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

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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 to 66.3 % of the samples, with a median of 46.1 %. Indirect immunofluorescence and ELISA tests were employed less frequently, but led to 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.

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.

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

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). *Chlamyaphila psittaci* (Everett et al., 1999; Garrity et al., 2004), an obligate intracellular bacterium which is the agent of avian chlamydirosis 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., pers. comm. 2006). 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 over-population 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 harbourred 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; 2006b).

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 \textit{ompA} and \textit{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 \textit{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
\textit{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 \textit{Chlamydiaceae} family and allows species identification (Sachse et al.,
2005), while the other has been specifically developed for \textit{ompA}-based \textit{C. psittaci} genotyping
(Sachse et al., 2008).
Among all chlamydial species, only \textit{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
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 eight distinct genomic areas dispersed throughout the genome, none of them being localised 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 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; 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 30 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-culturale 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 theompA, 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 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 needs 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 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. Sensitivities 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 4 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; 2006b).

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, pers. comm. 2008).
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, pers. comm. 2007).

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 this risk 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.

12. Conflict of interest statement

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”.

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

<table>
<thead>
<tr>
<th>Country</th>
<th>Serovars</th>
<th>Genotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>B, E</td>
<td>B, E</td>
<td>Andersen, 1997; Vanrompay <em>et al.</em>, 1997; Geens <em>et al.</em>, 2005a</td>
</tr>
<tr>
<td>France</td>
<td>B</td>
<td>B, E</td>
<td>Duan <em>et al.</em>, 1999; Laroucau <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Italy</td>
<td>A, B, E</td>
<td>A, B, D, E, E/B</td>
<td>Geens <em>et al.</em>, 2005a; Laroucau <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Japan</td>
<td>n.d.</td>
<td>C</td>
<td>Sayada <em>et al.</em>, 1995</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>n.d.</td>
<td>B</td>
<td>Heddema <em>et al.</em>, 2006b</td>
</tr>
<tr>
<td>Switzerland</td>
<td>n.d.</td>
<td>B</td>
<td>Hoop <em>et al.</em>, 2002</td>
</tr>
</tbody>
</table>

n.d. = not determined
Table 2 - A summary of surveys for detecting antibodies to *C. psittaci* in feral pigeon populations in some European towns and cities

