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SHORT PAPER

PCR detection of *Chlamydia psittaci* in faecal samples from passerine birds in Sweden

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SUMMARY

To investigate to what extent wild passerine birds are carriers of *Chlamydia psittaci*, 312 faecal samples from 18 bird species were collected. Using the PCR technique and subsequent DNA sequencing, *C. psittaci* DNA was demonstrated in faecal samples from 9 (2·9%) birds of 6 different species. Sera from 65 bird-ringers, highly exposed to wild birds, were tested by microimmunofluorescence assay for the occurrence of IgG and IgM antibodies to *C. psittaci*. No such antibodies were found. This results indicate that a significant proportion of wild passerine birds are carriers of *C. psittaci*, but rarely infectious to humans.

Chlamydia psittaci, the causative agent of psittacosis or ornithosis, has been recognized world-wide since 1929 when about 800 cases of pneumonias were reported in North America and Europe. The source of the outbreak was traced to parrots imported from Argentine [1]. The term psittacosis reflects the initial belief that the human disease is acquired exclusively from psittacine birds. However, during studies of frequently fatal pneumonias in the Faeroe Islands from 1930–8, *C. psittaci* was isolated both from the seabird fulmar (*Fulmarus glacialis*) and from the Faeroes people themselves [1]. Since then, *C. psittaci* has been isolated from around 140 different bird species within 17 orders [2]. Passerine birds have formerly not been considered to play an important role as carriers of the agent, but more recent investigations show that even in this order many species are infected [3, 4].

Around 300000 birds, mostly passerines, are ringed every year in Sweden [5]. Most of them are caught during the spring and autumn passages at bird observatories and ringing stations. A considerable part is also ringed by the around 200 private bird ringers spread over the country. Occasionally during bird-ringing, birds show clinical symptoms; serous conjunctivitis, purulent rhinitis and green diarrhoea, which could be consistent with *C. psittaci* infection [6]. Still, there are to our knowledge, no reports of increased risk for contracting ornithosis among birdringers or others working with wild birds.

In this study we investigated the shedding of *C. psittaci* in faecal samples from wild passerine birds using PCR. The result of a sero-epidemiological survey among Swedish bird-ringers is also presented.

We collected faecal samples from 312 passerine birds of 18 different species (Table 1) caught in mistnets at the Swedish bird-observatory Stora Fjäderägg, situated in the northern Bothnian Gulf (63·00 N/21·05 E). The droppings were put into plastic vials with 500 μl of 0·01% formaline and stored at room temperature before DNA preparation. After removal of the formaline approximately 0·1 g of the sample was boiled in 500 μl autoclaved water for 3 min. After boiling, 100 μl of the supernatant was removed and subjected to the DNA preparation method of Ishizawa and colleagues [7]. The final pellet was dissolved in 50 μl of sterile water and 10 μl of this solution was used as a template for DNA amplification by PCR. The primers Or1

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Table 1. C. psittaci *detected by PCR in faecal samples from passerine birds in Sweden*

Bird species	Number of birds with *C. psittaci* DNA/ number examined
Tree pipit (*Anthus trivialis*)	2/27
Meadow pipit (*Anthus pratensis*)	1/10
Yellow wagtail (*Motocilla flava*)	0/6
Willow warbler (*Phylloscopus trochilus*)	0/76
Pied flycatcher (*Ficedula hypoleuca*)	0/9
Wheatear (*Oenanthe oenanthe*)	0/7
Whinchat (*Saxicola rubetra*)	0/6
Redstart (*Phoenicurus phoenicurus*)	0/15
Robin (*Erithacus rubecula*)	2/29
Blackbird (*Turdus merula*)	0/6
Redwing (*Turdus iliacus*)	0/18
Song thrush (*Turdus philomelos*)	1/12
Fieldfare (*Turdus pilaris*)	2/26
Great tit (*Parus major*)	1/21
Chaffinch (*Fringilla coelebs*)	0/12
Reed bunting (*Emberiza schoeniclus*)	0/22
Yellow hammer (*Emberiza citrinella*)	0/9
Lappland bunting (*Calcarius lapponicus*)	0/1
Total	9/312

