Chlamydophila psittaci in homing and feral pigeons and zoonotic transmission

V. Dickx,¹ D. S. A. Beeckman,¹ L. Dossche,¹ P. Tavernier^{2,3} and D. Vanrompay¹

Correspondence

V. Dickx

veerle.dickx@ugent.be

¹Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

²Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

³Wildsurv Project, Operational Direction Interactions and Surveillance, Veterinary and Agrochemical Research Center, Groeselenberg 99, 1180 Brussels, Belgium

Chlamydiosis is a zoonotic disease in birds caused by *Chlamydophila psittaci*, an obligate intracellular bacterium. There are seven known avian outer-membrane protein A genotypes, A–F and E/B. The importance of genotyping lies in the fact that certain genotypes tend to be associated with certain hosts and a difference in virulence. Genotype B is the most prevalent in pigeons, but the more virulent genotypes A and D have also been discovered. The current study assessed the prevalence of *C. psittaci* in 32 Belgian homing-pigeon facilities and in 61 feral pigeons captured in the city of Ghent, Belgium. Additionally, zoonotic transmission of *C. psittaci* was investigated in the homing-pigeon facilities. Homing pigeons were often infected, as at least one of the lofts was positive in 13 of the 32 (40.6%) pigeon breeding facilities. Genotypes B, C and D were detected. Zoonotic transmission was discovered in 4 of the 32 (12.5%) pigeon fanciers, revealing genotype D in two of them, whilst genotyping was unsuccessful for the other two human pharyngeal swabs. This study clearly demonstrates the possible risk of *C. psittaci* zoonotic transmission from homing pigeons. Pigeon fanciers often (37.5%) used antibiotics for prevention of respiratory disease. Because of the risk of developing drug-resistant strains, regular use of antimicrobial drugs must be avoided. This study is believed to be the first to detect *C. psittaci* in Belgian feral pigeons. The prevalence rate in the city of Ghent was extremely low, which is beneficial for public health.

Received 23 June 2010

Accepted 9 August 2010

INTRODUCTION

Chlamydiosis is a zoonotic disease in birds caused by *Chlamydophila psittaci*, an obligate intracellular bacterium. Symptoms include abnormal respiratory signs, nasal discharge, diarrhoea, polyuria and dullness (Vanrompay *et al.*, 1995a). Zoonotic transmission happens through direct contact or inhalation of infected aerosols. The clinical course in humans varies from asymptomatic to severe systemic disease with interstitial pneumonia. Symptoms frequently include high fever (up to 40.5 °C) accompanied by a relatively low pulse, chills, headache, myalgia, non-productive coughing and difficulty breathing (Beeckman & Vanrompay, 2009). The incubation period ranges from 5 to 14 days.

Currently, there are seven *C. psittaci* outer-membrane protein A (*ompA*) genotypes designated A–F and E/B. The importance of genotyping lies in the fact that certain genotypes tend to be associated with certain hosts and differ in virulence. Genotypes A and D are more virulent

than genotype B (Vanrompay *et al.*, 1994, 1995b). Genotypes A–E and E/B have been found in pigeons (Geens *et al.*, 2005; Laroucau *et al.*, 2008; Sayada *et al.*, 1995). However, in Belgium and neighbouring countries, only genotypes A, B and D have been discovered so far (Vanrompay *et al.*, 1993, 1997), with genotype B being the most prevalent (Magnino *et al.*, 2009). Recently, genotype B has been discovered in three human samples in The Netherlands and may be an underestimated source of disease (Heddema *et al.*, 2006b).