<table>
<thead>
<tr>
<th>Country</th>
<th>Town or city</th>
<th>Year of sampling</th>
<th>Laboratory test</th>
<th>Results positive/total (% positive)</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosnia and Herzegovina</td>
<td>Sarajevo</td>
<td>2005</td>
<td>IIF</td>
<td>53/176 (30.1%)</td>
<td>sera from captured pigeons with no clinical signs</td>
<td>Rešidbegović <em>et al.</em>, 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2006</td>
<td>IIF</td>
<td>62/234 (26.5%)</td>
<td>sera from captured pigeons with no clinical signs</td>
<td>Rešidbegović <em>et al.</em>, 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1988-1993</td>
<td>CFT</td>
<td>410/834 (49.2 %)</td>
<td>higher antibody titers were detected in pigeons with lesions at necropsy cut-off: 1:8</td>
<td>Pavlak <em>et al.</em>, 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000-2003</td>
<td>Ab-ELISA (LPS)</td>
<td>174/182 (95.6 %)</td>
<td>high antibody titers were detected in 57/182 sera (32.8 %)</td>
<td>Prušker-Radović <em>et al.</em>, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CFT, IIF</td>
<td></td>
<td>46/101 (45.5 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1984</td>
<td>CFT, IIF</td>
<td>315/475 (66.3 %)</td>
<td>CFT cut-off: 1:8 IIF cut-off: 1:40</td>
<td>Trap <em>et al.</em>, 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1990</td>
<td>CFT</td>
<td>176/415 (42 %)</td>
<td>cut-off: 1:8</td>
<td>Laroucau, pers. comm. 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1999</td>
<td>CFT</td>
<td>316/658 (48 %)</td>
<td>pigeons sampled in the 20 districts, in the Bois de Boulogne and in the Bois de Vincennes</td>
<td>Laroucau <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>Country</td>
<td>Site</td>
<td>Year</td>
<td>Test</td>
<td>Cut-off</td>
<td>Reference</td>
<td></td>
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<td>---------</td>
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<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Giessen</td>
<td>1979-2004</td>
<td>CFT</td>
<td>46/81 (56.8%)</td>
<td>sera from diseased and necropsied feral pigeons</td>
<td>Kaleta and Hönicke, 2004; Kaleta, pers. comm. 2007: Helmecke, 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CFT</td>
<td>286/1,474 (19.4%)</td>
<td>sera from feral pigeons with no clinical history</td>
<td>cut-off: 1:8</td>
</tr>
<tr>
<td>Italy</td>
<td>Pisa</td>
<td>1988</td>
<td>CFT</td>
<td>263/495 (53.1%)</td>
<td>cut-off: 1:16</td>
<td>Cerri et al., 1989</td>
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<tr>
<td></td>
<td>Bolzano</td>
<td>2006</td>
<td>CFT</td>
<td>38/68 (55.9%)</td>
<td>cut-off: 1:10</td>
<td>Ceglie et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Venice</td>
<td>2006</td>
<td>CFT</td>
<td>111/267 (41.6%)</td>
<td>cut-off: 1:10</td>
<td>Ceglie et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Padua</td>
<td>2006</td>
<td>CFT</td>
<td>65/100 (65%)</td>
<td>cut-off: 1:10</td>
<td>Ceglie et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Verona</td>
<td>2006</td>
<td>CFT</td>
<td>78/167 (46.7%)</td>
<td>cut-off: 1:10</td>
<td>Ceglie et al., 2007</td>
</tr>
<tr>
<td>Spain</td>
<td>Murcia</td>
<td>1991</td>
<td>CFT</td>
<td>36/128 (36.8%)</td>
<td>CFT cut-off: 1:8</td>
<td>Salinas et al., 1993a</td>
</tr>
<tr>
<td>Country</td>
<td>Location</td>
<td>Year</td>
<td>Test Type</td>
<td>Positive Cases</td>
<td>Cut-off</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2000</td>
<td>IIF</td>
<td>33/139</td>
<td>1:40</td>
<td>Dovč et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2006</td>
<td>IIF</td>
<td>26/86</td>
<td>1:40</td>
<td>Dovč, pers. comm. 2006</td>
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<tr>
<td>Switzerland</td>
<td>Luzern</td>
<td>2001</td>
<td>Ab-ELISA (LPS)</td>
<td>33/59</td>
<td>1:40</td>
<td>Haag-Wackernagel, 2006a</td>
</tr>
</tbody>
</table>

