INFEKTOL GLASN 2019;39(1):3-7
https://doi.org/10.37797/ig.39.1.1

Izvorni znanstveni rad | Original scientific article

# Prevalence and Zoonotic Potential of Avian Chlamydia in Croatia

## Pojavnost i zoonotski potencijal klamidija ptica u Hrvatskoj

Danijela Horvatek Tomić[1], Željko Gottstein[1], Maja Lukač[1], Iva Pem Novosel[2], Estella Prukner-Radovčić[1]

[1] University of Zagreb, Faculty of Veterinary Medicine, Department of Poultry Diseases at the Clinic, Zagreb, Croatia
[2] Croatian Institute of Public Health, Department of Epidemiology, Zagreb, Croatia

**Keywords:**
*Chlamydia sp.*
*avian*
*zoonosis*
*Croatia*

**Ključne riječi:**
*Chlamydia sp.*
*ptičja*
*zoonoza*
*Hrvatska*

**Primljeno**: 8.6.2018.
**Received:** 8.6.2018.

**Prihvaćeno:** 30.7.2018.
**Accepted:** 30.7.2018.

### Abstract

Background: Zoonotic *Chlamydia (C.) psittaci,* can be found in different bird species, but also in other animals and humans. Recently, new species have been described, as *C. gallinacea* and *C. avium,* changing the perspective of a single causative agent of avian chlamydiosis. Genotypes of *C. psittaci* differ, according to their pathogenicity, and the ones isolated from parrots, turkeys and ducks are particularly pathogenic to bird breeders or owners, veterinarians, poultry farms and slaughterhouse workers. Infections in humans may pass unapparent or to the onset of systemic lung inflammation. Asymptomatic infection is most commonly found in birds, but acute systemic or chronic infections are also possible.

Material and Methods: Presence of *C. psittaci* in birds in Croatia must be reported by law, and all registered flocks of pigeons, parrots and other birds must be examined once, and pet shops at least twice a year. In the period from 2008 to 2017, a total of 3283 avian samples were examined by real-time PCR and the presence of *Chlamydiaceae, C. psittaci* and *C. gallinacea* were detected in faecal samples or swabs.

Results and Conclusions: In total, 12% of the samples were found positive for *Chlamydiaceae.* From the 2015, the presence of *C. psittaci* has also routinely been determined, whereas 34.01% positive avian samples proved to be *C. psittaci.* Findings of chlamydia in various avian species indicates the importance of proper implementation of the protective measures, by taking into account the zoonotic potential of chlamydia originating from birds and poultry.

### Sažetak

Uvod: Bakteriju *C. psittaci* moguće je pronaći kod različitih vrsta ptica, ali i kod drugih životinja i ljudi. Nedavno su opisane nove vrste, *C. gallinacea* i *C. avium*, čijim se nalazom mijenja slika jednog od uzročnika klamidioze u ptica. Genotipovi *C. psittaci* razlikuju se prema svojoj patogenosti, a oni izdvojeni iz papiga, purana i pataka posebno su patogeni za uzgajivače ili vlasnike ptica, veterinare, radnike na peradarskim farmama i u klaonicama. Infekcije kod ljudi mogu proći inaparentno ili uz teške kliničke znakove upale pluća. Asimptomatska infekcija se najčešće javlja kod ptica, no moguće su i akutne sistemske ili kronične infekcije.

Materijali i metode: Prisutnost *C. psittaci* kod ptica u Hrvatskoj mora se prijaviti prema zakonu, a sva registrirana jata golubova, papiga i drugih ptica moraju se pregledati jednom, a trgovine za kućne ljubimce najmanje dva puta godišnje. U razdoblju od 2008. do 2017. godine ukupno je pregledano 3283 uzoraka ptica primjenom metode PCR-a u stvarnom vremenu, a prisutnost *Chlamydiaceae, C. psittaci* i *C. gallinacea* dokazana je u uzorcima izmeta ili obriscima organa.

Rezultati i rasprava: Ukupno je 12% uzoraka bilo pozitivno na *Chlamydiaceae.* Od 2015. godine, prisutnost *C. psittaci* također je rutinski određena te se 34.01% pozitivnih uzoraka identificiralo kao *C. psittaci.* Nalazi klamidije kod različitih ptičjih vrsta ukazuju na važnost pravilne provedbe zaštitnih mjera, uzimajući u obzir zoonotski potencijal klamidije koja potječe od ptica i peradi.

