paris	1984	EI	4/7 (57.1%)	Tissue samples from necropsied birds	Trap et al. (1986)
		2003 (March)	Rt PCR (23S rRNA)	5/33 (15.2%)	Cloacal swabs	Laroucau (2007, personal communication)
		2003 (December)	Rt PCR (23S rRNA)	4/20 (20%)	Cloacal swabs	Laroucau (2007, personal communication)
	Troyes	2007 (March)	Rt PCR (23S rRNA)	5/33 (15.2%)	Cloacal swabs	Laroucau (2007, personal communication)
		2007 (June)	Rt PCR (23S rRNA)	1/29 (3.4%)	Cloacal swabs	Laroucau (2007, personal communication)
Germany	Giessen	1979–2004	TC	10/77 (13%)	Tissue samples from necropsied birds	Kaleta and Hönicke (2004); Kaleta (2007, personal communication); Helmecke (2007)
Italy	Pisa	1988	EI	14/35 (40%)	Pool of viscera (lung, liver and spleen) from necropsied birds	Cerri et al. (1989)
	Trento	1995	TC	12/35 (34.3%)	Intestinal content from necropsied birds with no clinical history	Manfredi et al. (1997)
	Milan	1996–1997	TC	30/163 (18.4%)[a]	Intestinal content from necropsied birds with no clinical history	Rampin et al. (1998)
	Bologna and Ferrara	1997	TC	34/178 (19.1%)[b]	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 et al. (2000)
	Venice	2006	PCR (16S rRNA)	7/50 (14%)	Liver and spleen collected from necropsied birds	Ceglie et al. (2007)
The FYR of Macedonia	Skopje	2004–2005	Ag-ELISA	10/25 (40%)	Conjunctival, choanal and cloacal swabs	Mitevski et al. (2005)
		2006	Rt PCR (23S rRNA)	2/36 (5.6%)[c]	Cloacal swabs	Ilieski et al. (2007)
	Prilep	2004–2005	Ag-ELISA	4/16 (25%)	Conjunctival, choanal and cloacal swabs	Mitevski et al. (2005)

Table 3 (*Continued*)

Country	Town or city	Year of sampling	Laboratory test	Results positive/total (% positive)	Notes on the type of sample(s)	Reference
	Kumanovo	2004–2005	Ag-ELISA	2/10 (20%)	Conjunctival, choanal and cloacal swabs	Mitevski et al. (2005)
	Bogdanci	2004–2005	Ag-ELISA	2/12 (16.7%)	Conjunctival, choanal and cloacal swabs	Mitevski et al. (2005)
	Vinica	2006	Rt PCR (23S rRNA)	10/20 (50%)[d]	Cloacal swabs	Ilieski et al. (2007)
	Stip	2006	Rt PCR (23S rRNA)	4/60 (6.7%)[e]	Cloacal swabs	Ilieski et al. (2007)
The Netherlands	Amsterdam	2005	Rt PCR (*ompA*)	26/331 (7.9%)[f]	Fresh faecal droppings	Heddema et al. (2006b)
Spain	Murcia	1991	TC	7/39 (18%)	Cloacal swabs	Salinas et al. (1993a)
			EI	5/39 (12.8%)	Cloacal swabs	
Slovenia	Ljubljana	2006	DIF	2/86 (2.3%)	Cloacal swabs	Dovč (2006, personal communication)
			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	

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).

[a] Three isolates were genotype B.
[b] Four isolates were genotype E and one was genotype E/B.
[c] One PCR product was genotype E.
[d] Four PCR products were genotype E.
[e] One PCR product was genotype B, and one was genotype E.
[f] Ten PCR products were genotype B.

isolation of *C. psittaci* in cell culture or embryonated eggs. The cultural method is highly sensitive provided that the viability of chlamydiae in the sample has been preserved, while the use of non-culturable antigen detection methods has been questioned in the last few years due to the possible occurrence of false-positive results arising from cross-reactivity with other bacterial antigens. On the other hand, some of the recently developed molecular methods, i.e. PCR-RFLP, real-time PCR and DNA microarrays, look particularly attractive for their specificity, sensitivity and flexibility, since they allow more rapid detection and typing of *C. psittaci* without the need for culturing the organism, which also makes them safer. There is no consensus at the moment for recommending a single PCR assay for the diagnosis of avian (including pigeon) chlamydiosis. However, in a comparison of different conventional PCR protocols, the PCR assay targeting the *pmp* gene family has been recently found highly specific and more sensitive, up to 10 times, than assays targeting other chlamydial genes (Laroucau et al., 2007). Real-time PCR protocols are characterized by high sensitivity and also allow quantification of genome copy numbers in the samples. A validation study has recently compared conventional PCR, real-time PCR, immunohistochemistry, cell culture and a DNA microarray assay. Sensitivies of microarray testing and real-time PCR have been found to be equivalent (Borel et al., 2008).

10. The zoonotic relevance of chlamydiae acquired from feral pigeons

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

In 1941, Meyer described the first case of transmission of *C. psittaci* from feral pigeons to humans. A mother and her daughter had picked up a sick feral pigeon in the street in New York City. The pigeon died after four days and, two weeks later, both mother and daughter developed ornithosis with fever and pneumonia. Two thirds of the feral pigeons examined in their environment were positive for *C. psittaci* (Meyer, 1941). Since the first description, a number of case reports have demonstrated the successful transmission of chlamydiae from pigeons to humans (for references, see Süss et al., 1996). A recent extensive search of the literature identified 101 case reports of ornithosis in humans where the route of transmission could be traced to a contact with feral pigeons (Haag-Wackernagel and Moch, 2004).

In most cases (95%), detailed information has been provided as to the circumstances of interaction between humans and pigeons (supplemented data according to Haag-Wackernagel, 2006a,b). Overall, 53% of all reported cases of disease in humans could be referred to a close contact with feral pigeons or their excreta as detailed hereafter. About one fourth (27%) of cases was related to occupational exposure to contaminated dust, while fewer cases (15%) followed the handling of sick or dead pigeons. Fatalities – one case each – following both exposures were

reported. Few cases (11%) were linked to the habit of feeding pigeons. On the other hand, loose or transient contact with feral pigeons were mentioned in 43 cases (42%) of human disease, of which 11 were children and 6 were immunosuppressed patients. In this case, the activities leading to infection included, e.g., eating lunch in a park frequented by pigeons, walking through a pigeon flock, and living in a neighbourhood frequented by pigeons. Only in a limited percentage of cases (5%) no information was provided as to the nature and circumstances of the contact with pigeons.

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

Loose or transient contact with feral pigeons leading to disease in humans is difficult to identify and document, but may indeed be relevant, as shown by the analysis of the occurrence of the disease in people only temporarily exposed to infected birds or contaminated aerosols, e.g. customs officers transiently exposed to imported parrots (De Schrijver, 1995) and veterinarians visiting a duck processing plant on a single occasion (Palmer et al., 1981; Kaleta, 2008, personal communication).

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

On the other hand, it may be difficult to unequivocally trace a human case of ornithosis to contact with feral pigeons, since contact with other *C. psittaci*-infected free-living birds that dwell close to humans may have simultaneously occurred. For example, free-living tits (*Parus major* and other *Paridae*) are frequently infected with chlamydiae (Holzinger-Umlauf et al., 1997) and they too might be a source of infection for humans.

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

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

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

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

It may be also speculated that other workers in the urban environment, such as street sweepers and traffic wardens might be particularly exposed to *C. psittaci* through

inhalation of dust contaminated with pigeon excreta. However, no information is available as to an increased risk of infection in this group compared to the general population. Targeted studies might be helpful to clarify this issue.

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

In many European towns and cities, a reduced and healthier population of feral pigeons should be included among the aims of administrators and health officers, as a general intervention for preserving urban hygiene. The management of feral pigeon populations in the urban environment is a complex issue that requires careful planning. Before any intervention, an evaluation of the local situation as to the number of birds and their aggregation sites is mandatory. Fencing of buildings with pigeon deterring systems such as net-like structures and other mechanical devices represents a first-line intervention measure for preventing fouling. Administration of contraceptive drugs may be useful for reducing the bird population, but this measure is unlikely to lead to a permanent solution and should be coupled with others, in particular with a feeding ban. Pigeon feeders should be encouraged to stop or limit their activity by at least enforcing a feeding ban in defined urban areas that are close to hospitals, railway stations, kindergardens and prisons, where avoidance of pigeon aggregation is considered as a priority. Building dovecotes and artificial breeding facilities may be also considered for providing a balanced diet to the pigeons and a chance of interaction between pigeon lovers and the birds in a hygienically controlled environment. The personnel attending dovecotes should be adequately informed about the health risks arising from contact with pigeons and be regularly monitored for *C. psittaci* infections by DNA or antigen detection methods and/or by antibody testing. For the sake of animal protection, overtly sick birds should be captured and taken into veterinary care. In case chlamydiosis is confirmed, the birds should be appropriately treated with effective drugs such as tetracyclines (chlortetracycline, doxycycline), quinolones (enrofloxacin, difloxacin) or macrolides (clarithromycin) (Theis, 2007; Kinndle, 2007). In the case of very poor conditions, the birds should be euthanized in order to adapt the population to the reduced food supply resulting from public restriction of feeding. Education initiatives directed to the general public are strongly encouraged to illustrate the relationship between feeding, overcrowding, and the deterioration of living conditions of pigeons. In this context, reliable and unbiased information concerning the health hazards arising from the uncontrolled increase of feral pigeon populations should also be provided to the citizens through a variety of media. Regular interaction with the associations involved in the protection of animal welfare and health, such as the Society for the Protection of Animals, is recommended in order to illustrate the implementation of regulatory measures which need to be adopted. Education and information are fundamental, since the imposition of feeding bans usually does not prove successful given the solidarity that pigeon feeders tend to get from the general population. In this scenario, the usefulness of sanctions for those who defy the ban is questionable, since they might actually prove ineffective as to their intended scope of controlling the bird populations.

Acknowledgements

This paper is dedicated to the memory of Sergio Vigo who tragically passed away in April 2007, and whose extraordinary technical assistance in the NRL for Animal Chlamydioses of Pavia is gratefully acknowledged.

This review article has been prepared in the context of the collaboration promoted by the European COST Action 855 "Animal chlamydioses and the zoonotic implications".

Conflict of interest

None of the authors (Magnino, Haag-Wackernagel, Geigenfeind, Helmecke, Dovč, Prukner-Radovčić, Residbegović, Ilieski, Laroucau, Donati, Martinov, Kaleta) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the paper entitled "Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications".

References

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

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

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

Andersen, A.A., 1998. Chlamydiosis. In: Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E., Reed, W.M. (Eds.), A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th editon. American Association of Avian Pathologists, University of Pennsylvania, New Bolton Center, Kennett Square, PA, pp. 81–88.

Andersen, A.A., 2004. Avian chlamydiosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual), 5th edition. Office International des Epizooties, Paris, France, pp. 856–867.

Andersen, A.A., 2005. Serotyping of US isolates of *Chlamydophila psittaci* from domestic and wild birds. J. Vet. Diagn. Invest. 17, 479–482.

Andersen, A.A., Vanrompay, D., 2003. Avian chlamydiosis (psittacosis, ornithosis). In: Saif, Y.M. (Ed.), Diseases of Poultry. 11th edition. Iowa State University Press, Iowa, USA, pp. 863–879.

Ballarini, G., Baldaccini, N.E., Pezza, F., 1989. Colombi in città, Aspetti biologici, sanitari e giuridici. Metodologie di controllo. Istituto Nazionale di Biologia della Selvaggina. Documenti Tecnici 6, 1–58.

Barbieri, F., Caldonazzi, M., Zanghellini, S., Zorzi, S., 1997. The Street Pigeon (*Columba livia* forma *domestica*) in the Municipality of Trento. Centro di Ecologia Alpina. Report no. 7. pp. 1–62.

Bassi, M., Chiatante, D., 1976. The role of pigeon excrement in stone biodeterioration. Int. Biodeterior. Bull. 12 (3), 73–79.

Bedson, S.P., 1935. The use of complement-fixation reaction in the diagnosis of human psittacosis. Lancet 2, 1277–1280.

Borel, N., Kempf, E., Hotzel, H., Schubert, E., Torgerson, P., Slickers, P., Ehricht, R., Tasara, T., Pospischil, A., Sachse, K., 2008. Direct identification of chlamydiae from clinical samples using a DNA microarray assay—a validation study. Mol. Cell. Probes 22, 55–64.

Bursi, E., Gelati, A., Ferraresi, M., Zannetti, G., 2001. Impiego della nicarbazina nel controllo della riproduzione del colombo randagio di città. Ann. Fac. Med. Vet. Univ. Parma 21, 97–115.

Ceglie, L., Lafisca, S., Guadagno, C., Dalla Pozza, G., Capello, K., Bano, L., Vicari, N., Donati, M., Mion, M., Giurisato, I., Lombardo, D., Pozzato, N., Cevenini, R., Natale, A., 2007. Serological surveillance in north-eastern Italy for the presence of *Chlamydophila* spp. from birds and molecular characterisation of PCR isolates within the area of Venice. In: Proceedings of the 5th Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Pulawy (Poland), September 10–11, pp. 62–67.

Cerri, D., Andreani, E., Salvi, G., Perelli, G., 1989. The pigeons as carriers of some pathogenic agents for man and animals. In: Proceedings of the International Symposium "Environmental pollution and animal populations", Pisa, Italy, October 3–4, pp. 195–202.

Del Monte, M., Sabbioni, C., 1986. Chemical and biological weathering of an historical building: Reggio Emilia Cathedral. Sci. Total Environ. 50, 165–182.

Dell'Omo, A., 1996. Il ruolo degli escrementi dei piccioni nella bioalterazione delle rocce. Tesina di Università degli Studi di Roma "La Sapienza", Facoltà di Scienze Matematiche, Fisiche e Naturali, Dipartimento di Biologia Animale e dell'Uomo: 18 pp.

De Schrijver, K., 1995. A psittacosis outbreak in Belgian customs officers. Euro Surveill. 0 (0), 3. Available online: http://www.eurosurveillance.org/em/v00n00/0000-222.asp.

Dobeic, M., 2003. Regulation of population size of street pigeons in Ljubljana, Slovenia. Acta Vet. (Beograd) 53 (2–3), 171–182.

Donati, M., Renzi, M., Magnino, S., Di Francesco, A., Rinieri, G., Pignanelli, S., Cevenini, R., 2006. In vitro detection of neutralizing antibodies to *Chlamydophila psittaci* in pigeon sera with a microneutralization test. In: Proceedings of the 4th Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Edinburgh, Scotland (UK), September 3–5, pp. 113–114.

Dovč, A., 1995. Introduction of immunofluorescence method in diagnosing chlamydiosis (*Chlamydia psittaci*) and the epidemiology of the disease in parrots and pigeons. Slov. Vet. Res. 32 (2), 257–276 (in Slovenian with English abstract and summary).

Dovč, A., Račnik, J., Pišot, J., Cestnik, V., Čebulj-Kadunc, N., Dobeic, M., 2003. Progesterone concentration in the sera of pigeon treated with Ornisteril. In: 7th European A.A.V. Conference. 5th ECAMS Scientific Meeting in cooperation with Loro Parque & Loro Parque Fundación, Tenerife: European Committee of the Association of the Avian Veterinarians, pp. 73–74.

Dovč, A., Zorman-Rojs, O., Vergles Rataj, A., Bole-Hribovsek, V., Krapez, U., Dobeic, M., 2004. Health status of free-living pigeons (*Columba livia domestica*) in the city of Ljubljana. Acta Vet. Hung. 52 (2), 219–226.

Duan, Y.J., Souriau, A., Mahé, A.M., Trap, D., Andersen, A.A., Rodolakis, A., 1999. Serotyping of chlamydial clinical isolates from birds with monoclonal antibodies. Avian Dis. 43, 22–28.

Ehricht, R., Slickers, P., Goellner, S., Hotzel, H., Sachse, K., 2006. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol. Cell. Probes 20, 60–63.

Eidebenz, C., 1990. Comparative studies on the demonstration of antibodies against *Chlamydia psittaci* using CF and ELISA. In: Proceedings, VII Tagung über Vogelkrankheiten, Munich, Federal Republic of Germany, pp. 290–294.

Everett, K.D., Bush, R.M., Andersen, A.A., 1999. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. 49 (Pt 2), 415–440.

Fudge, A.M., 1991. ELISA testing for avian chlamydiosis. Vet. Clin. North Am. Small Anim. Pract. 21 (6), 1181–1187.

Gaede, W., Reckling, K.-F., Dresenkamp, B., Kenklies, S., Schubert, E., Noack, U., Irmscher, H.-M., Ludwig, C., Hotzel, H., Sachse, K., 2008. *Chlamydophila psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. Zoonoses Publ. Health 55 (4), 184–188.

Gaffuri, A., Garbarino, C., Consoli, T., Carrara, M., Broussard, D., Pietra, V., Magnino, S., Paterlini, F., 2000. Study on the health status of pigeons in Bergamo province. Sel. Vet. 8–9, 827–830.

Garrity, G.M., Bell, J.A., Lilburn, T.G., 2004. Taxonomic Outline of the Prokaryotes. Bergey's Manual of Systematic Bacteriology, Second Edition Release 5.0. Springer-Verlag, New York, doi:10.1007/bergeysoutline200405, p. 302.

Geens, T., Desplanques, A., Van Loock, M., Bönner, B.M., Kaleta, E.F., Magnino, S., Andersen, A.A., Everett, K.D.E., Vanrompay, D., 2005a. Sequencing of the *Chlamydophila psittaci ompA* gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. J. Clin. Microbiol. 43 (5), 2456–2461.

Geens, T., Dewitte, A., Boon, N., Vanrompay, D., 2005b. Development of a *Chlamydophila psittaci* species-specific and genotype-specific real-time PCR. Vet. Res. 36, 787–797.

Giemsa, G., 1902. Färbemethoden für Malariaparasiten. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten I. Abteilung. Originale 32, 307–313.

Giemsa, G., 1904. Eine Vereinfachung und Vervollkommnung meiner Methylenazur-Methylenblau-Eosin-Färbemethode zur Erzielung der Romanowsky-Nacht'schen Chromatin-Färbung. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten I. Abteilung. Originale 37, 308–311.

Giménez, D.F., 1964. Staining Rickettsiae in yolk-sac cultures. Stain Technol. 39, 135–140.

Haag, D., 1984. Ein Beitrag zur Ökologie der Stadttaube. Dissertation, Phil.Nat. Fakultät der Universität Basel, Verlag Medizinische Biologie.

Haag-Wackernagel, D., 1993. Street pigeons in Basel. Nature 361, 200.