In 1940, *C. psittaci* was isolated for the first time from homing pigeons (Pinkerton & Swank, 1940). Since then, several serological surveys have been conducted in pigeons. However, most articles have focused on *C. psittaci* in feral pigeons (*Columba livia*). Feral pigeons are more commonly known as ‘urban’ or ‘city’ pigeons. Thirty-eight studies on the seroprevalence of *C. psittaci* in feral pigeons conducted from 1966 to 2005 revealed a seropositivity rate 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). More recent serological studies performed in feral pigeons in Italy, and Bosnia and Herzegovina, showed a seropositivity of 48.5 and 26.5 %, respectively (Ceglie *et al.*, 2007; Ilieski *et al.*, 2007). Fourteen studies on the culture of *C. psittaci* conducted in European feral pigeons from 1980 to 2007 revealed 1.6 to 57 % positivity (Magnino *et al.*, 2009). The highest percentage of culture positives was found in Paris (Trap *et al.*, 1986). Eleven studies using nucleic acid amplification assays performed from 2003 to 2007 demonstrated the presence of chlamydial DNA in 3.4–52.6 % of the feral pigeons examined (Magnino *et al.*, 2009; Vázquez *et al.*, 2010). Data on the occurrence of *C. psittaci* in racing pigeons are rare, but seropositivity has been found to range from 35.9 to 50.1 % (Alexander *et al.*, 1989; Salinas *et al.*, 1993).

Pigeons are present in urban and rural areas all over the world and get in close contact with people in public places (Magnino *et al.*, 2009). The first case of *C. psittaci* zoonotic transmission from pigeons was described in 1941 (Meyer, 1941). Since then, 47 zoonotic cases linked to pigeons have been reported (reviewed by Haag-Wackernagel & Moch, 2004).

Breeding homing pigeons is an important leisure activity in Belgium. In addition to the training and racing flights, pigeons are allowed a limited daily free flight activity. Contact with infected feral pigeons or other birds is therefore possible. Once infected, the infectious agent can easily spread in the loft through contaminated dust and aerosols or during transport of pigeons to the release location (Harkinezhad *et al.*, 2009a). The pigeon fanciers themselves, spending many hours in the lofts, are also exposed to *C. psittaci*. In the UK, 40 % of pigeon fanciers examined had antibodies against *C. psittaci* (Bourke *et al.*, 1992). The current study assessed the prevalence of *C. psittaci* in Belgian feral and homing pigeons. Additionally, we investigated zoonotic transmission of *C. psittaci* in homing-pigeon facilities.

METHODS

Study concept. In February 2008, we conducted a *C. psittaci* zoonotic risk study in Belgian pigeon fanciers. Participants were recruited through the Royal Belgian Society for Pigeon Fanciers (KBDB). Fifty-two facilities received a sampling package by regular mail. The package contained a questionnaire designed to assess information: on the pigeon fanciers' professional and non-professional activities, smoking habits, general health status, use of medication, allergies and clinical signs specifically related to psittacosis; on the birds' origin, housing, feeding, breeding, health status and medication; and on the presence of other bird species. The package also contained eight Copan rayon-tipped swabs (Fiers) and instructions for faecal (cage floor) sampling of the lofts and pharyngeal sampling in humans. We provided 2 ml transport medium (Vanrompay *et al.*, 1992) for culture and 2 ml DNA stabilization buffer (Roche) for PCR, to be added to the swabs after sampling. Packages returned by express mail 1 day after sampling

were stored at -80°C until use. The sampling packages were convenient and safe for investigators. All participants examined provided informed consent.

Each participant was asked to take two swabs from the floor of the breeder, young and adult racing-pigeon lofts. Thus, the middle and all four corners of each cage in the loft were sampled twice. The pigeon fanciers also took two pharyngeal swabs from themselves and completed the medical questionnaire. The first swab, for a nested PCR, was deposited in 2 ml DNA stabilization buffer, whilst the second one, for chlamydial culture, was submersed in 2 ml chlamydial transport medium (Vanrompay *et al.*, 1992). Incompletely returned packages were excluded from the study.