✉ **Corresponding author:**
Assoc. Prof. Danijela Horvatek Tomić
University of Zagreb
Faculty of Veterinary Medicine
Department of Poultry Diseases at the Clinic
Heinzelova 55, 10000 Zagreb
Tel: 012390281, fax: 012390410
E-mail: horvatek@vef.hr

## Introduction

Avian chlamydiosis, most commonly caused by *Chlamydia* (*C.*) *psittaci,* a mandatory intracellular bacterium with a unique biphasic developmental cycle, occurs in over 400 bird species, including poultry, other animals and humans [1-4]. In the last decade, due to the implementation of new molecular typing methods, two new species of avian chlamydia, *C. gallinacea* (present primarily in poultry) and *C. avium* (primarily in pigeons and other birds) are increasingly mentioned, changing the perspective of a single causative agent of chlamydiosis in birds [5-7]. The findings of *C. gallinacea* in poultry all over the world is surpassing the one of *C. psittaci* and authors even mention it as endemic chlamydial species in poultry flocks. Although much is still unknown about its pathogenicity and virulence [7-11], Laroucau et al.[12] described its association with atypical pneumonia among workers in a slaughterhouse in France. Other chlamydial agents such as *C. abortus*, *C. suis*, *C. muridarum*, *C. pecorum* and *C. trachomatis* were also isolated in rare occasions from birds [13-15].

On the other hand, it is well known that *C. psittaci* appears in 16 different serovars (genotypes) [16]. Genotype A primarily occurs in parrots, genotype B in pigeons, C in ducks and geese, D in turkey, E and E/B in pigeons and ducks, F in psittacines and turkeys, WC in cattle, and M56 in rodents [17-18]. Zoonotic potential particularly indicates genotypes A, B and E/B [19-20]. Although the spill-over of avian *C. psittaci* to other animal species is well recognized, new investigations also emphasise its role as equine abortion agent [21-23]. The transmission of the agent mainly occurs through contaminated air and dust. Depending on the serovar, asymptomatic infections are most commonly found in birds, but acute systemic or chronic infections are also possible. If clinical signs occur, they usually include anorexia, diarrhoea, conjunctivitis, respiratory symptoms or nervous system disorders [18]. From patoanatomical perspective, hepato- and splenomegaly are present, with fibrinous air sacculitis and peritonitis, while secondary infections aggravate clinical signs as well as patoanatomical findings [24]. Diagnosis is mainly based on the detection of specific antigen, rather than the evidence of antibodies against Chlamydia [25]. From the living birds, the so-called „triple swabs" (from conjunctiva, oropharynx and cloaca) or faecal sample could be used for diagnostic purposes [26]. Treatment of infected birds is sometimes unsuccessful, but in majority of cases, long term use of tetracycline or enrofloxacin is recommended [18].

It is well known that *C. psittaci* can cause a dangerous zoonosis (psittacosis or ornithosis), most commonly in bird breeders or owners, veterinarians, poultry farms, slaughterhouse and/or pet shops workers. Infections in humans range from unapparent subclinical to the onset of systemic inflammation and severe pneumonia. Other onset of the symptoms have scarcely been reported [27-30]. Main source of the disease are infected birds, rarely other animals [31-32]. Human to human transmission is extremely rare [33]. The zoonotic potential of *C. gallinacea* and/or *C. avium* is yet to be confirmed, as there is only one case reported of slaughterhouse workers exposed to chickens infected with chlamydia other than *C. psittaci* who developed atypical pneumonia [12].

In the Republic of Croatia, avian chlamydiosis caused by *C. psittaci* is notifiable disease and must be reported if present in the registered flocks of pigeons, parrots and other birds or in pet shops. This paper describes the prevalence of avian chlamydiosis in Croatia during the last decade, as well as its zoonotic potential.