Haag-Wackernagel, D., 1995. Regulation of the street pigeon in Basel. Wildlife Soc. Bull. 23 (2), 256–260.

Haag-Wackernagel, D., 1998. Die Taube. Vom heiligen Vogel der Liebesgöttin zur Strassentaube. Verlag Schwabe & Co, AG, Basel.

Haag-Wackernagel, D., 1999. The Pigeon. From the Holy Bird of the Goddess of Love to the Feral Pigeon. An English Supplementum Translated by Derek Goodwin. Verlag Schwabe & Co, AG, Basel, Switzerland.

Haag-Wackernagel, D., 2002. Feral pigeons: management experiences in Europe. In: Dinetti, M. (Ed.), Specie ornitiche problematiche: Biologia e gestione nelle città e nel territorio. Atti 2 (Convegno Nazionale sulla Fauna Urbana, Firenze, 10 Giugno 2000, ARSIA e LIPU. Regione Toscana, Firenze, 25–37.

Haag-Wackernagel, D., 2003. Die Strassentaube: Geschichte–Probleme–Lösungen. Der Ornithologische Beobachter 100, 33–57.

Haag-Wackernagel, D., 2005. Feral pigeons (*Columba livia*) as a potential source of human ornithosis. In: Proceedings of the 3rd Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Siena, Italy, September 22–23, pp. 15–17.

Haag-Wackernagel, D., 2006a. Human diseases caused by feral pigeons. In: Chris, J., Feare, David, P., Cowan, (Eds.), Advances in Vertebrate Pest Management, 4. Filander Verlag, Fürth, pp. 31–58.

Haag-Wackernagel, D., 2006b. Gesundheitsgefährdung durch die Strassentaube *Columba livia*. Amtstierärztlicher Dienst und Lebensmittelkontrolle 4, 262–272.

Haag-Wackernagel, D., Moch, H., 2004. Health hazards posed by feral pigeons. J. Infect. 48 (4), 307–313.

Harkinezhad, T., Verminnen, K., Van Droogenbroeck, C., Vanrompay, D., 2007. *Chlamydophila psittaci* genotype E/B transmission from African grey parrots to humans. J. Med. Microbiol. 56, 1097–1100.

Hartley, J.C., Kaye, S., Stevenson, S., Bennett, J., Ridgway, G., 2001. PCR detection and molecular identification of *Chlamydiaceae* species. J. Clin. Microbiol. 39 (9), 3072–3079.

Heddema, E.R., Beld, M.G.H.M., de Wever, B., Langerak, A.A.J., Pannekoek, Y., Duim, B., 2006a. Development of an internally controlled real-time PCR assay for detection of *Chlamydophila psittaci* in the LightCycler 2.0 system. Clin. Microbiol. Infect. 12 (6), 571–575.

Heddema, E.R., ter Sluis, S., Buys, J.A., Vandenbroucke-Grauls, C.M.J.E., van Wijnen, J.H., Visser, C.E., 2006b. Prevalence of *Chlamydophila psittaci* in fecal droppings from feral pigeons in Amsterdam, the Netherlands. Appl. Environ. Microbiol. 72 (6), 4423–4425.

Heddema, E.R., Van Hannen, E.J., Duim, B., Vandenbroucke-Grauls, C.M., Pannekoek, Y., 2006c. Genotyping of *Chlamydophila psittaci* in human samples. Emerg. Infect. Dis. 12 (12), 1989–1990.

Helmecke, S., 2007. Retrospektive Analyse diagnostizierter Untersuchungsbefunde zu Tauben (*Columba livia* forma *domestica*) aus dem Zeitraum 1979 bis 2004. Veterinary Dissertation, University of Giessen, 156 pp.

Hewinson, G.L., Griffiths, P.C., Bevan, B.J., Kirwan, S.E.S., Field, M.E., Woodward, M.J., Dawson, M., 1997. Detection of *Chlamydia psittaci* DNA in avian clinical samples by polymerase chain reaction. Vet. Microbiol. 54, 155–156.

Holzinger-Umlauf, H.A.-M., Marschnag, R.E., Gravendyck, M., Kaleta, E., 1997. Investigation of *Chlamydia* sp. infections in tits (*Paridae*). Avian Pathol. 26, 779–789.

Hoop, R.K., Schiller, I., Fraefel, D., 2002. Serotyping of avian *Chlamydophila psittaci* isolates. Vet. Rec. 150, 315–316.

Ilieski, V., Naletoski, I., Pendovski, L., Sachse, K., Laroucau, K., Dovč, A., 2007. Detection of *Chlamydiaceae* spp. in clinical samples from pigeons by real time PCR in Macedonia. In: Proceedings of the 5th Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Pulawy (Poland), September 10–11, p. 48.

Kaleta, E.F., Hönicke, A., 2004. Review of the literature on avian influenza A viruses in pigeons and experimental studies on the susceptibility of domestic pigeons to influenza A viruses of the haemagglutinin subtype H7. Dtsch Tierarztl Wochenschr. 111 (12), 467–472.

Kaleta, E.F., Taday, E.M.A., 2003. Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. Avian Pathol. 32, 435–462.

Kaltenböck, B., Schmeer, N., Schneider, R., 1997. Evidence for numerous allels of porcine *Chlamydia trachomatis* and novel *Chlamydia* species obtained by PCR. J. Clin. Microbiol. 35, 1835–1841.

Kinndle, M.B.P., 2007. Untersuchung der Sensitivität von Chlamydien-Isolaten unter Verwendung verschiedener antibakterieller Wirkstoffe (chlortetracycline, doxycycline, enrofloxacine, diflo-xacine, clarithromycine, erythromycine). Veterinary Dissertation, University of Giessen. 135 pp.

Kösters, J., Döring, N., Grimm, F., 1994. Bemühungen zur Reduzierung freilebender Stadttaubenpopulationen Verh. Ber. Erkrg. Zootiere 36, 352–357.

Kösters, J., Kaleta, E.F., Monreal, G., Siegmann, O., 1991. Das Problem der Stadttauben. Deutsches Tierärzteblatt 39 (4), 272–276.

Kukowka, A., Stephan, H., Krebs, W., 1960. Entenfarm als Ausgangspunkt von Ornithoseerkrankungen beim Menschen. Dtsch Gesundheitsw. 15, 2477–2484.

Laroucau, K., Mahé, H., Bouillin, C., Deville, M., Gandouin, C., Touati, F., Guillot, J., Boulouis, H.J., 2005. Health status of free-living pigeons in Paris. In: Proceedings of the 3rd Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Siena, Italy, September 22–23, pp. 17–18.

Laroucau, K., Souriau, A., Rodolakis, A., 2001. Improved sensitivity of PCR for *Chlamydophila* using *pmp* genes. Vet. Microbiol. 82, 155–164.

Laroucau, K., Thierry, S., Vorimore, F., Aaziz, R., Blanco, K., Kaleta, E.F., Hoop, R., Magnino, S., Vanrompay, D., Sachse, K., Myers, G.S.A., Bavoil, P., Vergnaud, G., Pourcel, C., 2008. High resolution typing of *Chlamydophila psittaci* by Multilocus VNTR Analysis (MLVA). Infect. Genet. Evol. 8 (2), 171–181.

Laroucau, K., Trichereau, A., Vorimore, F., Mahé, A.M., 2007. A *pmp* genes-based PCR as a valuable tool for the diagnosis of avian chlamydiosis. Vet. Microbiol. 121 (1–2), 150–157.

Leopold, P.G., 1965. Ornithose als Berufskrankheit. Monatsh. Veterinarmed. 20 (23), 964–969.

Machiavello, A.M., 1937. Estudios sobre tifos exantimatico. II. Un nuevo método para tenir Rickettsia. Rev. Chil. Hig. Med. Prev. 1, 101–106.

Manfredi, M.T., Fabbi, M., Magnino, S., Rizzoli, A., Genchi, C., 1997. Survey of health status of the street pigeons (*Columba livia* forma *domestica*) in Trento. Centro di Ecologia Alpina. Report n. 7, pp. 63–68.

Martinov, S.P., 2006. Avian chlamydiosis in Bulgaria and zoonotic implications. In: Proceedings of the 4th Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications". Edinburgh, Scotland (UK), September 3–5, pp. 159–169.

Martinov, S.P., Kazachka, D., Shoilev, H., Runevska, P., 1997. Studies on the *Chlamydia psittaci* infection in birds and humans. Biotechnol. Biotechnol. Equip. 11 (1), 74–80.

Mendez-Tovar, L.-J., Mainou, L.-M., Pizarro, S.-A., Fortoul-Vandergoes, T., Lopez-Martinez, R., 1995. Fungal biodeterioration of colonial facades in Mexico City. Rev. Mex. Micol. 11 (0), 133–144.

Meyer, K.F., 1941. Pigeons and barn yard fowls as possible sources of human psittacosis or ornithosis. Schweiz. Med. Wochenschr. 44, 1377–1379.

Milon, A., Geral, M.F., Pellerin, J.L., Thises, I., Lautie, R., 1983. Enquête sur le portage et l'excrétion de *Chlamydia psittaci* par les pigeons semi-domestiques (*Columba livia*) de l'agglomeration toulousaine. Rev. Med. Vet. 134 (10), 559–565.

Mitevski, D., Pendovski, L., Naletoski, I., Ilieski, V., 2005. Surveillance for the presence of *Chlamydophila psittaci* in pigeons and doves from several towns in Macedonia. In: Proceedings of the 3rd Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Siena, Italy, September 22–23, pp. 141–145.

National Association of State Public Health Veterinarians (NASPHV), 2006. Compendium of measures to control *Chlamydophila psittaci* infection among humans (psittacosis) and pet birds (avian chlamydiosis), 2006. Available at http://www.nasphv.org/83416/index.html.

Ossewaarde, J.M., Meijer, A., 1999. Molecular evidence for the existence of additional members of the order *Chlamydiales*. Microbiology 145, 411–417.

Page, L.A., 1975. Chlamydiosis (ornithosis-psittacosis). In: Hitchner, S.B., Domermuth, C.H., Purchase, H.G., Williams, J.E. (Eds.), Isolation and Identification of Avian Pathogens. Arnold Printing Corporation, Ithaca, New York, pp. 118–131.

Palmer, S.R., Andrews, B.E., Major, R., 1981. A common-source outbreak of ornithosis in veterinary surgeons. Lancet ii 8250, 798–799.

Pantchev, A., et al. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. Vet. J. (2008), doi:10.1016/j.tvjl.2008.02.025.

Pavlak, M., Vlahović, K., Gregurić, J., Župančić, Ž., Jerčić, J., Božikov, J., 2000. An epidemiologic study of *Chlamydia* sp. in feral pigeons (*Columba livia domestica*). Z. Jadgdwiss. 46, 84–95.

Prukner-Radovčić, E., Horvatek, D., Gottstein, Z., Ciglar Grozdanić, I., Mazija, H., 2005. Epidemiological investigation on *Chlamydophila psittaci* in pigeons and free living birds in Croatia. Vet. Res. Commun. 29 (Suppl. 1), 17–21.

Quinn, P.J., Carter, M.E., Markey, B.K., Carter, G.R., 1994. Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited, London, UK.

Rampin, T., Grilli, G., Sironi, G., Volorio, A., Schmitz, G., Magnino, S., Vigo, P.G., Fabbi, M., Schiavini, N., 1998. Observations on the sanitary conditions of feral pigeons in Milan. Sel. Vet. 8–9, 667–684.

Renzi, M., Magnino, S., 1998. Isolamento di *Chlamydia psittaci* da piccioni urbanizzati (*Columba livia*) della provincia di Bologna. Final Report of a Research Funded by the Italian Ministry of Health.

Rešidbegović, E., Kavazović, A., Abduzaimović-Maglajlić, J., Dizdarević, Z., Kustura, A., Gagić, A., Dovč, A., 2006. Some bacterial and viral pathogens in free-living pigeons from the city of Sarajevo. In: Proceedings of the 4th Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Edinburgh, Scotland, September 3–5, p. 9.

Rešidbegović, E., Kavazović, A., Satrović, E., Alibegović-Zečić, F., Keše, D., Dovč, A., 2007. Detection of antibodies and isolation of *Chlamydophia psittaci* in free-living pigeons. In: Proceedings of the 5th Annual Workshop of COST Action 855 "Animal chlamydioses and its zoonotic implications", Pulawy, Poland, September 10–11, pp. 81–85.

Sachse, K., Hotzel, H., 2003. Detection and differentiation of chlamydiae by nested PCR. Methods Mol. Biol. 216, 123–136.

Sachse, K., Hotzel, H., Slickers, P., Ellinger, T., Ehricht, R., 2005. DNA microarray-based detection and identification of *Chlamydia* and *Chlamydophila* spp. Mol. Cell. Probes 19, 41–50.

Sachse, K., Laroucau, K., Hotzel, H., Schubert, E., Ehricht, R., Slickers, P., 2008. Genotyping of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of *omp*A genes. BMC Microbiol. 8, 63.

Salinas, J., Caro, M.R., Cuello, F., 1993a. Antibody prevalence and isolation of *Chlamydia psittaci* from pigeons (*Columba livia*). Avian Dis. 37 (2), 523–527.

Salinas, J., Caro, M.R., Cuello, F., 1993b. Comparison of different serological methods for the determination of antibodies to *Chlamydia psittaci* in pigeon sera. J. Vet. Med. B 40, 239–244.

Sayada, C.H., Andersen, A.A., Storey, C.H., Milon, A., Eb, F., Hashimoto, N., Hirai, K., Elion, J., Denamur, E., 1995. Usefulness of *omp1* restriction mapping for avian *Chlamydia psittaci* isolate differentiation. Res. Microbiol. 146. 155–165.

Schmeer, N., 1983. Enzyme immunoassay for detection of IgG and IgM antibodies against *Chlamydia psittaci* in the pigeons. Zentralbl. Veterinarmed. B 30, 356–370.

Simms, E., 1979. The Public Life of the Street Pigeon. Hutchinson & Co. Ltd., London, p. 144.

Stamp, J.T., McEwen, A.D., Watt, J.A., Nisbet, A.D.I., 1950. Enzootic abortion in ewes. I. Transmission of the disease. Vet. Rec. 62, 251–264.

Süss, A., Reetz, J., Schulze, P., Kretzschmar, M., Schirrmeistr, W., Süss, J., 1996. Schwerer Verlauf einer Ornithose und ihre intensivmedizinische und diagnostische Problematik–eine Kasuistik. Anaesthesiol. Reanim. 21, 97–102.

Theis, H.-P., 2007. *In-vitro*-Prüfung der Empfindlichkeit von aviären Chlamydien gegen Difloxazin im Vergleich zu den als "wirksame Mittel" eingestuften Arzneimitteln Chlortetrazyklin, Doxizyklin und Enrofloxazin. Veterinary Dissertation. University of Giessen, 124 pp.

Tiefbau-Berufsgenossenschaft, 2006. Gesundheitsgefährdungen durch Taubenkot. Handlungsanleitung zur Gefährdungsbeurteilung nach Biostoffverordnung (BioStoffV). Tiefbau-Berufsgenossenschaft, Technischer Aufsichtsdienst. Available online: http://www.bgbau.de/d/pages/koop/forschung/Handlungsanleitung_Tk.pdf.

Trap, D., Louzis, C., Gourreau, J.M., Gaumont, R., 1986. Chlamydiose aviaire chez les pigeons de Paris. Rev. Med. Vet. 137 (11), 789–796.

Vanrompay, D., Andersen, A.A., Ducatelle, R., Haesebrouck, F., 1993. Serotyping of European isolates of *Chlamydia psittaci* from poultry and other birds. J. Clin. Microbiol. 31 (1), 134–137.

Vanrompay, D., Butaye, P., Sayada, C., Ducatelle, R., Haesebrouck, F., 1997. Characterization of avian *Chlamydia psittaci* strains using *omp*1 restriction mapping and serovar-specific monoclonal antibodies. Res. Microbiol. 148, 327–333.

Vanrompay, D., Ducatelle, R., Haesebrouck, F., 1992. Diagnosis of avian chlamydiosis: specificity of the modified Giménez staining on smears

and comparison of the sensitivity of isolation in eggs and three different cell cultures. J. Vet. Med. B 39, 105–112.
Vanrompay, D., Van Nerom, A., Ducatelle, R., Haesebrouck, F., 1994. Evaluation of five immunoassays for detection of *Chlamydia psittaci* in cloacal and conjunctival specimens from turkeys. J. Clin. Microbiol. 32 (6), 1470–1474.
Verminnen, K., Duquenne, B., De Keukeleire, D., Duim, B., Pannekoek, Y., Braeckman, L., Vanrompay, D., 2008. Evaluation of a *Chlamydophila psittaci* diagnostic platform for zoonotic risk assessment. J. Clin. Microbiol. 46 (1), 281–285.
Vlahović, K., Župančić, Ž., Gregurić, J., Pavlak, M., 1998. Zur Zuverlässigkeit diagnostischer Verfahren beim Nachweis von Infektionen mit *Chlamydia* sp. bei Vögeln. Z. Jadgdwiss. 44 (3), 133–139.
Vlahović, K., Matica, B., Bata, I., Pavlak, M., Pavičić, Ž., Popović, M., Nejedli, S., Dovč, A., 2004. Campylobacter, salmonella and chlamydia in free-living birds of Croatia. Eur. J. Wildl. Res. 50, 127–132.
Yoshida, H., Kishi, Y., Shiga, S., Hagiwara, T., 1998. Differentiation of *Chlamydia* species by combined use of polymerase chain reaction and restriction endonuclease analysis. Microbiol. Immunol. 42, 411–414.

Chapter 3

Detection of *Chlamydophila psittaci* from feral pigeons in environmental samples: problems with currently available techniques

Ila Geigenfeind & Daniel Haag-Wackernagel

Department of Biomedicine, Institute of Anatomy, University of Basel,
Pestalozzistrasse 20, CH-4056 Basel, Switzerland

Geigenfeind I, Haag-Wackernagel D. (2010). Detection of *Chlamydophila psittaci* from feral pigeons in environmental samples: problems with currently available techniques. *Integrative Zoology*. 1, 63–69.