**Legenda**
CFT = complement fixation test; Ab-ELISA (EB) = antibody-detection ELISA based on crude antigen from chlamydial elementary bodies; Ab-ELISA (LPS) = antibody-detection ELISA based on chlamydial lipopolysaccharide; IIF = indirect immunofluorescence assay; MIF = microimmunofluorescence assay
<table>
<thead>
<tr>
<th>Country</th>
<th>Town or city</th>
<th>Year of sampling</th>
<th>Laboratory test</th>
<th>Results positive/total ( % positive)</th>
<th>Notes on the type of sample(s)</th>
<th>Reference</th>
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<tr>
<td>Bosnia and Herzegovina</td>
<td>Sarajevo</td>
<td>2006</td>
<td>PCR (ompA)</td>
<td>3/8 (37.5 %)</td>
<td>tissue samples from dead birds</td>
<td>Rešidbegović et al., 2007</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>Sofia</td>
<td>2006</td>
<td>EI</td>
<td>2/15 (13.3 %)</td>
<td>pool of spleen, liver and lung from necropsied birds</td>
<td>Martinov, 2006</td>
</tr>
<tr>
<td>Croatia</td>
<td>Zagreb</td>
<td>1992-1997</td>
<td>DIF</td>
<td>4/39 (10.2 %)</td>
<td>tissue samples from necropsied birds</td>
<td>Vlahović et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000-2003</td>
<td>ICT</td>
<td>3/107 (2.8 %)</td>
<td>cloacal swabs</td>
<td>Vlahović et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EI</td>
<td>0/3 (0 %)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2000-2003</td>
<td>Ag-ELISA</td>
<td>44/278 (15.8 %)</td>
<td>cloacal swabs</td>
<td>Prukner-Radovčić et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2006</td>
<td>Ag-ELISA</td>
<td>120/787 (15.3 %)</td>
<td>cloacal swabs</td>
<td>Prukner-Radovčić, pers. comm. 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TC</td>
<td>3/150 (2 %)</td>
<td>cloacal, intestinal and pharyngeal swabs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paris</td>
<td>1984</td>
<td>EI</td>
<td>4/7 (57.1 %)</td>
<td>tissue samples from necropsied birds</td>
<td>Trap et al., 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2003 (March)</td>
<td>Rt PCR (23S rRNA)</td>
<td>5/33 (15.2 %)</td>
<td>cloacal swabs</td>
<td>Laroucau, pers. comm. 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2003 (December)</td>
<td>Rt PCR (23S rRNA)</td>
<td>4/20 (20 %)</td>
<td>cloacal swabs</td>
<td>Laroucau, pers. comm. 2007</td>
</tr>
<tr>
<td>Location</td>
<td>Years</td>
<td>Method</td>
<td>Sample Type</td>
<td>Detection Rate</td>
<td>Source</td>
<td></td>
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<td></td>
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<tr>
<td>Troyes</td>
<td>2007</td>
<td>RT-PCR (23S rRNA)</td>
<td>Cloacal swabs</td>
<td>5/33 (15.2%)</td>
<td>Laroucau, pers. comm. 2007</td>
<td></td>
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<tr>
<td></td>
<td>2007</td>
<td>RT-PCR (23S rRNA)</td>
<td>Cloacal swabs</td>
<td>1/29 (3.4%)</td>
<td>Laroucau, pers. comm. 2007</td>
<td></td>
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<tr>
<td>Germany</td>
<td>Giessen</td>
<td>TC</td>
<td>Tissue samples from necropsied birds</td>
<td>10/77 (13%)</td>
<td>Helmecke, 2007</td>
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<tr>
<td>Italy</td>
<td>Pisa</td>
<td>EI</td>
<td>Pool of viscera (lung, liver and spleen) from necropsied birds</td>
<td>14/35 (40%)</td>
<td>Cerri et al., 1989</td>
<td></td>
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<tr>
<td>Trento</td>
<td>1995</td>
<td>TC</td>
<td>Intestinal content from necropsied birds with no clinical history</td>
<td>12/35 (34.3%)</td>
<td>Manfredi et al., 1997</td>
<td></td>
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<tr>
<td>Milan</td>
<td>1996-1997</td>
<td>TC</td>
<td>Intestinal content from necropsied birds with no clinical history</td>
<td>30/163 (18.4%)</td>
<td>Rampin et al., 1998</td>
<td></td>
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<tr>
