

## STUDY OF *CHLAMYDOPHILA PSITTACI* OUTBREAK IN BUDGERIGARS

ALENKA DOVČ, BRIGITA SLAVEC, RENATA LINDTNER-KNIFIC, OLGA ZORMAN-ROJS,  
JOŠKO RAČNIK, JANA GOLJA<sup>1</sup>, AND KSENIJA VLAHOVIĆ<sup>2</sup>

Institute for Health Care of Poultry, Veterinary Faculty,  
University of Ljubljana, 1000 Ljubljana, Slovenia

<sup>1</sup>Vetconsult Pharma d.o.o., 1000 Ljubljana, Slovenia

<sup>2</sup>Department of Biology, Veterinary Faculty,  
University of Zagreb, 10000 Zagreb, Croatia  
Alenka.dovc@vf.uni-lj.si

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### Abstract

In this paper, some facts have been discussed that could be important for the understanding of how the chlamydial pathogen spreads within the bird flock and to humans. The presented report has been based on pathological findings and interpretation of the results of diagnostic tests, obtained at chlamydial infection in a flock of parrots. In a two-week period, a high mortality in one flock of budgerigars (*Melopsittacus undulatus*) was reported. Adults as well as young older than 14 d died. The laboratory investigation confirmed the infection with *Chlamydophila psittaci*. In the same period two members of the owner's family showed signs of atypical pneumonia. The owner decided to eliminate the whole flock. Samples of blood and swabs from cloaca were taken before the birds were euthanised. A post-mortem examination was performed and samples from embryos and eggs were taken. For the confirmation of chlamydia infection, several different diagnostic methods were used: direct and indirect immunofluorescence, commercial immuno-enzymatic tests, isolation on chicken embryos and laboratory mice, as well as molecular detection. Avian chlamydiosis represents an important zoonosis in Europe. This is the reason for the necessity of developing more efficient methods for chlamydial disease control, and for setting up generally accepted rules in European and non-European countries.

**Key words:** parrots, *Chlamydophila psittaci*, chlamydiosis, diagnosis, disease outbreaks.

Avian chlamydiosis is caused by *Chlamydophila psittaci* (*C. psittaci*), formerly known as *Chlamydia psittaci* (4). It is a very important infectious disease in psittacines. It is found in wild and domestic birds and occurs in more than 469 different avian species causing considerable economic losses in the poultry industry. *C. psittaci* is a unique obligate intracellular bacterium known to be transmissible from birds to humans (9).

*C. psittaci* can be present in droppings and nasal discharges of infected birds in large numbers. The organism can remain infectious in the environment for months. Human infection usually occurs when a person inhales the bacterium shed from faeces and secretions of birds (12). Some birds can appear healthy and shed the organism intermittently. Shedding can be activated by stress factors, including relocation, shipping, crowding, chilling, and breeding (11).

The usual duration between exposure to *C. psittaci* and onset of illness ranges from 3 d to several weeks. However, the active disease can appear with no identifiable exposure. Whether a bird has acute or chronic disease or dies, it depends on the bird species, virulence, and dose of the strain, stress factors, age, and extent of treatment or prophylaxis (5). Signs of avian chlamydiosis are non-specific and include ruffled feathers, lethargy, anorexia, emaciation, dehydration, and death. Other signs include serous or mucopurulent ocular or nasal discharge, diarrhoea, and excretion of green to yellow-green urates. Presently, doxycycline is the drug of choice for treating birds with avian chlamydiosis (11).

The disease in humans causes typical influenza-like symptoms and can lead to severe pneumonia and non-respiratory health problems. Infection with this pathogen is also known as psittacosis, parrot fever, or ornithosis (11). Breeders of pet birds (mostly parrot breeders), breeders of pigeons, wildlife rehabilitators, zoo workers, employees in shops for pet birds, and persons in specific occupations (employees in poultry slaughterhouses, veterinarians, veterinary technicians, laboratory workers) are more often exposed (3, 11).