3.1. ABSTRACT

Chlamydophila psittaci, the pathogenic agent of human ornithosis, is widespread in feral pigeon populations and many cases of transmission from feral pigeons to humans have been reported. The aim of this study was to detect *Chlamydophila psittaci* in environmental samples to find out more about possible transmission routes and thus to assess the zoonotic risk for humans. Faecal samples were collected from nest boxes in a feral pigeon loft. Additionally, samples were taken from the feather dust film covering the water surface of public fountains where pigeons regularly bathe. The samples were tested for the presence of chlamydial antigen using an antigen-ELISA (enzyme-linked immunosorbent assay) to prove shedding of *Chlamydophila psittaci* by feral pigeons. This test detects a genus specific lipopolysaccharide in the outer membrane of the chlamydial bacteria. Samples were tested using the IDEIA™ *PCE* Chlamydia Test kit (DakoCytomation) and positive results were verified with IDEIA™ Chlamydia Blocking Reagents (DakoCytomation). The IDEIA™ *PCE* Chlamydia Test yields a high proportion of positive results. However, when IDEIA™ Chlamydia Blocking was performed, most of the positive results turned out to be negative or could not be interpreted. We conclude, that antigen-ELISA tests are not suitable for detecting *Chlamydophila psittaci* in environmental samples. Previous publications where no blocking test was used should be reconsidered critically.

KEY WORDS: antigen-ELISA, *Chlamydophila psittaci, Columba livia*, environmental samples, zoonosis

3.2. INTRODUCTION

Chlamydophila (*C.*) *psittaci* is the pathogenic agent of avian chlamydiosis and human ornithosis/psittacosis. Avian chlamydiosis is prevalent in wild birds, pet birds and poultry and causes considerable losses in poultry farming and the pet bird trade. *C. psittaci* has been detected in 469 bird species (Kaleta & Taday, 2003). Infections are acquired by inhalation of infectious aerosolized faecal dust, feather particles and dried excreta from infected birds (Leopold, 1965). The severity of avian chlamydiosis in birds can vary greatly. Most birds are chronically infected, showing no clinical signs of infection, whereas clinically ill birds show a wide range of symptoms, including ruffled feathers, swollen eyelids, conjunctivitis, rhinitis, respiratory distress, diarrhoea, emaciation, and even death (Andersen & Vanrompay, 2003). At necropsy, typical findings are fibrinous exudates in lung- and airsac tissues, enlarged liver and spleen, pericarditis and enteritis (Andersen & Vanrompay, 2003). Symptoms of ornithosis in humans range from mild influenza-like symptoms to severe atypical pneumonia, myocarditis, endocarditis and encephalitis. Due to good treatment options, infections are now rarely fatal. *C. psittaci* infections can be treated with appropriate concentrations of tetracyclines, chloramphenicol and erythromycin (Andersen & Vanrompay, 2003).

Chlamydophila psittaci in feral pigeons

Feral pigeons (*Columba livia*, Gmelin 1789) live in most of the world's large cities, where they often live in close contact with humans. Originating from the domesticated descendants of the wild Rock Dove, they are well adapted to surviving in the urban environment. To date, 110 microorganisms that are known to be pathogenic for humans have been detected in feral pigeons. However, only seven of these have evidentially been transmitted from feral pigeons to humans, causing 230 cases of illness, 13 of them fatal (Haag-Wackernagel & Moch, 2004; Haag-Wackernagel, 2006). Of these published 230 cases, 101 (44%) were attributed to *Chlamydophila psittaci*, two of them fatal. *C. psittaci* is apparently the most important zoonotic agent found in feral pigeons due to its worldwide distribution with a high prevalence in many populations (Magnino *et al*., 2009). In 58 (57.5%) of the 101 cases the contact with feral pigeons was intentional and infections could have been avoided in many cases by taking appropriate preventive measures, e.g. disinfection

and wearing a dust mask and coverall. In these avoidable cases of transmission the activities leading to an infection were handling of sick or dead feral pigeons, pigeon feeding and occupational dust exposure. However, in 43 (42.5 %) of the infections, the persons involved merely had loose or transient contacts with urban feral pigeons (Haag-Wackernagel, 2006), which were unintentional, such as breeding pigeons on windowsills or walking through a pigeon flock. In these cases the infections could hardly have been avoided by any preventive measures and therefore are of special interest, because the exact transmission routes are unclear.

The aim of this study is to assess the health hazard posed by feral pigeons for the transmission of *C. psittaci* to humans in the urban environment. Epidemiological investigations confirmed that feral pigeons are commonly infected with *C. psittaci* (Magnino *et al*., 2009). Infected pigeons shed the pathogen intermittently in their faeces and their ocular and respiratory exudates (Andersen & Vanrompay, 2003). Chronically infected feral pigeons may appear clinically healthy but they can shed the pathogen all the same. Clinically apparent illness can emerge when birds are exposed to stress factors (Andersen & Vanrompay, 2003). The potentially high number of apparently healthy pigeons that are shedding the pathogens makes it very difficult to assess the real risk of infection for other animals and humans. In this study, we investigated the occurrence of *C. psittaci* in faecal samples taken in a pigeon loft and water samples from public fountains. Faecal samples were taken from nest boxes in our feral pigeon loft in the St. Matthäus Church in Basel (Switzerland). Feral pigeons like to bathe in the public fountains of Basel, preferably in the early morning. Dried faecal dust and feather powder are washed off and form a dust film on the water surface. This potentially contaminated dust film could be a source of *C. psittaci* infections for feral pigeons and other city birds such as sparrows and crows. Humans could be infected by inhalation of contaminated water droplets. Children are particularly at risk, since in Basel they often bathe in large fountains on hot summer days.

Characteristic features of *Chlamydophila psittaci*

The members of the order Chlamydiales include the genera *Chlamydia* and *Chlamydophila* (Everett *et al*., 1999). The Chlamydiales are obligate intracellular gram-negative bacteria. The organisms belonging to these two genera are commonly

referred to as “chlamydiae”. Three morphologically distinct forms exist in the chlamydial life cycle. The elementary bodies (EB), the infectious form, are 0.2–0.3 µm in diameter and are among the smallest procaryotes. Elementary bodies can remain infectious for several months in the environment (CDC, 2000; Albrecht *et al*., 2003). After the EB has entered a host cell, it differentiates into a reticulate body (RB), which is the metabolically active, reproducing form (0.5–2.0 µm). RBs divide by binary fission and can form new EBs. During the maturation of the new EBs, the intermediate bodies (IB) are formed (0.3–1.0 µm). The elementary bodies are released from the lysed host cell and can infect other cells (Vanrompay *et al*., 1995; Everett *et al*., 1999; Andersen & Vanrompay, 2003). *C. psittaci* is currently grouped into seven avian genotypes (A, B, C, D, E, F, and E/B) and two mammalian genotypes (M56 and WC). The genotypes B and E are commonly found in feral pigeons, genotype B being endemic among pigeons and doves. However, the genotypes A, D and E/B have been isolated from feral pigeons as well (Geens *et al*., 2005a). All *C. psittaci* genotypes have been proved to be transmissible to humans (Heddema *et al*., 2006a; Harkinezhad *et al*., 2007).

Diagnostic Methods

The diagnosis of chlamydial infections is difficult. An overview of diagnostic methods is given by Andersen & Vanrompay (2003). Due to their obligate intracellular reproduction cycle, chlamydiae cannot be multiplied by standard bacteriological methods. Isolation of the organism can only be performed using cell culture or embryonated chicken eggs, which is time consuming and requires special sampling and transport conditions to keep the chlamydiae viable. Furthermore, multiplication of chlamydiae needs to be performed in a specialized laboratory with high bio-safety standards. Additionally, a high number of viable chlamydiae are needed to produce positive findings (Kaltenboeck *et al*., 1991).

The detection of anti-chlamydial antibodies in sera is frequently performed to detect acute chlamydial infections. The complement fixation test (CFT) is still the standard test used for diagnosis of chlamydial infections in most laboratories. Paired sera taken at two different times are used to confirm a positive diagnosis by measuring the rise in antibody titre. Positive findings in sera taken only at one time (single point sera) do not reveal acute infections. Due to the chronic character of chlamydial

infections, merely either a carrier state or a past contact to *C. psittaci* can be detected with this method. However, serological methods do not provide any information about whether an animal is shedding the pathogen into the environment or not. Shedding can occur with or without positive serological findings. Chlamydial shedding in birds can be detected by analysis of pharyngeal, cloacal and conjunctival swabs, as well as faeces. The detection rate of infections depends strongly on the kind of sample used (Anderson, 1996). The enzyme-linked immunosorbent assay (ELISA) is still widely used as a diagnostic method, both for detection of anti-chlamydial antibodies in sera and for detection of chlamydial antigen in a wide range of samples. The advantages of ELISA methods compared to CFT are described by Fudge (1991). Using the RIDASCREEN® *Chlamydia psittaci* ELISA (R-Biofarm, Darmstadt, Germany), Prukner-Radovčić *et al*. (2005) found 174 (95. 6%) of 182 feral pigeons to be seropositive for *C. psittaci*. Such findings are realistic, since feral pigeons are chronically infected carriers. Presumably most pigeons are exposed to *C. psittaci* at least once in their lifetime. The worldwide mean seroprevalence of *C. psittaci* in feral pigeon populations is 48.6 % and antigen detection of *C. psittaci* was successful in 11.9 % of feral pigeon specimens (Haag-Wackernagel, 2005).

The commercially available antigen-ELISAs were originally developed to detect *Chlamydia trachomatis* in human urogenital specimens. Since these tests detect the common lipopolysaccharide (LPS) in the outer bacterial membrane of the genera *Chlamydia* and *Chlamydophila*, the tests have been adapted to detect different chlamydial organisms of these two genera, apart from *C. trachomatis*. To determine the species or even the genotype of the chlamydial organism found, other methods must be used. ELISA-tests are relatively cheap, fast and easy to perform. There are a wide variety of commercially available antigen-ELISA tests that have been evaluated and used by different authors (Gerbermann, 1989; Fudge, 1991; Wittenbrink, 1991; Gerbermann & Korbel, 1993; Vanrompay *et al*., 1994; Guscetti *et al*., 2000; Mitevski *et al*., 2005; Prukner-Radovčić *et al*., 2005). In the last few years, new methods have been developed to detect *C. psittaci* DNA in different sample types. Common methods are the polymerase chain reaction (PCR), nested-PCR and real-time PCR, DNA microarrays, as well as sequencing of specific genes. Everett *et al*. (1999) give an overview of detection methods for different chlamydial organisms. Fast and reliable detection at the species-level and even at the genotype-level has

become possible (Kaltenboeck *et al*., 1991; Geens *et al*., 2005b; van Loock *et al*., 2005; Heddema *et al*., 2006a). Some of these methods are widely used in routine diagnostics in veterinary medicine. However, they are costly and can only be performed in appropriately equipped laboratories.

3.3. MATERIALS AND METHODS

Thirty-four water film samples were collected from fourteen different public fountains located in the city of Basel. We chose fountains where we either observed bathing feral pigeons directly, or where we found pigeon feathers and a feather powder film on the water surface as an evidence of former visits of feral pigeons. About 40 ml of the dust film covering the water surface of each fountain were aspirated with a sterile syringe and transferred into sterile flasks. Specimens were processed immediately after collection according to an adapted protocol of Gerbermann (1989). Specimens were thoroughly vortexed and ultrasonicated for 2 minutes. Each water film sample was subdivided into four centrifuge tubes, 10 ml each. Specimens were centrifuged at 4° C and 600 × *g* for 10 minutes. The pellets were discarded and the supernatants were ultracentrifuged at 4° C and 49´000 × *g* for 30 minutes. The supernatants were discarded and the pellets were resuspended in 0.5 ml of freshly prepared chlamydia transport medium at working strength provided in the IDEIA™ *PCE* Chlamydia test kit. The four resuspended pellets derived from a single sample were merged in a 2 ml heat resistant tube. Specimens were boiled at 100° C for 15 minutes in a heating block (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) and subsequently stored at -20° C until further processing.

Faecal samples were collected in the feral pigeon loft in the St. Matthäus Church in Basel. Thirty-nine nest boxes are installed in the loft, where the feral pigeons can breed and raise their young. However, not all the boxes are occupied by breeding pigeons at the same time. The number of feral pigeons living in the loft varies around a hundred. The pigeons are free-ranging in the city of Basel and have to search their food themselves. Therefore, the feral pigeons in this loft can be considered a representative cross section of the feral pigeon population of Basel.

Forty-seven faecal samples from twenty-four occupied nest boxes were collected using sterile collection tubes (FECON®, Medical Wire & Equipment Co. (Bath) Ltd., UK). Immediately after collection, 1–2 g of fresh faecal material were suspended in 10 ml of chlamydia transport medium at working strength to obtain a 10–20 % faeces suspension, as described by Gerbermann (1989). Each specimen was thoroughly vortexed, ultrasonicated for 2 minutes and transferred to a centrifuge tube. Specimens were centrifuged at 4° C and 600 × *g* for 10 minutes. The pellets were discarded and the supernatants were ultracentrifuged at 4° C and 49´000 × *g* for 30 minutes. The supernatants were discarded and the pellets were resuspended each in 2 ml of freshly prepared chlamydia transport medium at working strength. Specimens were transferred to heat resistant tubes and boiled at 100° C. All specimens were tested for the presence of chlamydial LPS using the IDEIA™ *PCE* Chlamydia Test (DakoCytomation Ltd., Cambridgeshire, CB7 4ET, UK), which is a new improved version of the formerly used IDEIA™ Chlamydia Test. All tests were performed according to the instructions of the manufacturer. Optical density (OD) values were measured at 490 nm using a microtiterplate reader (Dynatech AG, Embrach-Embraport, Switzerland). Evaluation of the OD values was performed following the calculation instructions provided by the manufacturer. Additionally, the IDEIA™ Chlamydia Blocking Reagents (DakoCytomation Ltd., Cambridgeshire, CB7 4ET, UK) was used to verify positive results. According to the instructions of the manufacturer, initially positive specimens were retested in parallel, once with the addition of a murine monoclonal antibody (blocking reagent), which binds specifically to chlamydial LPS, and at the same time with an antibody lacking any anti-chlamydial activity (control reagents). The monoclonal antibody in the blocking reagent competitively inhibits chlamydia-specific binding by the enzyme-labelled antibody in the coated wells of the IDEIA™ *PCE* Chlamydia Test. A negative result with the blocking reagent was interpreted as a verification of the initial positive test result, provided that the same specimen was positive with the control reagents. The single positive result obtained was sent to the Institute of Veterinary Pathology (University of Zurich) for identification of the chlamydial species using the Array Tube™ system (CLONDIAG Chip Technologies GmbH, Jena, Germany) described by Sachse *et al*. (2005).

3.4. RESULTS AND DISCUSSION

In 34 water film samples from public fountains, 26 (76.5 %) were positive for chlamydial antigen using the IDEIA™ *PCE* Chlamydia Test; 8 (23.5 %) were tested negative. After retesting each of the 26 initially positive specimens with the IDEIA™ Chlamydia Blocking Reagents, none of the results were interpretable. Neither with the blocking reagent, nor with the control reagent, any ODs were found to be above the calculated OD cut-off value for positive samples. Out of 47 faecal samples, 9 (19.1 %) were positive for chlamydial antigen, 38 (80.9 %) were negative. After retesting of the positive samples with the blocking test, only one sample could be confirmed to be a true positive. In two samples an unspecific reaction with bacterial LPS was proved, one result was equivocal. The remaining five initially positive results could not be reproduced with blocking reagents or control reagents. Retesting of the single true positive specimen with the Array Tube™ system yielded no clear result. The probe for the genus *Chlamydophila* was detected, but the probe for the species did not appear. This probe should normally appear at the same time as the probe indicating the genus. Sequence analysis of the sample was performed, but yielded no interpretable results (Borel N., 2006, personal communication).

Our findings show that the antigen-ELISA method we used is not suitable to detect chlamydial antigen in water samples. None of the 26 initially positive results could be reproduced using the control reagent from the blocking test. Normally an initially positive sample should be positive again with the control reagents, regardless of the origin of the detected bacterial LPS. The positive control and negative controls provided in the test kit worked normally and showed that the test had been conducted properly. From 9 initially positive faecal samples, one true positive result was obtained and could be attributed to the genus *Chlamydophila* using the Array Tube™ System. However, the exact species could not be determined.

The two unspecific reactions revealed by the blocking test had to be expected. One result was equivocal and five of the initially positive results could not be reproduced, thus corresponding to the results from the water samples. We conclude that the antigen-ELISA worked at least partially for analyzing the faecal samples, but due to the high proportion of non-interpretable results in the blocking test, we decided to not

further use this test for our environmental samples. Detecting chlamydial organisms such as *Chlamydophila psittaci* has always been a challenge. In environmental samples, like faecal samples and water samples, there are multiple unknown factors, such as other microorganisms that can potentially confound the testing procedure. It is well known that bacterial LPS, deriving from gram-negative bacteria other than chlamydiae, can give rise to false positive results in antigen-ELISAs such as the IDEIA™ *PCE* Chlamydia Test and the formerly used IDEIA™ Chlamydia Test (Vanrompay *et al*., 1994). To circumvent this problem the new IDEIA™ Chlamydia Blocking Reagent was developed. Using this blocking test to verify positive results provides a good means to get rid of unspecific reactions causing false positives.

In most of the previous studies of other researchers who used these antigen ELISAs the blocking test has not been performed to verify initially positive results. Other methods, such as histopathology, do not detect chlamydial antigen and do not allow a precise statement about the real shedding status of an individual bird (Mitevski *et al*., 2005). These results should therefore be reconsidered critically. The antigen-ELISAs originally developed for chlamydial testing in human urogenital specimens have never been officially certified for use in birds and other animals. The use of these tests for veterinary purposes has therefore been controversial. Vanrompay *et al*. (1994) found that different commercially available ELISA tests showed considerable differences in sensitivity and specificity compared to cell culture. The detection limit of these tests can be insufficient for certain categories of field samples, particularly those from asymptomatic carriers and chronically infected animals (Sachse *et al*., 2003). Gaede *et al*. (2005) tested the IDEIA™ Chlamydia Test, the new IDEIA™ *PCE* Chlamydia Test and the new IDEIA™ Chlamydia Blocking Reagents in various samples obtained from swine, sheep and poultry. All of these tests were characterized by a high number of false-positive results, compared to cell culture and PCR. They found the new IDEIA™ *PCE* Chlamydia Test to be less sensitive and highly unspecific compared to the previous test, in contradiction to the information of the manufacturer. The IDEIA Chlamydia Blocking Reagents was also found to have insufficient sensitivity. Therefore we intend to evaluate appropriate alternative methods to detect *C. psittaci* in environmental samples deriving from feral pigeons. For epidemiological studies it is crucial to determine the exact species and genotype of the detected chlamydiae to trace back human infections to particular bird

species as the infection source. Recent studies used promising methods to detect *C. psittaci* in feral pigeon faeces. Tanaka *et al*. (2005) tested 463 faecal samples from feral pigeons in Japan by nested PCR. They found 106 (22.9 %) of the samples to be positive for *Chlamydophila ssp*.. Sequencing revealed *C. psittaci* in 103 cases and in three cases *Chlamydophila pecorum* was found. Heddema *et al*. (2006b) tested faecal samples from feral pigeons in the city of Amsterdam (The Netherlands) using a real-time PCR assay. They found that 5–10 % of the 331 pigeons tested were shedding *C. psittaci* with their faeces. They suggest that PCR methods, as well as sequencing of the *ompA* gene for genotype determination, should be used as a reliable method for diagnosis of chlamydial infections. In future we plan to evaluate different PCR methods to test environmental samples of different origin. These results should allow an accurate assessment of the real zoonotic health risk posed by feral pigeons.