<td>Bologna and Ferrara</td>
<td>1997</td>
<td>TC</td>
<td>Intestinal content from necropsied birds with no clinical history</td>
<td>34/178 (19.1%)</td>
<td>Renzi and Magnino, 1998</td>
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<tr>
<td>Bergamo</td>
<td>1998-1999</td>
<td>TC</td>
<td>Tissue samples from necropsied birds with no clinical history</td>
<td>11/26 (42.3%)</td>
<td>Gaffuri et al., 2000</td>
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<tr>
<td>Venice</td>
<td>2006</td>
<td>PCR (16S rRNA)</td>
<td>Liver and spleen collected from necropsied birds</td>
<td>7/50 (14%)</td>
<td>Ceglie et al., 2007</td>
<td></td>
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<tr>
<td>The FYR of Macedonia</td>
<td>Skopje</td>
<td>Ag-ELISA</td>
<td>Conjunctival, choanal and cloacal swabs</td>
<td>10/25 (40%)</td>
<td>Mitevski et al., 2005</td>
<td></td>
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<tr>
<td></td>
<td>2006</td>
<td>RT-PCR (23S rRNA)</td>
<td>Cloacal swabs</td>
<td>2/36 (5.6%)</td>
<td>Ilieski et al., 2007</td>
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<tr>
<td>Prilep</td>
<td>2004-2005</td>
<td>Ag-ELISA</td>
<td>Conjunctival, choanal and cloacal swabs</td>
<td>4/16 (25%)</td>
<td>Mitevski et al., 2005</td>
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<tr>
<td>Location</td>
<td>Year(s)</td>
<td>Method</td>
<td>Positive Samples</td>
<td>Sample Type</td>
<td>Reference</td>
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<tr>
<td>Kumanovo</td>
<td>2004-2005</td>
<td>Ag-ELISA</td>
<td>2/10 (20%)</td>
<td>conjunctival, choanal and cloacal swabs</td>
<td>Mitevski et al., 2005</td>
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<td>Bogdanci</td>
<td>2004-2005</td>
<td>Ag-ELISA</td>
<td>2/12 (16.7%)</td>
<td>conjunctival, choanal and cloacal swabs</td>
<td>Mitevski et al., 2005</td>
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<tr>
<td>Vinica</td>
<td>2006</td>
<td>Rt PCR (23S rRNA)</td>
<td>10^4/20 (50%)</td>
<td>cloacal swabs</td>
<td>Ilieski et al., 2007</td>
<td></td>
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<tr>
<td>Stip</td>
<td>2006</td>
<td>Rt PCR (23S rRNA)</td>
<td>4^7/60 (6.7%)</td>
<td>cloacal swabs</td>
<td>Ilieski et al., 2007</td>
<td></td>
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<tr>
<td>The Netherlands</td>
<td>Amsterdam</td>
<td>Rt PCR (ompA)</td>
<td>26^6/331 (7.9%)</td>
<td>fresh faecal droppings</td>
<td>Heddema et al., 2006b</td>
<td></td>
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<tr>
<td>Spain</td>
<td>Murcia</td>
<td>1991 TC</td>
<td>7/39 (18%)</td>
<td>cloacal swabs</td>
<td>Salinas et al., 1993a</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>EI</td>
<td>5/39 (12.8%)</td>
<td>cloacal swabs</td>
<td></td>
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<tr>
<td>Slovenia</td>
<td>Ljubljana</td>
<td>2006 DIF</td>
<td>2/86 (2.3%)</td>
<td>cloacal swabs</td>
<td>Dovč, pers. comm. 2006</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>EI</td>
<td>1/86 (1.2%)</td>
<td>cloacal swabs</td>
<td></td>
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<tr>
<td>Switzerland</td>
<td>Luzern</td>
<td>2001 Ag-ELISA</td>
<td>2/60 (3.3%)</td>
<td>cloacal swabs</td>
<td>Haag-Wackernagel, 2006a</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>EI</td>
<td>1/60 (1.6%)</td>
<td>cloacal swabs</td>
<td></td>
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</tbody>
</table>

Notes
1. Three isolates were genotype B
2. Four isolates were genotype E and one was genotype E/B
3. One PCR product was genotype E
4. Four PCR products were genotype E
5. One PCR product was genotype B, and one was genotype E
Ten PCR products were genotype B

**Legenda**

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

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