In the same period, a study of 61 feral pigeons was conducted taking two pharyngeal swabs from each individual pigeon. The pigeons were captured on several days during the winter period of 2008 in the city of Ghent, Belgium. The captured pigeons were all adults and of both sexes. After capture, the pigeons were euthanized as part of pest control. The swabs were taken from the euthanized animals. Again, one swab was placed in DNA stabilization buffer for nested PCR and one in chlamydial transport medium for culture. All swabs were kept on ice during transport and stored at -80°C until use.

C. psittaci nested PCR. Bacterial DNA was prepared by the standard extraction method, as described previously (Van Loock *et al.*, 2005b). The DNA was purified further by extraction in phenol/chloroform/isoamylalcohol (25:24:1, v/v; Invitrogen). Purified DNA was precipitated by adding ethanol (100 %) and sodium acetate (5 M), and incubated at -80°C for 45 min. The DNA was centrifuged for 15 min (15 490 g) at 4°C . Pellets were washed with 70 % ethanol and suspended in 50 μl MilliQ water.

The presence of the *C. psittaci ompA* gene was examined using a *C. psittaci*-specific nested PCR and the results analysed by agarose gel electrophoresis (Van Loock *et al.*, 2005b). Briefly, nested PCR generated a product of 472 bp. Negative samples were retested to exclude false negatives by adding 10 ng internal inhibition control to assess possible polymerase inhibition. The inhibition control consisted of a pcDNA1 vector in which the *ompA* gene of *C. psittaci*, inserted as a 231 bp fragment, was cloned (Van Loock *et al.*, 2005b).

ArrayTube DNA microarray for ompA genotyping. For *C. psittaci* genotype determination, sample DNA was amplified and biotin-labelled *ompA* fragments of 418 and 530 bp were generated for the ArrayTube microarray (Clontia Chip Technologies) using 40 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 30 s, with primers VD1-f (5'-ACTACGGAGATTATGTTTCGATCGTGT-3'), Bio-VD2-r (5'-biotin-CGTGCACCYACGCTCCAAGA-3'), 201CHOMP (5'-GGIGCWGMITTCCAATAYGCICARTC-3') and Bio-ompA-rev (5'-biotin-TCCTTAGAATCTGAATTGAGC-3'). The results were visualized by agarose gel electrophoresis (Sachse *et al.*, 2008). Hybridization was performed at 53°C for 60 min. Subsequently, the ArrayTube was incubated with streptavidin-conjugated horseradish peroxidase (ArrayTube staining kit; Clontia). Finally, peroxidase substrate (3,3',5,5'-tetramethyl benzidine derivative from the ArrayTube staining kit) was added. Hybridization signals were analysed using an ATR-03 array tube reader (Clontia) and Iconoclust software version 2.3 (Clontia).

C. psittaci culture. Samples that were positive by PCR or showed signs of inhibition were tested by culture. The presence of viable *C. psittaci* was examined by isolation in buffalo green monkey kidney cells identifying the organism by a direct immunofluorescence staining (IMAGEN; Oxoid) at 6 days post-inoculation, as described previously (Vanrompay *et al.*, 1992). The number of *C. psittaci*-positive cells was counted (magnification $\times 600$; Nikon Eclipse TE2000-E microscope) and a score from 0 to 4 was given (Table 1).

Table 1. Culture scoring system

Score	Meaning
0	No EBs or inclusions
0.5	1–3 EBs
1	>3 EBs and/or 1–2 IPCs
1.5	3–5 IPCs
2	>5 IPCs in less than a quarter of the fields
2.5	IPCs in a quarter of the fields
3	IPCs (small inclusions) in half of the fields
3.5	Large IPCs in half of the fields
4	IPCs in all fields

EB, Elementary body; IPC, inclusion-positive cell.