3.5. ACKNOWLEDGEMENTS

We would like to thank Andreas Pospischil and Nicole Borel of the Institute of Veterinary Pathology (University of Zurich) as well as Erhard F. Kaleta of the University of Giessen, Germany, and Wolfgang Gaede of the State Institute for Consumer Protection Saxony-Anhalt (Stendal, Germany) for their valuable advice. We are especially grateful to Andreas Ochsenbein for technical support and assistance in the laboratory.

3.6. REFERENCES

Albrecht A, Schies U, Kämpfer P, Scholbeck R (2003). Gesundheitsgefährdung durch Taubenkot [Health hazards posed by pigeon droppings]. *Sonderdruck aus TIEFBAU, Tiefbau-Berufsgenossenschaft, München*; Heft 5/2001 und 3/2002, 1–14.

Andersen AA (1996). Comparison of pharyngeal, faecal, and cloacal samples for the isolation of *Chlamydia psittaci* from experimentally infected cockatiels and turkeys. *J Vet Diagn Invest*. 8(4), 48–50.

Andersen AA, Vanrompay D (2003). Avian Chlamydiosis (psittacosis, ornithosis). In: Saif YM, ed. *Diseases of Poultry*, 11th edition, Iowa State Press, Iowa, USA, pp. 863–879.

Centres for Disease Control and Prevention (2000). Compendium of measures to control *Chlamydia psittaci* Infection among humans (Psittacosis) and pet birds (Avian Chlamydiosis). *MMWR Recomm Rep*. 49 (RR-8), 3–17.

Everett KD, Bush RM, Andersen AA (1999). Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae fam. nov.* and *Simkaniaceae fam. nov.*, each containing a monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. *Int J Syst Bacteriol.* 49, 415–440.

Fudge AM (1991). ELISA testing for avian chlamydiosis. *The Veterinary Clinics of North America. Small Animal Practice*. 21(6),1181–1187.

Gaede W, Kenklies S, Dresenkamp B (2005). Effectiveness of PCR, antigen-ELISA and cell cultivation for the diagnosis of chlamydia-infections in different animal species. *Proceedings of the third annual workshop of the COST Action 855, Diagnosis and Pathogenesis of Avian Chlamydiosis,* Bononia University Press, Bologna, Italy, pp. 95–105.

Geens T, Desplanques A, Van Loock M, Bönner BM, Kaleta EF, Magnino S, Andersen AA, Everett KDE, Vanrompay D. (2005a). Sequencing of the *Chlamydophila psittaci ompA* gene reveals a new genotype E/B and, and the need for a rapid discriminatory genotyping method. *J Clin Microbiol*. 43 (5), 2456–2461.

Geens T, Dewitte A, Boon N, Vanrompay D (2005b). Development of a *Chlamydophila psittaci* species-specific and genotype-specific real-time PCR. *Vet Res.* 36(5-6), 787–797.

Gerbermann H (1989). Current situation and alternatives for diagnosis and control of chlamydiosis in the Federal Republic of Germany. *J Am Vet Med Assoc* 195(11), 1542–1547.

Gerbermann H and Korbel R (1993). The occurrence of *Chlamydia psittaci* infections in raptors from wildlife preserves. *Tierärztliche Praxis* 21(3), 217–224.

Guscetti F, Hoop R, Schiller I, Corboz L, Sydler T, Pospischil A (2000). Experimental enteric infection of gnotobiotic piglets with a *Chlamydia psittaci* strain of avian origin. *J Vet Med B Infect Dis Vet Public Health.* 47(8), 561–572.

Haag-Wackernagel D (2005). Feral pigeons (*Columba livia*) as a potential source of human ornithosis (Abstract). *Proceedings of the third annual workshop of the COST Action 855, Diagnosis and Pathogenesis of Avian Chlamydiosis,* Bononia University Press, Bologna, Italy, pp.15–16.

Haag-Wackernagel D (2006). Gesundheitsgefährdung durch die Strassentaube *Columba livia* [Health hazards by the feral pigeon *Columba livia*]*. Amtstierärztlicher Dienst und Lebensmittelkontrolle* 4, 262–272.

Haag-Wackernagel D, Moch H (2004). Health hazards posed by feral pigeons. *J Inf*. 48(4), 307–313.

Harkinezhad T, Verminnen K, Van Droogenbroeck C, Vanrompay D (2007). *Chlamydophila psittaci* genotype E/B transmission from African grey parrots to humans. *J Med Microbiol*. 56(Pt 8), 1097–1100.

Heddema ER, Van Hannen EJ, Duim B, Vandenbroucke-Grauls CM, Pannekoek Y (2006a). Genotyping of *Chlamydophila psittaci* in human samples. *Emerg Infect Dis.* 12(12), 1989–1990.

Heddema ER, ter Sluis S, Bujis JA, Vandenbroucke-Grauls CM, van Wijnen JH, Visser CE (2006b). Prevalence of *Chlamydophila psittaci* in fecal droppings of feral pigeons in Amsterdam, The Netherlands. *App Environ Microbiol.* 72, 4423–4425.

Kaleta EF, Taday EMA (2003). Avian host range of *Chlamydophila ssp.* based on isolation, antigen detection and serology. *Avian Pathol.* 32(5), 435–462.

Kaltenboeck B, Kousoulas KG, Storz J (1991). Detection and strain differentiation of *Chlamydia psittaci* mediated by a two-step polymerase chain reaction. *J Clin Microbiol.* 29(9), 1969–1975.

Leopold PG (1965). Ornithose als Berufskrankheit [Ornithosis as an occupational disease] *Monatshefte für Veterinärmedizin* 20(23), 964–969.

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovč A, Prukner-Radovčić E., Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF. (2009). Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Vet Microbiol*. 135, 54–67.

Mitevski D, Pendovski L, Naletoski I, Ilieski V (2005). Surveillance for the presence of *Chlamydophila psittaci* in pigeons and doves from several towns in Macedonia. *Proceedings of the third annual workshop of the COST Action 855, Diagnosis and Pathogenesis of Avian Chlamydiosis*. Bononia University Press, Bologna, Italy, pp. 95–105.

Prukner-Radovčić E, Horvatek D, Gottstein Ž, Ciglar-Grosdanić I, Mazija H (2005). Epidemiological investigation of *Chlamydophila psittaci* in pigeons and free-living birds in Croatia. *Vet Res Commun.* 29(Suppl.1), 17–21.

Sachse K, Hotzel H, Slickers P, Ellinger T, Ehricht R (2005). DNA microarray-based detection and identification of *Chlamydia* and *Chlamydophila spp*. *Mol Cell Probes* 19(1), 41–50.

Sachse K, Grossmann E, Jäger C. Diller R. Hotzel H (2003). Detection of *Chlamydia suis* from clinical specimens: comparison of PCR, antigen ELISA, and culture.*J of Microbiol Methods* 54(2), 233–8.

Tanaka C., Miyazawa T., Watarai M., Ishiguro N (2005). Bacteriological survey of feces from feral pigeons in Japan. *J Vet Med Sci.* 67(9), 951–953.

Van Loock, M, Verminnen, K, Messmer, TO, Volckaert, G, Goddeeris, BM, Vanrompay D (2005). Use of a nested PCR-enzyme immonoassay with an internal control to detect *Chlamydophila psittaci* in turkeys. *BMC Infect Dis.* 26, 5:76.

Vanrompay D, Van Nerom A, Ducatelle R, Haesebrouck F (1994). Evaluation of five immunoassays for detection of *Chlamydia psittaci* in cloacal and conjunctival specimens from turkeys. *J Clin Microbiol* 32(6), 1470–1474.

Vanrompay D, Ducatelle R, and Haesebrouck F (1995). *Chlamydia psittaci* infections: a review with emphasis on avian chlamydiosis. *Vet Microbiol.* 45, 93–119.

Wittenbrink, MM (1991). Use of an amplified enzyme immunoassay for the detection of chlamydiae in a suppurative vaginal discharge from cattle with a chlamydia-induced endometritis. *Berliner und Münchener Tierärztliche Wochenschrift* 104(4), 125–130.

Chapter 4

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

Ila Geigenfeind [(1)], Daisy Vanrompay [(2)], Daniel Haag-Wackernagel [(1)]

[(1)]Department of Biomedicine, Institute of Anatomy, University of Basel, Pestalozzistrasse 20, CH-4056 Basel, Switzerland

[(2)]Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

---

Geigenfeind I, Vanrompay D, Haag-Wackernagel D. (2012). Prevalence of *Chlamydia psittaci* in the feral pigeon population of Basel, Switzerland. *Journal of Medical Microbiology*. Feb; 61 (Pt 2), 261–5, Epub 2011 Sep 15.

4.1. ABSTRACT

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 17 (8.4 %) of the 202 feral pigeons in the loft. *Chlamydia psittaci* DNA was present in nine (2.0 %) of the 447 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.

KEYWORDS

Feral pigeons, *Columba livia*, *Chlamydia psittaci*, chlamydiosis, ornithosis, psittacosis, zoonosis

4.2. 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 to humans, they pose a potential threat to public health, since they are carriers of at least 110 zoonotic pathogens (Haag-Wackernagel & Moch, 2004; Haag-Wackernagel, 2006a,b; Haag-Wackernagel & Bircher, 2009). The most significant pathogen that can be transmitted from feral pigeons to humans is *Chlamydia psittaci,* formerly also named *Chlamydophila 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 presumed or proven transmission of *C psittaci* from feral pigeons to humans have been reported (supplemented data according to 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, or 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 o study individual resident birds repeatedly. Additionally, we examined 620 feral pigeon faecal samples collected at six public sites in Basel taking into 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.

4.3. 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 feral pigeons. A reduction of the population size can 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 to 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 > 20 000 birds to about 5 000–8 000 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 studied. 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 loft population 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 m^2 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.

In the second part of this study (Nov 2008–Nov 2009), 520 faecal droppings were examined, which were collected at 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 *C. psittaci* between pigeons and/or from pigeons to humans could occur (Rose *et al.,* 2006). The Marketplace with its restaurants, take-aways, and market stands represents a reliable food source for the birds; moreover, it increases the likelihood of close contacts close contacts between humans and feral pigeons. Therefore, we focused on the Marketplace as our main testing site and took 10 faecal samples weekly during one year. In May 2009, an additional 20 faecal samples were taken from each of five other public sites where feral pigeons regularly feed, including the Theaterplatz, the Claraplatz, the Centralbahnplatz and the Barfuesserplatz all of which have high human presence, and the Rhine harbour St. Johann, where human presence is low.

Sampling. During the first part of the research project, pigeons were trapped in the loft by closing the entrance after nightfall. Pigeons were individually caught with a hand net, weighted, clinically examined before sampling. Sampling took place when breeding activity was low, during February 2007 and February 2008 and when breeding activity was high, in July 2008 and May 2009. The numbers of birds tested during these periods were 99, 104, 124 and 120, respectively. Both pharyngeal and cloacal samples were collected, except for on the first sampling date, when only pharyngeal swabs were taken, resulting in a total of 447 pharyngeal and 348 cloacal swabs. Sterile, rayon-tipped, aluminium-shafted swabs (Copan) were used and placed in 1 ml RNA/DNA Stabilisation Reagent for Blood and Bone Marrow (Roche Diagnostics). Swabs were transported on ice and stored at -80 °C until further processing. During the second part of the research, fresh faecal droppings were collected at six public sites using the same sampling method. All experiments were

performed with the permission of the Cantonal Veterinary Office of Basel-Stadt, Switzerland (authorization no. 2120) and conformed to Swiss law on animal welfare.

Detection and molecular characterization of *C. psittaci*. Samples collected in the loft (447 pharyngeal and 348 cloacal swabs) were examined separately. Faecal droppings were pooled according to sampling time and sampling site. 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 a using a *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 analyzed 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 to F and E/B (Geens *et al.*, 2005a).

4.4. 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 to 57 % of the investigated feral pigeons (Magnino *et al.*, 2009). The highest percentage of culture positives was found in Paris (Trap *et al.*, 1986). In eleven studies conducted from 2003 to 2007, the presence of *C. psittaci*

DNA could be proven in 3.4 to 52.6 % of the examined feral pigeons by use of nucleic acid amplification assays (Magnino *et al.*, 2009; Vásquez *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 (reviewed in Magnino *et al.*, 2009). This could be due to the use of more specific diagnostic techniques like nucleic acid amplification tests. Research on optimal strategies for the management of feral pigeon (reviewed in Magnino *et al.*, 2009) and increased implementation of such strategies in cities could also play 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 and recently also in Lucerne and Lausanne, Switzerland (Cuendet & Beaud, 2009; Haag-Wackernagel, 1993, 1995; Keller, 2007).

From 2007 to 2009, the *ompA* gene of *C. psittaci* could be detected in 17 (8.4 %) of 202 feral pigeons sampled in the loft. *C. psittaci* DNA was present in 9 (2.0 %) of the 447 pharyngeal swabs and 11 (3.2 %) of the 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.

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

Sampling date	Number of positive samples/ number of feral pigeons tested (% positives)	
	Pharyngeal swab	Cloacal swab
1 (1.2.2007)	5/99 (5.1 %)	-
2 (7.2.2008)	1/104 (1.0 %)	1/104 (1.0 %)
3 (8.7.2008)	3/124 (2.4 %)	7/124 (5.6 %)
4 (6.5.2009)	0/120 (0.0 %)	3/120 (2.5 %)
Total	9/447 (2.0 %)	11/348 (3.2 %)

By retesting individual birds, we were able to document intermittent shedding of *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 *C. psittaci* could be demonstrated in feral pigeons of all ages and was not limited to young birds present in the loft (Table 2).

Outer membrane protein A (*ompA)* genotyping using real-time PCR was successful in 8 (47 %) of 17 nested PCR-positive pigeons, revealing genotype B in seven pigeons and a mixed infection with genotypes A, B, and E/B in one pigeon (B0054) (Table 2). To date, 7 genotypes (A, B, C, D, E, F, and E/B) of the *ompA* gene of *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 B is commonly found in feral pigeons, but infection with genotypes A, C, D, E, and 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 A, which is associated with a more severe disease in humans than that caused by the genotypes B and E/B (Magnino *et al*. 2009). Seven of the birds positive for *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 (Table 2). These data contribute to the understanding of of the epidemiology of *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, it could also be due to the presence of unknown avian *ompA* genotypes.

Table 2. Ring-number and age (in years) of 17 feral pigeons sampled in the loft, which tested positive for *C. psittaci* in either pharynx or cloaca. *ompA* genotyping results are presented.

Ring-number	Age at 1.2.2007	Age at 7.2.2008	Age at 8.7.2008	Age at 6.5.2009	*ompA* Genotype
A 180	8.1*	9.2	9.6	-**	B
A 255	7.7	8.8	9.2	10.0	-
A 449	6	-	7.5	8.3	-
A 756	2.8	3.9	4.3	5.1	-
A 773	2.7	-	4.2	-**	B
A 776	2.6	3.7	4.1	4.9	B
A 804	2.4	3.5	3.9	4.7	-
A 819	1.7	2.8	3.2	4	-
A 905	0.6	1.7	2.1	2.9	B
A 964	0.6***	1.4	1.8	2.8	-
A 965	0.6***	1.4	-**	-**	-
A 982	-	0.9	1.3	-**	B
B 0014	-	-	0.6***	1.1	B
B 0030	-	-	0.6***	1.1	-
B 0054	-	-	0.6***	1.1	A, B and E/B
B 0058	-	-	0.6***	1.1	B
B 0106	-	-	-	0.6***	-

* The age of the bird at the time of a positive PCR is underlined. ** Pigeon no longer present in the loft, ***Adult birds of unknown age immigrating into the loft. Their age was estimated to be at least 7 months old (0.6 years).

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 *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 (33.3%) feral pigeons in the city of Lucerne, were positive for *C. psittaci*. Interestingly, in the same study, the prevalence of *C. psittaci* in feral pigeons in Zurich was found to be significantly higher than in Lucerne. In Zurich, 10 (41.7%) out of 24 clinically healthy feral pigeons tested positive by analysis of cloacal swabs. Genotyping revealed genotype B in one sample from Lucerne and five of the samples from Zurich. Genotype E was detected in one sample from Zurich. The authors found no explanation for the remarkably differing prevalence of *C. psittaci* in these two Swiss cities. However, in Lucerne, a feral pigeons 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 *C. psittaci* genotypes in different feral pigeon populations.

C. psittaci 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 *C. psittaci* by analysis of cloacal swabs. In a similar setting in Amsterdam, Heddema *et al*. (2006b) detected the *C. psittaci ompA* gene in 7.9 % of examined faecal droppings. According to Buijs & Van Wijnen (2001), there are ~ 30 000 feral pigeons are in Amsterdam, most of which 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”, in the feral pigeon population in Basel decreased to around two thirds its previous size and is stable at a level of up to 8 000 birds, of which ~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 1 265 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 the amount of potentially infectious feral pigeon droppings in the 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 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, streets and squares are periodically cleaned with water to avoid dust formation. 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 minutes 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 was reported previously (Haag-Wackernagel, 2006a). This highlights the importance of using personal protective clothing during the handing 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 (Magnino *et al*., 2009).

4.5. CONCLUSIONS

Feral pigeons can become infected with *C. psittaci* and thus present a 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 as the detrimental effect it has on public 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.

4.6 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 Ochsenbein, Simone Probst and Alex Rufle (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.

4.7. REFERENCES

Andersen AA, Vanrompay D. (2003). Avian chlamydiosis (psittacosis, ornithosis). In *Diseases of Poultry*, pp. 863–879. Edited by Y. M. Saif, H. J. Barnes, A. M. Fadly, J. R. Glisson, L. R. McDougald & D. E. Swayne. Ames, IA: Iowa State Press.

Buijs JA, Van Wijnen JH. (2001). Survey of feral rock doves (*Columba livia*) in Amsterdam, a bird-human association. *Urban Ecosyst.* 5 (4), 1573–1642.

Cuendet G, Beaud P. (2009). Le pigeon biset domestique. In: *Les oiseaux nicheurs de Lausanne et enivirons*. pp. 213–222. Edited by P. Beaud. Nos oiseaux, Montmollin.