The aim of this study is to obtain a deeper understanding into an outbreak of chlamydiosis among the breeding parrots. The obtained data, based on pathological findings and results of diagnostic tests, can represent important facts about spreading of the disease

within the breed and indicate a mode of prevention of human infection in the future.

## Material and Methods

**Birds.** During breeding season in January an outbreak of avian chlamydiosis in Slovenia was diagnosed in one breeding flock of 12 pairs of sexually mature budgerigars (*Melopsittacus undulatus*), their 15 young, and two adult females without partners. Two members of the owner's family showed signs of atypical pneumonia, so the owner decided to eliminate the whole flock. All adult birds and young budgies were euthanised. In 7 nests there were 28 eggs. In these nests, 3 pairs had both, eggs and young and in another 4 nests young were found. Only one pair of budgerigars had not bred yet. One pair and one male of cockatiels (*Nymphicus hollandicus*) and one pair of zebra finches (*Taeniopygia guttata*) were part of this flock and were also included in this study. Post-mortem examination was performed on the dead young birds. Blood from right jugular vein and cloacal swabs were taken. Fertilised and not fertilised eggs were also tested. One day after euthanasia, the necropsy was performed and then the samples were collected for further investigation.

In further study, the diagnostic procedure on 46 cloacal swabs, 16 swab samples from different places of eggs or embryos, and 6 swabs from lumen of the oviduct taken from females with functional reproductive organs was performed. The swabs were examined for the presence of chlamydial antigen by immunoassay IDEIA™ PCE Chlamydia (DAKO Diagnostics Ltd., UK) (IDEIA) with the 620 nm filter.

Forty-two sera (24 adult budgerigars, 13 young budgerigars, 3 cockatiels, and 2 zebra finches) were analysed for the presence of *C. psittaci* antibodies. Blood of four budgerigars was not convenient for testing. Immune reactivity of the birds was tested using indirect immunofluorescence assay (Chlamydia psittaci spot, BioMerieux, France) (IIFA) according to manufacturer's recommendation with one modification. FITC conjugated rabbit anti-parrot IgG (homemade

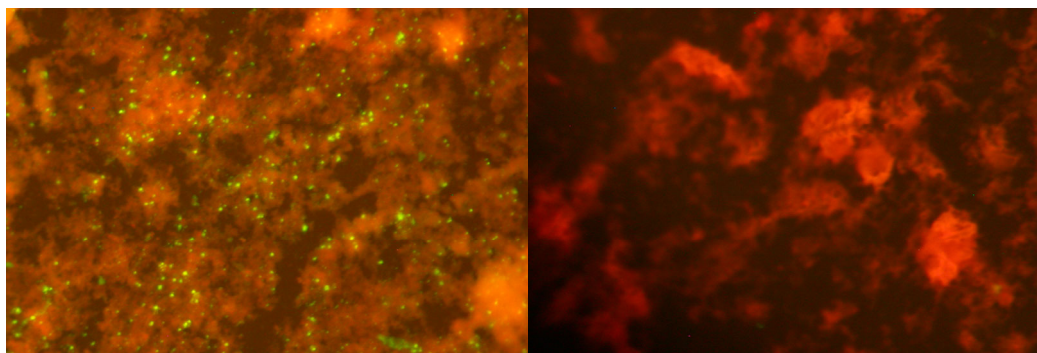
production) was used instead of human conjugate. Dilutions of 1:40 were done for each serum.

Differential diagnostic tests were included to eliminate other possible infections (*Mycoplasma* sp., polioma infection virus, or psittacine beak and feather disease virus). A rapid serum agglutination test and cultivation on media were performed. Diagnostic tests for viral infections were done in other laboratory (Avian Biotech International, UK).