Statistics. The data were examined statistically using a non-parametric test, as the sample populations were smaller than 30. The medical questionnaire enquired about respiratory complaints in the human participants, such as wheezing, dyspnoea, shortness of breath, sore throat, chest pain, runny nose, coughing, sneezing and coughing up mucus. For each of these complaints, the reporting frequency was scored as follows: 0, when the complaint was never reported; 1, if the complaint was reported once; 2, if it was reported several times; and 3, if the complaint was regularly reported. Scores were added to attain one overall respiratory complaint score per person. The same scoring method was applied to the reported respiratory symptoms and conjunctivitis in pigeons. The questionnaire also enquired about treatment with antibiotics in the pigeons as well as in the pigeon fanciers. A score of 0 or 1 was given when no treatment or recent treatment (in the last 3 months) was given, respectively.

RESULTS AND DISCUSSION

C. psittaci in homing pigeons

A total of 32 (61.5 %) out of 52 breeding facilities returned complete packages, resulting in samples from 96 lofts (32 young, 32 breeder and 32 racing-pigeon lofts) and 32 humans. The remaining 20 facilities returned an incomplete package and were therefore excluded from further examination. The results are presented in Table 2. A total of 2 of the 32 lofts (6.3 %) of young pigeons were PCR positive, whilst 11 of the 32 samples (34.4 %) contained PCR inhibitors. None of the 32 samples of the breeder pigeons were positive, whilst 9 (28.1 %) contained polymerase inhibitors. Adult racing pigeons were all PCR

negative. However, 10 of the 32 samples (31.3 %) contained polymerase inhibitors and could not be examined by PCR. Overall, PCR inhibition was frequently observed when examining floor swabs, as 30 of the 96 floor samples (31.3 %) contained enzyme inhibitors.

All positive samples and those with inhibitors (a total of 32 samples) were examined by culture. Nine out of thirteen (69.2 %), two out of nine (25 %) and seven out of ten (70 %) of the young, breeder and racing-pigeon lofts, respectively, were culture positive. All PCR-positive samples contained viable *C. psittaci*. The mean isolation scores for young, breeder and racing-pigeon lofts were 2.17, 1.25 and 1.5, respectively. Lofts were considered positive if PCR or culture was positive. Nine (28.1 %), two (6.3 %) and seven (21.9 %) of the thirty-two young, breeder and racing-pigeon lofts were positive, respectively.

Lofts of young birds were more often positive, showing higher mean culture scores, followed by lofts of racing and finally breeder pigeons. Young and racing-pigeon lofts were found to be positive significantly more often than breeder-pigeon lofts, but the mean culture scores were not significantly different. However, mean culture scores need to be interpreted with care, as the amount of faeces and medium present in the returned swab tubes differed. This inconvenience is inherent to the methodology of the do-it-yourself sampling package that was used. Nevertheless, our results for young birds are in accordance with other studies demonstrating more frequent *C. psittaci* infections in young birds (Bedson, 1955; Schettler *et al.*, 2001). Young birds are partially protected by maternal antibodies. As soon as maternal antibody titres decline, young birds may experience a primary infection, which is often accompanied by clinical signs due to the absence of an immunological memory (Van Loock *et al.*, 2005a).

Racing-pigeon lofts were more often positive than breeder-pigeon lofts, showing higher mean culture scores. Racing pigeons are transported, which creates stress, and crowding augments the infection pressure. During races, contact with wild birds, a reservoir for *C. psittaci*, may occur. Tiring could also render the homing pigeons more prone to infection. Whilst breeder pigeons suffer reproductive stress, which could render them more prone to infection, our results indicated that young and racing pigeons are more sensitive to *C. psittaci* infection (Andersen & Vanrompay, 2003). Overall, in 13 (40.6 %) of the 32 pigeon breeding facilities, at least one of the floor swabs was positive,

Table 2. *C. psittaci* detection in pharyngeal swabs of pigeon fanciers and in floor swabs of pigeon lofts

Test	No./total (%)			
	Young pigeons	Breeder pigeons	Racing pigeons	Humans
PCR positive	2/32 (6.3)	0/32 (0)	0/32 (0)	1/32 (3.1)
Inhibition in PCR	11/32 (34.4)	9/32 (28.1)	10/32 (31.3)	5/32 (15.6)
Culture positive	9/13 (69.2)	2/9 (25)	7/10 (70)	4/6 (66.7)

demonstrating the high prevalence of *C. psittaci* in Belgian homing pigeons.