Dickx V, Beeckmann DAS, Dossche L, Tavernier P & Vanrompay D. (2010). *Chlamydophila psittaci* in homing and feral pigeons and zoonotic transmission. *J Med Microbiol*. 59, 1348–1353.

Everett KD, Bush RM, Andersen AA. (1999). Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. *Int J Syst Bacteriol*. 49 (Pt 2.), 415–440.

Fritzsche K (1961). *Aktuelle Fragen der Ornithose*. Jahreskongress 1960 für Ärztliche Fortbildung vom 26. Bis 30. September 1960 in Leipzig für Ärzte und Fachärzte aller Fachgebiete. 16, 5–86. Berlin Verlag Volk und Gesundheit.

Geens T, Dewitte A, Boon N, Vanrompay D. (2005a) Development of a *Chlamydophila psittaci* species-specific and genotype-specific real-time PCR. *Vet Res*. 36, 787–97.

Geens T, Desplanques A, Van Loock M, Bönner BM, Kaleta EF, Magnino S, Andersen AA, Everett KDE, Vanrompay D. (2005b). Sequencing of the *Chlamydophila psittaci ompA* gene reveals a new genotype E/B and, and the need for a rapid discriminatory genotyping method. *J Clin Microbiol*. 43 (5), 2456–2461.

Haag D. (1984). Ein Beitrag zur Oekologie der Stadttaube. Dissertation. Phil.Nat. Fakultät der Universität Basel, Verlag Medizinische Biologie.

Haag D, Gurdan P. (1990). Über den hygienischen Zustand der Strassentauben in Basel. *Swiss Vet – Schweizerische Zeitschrift für Veterinärmedizin*. 7/6, 19–22.

Haag-Wackernagel, D. (1993). Street Pigeons in Basel. *Nature*. 361, 200.

Haag-Wackernagel D. (1995). Regulation of the street pigeon in Basel. *Wildl Soc Bull*. 23 (2), 256–260.

Haag-Wackernagel D. (2005). Feral pigeons (*Columba livia*) as potential source for human ornithosis. In *Proceedings of the 3rd Workshop for Diagnosis and Pathogenesis of Animal Chlamydioses, Siena, Italy*, pp. 15–16. Edited by R. Cevenini & V. Sambri. Bologna: Bononia University Press.

Haag-Wackernagel D. (2006a). Human diseases caused by feral pigeons. In: *Advances in Vertebrate Pest Management, 4.* pp. 31–58. Edited by C. J. Feare & D. P. Cowan, Fürth: Filander Verlag.

Haag-Wackernagel D. (2006b). Gesundheitsgefährdungen durch die Strassentaube *Columba livia*: Krankheiten. *Amtstierärztlicher Dienst und Lebensmittelkontrolle*. 4, 262–272.

Haag-Wackernagel D, Bircher A. (2010). Ectoparasites from feral pigeons affecting humans. Dermatology. 220, 82–92.

Haag-Wackernagel D, Moch H. (2004). Health hazards posed by feral pigeons. *J Infect.* 48 (4), 307–313.

Harkinezhad T, Geens T, Vanrompay D. (2009). *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. *Vet Microbiol*. 135 (1–2), 68–77.

Heddema, E. R., Van Hannen, E. J., Duim, B., Vandenbroucke-Grauls, C. M. & Pannekoek, Y. (2006a). Genotyping of *Chlamydophila psittaci* in human samples. *Emerg Infect Dis*. **12**, 1989–1990.

Heddema E. R., ter Sluis, S., Bujis, J. A., Vandenbroucke-Grauls, C. M., van Wijnen, J. H. & Visser, C. E. (2006b). Prevalence of *Chlamydophila psittaci* in fecal

droppings of feral pigeons in Amsterdam, The Netherlands. *Appl Environ Microbiol*. **72**, 4423–5442.

Johnston, RF, Janiga M. (1995). *Feral Pigeons*. New York, Oxford University Press.

Kaleta EF, Taday EMA. (2003). Avian host range of *Chlamydophila ssp*. Based on isolation, antigen detection and serology. *Avian Pathol*. 32, 435–62.

Keller, M. (2007). Projekt „Stadttauben Luzern“. *Schlussbericht*. Stadt Luzern, Umweltschutz, accessible at www.stadtluzern.ch/de.

Kuo C, Stephens R. (2011). Family I. Chlamydiacaea. in *Bergey's Manual of Systematic Bacteriology. Second Edition.* pp. 845. Edited by William B. Whitman. Springer Science+Business Media, New York, USA.

Laroucau K, Mahe AM, Bouillin C, Deville M, Gandouin C, Touati F, Guillot J, Boulouis HJ. (2005). Health status of free-living pigeons in Paris. In *Proceedings of the 3rd Workshop on Diagnosis and Pathogenesis of Animal Chlamydioses, Siena, Italy*, pp.17–18. Edited by R. Cevenini & V. Sambri. Bologna: Bononia University Press

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovč A, Prukner-Radovčić E., Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF. (2009). Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Vet Microbiol*. 135, 54–67.

Meyer KF. (1941). Pigeons and barn yard fowls as possible sources of human psittacosis or ornithosis. *Schweiz Med Wochenschr*. 44, 1377–1379.

Mitevski D, Pendovski L, Naletoski I, Ilieski V. (2005). Surveillance for the presence of *Chlamydophila psittaci* in pigeons and doves from several towns in Macedonia. In *Proceedings of the 3rd Workshop on Diagnosis and Pathogenesis of Animal Chlamydioses, Siena, Italy*, pp. 141–145. Edited by R. Cevenini & V. Sambri. Bologna: Bononia University Press.

Prukner-Radovčić E, Horvatek D, Gottstein Z, Grozdanić IC, Mazija H. (2005). Epidemiological investigation of *Chlamydophila psittaci* in pigeons and free-living birds in Croatia. *Vet Res Commun*. 29 (Suppl. 1), 17–21.

Rose E, Nagel P, Haag-Wackernagel D. (2006). Spatio-temporal use of the urban habitat by feral pigeons (*Columba livia*). *Behav Ecol Sociobiol*. 60, 242–254.

Tanaka C, Miyazawa T, Watarai M, Ishiguro N. (2005). Bacteriological survey of feces from feral pigeons in Japan. *J Vet Med Sci.* 67, 951–953.

Trap D, Louzis C, Gourreau JM, Gaumont R. (1986). Chlamydiose aviaire chez les pigeons de Paris. *Rev Med Vet (Toulouse)* 137, 789–796.

Van Loock M, Verminnen K, Messmer TO, Volkaert G, Godderis BM, Vanrompay D. (2005). Use of a nested PCR-enyme immunoassay with an internal control to detect *Chlamydophila psittaci* in turkeys. *BMC Infect Dis*. 5, 76.

Vázquez B, Esperón F, Neves E, López J, Ballesteros C, Muñoz MJ. (2010). Screening for several potential pathogens in feral pigeons (*Columba livia*) in Madrid. *Acta Vet Scand*. 52, 45.

Wreghitt T. (2003). Ornithosis. *Presentation, Seminar on Birds a2.5nd Public Health*, British Ornithologists's Union held at The British Academy, London, 27 November 2003 :14.

Yuan, Y, Lyng, K, Zhang, Y, Rockey, D D & Morrison, R P (1992). Monoclonal Antibodies Define Genus-Specific, Species-Specific, and Cross-Reactive Epitopes of the Chlamydial 60-Kilodalton Heat Shock Protein (hsp6O): Specific Immunodetection and Purification of Chlamydial hsp6O. *Infect Immun* 60, 2288-22

Zweifel, D, Hoop, R, Sachse, K, Pospischil, A & Borel, N. (2009). Prevalence of *Chlamydophila psittaci* in wild birds – potential risk for domestic poultry, pet birds, and public health? Eur J Wildl Res. 55, 575–581.

Chapter 5

Protecting Buildings against Feral Pigeons

Daniel Haag-Wackernagel & Ila Geigenfeind

Department of Biomedicine, Institute of Anatomy, University of Basel,
Pestalozzistrasse 20, CH-4056 Basel, Switzerland

Haag-Wackernagel D, Geigenfeind I. (2008) Protecting buildings against feral pigeons. *European Journal of Wildlife Research*. 54 (4), 715–721.

5.1. ABSTRACT

Feral pigeons (*Columba livia*, Gmelin 1789) cause different problems for building owners when using structures for daytime perching, sleeping, and breeding. Problems include fouling of building facades and pavements, transmission of allergens and pathogenic microorganisms, and infestations with ectoparasites emanating from breeding sites. Owners are primarily interested in keeping unwanted away pigeons from their property. Pest control companies offer different deterrent systems, of widely varying efficacy, for proofing buildings against feral pigeons. A better solution is avoiding attractive structures during building design or subsequent alterations of existing structures used by feral pigeons. With our study, we elaborate the relevant structural data to help to maintain a building free of pigeons. We performed experiments with free ranging feral pigeons in a feral pigeon loft in the City of Basel, Switzerland. The maximum outlet width a pigeon is not able to pass through is 4 cm, the respective outlet height is 5 cm and a pigeon-safe square opening is not larger than 6 × 6 cm. The maximum ledge width a pigeon is not able to sit on is 4 cm. The pigeon-safe angle of inclination for smooth construction materials (tinplate, glass, plastics) is 25°, for medium rough materials (wood, plane concrete) 35°, and for rough materials (sandstone, rough concrete) at least 50°. Additionally, we studied the behavioural strategies used by feral pigeons to surmount our experimental constructional restrictions, ledge width, and ledge inclinations. Our data provide the essential data to prevent feral pigeons from using building structures.

KEYWORDS *Columba livia*, behaviour, building structures, deterrent systems, constructional restrictions

5.2. INTRODUCTION

Feral pigeons are descendants of the domesticated form of the wild rock dove (*Columba livia*, Gmelin 1789). After World War II, feral pigeon populations increased worldwide in most large cities due to feeding by pigeon enthusiasts, food discarded by humans, accidental food spillage, and on a lesser scale by seasonally occurring natural food (Simms, 1979; Haag-Wackernagel, 1995). Regular food supply allows pigeons extra time for breeding, so that some individuals are able to breed throughout the year (Murton *et al*., 1972; Johnston & Janiga, 1995). Pigeons living in urban areas have expanded their originally granivorous diet to the extent that feral pigeons are now omnivorous (Haag, 1984). Large feral pigeon populations cause various problems. Their vocalization may cause hysteric reactions (Carle, 1959) and insomnia in sensitive persons when occupying buildings (Wormuth, 1994). Excessive population density activates density-dependent regulation mechanisms (Haag, 1991a). An individual pigeon produces around 12 kg of excreta yearly (Kösters *et al*., 1991) which fouls breeding sites, house facades, monuments, pavements, sidewalks, and other public areas and is able to deface and deteriorate calcareous stone (Del Monte & Sabbioni, 1968; Dell'Omo, 1996).

Feral pigeons living close to humans can present a health risk. Ectoparasites can migrate from breeding sites into human living space when they lose their natural hosts and infest humans causing traumatic experiences to the persons concerned (Haag-Wackernagel, 2005). Feral pigeons harbour at least 110 different human pathogenic microorganisms (Haag-Wackernagel & Moch, 2004). In fact, of these human pathogens harboured by feral pigeons, up to the present, only seven caused a total of 230 human infections worldwide, 13 with a fatal course (Haag-Wackernagel & Moch, 2004; Haag-Wackernagel, 2006). Recently, the risk of pigeon breeders' disease (allergic alveolitis) due to exposure to feral pigeons emerged and so far nine cases, of which one was fatal, have been reported worldwide since 2000 (Haag-Wackernagel, 2006). Many cases of parasitic infestations and disease transmissions could be traced back to feral pigeons breeding at house facades or in attics. Facing these problems, house owners and city authorities want to avoid pigeon infestations. Pest control companies offer a wide range of deterrent systems but these vary in efficacy and are costly and often difficult to install and maintain. Conspicuous

systems can detract from the architectural impression and many systems confer only limited or transient protection. Experiments with free-living feral pigeons demonstrated that highly motivated individuals are able to surmount almost every deterrent system (Haag-Wackernagel, 2000). Pigeons are able to use very small spaces to build their nests and can squeeze through tight passages to reach their favourite sites (Fig. 1) and they are able to sit on steeply inclined and small ledges. The exclusion of pigeons from buildings is the best option to solve this problem. However, in many cases aesthetic or technical needs do not allow complete sealing. Feral pigeons can be discouraged from roosting on ledges by installing sloping surfaces over the flat surface or downsizing openings to the extent preventing a feral pigeon from passing. This can be as simple as a board or metal sheet installed with a steep angle. Problems could be more efficiently prevented by incorporating deterrent features into building design at the planning stage. This requires knowledge of the physical features that will exclude pigeons. In the literature, only few and more general data on structural measures have been published and data on the minimal dimensions of openings feral pigeons can squeeze through are completely missing. In this paper, we describe experiments undertaken to determine the minimum apertures that pigeons can enter when they are highly motivated to do so and the maximum slopes that they can tolerate. These data provide sound guidance for the avoidance of pigeon infestations in building design.

Figure 1. Feral pigeons are able to use small hollows within building facades as breeding sites.

5.3. MATERIALS AND METHODS

The pigeons used for this study were free-living feral pigeons breeding in nest boxes in the loft of the St. Matthäus Church in Basel, Switzerland. The loft lies above the nave of the church and has a floor space of 31 m^2. Around 120 feral pigeons are residents in this loft. Experiments were performed under natural conditions, the birds obtaining all their food and water from their normal resources in the city. The loft is cleaned every 14 days and in the event of the occurrence of ectoparasites (red blood mite *Dermanyssus gallinae* or pigeon tick *Argas reflexus*) the nests are treated with an acaricide (Vapona pest strips with dichlorvos). The 39 breeding boxes are 48 cm wide, 28 cm high, and 41 cm deep and can be closed from outside the loft to catch breeding birds. Body masses of 206 feral pigeons were recorded during dissections performed for an earlier study (Haag, 1984). Morphological data were recorded with 20 adult breeding feral pigeons caught in the loft. With a ruler, we measured the widest part of the chest with closed wings (max chest width), the widest part of the chest without wings (minimal chest width), and the circumference of the chest with closed wings (maximal chest circumference). To investigate the minimal area required for a feral pigeon nest, the inner flat part of the nest where the birds are able to lie (minimal nest diameter) and the outer diameter that includes most of the nesting material, preventing the eggs from rolling out (maximal nest diameter), were measured in 16 nests.

The pigeons enter and leave the loft at will. The experiments to investigate the minimal openings were performed at the single entrance to the loft, constructed as a trapdoor of 42 cm wide 40 cm high. The trapdoor can be opened and closed by a linkage from outside the loft. In the experimental design, a tunnel 38.5 cm high, 38.5 cm wide and 60 cm long was placed in front of the loft entrance for the duration of the experiments (Fig. 2). For one month before the experiments started, the pigeons had the opportunity to habituate to the altered entrance. Pigeons are highly motivated to enter or leave the loft even when obstructed with an experimental reduction of the opening to reach their nesting sites or to leave the loft to forage. During four 4-h intervals, the frequency of pigeons passing through the tunnel without the experimental restriction was recorded twice in the morning between 0800 and 1200 h and twice in the afternoon between 1400 and 1800 h. To investigate the

minimal outlet height and width and the minimum outlet square opening that allowed feral pigeons to squeeze through, a restriction of the outlet was constructed with adjustable wooden boards fixed in tracks that were attached to the inner front opening of the tunnel. A video camera was used to record all pigeons entering and exiting the loft during four 4-h recordings. The openings were restricted in decreasing steps of 1 cm until no pigeon was recorded successfully negotiating the experimental restriction.

Figure 2. Experimental design with a tunnel and adjustable wooden boards to test the minimal restriction a feral pigeon is able to pass through. In this experimental design the width can be varied to the extent that even a highly motivated pigeon is not able to squeeze through the opening.

To investigate the minimal ledge width a pigeon can sit on, a nest box not occupied by a breeding pair, and therefore used briefly by different individuals, was observed. During four 4-h intervals recorded with video, the frequency of pigeons staying in the unaltered box was recorded. With an adjustable construction of a vertically erected wooden board (42.4 × 26.5 cm), the nest box ledge was decreased in 1-cm steps beginning with an “experimental ledge” of 10 cm to the width on which no pigeon was able to sit for > 1 s (Fig. 3). Inclination tests were performed in nest boxes that were occupied by breeding pairs. The birds were highly motivated to return to their nest

and therefore attempted to sit on the test installation even if very uncomfortable. The same method was already successfully used for an earlier study to test pigeon deterrent systems (Haag-Wackernagel, 2000). On an adjustable retainer of 42 × 30 cm, test materials (a tinplate slab, a plywood board, a washed out concrete board, and a ground sandstone slab) of the same size were fixed and then placed in the nest box (Fig. 4). The inclination was increased in steps of 5° to the angle the pigeon was unable to stay for > 1 s. An inclination was assessed as unusable (negative) when in five different occupied nest boxes no pigeon was able to stay on the test slope for > 1 s. After each recording interval of all experiments, the restrictions were removed to allow the pigeons to rehabituate to their familiar environment. Video recordings of all experiments were subsequently analyzed on a video recorder. Selected scenes were imported into the video program iMovie on a Macintosh Computer MacPro and analyzed using slow motion and detail screen function.

Figure 3. Experimental design to test the ledge width with a variable holder placed in an unoccupied nest box.

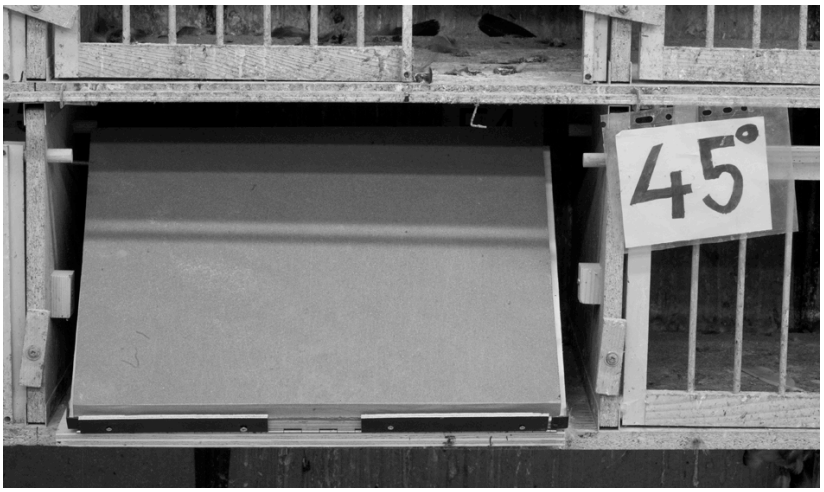


Figure 4. With a holder fixing the test material, here a sandstone slab, the maximal inclined slope a feral pigeon can sit on was tested.