## Results

**Routine diagnostic procedure.** From 15 dead young birds four from two nests were examined. At necropsy, enlarged liver and spleen, hyperaemia of lung, catarrhal to haemorrhagic enteritis, crops full of soft feed, and serohaemorrhagic sinusitis were found. In two of the birds, fibrous airsacculitis and soft bones were diagnosed too. Ectoparasites (*Cnemidocoptes pilae*) were found in 6 out of 26 adult birds (23.1%). Cultures of *Aerococcus viridans* were isolated bacteriologically from liver (diffuse growth) and intestines. Sera of their parents were tested for *Mycoplasma gallisepticum* using a rapid serum agglutination test. The results were negative. Some birds in the flock had rough plumage and ruffled feathers, therefore they were tested for PIV and PBDFV. The results were negative. Swabs of the air sacs of birds with airsacculitis were positive for antigen in two methods: CWT and DIFA (Fig. 1). The chorioallantoid fluid of eggs inoculated with samples taken from these birds was positive in PCR for *C. psittaci* antigen after 5 d. In biological experiment, 2 mice showed clinical signs of illness 3 d after inoculation and they died 6 d after the onset of clinical signs, *i.e.* 9 d after inoculation. Ascites fluid from both infected laboratory mice was positive 3 d (samples were taken from live mice) and 9 d after the inoculation in CWT and DIFA. On post mortem examination perihepatitis, perilienitis, peritonitis, and pneumonia were seen.

**Post-mortem examination of euthanised birds.** Results of some important post-mortem findings, suspicious for *C. psittaci* infection are seen in Table 1.



**Fig. 1.** *C. psittaci* antigen positive sample of air sac (left) and negative control sample (right) using DIFA.

**Table 1**  
Post-mortem findings in 46 euthanised birds

Necropsy results	Number of findings	Necropsy results	Number of findings	Necropsy results	Number of findings
icterus	14	bronchopneumonia	4	oophoritis	3
gastroenteritis	13	haemorrhagic enteritis	4	atrophia universalis	3
enlarged spleen	12	peritonitis, perihepatitis	4	synusitis	2
enlarged liver	11	enlarged testes	4	liver necrosis	2
atrophia of spleen	7	airsacculitis	3	spleen necrosis	1

**Chlamydial antigen detected by immunoassay IDEIA.** *C. psittaci* antigen from cloacal swabs of 46 birds was confirmed in 7 (15.2%) birds and suspicious results were established in 10 (21.7%) birds. Among 12 pairs and their young in 7 (58.3%) families, antigen *C. psittaci* was detected at least in one bird from each family, while at least one bird in 4 (33.3%) families were suspicious for infection with *Cp. psittaci*. One pair (8.4%) of budgerigars, which had scaly face mites (*Cnemidocoptes pilae*) and had neither eggs nor progeny, was negative. Two single female budgerigars and five another birds, cockatiels and zebra finches were negative.

Six swabs from the lumen of the oviduct taken from females with active reproductive organs were positive in two cases. In both females cloacal swabs were also positive and six eggs from one of them were also tested. All swabs from yolks were negative.

From twenty-eight eggs found in nests, 19 were either not fertilised or early embryonal death was established, 6 embryos were dead, and only 3 stayed alive. From two females, in which one cloacal swab was positive and another negative, 16 different samples from their eggs and embryos were tested. Out of the samples taken from the surface of eggs, chorioallantoic fluid, yolk, and umbilical vein (each sample from two different eggs) originated from the positive female, only two samples taken from the yolk were positive. Among the same samples taken from progeny of the negative female, only samples taken from the yolk were positive.

**Serological tests.** All 42 tested sera were negative in IIFA for IgG *C. psittaci* antibodies.

**Occurrence of chlamydiosis in owner's family.** Two out of three members of the family fell ill with signs of atypical bronchopneumonia and were hospitalised. A fourfold rise of specific IgG antibody titre in paired sera was confirmed in one person. In other two family members diagnostic tests for the confirmation of infection with *C. psittaci* were performed only once within the first 14 d after the onset of clinical signs. The results of serological tests were negative. Paired sera were not taken.