Thirteen out of eighteen positive floor samples (72.2 %) could not be genotyped directly on clinical samples. They all contained polymerase inhibitors generating negative microarray results. They were positive by culture but unfortunately did not grow efficiently enough to extract sufficient DNA for the genotyping microarray. Samples of five young-pigeon lofts were successfully genotyped, revealing *ompA* genotypes B (three lofts), C (one loft) and D (one loft). So far, genotype C has been detected only in a Japanese pigeon (Sayada *et al.*, 1995). This genotype is found mostly in waterfowl. It is highly pathogenic to domestic waterfowl, and is an admitted occupational hazard for slaughterhouse workers and generally for all people in contact with ducks.

A *C. psittaci*-positive result in birds was not statistically correlated with the presence or absence of respiratory disease ($\sigma=0.821$), nor with antibiotic administration ($\sigma=0.563$). *C. psittaci* can be detected in carriers showing no clinical disease. In addition, respiratory disease can also be due to other systemic bacterial infections, viral infections such as herpesviruses and even fungal infections (Marlier & Vindevogel, 2006).

As in psittacine breeding facilities, antibiotics were also used prophylactically (Harkinezhad, 2008). In our study, 12 of the 32 pigeon fanciers (37.5 %) used tetracyclines for prevention of respiratory disease. In Belgium, a prescription by a veterinarian is required to obtain these drugs. However, tetracyclines are sold on the Internet without a prescription as a prescription is not needed in every country. A vaccine as well as information on the sensible use of antimicrobial drugs in homing pigeons are needed to prevent psittacosis in humans and the development of drug-resistant chlamydial strains.

***C. psittaci* zoonotic transmission from homing pigeons to humans**

Only 1 of the 32 pigeon fanciers (3.1 %) tested positive by nested PCR. A total of 5 of the 32 human pharyngeal samples (15.6 %) contained polymerase inhibitors. The one PCR-positive sample and the five containing inhibitors were examined for the presence of viable *C. psittaci*. Four of the six samples (66.7 %) were positive by culture, including the one that was positive by nested PCR, with a mean isolation score of 2.13. Overall, 4 of the 32 pigeon fanciers (12.5 %) were positive for *C. psittaci*. Two of the four positive samples (50 %) could be genotyped. They both revealed genotype D, which is a virulent genotype often found in turkeys and known to be transmitted to humans (Van Droogenbroeck *et al.*, 2009). One person testing positive had been treated with doxycycline until 2 weeks before the start of the study. He suffered from respiratory disease (sneezing, coughing up mucus and head cold). His pigeons had respiratory symptoms and were still being

treated with doxycycline. Probably due to this treatment, neither viable *C. psittaci* nor chlamydial DNA could be detected in the loft samples. This pigeon fancier had no contact with other birds. The other person testing positive reported mild respiratory symptoms (shortness of breath and sneezing) but took no medication. Although his pigeons showed respiratory problems and were being treated with enrofloxacin, we detected chlamydial DNA as well as viable *C. psittaci* genotype D. This pigeon fancier was also infected with genotype D.

Harkinezhad *et al.* (2009b) showed that 7 % of apparently healthy people having daily contact with homing pigeons were positive by PCR and/or by a recombinant major outer-membrane protein antibody ELISA. Our study revealed a slightly higher percentage (12.5 %) of positive persons. The relatively small number of humans included in both studies (both ~30) makes a robust comparison difficult. However, both studies indicated the risk of zoonotic transmission from homing pigeons to humans.

Interestingly, infection in pigeon fanciers occurred statistically more frequently when pigeons experienced respiratory disease and were not simply *C. psittaci* positive ($\sigma=0.042$). Thus, the presence of positive animals in itself did not lead to a higher infection rate in humans ($\sigma=0.648$). Respiratory disease in pigeons most often reflects an acute infection accompanied by excretion of a higher amount of infectious *C. psittaci* than in birds without respiratory symptoms, which most often have intermittent shedding (Harkinezhad *et al.*, 2009a).