5.4. RESULTS

Morphological Traits

The minimal opening a pigeon can pass through depends on its body dimensions. The most relevant morphological factors are (a) distance between keel of sternum and the thoracic vertebrae of the back and (b) chest width (Fig. 5). The average body mass for adult males in Basel was 345.5 g (SD 30.5, n=76) and for females 307.4 g (SD 35.2, n=35). The average widest part of the chest with closed wings of 20 adult feral pigeons was 8.6 cm (SD 0.53 m), the widest part of the chest without wings 6.13 cm (SD 0.41 cm), and the circumference with closed wings 25.25 cm (SD 1.4 cm) resulting in a mean chest diameter of 8.04 cm. Theoretically, a pigeon should not be able to squeeze through an opening < 6 cm as a result of these morphological restrictions (widest chest width).

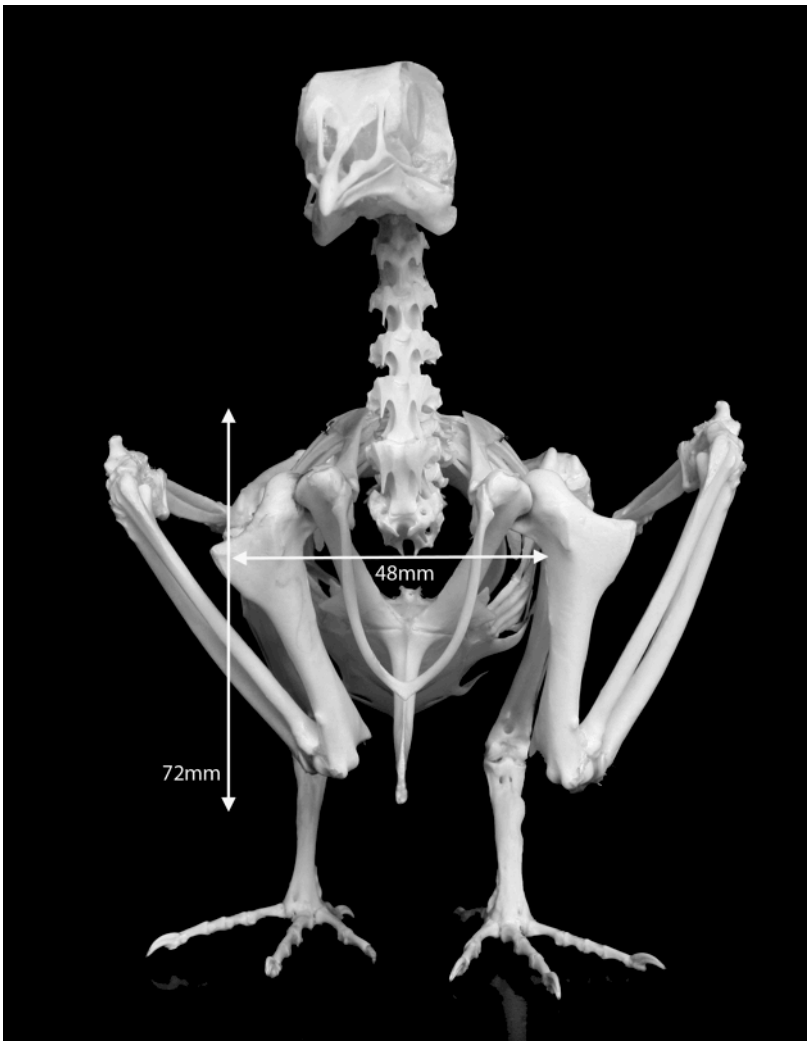


Figure 5. The skeleton of a pigeon shows the morphological traits relevant for the ability to pass through structural restrictions. Chest width and distance between keel of sternum and the thoracic vertebrae of the back are outlined.

Nest dimensions

The 16 measured nests had an average outer diameter of 20.0 cm (SD 5.3 cm) and an inner diameter of 10.9 cm (SD 1.57 cm). The inner diameter of 10.9 cm seems to be the minimum dimension a feral pigeon needs to perform its normal behaviour, including space demanding breeding behaviours such as nest building, moulding (while lying down at the nest site, the bird rotates its body in a horizontal plane and often performs scratching movements with the feet on the substratum to build a mould in the nest), and incubation (Fabricius and Jansson, 1963; Haag, 1991b).

Entrance restriction

The tunnel without an experimental restriction was passed by an average of 550.3 pigeons per 4-h interval (min 214, max 944, SD 375.3). There is no evidence that the tunnel, compared with the normal 42 × 40-cm trapdoor entrance, had any deterrent effect on the pigeons.

Restriction in width Figure 6 shows the numbers of pigeons that passed the restrictions of 7–4 cm. With a width of 5 cm, only few passages were recorded (mean 3 pigeons per 4-h interval, min 1, max 9, SD 4). With a restriction of 4 cm, no pigeon was able to pass during four 4-h intervals. Figure 7 shows sketches of a video analysis of a feral pigeon squeezing through a restriction width of 6 cm. The bird had to turn the chest diagonally to attain its smallest chest radius and to position the wings from horizontal to vertical. The optimal position is realized by an angle of 45° between the vertical of the restriction and the direction of the back to keel axis. This position corresponds to the diagonal of the chest (Fig. 5). Simultaneously, the bird rests upon the elbow (Fig. 7, 3 and 4). After having passed the chest, the body axis is turned back to a horizontal position and the bird slips through the restriction (Fig. 7, 5 and 6).

Restriction in height Figure 8 shows the number of pigeons passing a height restriction from 7 to 5 cm. A height restriction of 7 cm with an average of 119 pigeons per 4-h interval (min 42, max 196, SD 72.8) seemed not to be a problem whereas a 6-cm restriction led to a considerable decrease in passages with an average of 4.5 pigeons per 4-h interval (min 1, max 11, SD 4.5). With a 5-cm restriction, no pigeon was able to pass. Video analysis of the behaviour revealed a lateral torsion of the

chest of 25° combined with a simultaneous crouching allowing the bird to pass.

Square restriction Figure 9 shows the number of pigeons passing a square restriction with an edge length ranging from 8 to 6 cm. With a square of 8 × 8 cm, an average of 12.4 pigeons per 4-h interval (min 9, max 35, SD 12.4) were able to pass. A square restriction of 7 × 7 cm led to a significant decrease in passages (mean 4.5, min 1, max 11, SD 4.5) while a square restriction of 6 × 6 cm prevented pigeons from passing. With a square restriction, the pigeons did not apply special behaviours to fit their body to the experimental restrictions.

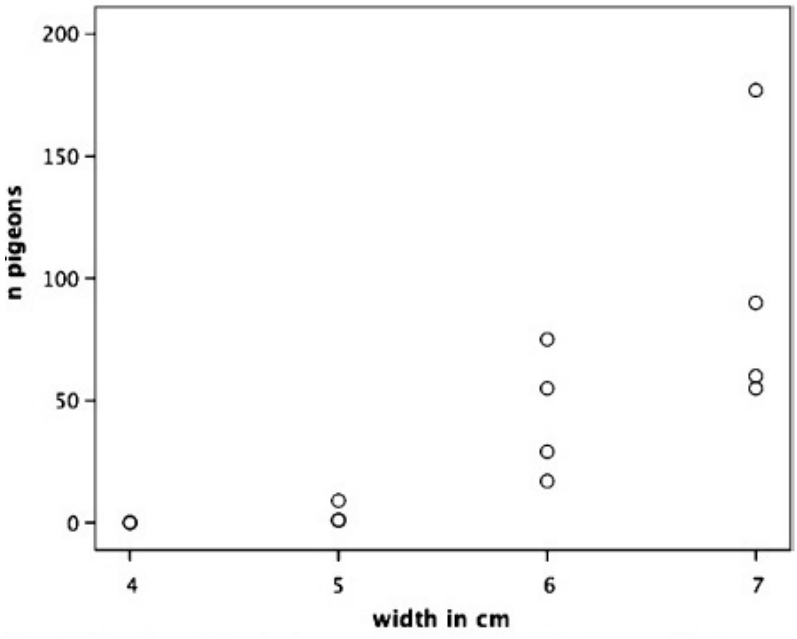


Figure 6. Number of feral pigeons per 4hr-interval that were able to pass a restriction in width.

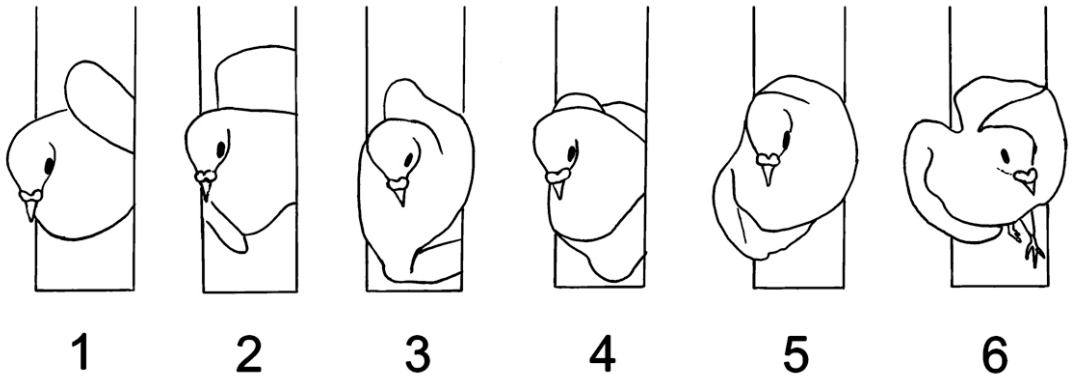


Figure 7. Behavior shown by a feral pigeon to squeeze through a restriction with a restriction width of 6 cm.

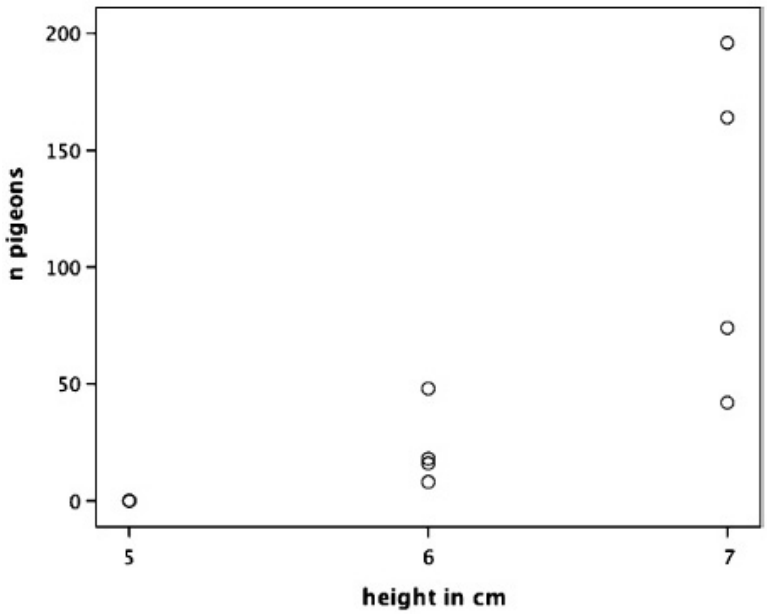


Figure 8. Number of feral pigeons per 4hr-interval that were able to pass a restriction in height.

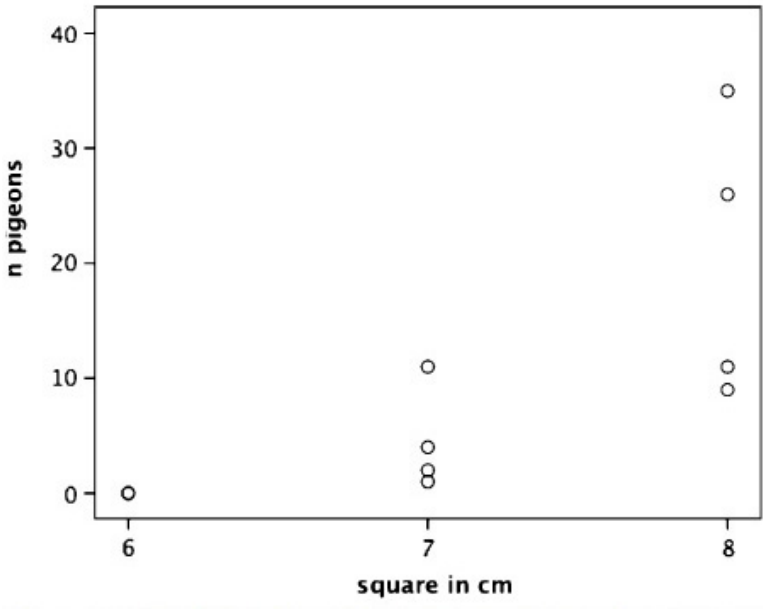


Figure 9. Number of feral pigeons per 4hr-interval that were able to pass a square restriction with an edge length ranging from 8–6 cm.

Ledge width

Figure 10 shows the number of pigeons that were able to sit on a variable ledge width of 10–4 cm. A ledge width of 10–6 cm seemed not to be a problem for a feral pigeon to sit on. On a ledge width of 5 cm, only few pigeons are able to sit (mean 3.25 pigeons per 4-h interval, min 1, max 5, SD 2.06). A ledge is pigeon safe with a width of 4 cm.

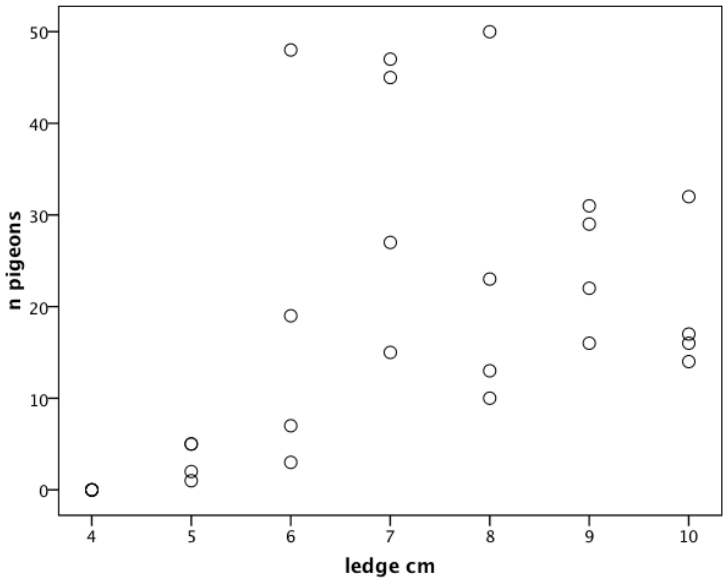


Figure 10. Number of feral pigeons per 4hr-interval that were able to sit on a ledge of restricted width.

Angle of inclination

Table 1 shows the results of the experiments with inclined slopes of four different construction materials. On an inclined tinplate slab angled at 20°, pigeons were not able to stay for >1 s. Plywood and concrete slopes had to be inclined further, with an angle of at least 30°, to prevent feral pigeons from staying. Sandstone, with a surface texture, needed to be inclined at least to 45° to exclude pigeons.

Table 1. Identification of the angle of inclination of a slope a feral pigeon is able to sit on depending on different construction materials

Angle of inclination	Tinplate	Plywood	Concrete	Sandstone
15°	1[a] pos			
20°	5 neg	1 pos	1 pos	
25°		1 pos	1 pos	
30°		5 neg	5 neg	1 pos
35°		5 neg	5 neg	1 pos
40°				1 pos
45°				5 neg

[a] Number of experiments performed in an occupied breeding box, pos (positive) = pigeon is able to sit for >1 sec, neg (negative) = pigeon slips off. The grey part of the table designates the angles of the respective materials a feral pigeon is not able to sit on.

5.6. DISCUSSION

Body size limits a feral pigeon's ability to pass through a structural restriction. The body mass of feral pigeons varies geographically up to 30 % (Johnston & Janiga, 1995). The mean body mass of feral pigeons in Basel (males 345.4 g, SD 30.5 g; females 307.4 g, SD 35.2 g) lies between large strains from Lawrence, KS, USA (males 358.7 g, SD 38.6 g; females 340.1 g, SD 34.7 g) and small Rock-Dove-like strains from Fertilia, Sardinia, Italy (males 289.4 g, SD 16.8 g; females 242.5 g, SD 17.7 g; Johnston & Janiga, 1995). The midrange of the feral pigeons of Basel suggests that our results should be applicable to most other feral pigeon populations. Any restriction of the tunnel had a strong deterrent effect on the feral pigeons of our experimental loft. This demonstrates that feral pigeons react extremely carefully and shyly towards alterations of their familiar environment, a neophobic reaction. We assume that only birds under a high motivation tried to pass the experimental restrictions, such as breeding individuals that want to reach their nest. We additionally observed that pigeons that did not pass the experimental design waited until the end of the 4-h experiments outside the loft. Feral pigeons can squeeze through astonishingly narrow restrictions close to the limit determined by their skeletal anatomy. Due to special behavioural strategies, the birds were able to fit the body to extremely narrow restrictions in width by turning the chest diagonally to an angle of 45° and expanding the body to the vertical axis. Only a 4-cm-wide restriction excluded pigeons. With a horizontal restriction, the bird was unable to lift the wing in the same way to use the smallest chest radius. Therefore, the lateral torsion of the chest was limited to an angle of 25°, leading to a minimum height of 6 cm to allow passing compared with 5 cm in a width restriction. A square restriction does not allow lifting the wings to attain a lateral torsion. This led to an increased space demand of at least 7×7 cm. According to their behavioural possibilities, pigeon deterrent dimensions of openings can be achieved with a width of ≤ 4 cm, a height of ≤ 5 cm, and a square restriction of ≤ 6×6 cm.

The ability to sit on a narrow ledge depends on the standing width (distance between the legs) of a feral pigeon in relation to its centre of gravity. In our experiments, a 4-cm-wide ledge prevented pigeons from sitting on it. This width of 4 cm is 2 cm smaller than the single recommended literature value of 6 cm (Andelt & Burnham,

1993). Artificial restrictions preventing access to buildings for feral pigeons could also have an effect by excluding other city birds using the same sites as, e.g., kestrels. Bats and smaller birds as, e.g., swifts can still use openings that exclude feral pigeons (Thurston, 1983).