## Material and Methods

In the period from 2008 to 2017, a total of 3283 samples originating from different bird species were examined, as a routine diagnostic work at the Laboratory for Chlamydia (CHLAMlab) of the Department of Poultry Diseases at the Clinic, Faculty of Veterinary Medicine University of Zagreb. The received samples were in majority pooled faecal samples, collected during the three consecutive days, as stated in the national legislative. Other than faeces, veterinarians also collected different organs or swabs (mainly oropharyngeal and/or cloacal). The samples were obtained from the different avian species, but rarely representing only one single species or a single bird. As, according to the law, the pooled faecal sample should contain the faeces of at least 10% of individuals in the flock, in majority of cases, origin of a sample was a combination of pigeon/other birds' species from private breeders or parrots/passerines from pet shops. All the samples were examined by using real-time polymerase chain reaction (qPCR) assay. Genomic DNA was extracted by using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, USA), according to the manufacturer's instructions. The samples were further examined by specific *Chlamydiaceae* qPCR as described by Ehricht et al. [34], targeting the 23S rRNA gene, and in the case of positive reaction, by *C. psittaci* - specific qPCR (targeting the *incA* gene) as described by Menard et al. [35] or *C. gallinacea* - specific qPCR (targeting *enoA* gene) as described by Laroucau et al. [36].

## Results and Discussion

During the period of 2008-2017, altogether 3283 samples originating from different bird species were

examined for the presence of *Chlamydiaceae* (Table 1). During the last decade, the total of 12% of the samples were found positive for the *Chlamydiaceae*, ranging from 5.42% in 2017 to 21.16% in 2013 (Table 1). During the period from 2008 to 2014, determination to the species level was not done routinely, but only as a part of research activities. From 2015, the presence of *C. psittaci* must be recorded in avian samples, and it was determined in 134 of the 394 *Chlamydiaceae* positive samples (34.01%).

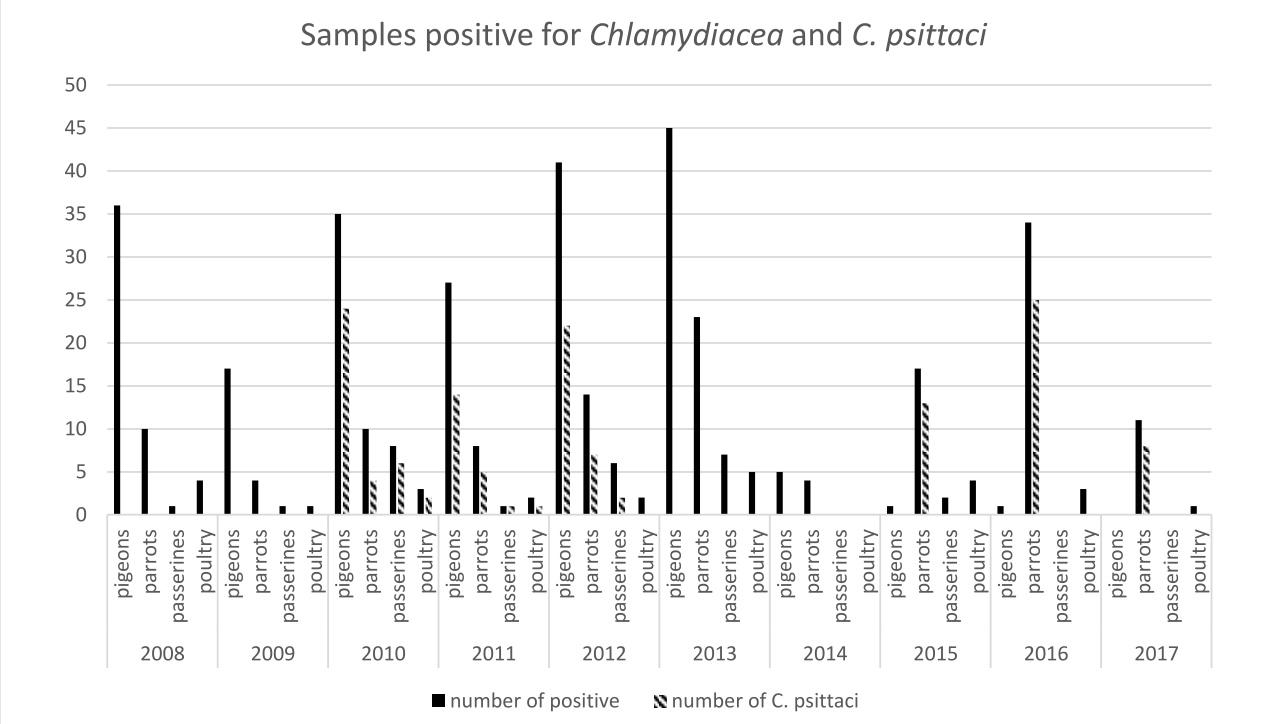
The samples originating from pigeons were, in the majority of cases, positive for *Chlamydiaceae* (52.79%