Three of the four culture-positive persons (75 %) showed respiratory symptoms. However, 10 of the 28 PCR- and culture-negative persons (35.7 %) also showed respiratory symptoms. Respiratory symptoms due to *C. psittaci* can easily be confused with pigeon fanciers' lung, which is due to inhalation of organic dust material (Calvert *et al.*, 1999).

***C. psittaci* in feral pigeons**

One of the sixty-one feral pigeons (1.6 %) from the city of Ghent was PCR positive and three of the samples (4.9 %) contained polymerase inhibitors. The PCR-positive sample and those containing inhibitors were subsequently examined by culture. Only the PCR-positive sample contained viable *C. psittaci* with a low culture score of 2. Passages on buffalo green monkey kidney cells and subsequent genotyping were unsuccessful. The latter was probably due to an insufficient amount of chlamydial DNA, rather than to the presence of a new *ompA* genotype.

A study in Amsterdam pigeons, conducted in the low breeding period, revealed 5 % PCR-positive pigeons (Heddema *et al.*, 2006a). Our study, also conducted when breeding activity was low, revealed only one *C. psittaci*-positive pigeon, which is comparable to the low infection rate in the city of Amsterdam. This low infection rate is beneficial to public health. However, caution is needed, as zoonotic transmission of *C. psittaci* from feral pigeons is

known to be an underestimated source of infection (Haag-Wackernagel & Moch, 2004). Other studies on feral pigeons in the city of Ghent should be conducted, including at different time periods (e.g. the high breeding season) and other locations (with high concentrations of pigeons). Feral pigeon populations should be managed carefully in the urban environment to obtain an appropriate-sized and healthy population.

For this study, pharyngeal swabs were used instead of faecal or cloacal swabs. Whilst this makes comparison with the pigeon breeder study less easy, this technique does not have the disadvantage of intermittent shedding (Harkinezhad *et al.*, 2009a). When cloacal swabs are taken, false negatives can be obtained because of intermittent shedding. We were unable to genotype the PCR-positive sample. In the city of Amsterdam, ten genotyped PCR-positive samples were all genotype B. Genotype B may be an underestimated source of zoonotic infections and human disease (Heddema *et al.*, 2006b).

Conclusions

We found that homing pigeons were often infected, as at least one of the lofts was positive in 13 of the 32 pigeon breeding facilities (40.6%). Genotypes B, C and D were detected. Zoonotic transmission was discovered in 4 of the 32 pigeon fanciers (12.5%), revealing genotype D in 2 of them, whilst genotyping was unsuccessful for the other 2 human pharyngeal swabs. This study demonstrates the possible risk of *C. psittaci* zoonotic transmission from homing pigeons. Further research on more cases is necessary to confirm this result. The pigeon fanciers often (37.5%) used antibiotics for prevention of respiratory disease. Because of the risk of developing drug-resistant strains, as described for *Chlamydia suis* (Dugan *et al.*, 2004), regular use of antimicrobial drugs must be avoided. Our study is believed to be the first to detect *C. psittaci* in Belgian feral pigeons. However, the prevalence rate in the city of Ghent was extremely low, which is beneficial for public health.

ACKNOWLEDGEMENTS

This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (convention RF-6177). D. S. A. B. is a post-doctoral fellow of the Research Foundation Flanders (FWO-Vlaanderen) and this institution is acknowledged for providing a grant. The Royal Belgian Society for Pigeon Fanciers (KBDB) is also acknowledged for supporting this study. We thank L. Braeckman (Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University) for providing the medical questionnaire.

REFERENCES

Alexander, D. J., Bevan, B. J., Lister, S. A. & Bracewell, C. D. (1989). *Chlamydia* infections in racing pigeons in Great Britain: a retrospective serological survey. *Vet Rec* **125**, 239.