The few recommendations in the literature indicate angles of inclined slopes for feral pigeons without respect to the texture of the material. Andelt and Burnham (1993) and Kern (2007) recommended angles exceeding 45°, and > 55° was recommended by the German Landesamt fur Arbeitsschutz (2000). We found that the ability of a feral pigeon to sit on an inclined slope depends on the construction material and on the angle of inclination of the respective materials. Accordingly, the rough and grainy surface of sandstone needs a steeper inclination (45°) than the slippery tinplate (20°) to prevent pigeons from sitting on it. All construction materials experience weathering and seasonal variation in temperature that can lead to erosion and increases the roughness of the surface. Additionally, we observed that pigeons with dirty feet deposited this material, mostly droppings, on the surface of the slope when trying to land. This led to improved foothold over time, allowing pigeons to sit on steeper slopes. Hence, we recommend adding a safety addition of 5° to our experimental data and to regularly clean smooth inclined surfaces intended to keep pigeons away. Field observations showed that feral pigeon are not able to use ledges with angles > 50° for more than a few seconds even if the material is heavily structured. Further studies should test other materials, including the aspects of erosion. New construction materials with slippery coatings will offer new options in making buildings and other structures inaccessible to pigeons.

Management implications

Buildings can be protected effectively against feral pigeons by avoiding attractive structures during building design or by subsequent alterations of existing structures used by feral pigeons. Openings can be reduced to dimensions that pigeons are not able to pass. Ledges used by pigeons can be made unusable by increasing the angle of inclination according to building material properties.

According to our experiment, we suggest to use the following pigeon safe dimensions:

Pigeon-safe openings:

- Restriction in width 4 cm
- Restriction in height 5 cm
- Square restriction 6 × 6 cm

Pigeon-safe ledges:

- Ledge width 4 cm

Angle of Inclination:

- Smooth material (tinplate, glass, plastics)
  angle of inclination 25°
- Medium rough material (wood, plane concrete):
  angle of inclination 35°
- Rough material (sandstone, rough concrete):
- Angle of inclination 50°

5.7. ACKNOWLEDGEMENTS

We are very grateful to Andreas Ochsenbein for technical support and assistance. We thank Chris Feare for his valuable comments and amendments. All experiments were performed with the animal experimental permission of the Cantonal Veterinary Office of Basel-Town, Switzerland (authorization no. 2121 of the 16 Feb. 2006) and conformed to Swiss law on animal welfare.

5.8. REFERENCES

Andelt WF, Burnham KP. (1993). Effectiveness of nylon lines for deterring Rock Doves from landing on ledges. *Wildl Soc Bull*. 21, 451–456.

Carle R. (1959). Die Vorbereitung zur Bestandsregelung unter den verwilderten Haustauben in den Städten. Städtehygiene 8:164–168 Del Monte M, Sabbioni C (1986) Chemical and biological weathering of an historical building: Reggio Emilia Cathedral. *Sci Total Environ*. 50, 165–18.

Dell'Omo A. (1996). Il ruolo degli escrementi dei piccioni nella bioalterazione delle rocce. Tesina di Università degli Studi di Roma "La Sapienza", Facoltà di Scienze Matematiche, Fisiche e Naturali, Dipartimento di Biologia Animale e dell'Uomo.

Fabricius E, Jansson AM. (1963). Laboratory observations on the reproductive behaviour of the pigeon (*Columba livia*) during the pre-incubation phase of the breeding cycle. *Anim Behav.* XI (4), 534–547.

Haag D. (1984). Ein Beitrag zur Oekologie der Stadttaube. *Dissertation*, Phil. Nat. Faculty of the University of Basel, Verlag Medizinische Biologie, Basel, Switzerland.

Haag D. (1991a). Population density as a regulator of mortality among eggs and nestlings of feral pigeons (*Columba livia domestica*) in Basel, Switzerland. In: *Proceedings of International Symposium of the Working Group on Granivorous Birds*, INTECOL, Slupsk, Poland, Sept 14–17 1989, Warzawa: J. Pinowski, B. P. Kavanagh and W. Gorski, PWN—Polish Scientific Publishers, Warzawa: 21–31.

Haag D. (1991b). Ethogramm der Taube. Ruhr University, Bochum Published in the series *"Orn-Projekt".*

Haag-Wackernagel D. (1995). Regulation of the street pigeon in Basel. *Wildl Soc Bull.* 23(2), 256–260.

Haag-Wackernagel D. (2000). Behavioural responses of the feral pigeon (*Columbidae*) to deterring systems. *Folia Zoologica.* 49 (2), 25–39.

Haag-Wackernagel D. (2005). Parasites from feral pigeons as a health hazard for humans. *Ann Appl Biol*. 147, 203–210.

Haag-Wackernagel D. (2006). Human diseases caused by feral pigeons. *Advances in Vertebrate Pest Management.* 4, 31–58.

Haag-Wackernagel D, Moch H. (2004). Health hazards posed by feral pigeons. *J Inf.* 48(4), 307–313.

Johnston RF, Janiga M (1995) *Feral Pigeons*. Oxford University Press, Oxford

Kern WH. (2007). Pigeons. Fact Sheet SS-WEC-117 (UW117) Department of Wildlife Ecology and Conservation, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu/UW117

Kösters J, Kaleta EF, Monreal G, Siegmann O. (1991). Das Problem der Stadttauben. *Deutsches Tierärzteblatt.* 4, 272–276.

Landesamt fur Arbeitsschutz, Gesundheitsschutz und Technische Sicherheit Berlin. (2000). Massnahmen der Taubenvergrämung. *Fachinfo 1/2000*, http://www.hygieneinspektoren.de/download/tauben.pdf

Murton RK, Thearle RJP, Thompson J. (1972). Ecological studies of the feral pigeon *Columba livia* var. I. Population, breeding biology and methods of control. *J Appl Ecol*. 9, 835–874.

Simms E. (1979). *The public life of the Street Pigeon*. Hutchinson, London

Thurston P. (1983). Bauen für Segler. *Gartenbauamt der Stadt Zürich*

Wormuth HJ. (1994). Zum Problem der verwilderten Haustauben. *Merkblatt des Robert von Ostertag-Instituts des Bundesgesundheitsamtes*, Berlin, p.6

Chapter 6

General Discussion and Conclusion

6. General Discussion and Conclusion

6.1. GENERAL DISCUSSION

This PhD thesis provides insight into the epidemiology of *C. psittaci* infections in feral pigeons in Basel. It could be documented that *C. psittaci* shedding occurs in the feral pigeon population of Basel and intermittent shedding of the pathogen was demonstrated in some of the birds. In addition, important architectural parameters were determined for the first time, which help to keep a building free from feral pigeons to reduce contacts leading to infection in the human population. The present thesis contributes to the prevention of the transmission of pathogens and parasites from feral pigeons to humans.

Numerous previous studies documented the abundance of *C. psittaci* in feral pigeon populations. The wide prevalence ranges described by Magnino *et al*. (2009) in Chapter 2, show how the prevalence of *C. psittaci* in feral pigeons seems to vary from one city to another. The prevalence of *C. psittaci* shedding in our investigated birds was constant at a very low level on all four sampling dates (Chapter 4, Table 1). In total, 9 out of 447 (2.0%) pharyngeal swabs were tested positive. The highest proportion of positive pharyngeal swabs was found in February 2006 with 5 out of 99, (5.1 %), the lowest in May 2009 with 0 out of 120 (0.0 %). Cloacal swabs were positive in 11 out of 348 (3.2 %) of the tested birds. The highest proportion of positives was found in July 2008 with 7 out of 124 (5.6 %) the lowest in February 2008 with 1 out of 104, (1.0%). The prevalence of *C. psittaci* in the tested feral pigeon population is low. These results are in accordance with other studies, as described in Chapter 4

At present, no other studies provide information about intermittent chlamydial shedding in free ranging feral pigeons. However, intermittent shedding is characteristic for *C. psittaci* infections in birds and has been proven in many bird species, including domestic pigeons (Andersen & Vanrompay, 2003; Harkinezhad *et al*., 2009). The majority of the positive feral pigeons were shedding *C. psittaci* intermittently. Intermittent shedding was documented in 11 of 17 (64.7 %) of the positive birds (Chapter 4, Table 2). The 6 other birds could not be recaptured in the

loft after positive testing. Their fate is unknown. Nine of the birds were tested positive once and remained negative when retested. Two of the birds were tested positive repeatedly. Bird A 180, a large male, was tested positive in February 2007 and July 2008, respectively. This bird was 9.6 years old at the last testing date. It could not be recaptured in the loft in 2009 and we assume that it died of old age. Bird A 819 was tested positive in February 2008 and in July 2008. In May 2009, the bird was recaptured at the age of 4 years and was tested negative. It could be clearly demonstrated that chlamydial infections in our feral pigeons are not fatal for the majority of the birds and that intermittent shedding of the pathogen can occur either by respiratory secretions or faeces. It can be concluded that a low percentage of the feral pigeon population of Basel are shedding *C. psittaci* into the environment at any time point.

Using the antigen-ELISA, one true positive faecal sample taken from a nesting box in the St. Matthäus Church loft was detected (Chapter 3). The single positive sample could be attributed to the former genus *Chlamydophila* by microarray. However, the species could not be determined and sequencing yielded no result. We were not able to detect *C. psittaci* from samples taken in the urban environment using *ompA* nested PCR (Chapter 4). The occurrence of PCR inhibitors in the faecal material has been ruled out by the use of an inhibition control plasmid. In spite of the low shedding prevalence detected in the tested feral pigeons, it could be expected to find at least some positive samples. However, it is possible that no *C. psittaci* shedding birds were present at the sampling sites when samples were taken or that chlamydiae have already been destroyed by adverse environmental influences. Heddema *et al*. (2006a) proved a mean *C. psittaci* prevalence of 7.9 % in faecal samples from feral pigeons in Amsterdam (The Netherlands). Prevalence in these faecal samples ranged from 5% to 10 %. Faecal swab samples were taken from nine locations in Amsterdam at two time points. Similar to our results, the authors were not able to detect *C. psittaci* in every location where samples were taken.

In a previous study conducted in the feral pigeon lofts in Basel, Rose *et al*. (2006) showed that feral pigeon subpopulations overlap at important feeding sites. The authors assumed that this overlap could favour the transmission of diseases and parasites and could contribute to their spread all over the city area. Although

C. psittaci was not documented at important feeding sites, it could be demonstrated that the interconnectedness of feral pigeon subpopulations by immigration and emigration favours the spread of the pathogen. Seven of the 17 (41 %) pigeons which tested positive for *C. psittaci* did not hatch in the St. Matthäus Church loft, but immigrated from other subpopulations in the city. They were caught in the loft at adult age. The birds were not ringed or marked otherwise, showing that they did not hatch in another pigeon loft. Genotyping of *C. psittaci* was successful in three of these immigrated birds. Two of them were found to harbour *C. psittaci* genotype B, which is predominantly found in feral pigeons. In one bird, however, a mixed infection with the chlamydial genotypes A, B, and E/B was documented for the first time. This is particularly important, since it shows the possibility of new chlamydial genotypes being carried into the loft by immigrating birds. Moreover, genotype A is associated with severe disease in birds and human and is commonly found in psittacine birds (Geens *et al*., 2005; Magnino *et al*., 2009).

At the beginning of the present studies, preliminary results obtained by antigen-ELISA indicated that the biofilm on the water surface of public fountains could serve as a transmission medium for *C. psittaci* (Chapter 3). Feral pigeons in Basel use public fountains for bathing, preferably in the early morning. Accumulations of feather dust particles are clearly visible on the water surface. However, the initially positive results were proven to be false positives or were equivocal after the additional blocking test was used to verify the results from the antigen-ELISA. Considering the low shedding prevalence we found in our feral pigeons by PCR, we conclude, it is unlikely for *C. psittaci* from feral pigeons in fountain water to pose a threat to public health.

Environmental physical influences can affect the survival of *C. psittaci* in faecal droppings. Chlamydiae can be destroyed by UV-light within three minutes (Fritzsche, 1961). The effect of sunlight on chlamydial survival has not been investigated. It can therefore only be speculated that chlamydiae are not able to survive a long-term exposure to sunlight. Interestingly, chlamydiae are highly susceptible to repeated cycles of freezing and thawing (Andersen & Vanrompay, 2003). In our feral pigeon lofts, freezing of faecal droppings can regularly be observed in winter. This applies even more to unprotected sites in the city. Thus, long-term survival of chlamydiae in

unprotected areas is not likely in Basel, since chlamydial elementary bodies are eliminated from the urban environment in the winter months. Moreover, at open, unprotected locations, pigeon faeces are regularly washed away by precipitation. In addition, during longer periods of dry weather streets and squares in Basel are cleaned with water to prevent dust formation.

C. psittaci was detected in at least 467 bird species of 30 different orders (Kaleta & Taday, 2003). Despite this fact, most studies investigating *C. psittaci* focus either on pet birds, livestock or pigeons. Investigations of *C.psittaci* in other species of wild living birds are rare. Zweifel *et al.* (2009) tested 527 songbirds and 442 waterfowl samples pecies, as well as feral pigeons by real-time PCR and other methods. They could not demonstrate the presence of *C. psittaci* in songbirds and waterfowl. The authors concluded, at present songbirds and waterfowl do not pose a health risk due to *C. psittaci*.

A study investigating the health risk posed by faecal accumulations from starlings (*Sturnus vulgaris*) was conducted in Basel, Switzerland (Odermatt *et al.*, 1998). In 40.5 % of the investigated faeces, the presence of *C. psittaci* could be demonstrated among other potentially pathogenic microorganisms. Despite the considerable contamination caused by these birds, investigations on the health status of children in a nearby nursery school showed no infections, which could be correlated to their presence. The authors concluded that the children probably avoided the obviously contaminated areas. Furthermore, they assumed that the climatic conditions in autumn could have had an impact for the prevention of pathogen transmission. This supports the hypothesis that physical environmental influences may have an effect on the health risk posed by bird faeces in the urban environment.

Transmissions of *C. psittaci* from feral pigeons to humans can be prevented by the use of appropriate personal protective equipment (PPE), such as dust masks, gloves and a coverall, when contacts to the birds or their faecal droppings are unavoidable. These measures are highly recommended when working in closed rooms like attics, where heavy dust formation can occur during cleaning. Faecal droppings should be kept damp to avoid dust formation (Magnino *et al*., 2009, Chapter 2).

The thorough cleaning of the city centre by the employees of the city cleaning department could play an essential role in the prevention of disease transmission due to feral pigeon faeces. By constantly removing potentially contaminated faecal droppings from sidewalks, streets and squares, the reservoir of infectious pathogens is minimized and thus potential routes of transmission are interrupted. In contrast, accumulation of faecal droppings in protected areas where no regular cleaning is performed is more likely to contain infectious chlamydiae. According to Rose *et al*. (2006), feral pigeons spend the majority of their time inside the loft, which they use for roosting and breeding. Therefore, feral pigeon droppings are mainly deposited at this breeding site. As described in Chapter 4, large amounts of feral pigeon droppings are removed from the feral pigeon lofts, which would otherwise be deposited in the public urban environment. Thus, these feral pigeon lofts contribute to public health, since feral pigeon droppings can be removed safely. Acquiring *C. psittaci* by respiratory secretions from birds is likely to occur due to very close contact to the birds, as e.g. handling. In contrast, contaminated faecal droppings from feral pigeons can accumulate in the public urban environment or at house facades. Regular safe removal of feral pigeon faeces is important to prevent disease transmission.

Another important prevention measure is the education of the public. Local newspapers, the Internet, radio, and television should point out the scientifically proven ecological relationship between feral pigeon feeding, overcrowding, and density-dependent regulation mechanisms, such as the occurrence of diseases and parasites (Haag, 1984). The public should be informed about possible risks arising from intentional or non-intentional contacts to feral pigeons and other birds. This risk awareness could contribute to a fast diagnosis in case of disease transmission or parasite infestation.

Many building owners and communities want to keep feral pigeons away from their buildings to prevent soiling and the transmission of pathogens and parasites to humans. In the study described in Chapter 5, it could be documented how feral pigeons are able to use very narrow ledges of 5 cm width for roosting. Furthermore, it was proven how feral pigeons are able to squeeze through very narrow openings, when they are highly motivated to reach their nests. One bird was even filmed while

flying through a vertical opening of 7 cm. Furthermore, the dependence of a pigeon safe angle of inclination on a surface and the smoothness of the material used, was demonstrated. This is the first study to provide these parameters.

The results of the present studies show the low prevalence of *C. psittaci* shedding in our feral pigeons. However, shedding occurs and therefore the risk of zoonotic transmission of the pathogen is ever present. The feral pigeon population in Basel has been found to be in a good state of health in 1990, despite a *C. psittaci* seroprevalence of 62 % (Haag & Gurdan, 1990). Since then, the feral pigeon population of Basel is stable at a level of up to 8 000 birds. The present studies documented the still very good health status of our feral pigeons. In spite of this verdict, an estimated number of 250 feral pigeons are shedding the agent in their faecal droppings at any time point, since *C. psittaci* was detected in 3.2% of the investigated cloacal swabs.

Despite the relatively low prevalence detected in our feral pigeon population, it is important to be aware of *C. psittaci* infections and the routes of transmission in the city. As mentioned before, a study by Dickx *et al*. (2010) demonstrated a very low prevalence of *C. psittaci* in feral pigeons in Ghent, Belgium. In contrast, in the same study the authors demonstrated *C. psittaci* in 13 of 32 (40.6 %) homing pigeons and they could demonstrate zoonotic transmission in 4 out of 32 (12.5 %) homing pigeon fanciers. Two of them were infected with genotype D, which is usually abundant in turkeys and ducks. These results demonstrate the seemingly widespread *C. psittaci* infections in homing pigeons and the health risk for pigeon fanciers is high. Moreover, the authors expressed their concern about the widespread prophylactic use of antibiotics by pigeon fanciers. These drugs can easily be purchased on the Internet, since a prescription is not needed in every country (Vanrompay *et. al*., 2007). The misuse of antibiotics could favour the development of drug-resistant *C. psittaci* strains in the future. Tetracycline resistance has already been proven in *Chlamydia suis* (Dugan *et al*. 2004). Lost homing pigeons can establish themselves in feral pigeon flocks. Between 1990 and 1995, 13 lost homing pigeons were recorded in the feral pigeon lofts in Basel, three of them paired with feral pigeon mates and reared young (Haag-Wackernagel, 1998). Thus, immigration of homing pigeons into feral pigeon flocks could lead to transmissions of drug-resistant

C. psittaci strains in the future.

The high numbers of feral pigeons in most of the larger cities in the world can become a serious problem. Despite the fact that the number of infected feral pigeons seems to be high in some cities, the number of reported human psittacosis cases due to feral pigeons is low. We are not able to assess the amount of psittacosis infections, which have been misdiagnosed or otherwise remained unreported. In contrast to *C. psittaci* infections in birds, human *C. psittaci* infections are not notifiable in Switzerland. Thus data on transmissions from birds to humans are lacking and likely to be incomplete. In Switzerland, no human *C. psittaci* infection has been reported from 2005 to 2009 (*BVET, 2009*). However, from 2000 to 2009 a total of 72 cases of avian chlamydiosis have been reported to the cantonal veterinary offices. According to the Federal Veterinary Office (FVO, BVET), avian chlamydiosis is very rare in Switzerland and thus the risk of infection for humans should be low (BVET, 2009).