## Discussion

Chlamydiosis is often a systemic disease and infection can be unapparent, severe, acute or chronic with intermittent shedding. Clinically unapparent, a latent infection is supposed to be the predominant state. The clinical form can be activated by stress factors, including overcrowding, poor nutrition, other bacterial and viral infections, and transportation. In our study, it seems that infection occurred in parrots imported approximately one month before the onset of the disease from Italy. Ectoparasites (*Cnemidocoptes pilae*) diagnosed in the budgerigar flock were also one of the stress factors and could affect the outbreak of chlamydiosis.

Young birds are more susceptible to infection than older ones, and some species seem to be more susceptible than others. Disease carriers may be often identified by the transmission of disease to other susceptible birds or by the sudden death of young nestlings with apparently healthy parents (8). This happened in our case. Most of young birds were cachectic and some had synusitis. Adults did not show any clinical signs except conjunctivitis in two females and one male.

Infectious *Chlamydiae* in respiratory secretions or faeces may remain viable for several months. The transmission of the disease is mainly through aerosols of faecal or feather dust, but oral infection is an alternate route. The transmission through eggs has been shown in chickens, ducks, seagulls, and psittacine birds (8). In our study, *C. psittaci* in mucous of the oviduct and in yolk sacs of embryos of budgerigars was confirmed. Early embryonal death was established in 19 (67.9%) eggs. However, the presence of *C. psittaci* antigen in the cloaca and consequently shedding pathogen in environment among the birds younger than 10 d was not confirmed using IDEIA. Cockatiels and zebra finches stayed negative. We could not confirm either infection with *C. psittaci* or specific immunoreaction against this pathogen in these species of birds.

Immune response of budgerigars was also tested and all birds were negative. This indicates that the outbreak was acute and IgG antibodies did not arise yet. The complete flock was euthanised two weeks after clinical signs appeared and at the same time two people fell ill. Fifty percent mortality (15/30) was established among the young. A high mortality was in young older than 14 d. At necropsy of young birds, enlarged liver and spleen, hyperaemia of lung, catarrhal to haemorrhagic enteritis, crops full of soft feed, and serohaemorrhagic sinusitis were seen. In post-mortem examinations of the euthanised birds, pathological findings were more severe in young than in adults.

Avian chlamydiosis is continuously present among birds in Slovenia (2). Investigation of risk groups of people for the presence of specific antibodies against *C. psittaci* infection noted in 6.5% of immunoreactive people in Slovenia 9 years ago. Correlation between infections in breeders and their flocks was also confirmed. Out of the examined birds owned by serologically positive breeders, 32.1% of the birds were immunoreactive (3).

Cases of zoonotic transmission to humans are regularly happening, but are under diagnosed and poorly documented throughout Europe. The infections can be life threatening to the affected individuals, particularly in the absence of *C. psittaci*-specific diagnosis. In Slovenia, none or only a few cases are reported every year. There are also not many articles concerning transmission of *C. psittaci* infection from birds to humans (6, 7). However, there are no solid and comprehensive data on cases of zoonotic transmission throughout Europe. Addressing the sources of the infection will considerably lower the risk of human infections, which can be achieved through epidemiological investigations in wild and domesticated birds and genetic characterisation of isolated strains. Genotype or serotype of *C. psittaci* isolated in our country has not been determined yet. This is a very important stage of our epidemiological study of chlamydiosis in the future.

The severity of the disease in humans ranges from the unapparent to systemic illness with severe pneumonia. In this case two family members were hospitalised for having severe pleuropneumonia with breathing disorders. A fourfold rise of specific IgG antibody titre in paired sera was confirmed in one person and considered as presumptive evidence of acute or recent infection with chlamydiae.

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