Andersen, A. A. & 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.

Bedson, S. P. (1955). Psittacosis. *Proc R Soc Med* **48**, 633–636.

Beeckman, D. S. & Vanrompay, D. C. (2009). Zoonotic *Chlamydia psittaci* infections from a clinical perspective. *Clin Microbiol Infect* **15**, 11–17.

Bourke, S. J., Carrington, D., Frew, C. E., McSharry, C. P. & Boyd, G. (1992). A comparison of the seroepidemiology of chlamydial infection in pigeon fanciers and farmers in the UK. *J Infect* **25**, 91–98.

Calvert, J. E., Baldwin, C. I., Allen, A., Todd, A. & Bourke, S. J. (1999). Pigeon fanciers' lung: a complex disease? *Clin Exp Allergy* **29**, 166–175.

Ceglie, L., Lafisca, S., Guadagno, C., Dalla Pozza, G., Capello, K., Bano, L., Vicari, N., Donati, M., Mion, M. & other authors (2007). Serological surveillance in north-eastern Italy for the presence of *Chlamydia* spp. from birds and molecular characterization of PCR isolates within the area of Venice. In *Proceeding of the 5th Annual Workshop of Cost Action 855: Animal Chlamydioses and Zoonotic Implications*, Pulawy, Poland, pp. 62–67. Edited by K. Niemczuk, K. Sachse & L. D. Sprague.

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

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

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. & Moch, H. (2004). Health hazards posed by feral pigeons. *J Infect* **48**, 307–313.

Harkinezhad, T. (2008). *Molecular epidemiology of Chlamydia psittaci in psittacine birds and humans and prevention by DNA vaccination*. PhD thesis, Ghent University.

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

Harkinezhad, T., Verminnen, K., De Buyzere, M., Rietzschel, E., Bekaert, S. & Vanrompay, D. (2009b). Prevalence of *Chlamydia psittaci* infections in a human population in contact with domestic and companion birds. *J Med Microbiol* **58**, 1207–1212.

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

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

Ilieski, V., Naletoski, I. & Pendovski, L., Sachse, K., Laroucau, K. & Dovc, A. (2007). Detection of Chlamydiaceae spp. in clinical samples from pigeons by real time PCR in Macedonia. In *Proceeding of the 5th Annual Workshop of Cost Action 855: Animal Chlamydioses and Zoonotic Implications*, Pulawy, Poland, p. 48. Edited by K. Niemczuk, K. Sachse & L. D. Sprague.