In 64 (57 %) of the 113 reported cases reviewed in Haag-Wackernagel (2006a,b), the epidemiological evidence that psittacosis was acquired from feral pigeons is strong or very strong, since close contact to feral pigeons could be proven. However, in 49 of the reported cases (43 %) the evidence of feral pigeons as the source of infection is either lacking or weak. In these cases the exact kind of contact to pigeons is not described or based on speculation. Levinson *et al*. (1944) reported the cases of two women and two men who were admitted to hospital showing symptoms of psittacosis. The diagnosis was confirmed by complement fixation test (CFT). The patients denied any direct contact with birds. The infections were attributed to feral pigeons living in the neighbourhood where the birds were roosting on roofs and window ledges. A serological survey proved that 42.0 % of these birds were seropositive for *C. psittaci*. However, this provides no sound epidemiological evidence that feral pigeons were in fact the source of infection. The relationship between positive serology in a bird and actual disease is not yet clarified. Thus, serology provides only limited diagnostic value (Vanrompay, 2008). Whether all of the 113 cases reported in the medical literature were in fact *C. psittaci* infections originating from feral pigeons cannot be assessed retrospectively. In addition, CFT used as a standard serological test in humans is not able to discriminate between

C. psittaci, *C. pneumoniae, C. trachomatis* and other chlamydial species. Moreover, cross reactions with hsp's or LPS originating from other bacteria can lead to false positive results, as already pointed out in Chapter 3. False negative results can also occur if patients have been treated with antibiotics 2–3 weeks before testing or if testing is performed before seroconversion (Beeckman & Vanrompay, 2009). A thorough revision of the literature raises the question whether all of the 113 reported infections were in fact due to *C. psittaci*. We suppose that at least some of the cases of presumed psittacosis diagnosed by CFT before 1986 could have been due to *C. pneumoniae*. Jansson (1960) and Babudieri (1956, 1964) reported of some family members of patients with positive CFT showing signs of respiratory infection in the same period of time, suggesting that person-to-person transmission occurred. This transmission mode is characteristic for *C. pneumoniae* infections but is considered to be extremely rare for *C. psittaci* (Hughes *et al*., 1997, Ito *et al*, 2002). It can therefore not be excluded that at least some of these reported cases were in fact due to *C. pneumoniae*, in spite of previous brief and transient contacts to birds. This particularly applies to cases published before 1986. The chlamydial species *C. pneumoniae* (strain TWAR) was first detected in 1986 and was at first described as a new strain of *C. psittaci* until it was recognized as a separate chlamydial species in 1989 (Grayston *et al*. 1886, 1989). In two studies conducted shortly after *C. pneumoniae* had been described, it was proven that 9–46 % of cases previously listed as "ornithosis" were in fact due to *C. pneumoniae* (Frydén *et al*., 1989; Persson *et al*. 1989). Signs and symptoms of human chlamydiosis due to *C. psittaci* and *C. pneumoniae* are very similar and differential diagnosis requires additional tests, preferably nucleic acid amplification techniques, which are expensive and not routinely used. All chlamydial species are susceptible to the same groups of antibiotics (Andersen & Vanrompay, 2003). Thus, for the treatment of acute chlamydial infections in humans, the exact identification of the chlamydial species is often not performed (Süss *et al*. 1996). Further investigations of presumed psittacosis/ornithosis due to feral pigeons should provide information about the chlamydial genotype involved by use of real-time PCR or microarray (Heddema *et al*. 2006b, Sachse *et al*. 2008, Harkinezhad *et al*., 2009).

Suitability of our methods

Validity. In the past few years, safe, fast and very sensitive detection methods based on nucleic acid amplification techniques have been developed, as e.g. PCR, real-time PCR, and microarrays (Sachse *et al*., 2008). However, these methods are costly and require appropriately equipped laboratories and experienced personnel. The *ompA* nested PCR we used has been developed by Van Loock *et al*. (2005) and has been successfully used by Daisy Vanrompay and colleagues (Ghent University). As described previously, the assay is able to detect 10^{-2} IFU of all *C. psittaci* reference strains. Specificity has been found to be 100%, since other chlamydial bacteria have not been detected. False negative results due to amplification inhibitors have been ruled out by the use of the internal control plasmid. This assay proved to be suitable for our purpose, since it is very sensitive and specific.

The antigen-ELISA we used for our preliminary study proved to be unsuitable for our purpose, as described in Chapter 3. The assay provides no information on the species or genotype of the detected chlamydiae.

Limitations. The major disadvantage of this nested PCR method is the high risk of carry-over contamination. Special care must be taken and each step has to be performed in separated locations, preferably separate rooms, using dedicated sets of pipettes, pipette tips with aerosol barrier and a unidirectional workflow. Additionally, the nested PCR method is very time-consuming, since two rounds of PCR have to be performed for each sample. The PCR technique cannot be used to confirm the viability of the chlamydial elementary bodies. Since chlamydial bacteria are obligate intracellular parasites, they are not able to replicate outside a host cell. Therefore, cell culture or inoculation into embryonated chicken eggs is required for multiplication of the pathogen (Andersen & Vanrompay, 2003). Cell culture of *C. psittaci* can only be performed in few selected laboratories, since it requires biosafety-level 3 facilities.

6.2. CONCLUSION

It could be demonstrated that *C. psittaci* infections occur in the feral pigeon population in Basel and intermittent shedding could be detected. Therefore, an infection with *C. psittaci* can never be completely ruled out. The feral pigeon loft of

the “Pigeon Action of Basel” has prov to be very useful for monitoring the health status of the local feral pigeon population, since it provides repeated access to the same individual free ranging birds. The interconnectedness of the subpopulations by immigration and emigration favours the spread of pathogens, such as *C. psittaci*. Similar studies could also be performed for investigating other infectious diseases as well as parasites, to contribute to a more complete picture of the epidemiological situation in feral pigeon populations. Further studies in other cities could adapt this method. One very important risk factor for the transmission of *C. psittaci* from feral pigeons to humans is the exposure to pigeons or their excreta in attics or on balconies, window ledges, and other building structures. Further studies could investigate the possible subclinical presence of *C. psittaci* infections in people with a high presence of feral pigeons near their homes. The relevant parameters that are required to proof a building against feral pigeons are provided. These parameters provide the basis for the development of practically applicable methods for feral pigeon management. Architects and building owners should use these parameters to minimize the risk of disease- and parasite transmissions and at the same time to prevent high costs due to fouling and biodeterioration

6.3. REFERENCES

Albrecht A, Schies U, Kämpfer P, Scholbeck R, (2003). Gesundheitsgefährdung durch Taubenkot [Health hazards posed by pigeon droppings]. *Sonderdruck aus TIEFBAU, Tiefbau-Berufsgenossenschaft, München*; Heft 5/2001 und 3/2002, 1–14.

Andersen AA, Vanrompay D (2003). Avian Chlamydiosis (psittacosis, ornithosis). In: Saif YM, ed. *Diseases of Poultry*, 11th edition, Iowa State Press, Iowa, USA, pp. 863–879.

Babudieri B. (1956). L’ornitosi: sua presenza e frequenza in Italia. *Terapia*. 317, 3–19.

Babudieri B. (1964). Epidemiologie und Virologie der Ornithose beim Menschen. *Archiv Exper Vet Med*. 18, 5–18.

Beeckman DSA, Vanrompay D. (2009) Zoonotic Chlamydophila psittaci infections from a clinical perspective. *Clin Microbiol Infect*. 15, 11–17.

BVET. (2010). *Swiss Zoonoses Report 2009*. Federal veterinary office FVO (Bundesamt für Veterinärwesen BVET). accessible online at www.bvet.admin.ch

Dickx V, Beeckman DSA, Dossche L, Tavernier P, Vanrompay D. (2010). *Chlamydophila psittaci* in homing and feral pigeons and zoonotic transmission. *J Med Microbiol*. 59, 1348–1353.

Dugan J, Rockey DD, Jones L, Andersen AA (2004). Tetracycline resistance in *Chlamydia suis* mediated by genomic islands inserted into the chlamydial inv-like gene. *Antimicrob Agents Chemoter*. 48, 3989–3995.

Fritzsche K (1961). Aktuelle Fragen der Ornithose. *Jahreskongress 1960 für Ärztliche Fortbildung*. 16, 75–86.

Frydén A, Kihlström E, Maller R, Persson K, Romanus V, Anséhn S. (1989). A clinical and epidemiological study of „ornithosis“ caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). Scand. *J Infect Dis.* 21(6), 681–91.

Geens T, Desplanques A, Van Loock M, Bönner BM, Kaleta EF, Magnino S, Andersen AA, Everett KDE, Vanrompay D. (2005b). Sequencing of the *Chlamydophila psittaci ompA* gene reveals a new genotype E/B and, and the need for a rapid discriminatory genotyping method. *J Clin Microbiol*. 43 (5), 2456–2461.

Grayston JT, Kuo CC, Wang SP, Altmann J. (1986). A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med*. 315 (3), 161–168.

Grayston JT, Kuo CC, Campbell LA, Wang SP. (1989). *Chlamydia pneumoniae sp. Nov.* For *Chlamydia sp*. Strain TWAR. *Int J System Bacteriol.* 39, 88-90

Haag D. (1984). Ein Beitrag zur Oekologie der Stadttaube. *Dissertation*.

Phil.Nat. Fakultät der Universität Basel, Verlag Medizinische Biologie.

Haag-Wackernagel D. (1998). Ecology of feral pigeons in Basel, Switzerland. INTECOL, VII International Congress of Ecology, Florence 19–25 July 1998, *Proceedings*, Farina A., Kennedy J., Bossu V. (eds.)

Haag-Wackernagel D. (2006a). Human diseases caused by feral pigeons. In: *Advances in Vertebrate Pest Management, 4.* pp. 31–58. Edited by C. J. Feare & D. P. Cowan, Filander Verlag, Fürth.

Haag-Wackernagel D. (2006b). Gesundheitsgefährdungen durch die Strassentaube *Columba livia*: Krankheiten. *Amtstierärztlicher Dienst und Lebensmittelkontrolle*. 4, 262–272.

Haag D, Gurdan P. (1990). Über den hygienischen Zustand der Strassentauben in Basel. *Swiss Vet – Schweizerische Zeitschrift für Veterinärmedizin*. 7/6, 19–22.

Harkinezhad T, Geens T, Vanrompay D. (2009). *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. *Vet Microbiol*. 135 (1–2), 68–77.

Heddema ER, ter Sluis S, Bujis JA, Vandenbroucke-Grauls CM, van Wijnen JH, Visser CE (2006a). Prevalence of *Chlamydophila psittaci* in fecal droppings of feral pigeons in Amsterdam, The Netherlands. *Appl Environ Microbiol.* 72, 4423–4425.

Heddema ER, Van Hannen EJ, Duim B, Vandenbroucke-Grauls CM, Pannekoek Y (2006b). Genotyping of *Chlamydophila psittaci* in human samples. *Emerg Infect Dis.* 12(12), 1989–1990.

Hughes C, Maharg P, Rosario P, Herrell M, Bratt D, Salgado J, Howard D. (1997). Possible nosocomial transmission of psittacosis. *Infect Control Hosp Epidemiol*. Mar, 18 (3), 165–168.

Ito I, Ishida T, Mishima M, Osawa M, Arita M, Hashimoto T, Kishimoto T. (2002). Familial cases of psittacosis: possible person-to-person transmission. *Intern Med*. Jul, 41 (7), 580–583.

Jansson E. (1960). Ornithosis in Helsinki and some other localities in Finland. *Annales medicinae experimentalis et biologiae Fenniae.* 38, suppl 4, 6–110.

Kaleta EF, Taday EMA (2003). Avian host range of *Chlamydophila ssp.* based on isolation, antigen detection and serology. *Avian Pathol.* 32(5), 435–462.

Levinson DC, Gibbs J, Beardwood JT. (1944). Ornithosis as a cause of sporadic atypical pneumonia. *JAMA J Am Med Assoc*. 126, 1079–1084.

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovč A, Prukner-Radovčić E, Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF. (2009). Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Vet Microbiol*. 135, 54–67.

Odermatt P, Gautsch S, Rechsteiner D, Ewald R Haag-Wackernagel D, Mühlemann R, Tanner M. (1998) Starenschwärme in Basel: ein Naturphänomen, eine Belästigung oder ein Gesundheitsrisiko?. *Gesundheitswesen.* Dec; 60(12), 749–754.

Persson K, Treharne J. (1989). Diagnosis of Infection Caused by *Chlamydia pneumoniae* (Strain TWAR) in Patients with “Ornithosis” in Southern Sweden 1981-1987. *Scand J Infect Dis*. 21, 675–679.

Rose E, Nagel P, Haag-Wackernagel D. (2006). Spatio-temporal use of the urban habitat by feral pigeons (*Columba livia*). *Behav Ecol Sociobiol*. 60, 242–254.

Sachse K, Laroucau K, Hotzel H, Schubert E, Ehricht R, Slickers P. (2008). Genotyping of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of ompA genes. *BMC Microbiology*. 8:63.

Süss A, Reetz J, Schulze P, Kretzschmar M, Schirrmeister W, Süss J. 1996. [Severe course of ornithosis and its intensive care and diagnostic problems – a case report]. *Anaesthesiol Reanim*. 21(4), 97–102.

Van Loock M, Verminnen K, Messmer TO, Volkaert G, Godderis BM, Vanrompay D. (2005). Use of a nested PCR-enyme immunoassay with an internal control to detect *Chlamydophila psittaci* in turkeys. *BMC Infect Dis*. 5, 76.

Vanrompay, D. (2008). Update on avian chlamydiosis and its public health significance. *EJCAP*. 18(3), 267–273.

Vanrompay D, Harkinezhad T, van de Walle M, Beeckman D, van Droogenbroeck C, Verminnen K, Leten R, Martel A, Cauwerts K. (2007). *Chlamydophila psittaci* transmission from pet birds to humans. *Emerg Infect Dis*. 13 (7), 1108–1110.

Vázquez B, Esperón F, Neves E, López J, Ballesteros C, Muñoz MJ. (2010). Screening for several potential pathogens in feral pigeons (*Columba livia*) in Madrid. *Acta Vet Scand*. 52, 45.

Warfolomeow J. (2001). Tauben – nicht nur Friedensboten. Tod eines Elektroinstallateurs durch massive Belastungen mit Taubenkot am Arbeitsplatz. *Tiefbau*. 5, 352–353.

Wreghitt T. (2003). Ornithosis. Presentation, Seminar on Birds and Public Health, British Ornithologists's Union held at The British Academy, London, 27 November 2003, 14.

Zweifel D, Hoop R, Sachse K, Pospischil A, Borel N. (2009). Prevalence of *Chlamydophila psittaci* in wild birds – potential risk for domestic poultry, pet birds, and public health? *Eur J Wildl Res*. 55, 575–581.

Curriculum vitae

PERSONAL DATA

Full Name:	Ila Germaine Geigenfeind
Place of birth:	Basel (BS)
Date of birth	14th of February 1978
Nationality:	Swiss

EDUCATION

2005–2011 PhD dissertation at the University of Basel, Switzerland: "On the Biology and Epidemiology of the Feral Pigeon (*Columba livia*)", supervised by Prof. Dr. Daniel Haag-Wackernagel, Department of Biomedicine, Institute of Anatomy, Integrative Biology. Assistance in teaching and research.

1998–2005 Studies in Biology at the University of Basel, focus on vertebrate biology, marine zoology, developmental biology and plant ecology. Diploma thesis in vertebrate biology (supervised by Prof. Dr. David G. Senn): "Zur Haltung des Laternenfisches *Anomalops katoptron* (Bleeker, 1856) im Aquarium."

1989–1997 Gymnasium Bäumlihof, Basel (BS), Switzerland

1985–1989 Primary School, Riehen (BS), Switzerland

TEACHING

2005–2011 Teaching Activities as a PhD Student

- Practical courses in microscopy for medical students
- Problem based learning tutorials for medical students
- Lectures in Ecology for medical students

2002–2010 Assistance in courses and excursions by Prof. Dr. David G. Senn

- Vertebrate Biology
- Marine Zoology
- Marine Biology Course in Banyuls-Sur-Mer (France)
- Excursions to Museums of Natural History in Basel, Zürich, Vienna, Paris, Solnhofen-Eichstätt, and Stuttgart

TUTORS IN BASEL

B. Bruderer, P. Duelli, H, D. Ebert, W.J. Gehring, D. Haag-Wackernagel, H.P. Hauri, H.-C. Im Hof, W. Keller, Ch. Körner, H. Kraft, J. Meier, Ch. Meyer, P. Odermatt, P. Oelhaven, U. Rahm, H. Reichert, V. Schmid, H. Schneider, D.G. Senn, U. Séquin, H. Sigel, J. Spiess, J. Spring, S.C. Stearns, J. Stöcklin, M. Tanner, C. Ullrich, N. Weiss, P. Vounatsou, A. Wetzel, A. Wiemken, J. Zinstag

PUBLICATIONS

Haag-Wackernagel D**, Geigenfeind I.** (2008) Protecting buildings against feral pigeons. *European Journal of Wildlife Research*. 54 (4), 715–721.

Magnino S, Haag-Wackernagel **D, Geigenfeind I,** Helmecke S, Dovč A, Prukner-Radovčić E, Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF. (2009). Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. *Veterinary Microbiology.* 135, 54–67.

Geigenfeind I, Haag-Wackernagel D. (2010). Detection of *Chlamydophila psittaci* from feral pigeons in environmental samples: problems with currently available techniques. *Integrative Zoology*. 1, 63–69.

Geigenfeind I, Vanrompay D, Haag-Wackernagel D. (2012). Prevalence of *Chlamydia psittaci* in the feral pigeon population of Basel, Switzerland. *Journal of Medical Microbiology*. Feb, 61 (Pt 2), 261–5, Epub 2011 Sep 15.

CONFERENCE PRESENTATIONS

“Transmission of *Chlamydophila psittaci* from Feral Pigeons to Humans”. Presentation at the COST-855 Meeting, April 2006, Pavia, Italy.

“Detection of *Chlamydophila psittaci* from Feral Pigeons in Environmental Samples”. Presentation at the 6th European Vertebrate Pest Management Conference, September 2007, Reading, UK

“Die Strassentaube als Überträgerin von *Chlamydophila psittaci”.* Presentation at the Cantonal Laboratory Basel-Stadt. May 2008.