(5′-TTT CGA TCG TGT ATT AAA AGTT-3′) and Or2 (5′-AGA AAA TGT CGA AGC GAT CCA-3′) amplifying a 245 bp long fragment in the early 5′ region of the gene encoding the major outer membrane protein, *ompA* [8, 9] were used for DNA amplification. The primers were selected to exclusively amplify the target sequence of *C. psittaci* and not DNA from prototype strains of *C. trachomatis* or *C. pneumoniae*. The samples were subjected to 35 cycles of 20 s at 95 °C, 1 min at 48 °C and 1·5 min at 72 °C. Amplified DNA was electrophoresed through a 1·5% agarose gel and visualized by ethidium-bromide staining. All PCR reagents were pipetted with aerosol-resistant tips and tested for PCR reactivity without the adding of DNA. In all DNA amplification experiments, *Borrelia burgdorferi* DNA was used as negative control and *C. psittaci* DNA from serovar CAL-10 was used as positive control. By PCR chlamydia DNA were detected in 9 (2·9%) faecal samples from 6 different bird species (Table 1). The PCR amplified fragment was isolated and ligated into pT7Blue T-Vector (Novagen, Madison, Wisconsin, USA) according to the instructions of the manufacturer. Competent *Escherichia coli* DH5α was transformed with the recombinant plasmids containing the *ompA* fragment according to standard protocols [10]. Both strands of positive clones containing the PCR-amplified *ompA* fragment were sequenced by primer-directed sequencing by the dideoxy chain termination method, using α-^{35}S-dATP (Pharmacia T7 sequencing kit, Uppsala, Sweden). Two different *C. psittaci* sequences were found (data not shown). When compared to other previously described *ompA* sequences of *C. psittaci* two were found identical to those of serovar MN and seven to serovar 6BC or A22/M [8]. They differed only in position 208, were G (MN) was substituted for a C (6BC or A22/M).

At a national bird-ringing conference in Sweden in 1991, serum samples were collected from 65 Swedish bird-ringers. The participants answered a questionnaire to find out the duration of contact with birds, preference for certain species or groups of birds and previous symptoms reminiscent of ornithosis. The mean age of the bird-ringers was 43 years (range 16–69 years). They had handled birds for 1–50 years (mean 20 years). The mean number of ringed birds/ringer/year was 2·700. Ringing of passerine birds dominated in the group (91%), followed by raptors/owls (59%) and water related species as gulls, waders and ducks (40%). There was no known case of ornithosis in the group.

In a microimmunofluorescence assay (MIF) [11] the egg-grown prototype strains *C. psittaci* 6BC, *C. pneumoniae* IOL-207 and a pool of egg-grown *C.*

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trachomatis serovars B through K were used as antigens. The 3 different antigens were applied as series of triplets on each slide. The sera were tested at a dilution of 1/16 and titrated to end point 1/1024 if positive. Specific IgM or IgG antibodies to *C. psittaci* were not detected in any of the serum samples. IgG antibodies to *C. pneumoniae* (TWAR) were demonstrated in 51 of the 65 sera (78%).

Since many bird species can harbour *C. psittaci*, it is surprising that all tested bird-ringers, with their long-term and high exposure to wild birds, were serologically negative. There may be a difference in virulence or pathogenicity for man between strains of *C. psittaci* associated with various birds. Certain orders of birds may be susceptible to and carriers of distinct serovars [3, 12]. Differences in the virulence of various strains carried by wild birds is also reported [13]. As with the parrots imported from Argentine, stress may be suspected to increase the risk of inducing disease in *C. psittaci* carrying birds. Many birds species change their behaviour during migration. The migratory restlessness and hormonal changes can be extremely stressful to the individual bird. Starvation or other catabolic conditions may also be important factors in activating a latent infection. Although bird ringing is stressful for the bird, the processing time is probably not sufficient for activating the disease. The question of why more stress-related morbidity or mortality from ornithosis is not seen in wild birds remains.

It is unlikely that the failure to find specific antibodies was due to technical problems. The MIF test has performed well for many years in the routine diagnosis of ornithosis. The validity of the data is strengthened by the lack of previous episodes reminiscent of ornithosis among the birdringers. Cross-reactive antibodies to all three antigens, *C. trachomatis*, *C. psittaci* and *C. pneumoniae* were observed in some cases. This is often the result of antibody reacting to the group antigen, the lipopolysaccharide (LPS) of the organism, and may occur after both *C. psittaci* and *C. pneumoniae* infections. *C. trachomatis* usually does not evoke a strong LPS antibody response. The prototype *C. psittaci* strain, 6BC, may not be immunologically similar to the strains from wild birds. However, the sequence data indicate clear similarities between the wild type strains and the 6BC strain used as antigen in MIF. Antibodies to *C. psittaci* may also disappear after some time. That could explain the lack of antibodies some years after a single exposure. If the birds infect humans regularly, antibody levels would be expected to be boostered by the repeated exposures.

The prevalence of antibodies to *C. pneumoniae* of 78% is equal to that described earlier among healthy blood donors in Sweden [14]. The antibody reactivity to *C. pneumoniae* was not seen as a dual reactivity to both *C. psittaci* and *C. pneumoniae*. The prevalence of *C. pneumoniae* antibodies is therefore not the result of cross-reactive antibodies to *C. psittaci*. This indicates that subclinical infections with *C. pneumoniae* are common, since past severe pneumonia was not reported among the bird-ringers.

In conclusion, no evidence was found in the present study of an increased risk for the acquisition of *C. psittaci* by birdringing, either as judged by questionnaire data or by serology. As *C. psittaci* is known to be carried by various wild birds and the 65 birdringers in the study had ringed a great number of birds, some factors beside the mere contact with *C. psittaci* carrying birds seems to be required for spread of ornithosis to humans.

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