- Laroucau, K., Mahe, A. M., 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 Workshop on Diagnosis and Pathogenesis of Animal Chlamydioses, Siena, Italy*, pp. 17–18. Edited by R. Cevenini & V. Sambri. Bologna: Bononia University Press.
- Laroucau, K., Thierry, S., Vorimore, F., Blanco, K., Kaleta, E., Hoop, R., Magnino, S., Vanrompay, D., Sachse, K. & other authors (2008). High resolution typing of *Chlamydomydia psittaci* by multilocus VNTR analysis (MLVA). *Infect Genet Evol* 8, 171–181.
- Magnino, S., Haag-Wackernagel, D., Geigenfeind, I., Helmecke, S., Dovc, A., Prukner-Radovčić, E., Residbegović, E., Ilieski, V., Laroucau, K. & other authors (2009). Chlamydial infections in feral pigeons in Europe: review of data and focus on public health implications. *Vet Microbiol* 135, 54–67.
- Marlier, D. & Vindevogel, H. (2006). Viral infections in pigeons. *Vet J* 172, 40–51.
- Meyer, K. F. (1941). Pigeons and barnyard fowls as possible sources of human psittacosis or ornithosis. *Schweiz Med Fortbildungszeitschr* 44, 1377–1379.
- Mitevski, D., Pendovski, L., Naletoski, I. & Ilieski, V. (2005). Surveillance for the presence of *Chlamydomydia 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.
- Pinkerton, H. & Swank, R. L. (1940). Recovery of virus morphologically identical with psittacosis from thiamin-deficient pigeons. *Proc Soc Exp Biol Med* 45, 704–706.
- Prukner-Radovčić, E., Horvatek, D., Gottstein, Ž., Grozdanić, I. C. & Mazija, H. (2005). Epidemiological investigation of *Chlamydomydia psittaci* in pigeons and free-living birds in Croatia. *Vet Res Commun* 29 (Suppl. 1), 17–21.
- Sachse, K., Laroucau, K., Hotzel, H., Schubert, E., Ehrlich, R. & Slickers, P. (2008). Genotyping of *Chlamydomydia psittaci* using a new DNA microarray assay based on sequence analysis of *ompA* genes. *BMC Microbiol* 8, 63.
- Salinas, J., Caro, M. R. & Cuello, F. (1993). Antibody prevalence and isolation of *Chlamydia psittaci* from pigeons (*Columba livia*). *Avian Dis* 37, 523–527.
- Sayada, C., Andersen, A. A., Storey, C., 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.
- Schettler, E., Langgemach, T., Sommer, P., Streich, J. & Frolich, K. (2001). Seroepizootiology of selected infectious disease agents in free-living birds of prey in Germany. *J Wildl Dis* 37, 145–152.
- 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, J. M. & Gaumont, R. (1986). Chlamydiose aviaire chez les pigeons de Paris. *Rev Med Vet (Toulouse)* 137, 789–796.
- Van Droogenbroeck, C. M., Beeckman, D. S. A., Verminnen, K., Marien, M., Nauwynck, H., Boesinghe, L. de T. & Vanrompay, D. (2009). Simultaneous zoonotic transmission of *Chlamydomydia psittaci* genotypes D, F and E/B to a veterinary scientist. *Vet Microbiol* 135, 78–81.
- Van Loock, M., Geens, T., De, S. L., Nauwynck, H., Van, E. P., Naylor, C., Hafez, H. M., Goddeeris, B. M. & Vanrompay, D. (2005a). Key role of *Chlamydomydia psittaci* on Belgian turkey farms in association with other respiratory pathogens. *Vet Microbiol* 107, 91–101.
- Van Loock, M., Verminnen, K., Messmer, T. O., Volckaert, G., Goddeeris, B. M. & Vanrompay, D. (2005b). Use of a nested PCR–enzyme immunoassay with an internal control to detect *Chlamydomydia psittaci* in turkeys. *BMC Infect Dis* 5, 76.
- 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. *Zentralbl Veterinarmed B* 39, 105–112.
- 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, 134–137.
- Vanrompay, D., Ducatelle, R. & Haesebrouck, F. (1994). Pathogenicity for turkeys of *Chlamydia psittaci* strains belonging to the avian serovars A, B and D. *Avian Pathol* 23, 247–262.
- Vanrompay, D., Ducatelle, R. & Haesebrouck, F. (1995a). *Chlamydia psittaci* infections: a review with emphasis on avian chlamydiosis. *Vet Microbiol* 45, 93–119.
- Vanrompay, D., Mast, J., Ducatelle, R., Haesebrouck, F. & Goddeeris, B. (1995b). *Chlamydia psittaci* in turkeys: pathogenesis of infections in avian serovars A, B and D. *Vet Microbiol* 47, 245–256.
- Vanrompay, D., Butaye, P., Sayada, C., Ducatelle, R. & Haesebrouck, F. (1997). Characterization of avian *Chlamydia psittaci* strains using *omp1* restriction mapping and serovar-specific monoclonal antibodies. *Res Microbiol* 148, 327–333.
- Vázquez, B., Esperón, F., Neves, E., López, J., Ballesteros, C. & Muñoz, M. J. (2010). Screening for several potential pathogens in feral pigeons (*Columba livia*) in Madrid. *Acta Vet Scand* 52, 45.