Table 1. Number of samples and the finding of Chlamydiaceae and C. psittaci in the period 2008.-2017.
Tablica 1. Broj uzoraka i nalaz Chlamydiaceae i C. psittaci u razdoblju 2008-2017.

| Year | Number of samples | Number of *Chlamydiaceae* positive samples | Number of *C. psittaci* positive samples | Number of *C. gallinacea* positive samples |
|---|---|---|---|---|
| 2008 | 329 | 51 | nd* | nd |
| 2009 | 232 | 23 | nd | nd |
| 2010 | 414 | 56 | 36 | nd |
| 2011 | 542 | 38 | 21 | nd |
| 2012 | 494 | 63 | 31 | nd |
| 2013 | 378 | 80 | nd | 6 |
| 2014 | 165 | 9 | nd | nd |
| 2015 | 225 | 24 | 13 | nd |
| 2016 | 283 | 38 | 25 | nd |
| 2017 | 221 | 12 | 8 | nd |
| Total | 3283 | 394 | 134 | 6 |

*nd= not done (Detection of *C. psittaci* was not done in 2008, 2009, 2013 and 2014; Detection of *C. gallinacea* was not done in 2008-2012 and 2014-2017).

Figure 1. Distribution of positive samples according to the avian species.
Slika 1. Raspodjela pozitivnih uzoraka prema vrsti ptica



of all positive samples), but unfortunately, the pigeons were excluded from the obligatory testing from 2013 (Figure 1). The samples originating from psittacines were positive for *Chlamydiaceae* in 34.26% of cases, while 45.92% of the positive samples were identified as *C. psittaci*. It is not obligatory to test the commercial poultry flocks for the presence of Chlamydia, but 25 samples received from the ornamental poultry flocks have been found positive for *Chlamydiaceae* over the years. *C. gallinacea* was detected in 6 positive samples (24%) and *C. psittaci* in 3 (12%) (Figure 1). This result is in accordance with Quilicot and Prukner-Radovčić [37] who found 46.3% of backyard poultry flocks to be positive for *C. gallinacea* in Croatia.

When dealing with public health, according to the Reference Center for Epidemiology of the Ministry of Health, Croatian Institute of Public Health, in the period from 2008 to 2017, there were only 5 cases of psittacosis reported in humans, one in 2008, 2011 and 2014, and two in 2010 (personal communication, undisclosed data). Although this isn't a high number, there is a clear evidence of the need for communication and collaboration between human and veterinary medicine professionals. A joint work on diagnosis of psittacosis up to the species level could be of significant advantage when dealing with atypical clinical cases [38] or other species than *C. psittaci*, whose pathogenicity in humans is rarely demonstrated [12]. In order to prevent the occurrence of avian chlamydiosis in birds and humans, it is necessary to follow the strict protection measures including biosecurity measures and the improvement of animal health conditions, but also a particular caution during the laboratory work if dealing with live microorganisms. Findings of chlamydia in various avian species indicates the importance of proper implementation of the protective measures, by taking into account the zoonotic potential of chlamydia originating from birds and poultry.

## Acknowledgments

The authors do not have an association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding). The authors wish to acknowledge the technicians working in the CHLAMlab – Aleksandar Gavrilovic, Maja Strkalj and Ivancica Ivana Hustic Prelogar for their contribution.

## REFERENCES

[1] Kaleta EF, Taday EM. Avian host range of *Chlamydophila spp*. based on isolation, antigen detection and serology. Avian Pathol, 2003; 32: 435-461.

[2] Polkinghorne A, Greub G. A new equine and zoonotic threat emerges from an old avian pathogen, *Chlamydia psittaci*. Clin Microbiol Infect, 2017; 23: 693-694.

[3] Degraves FJ, Gao D, Hehnen HR, Schlapp T, Kaltenboeck B. Quantitative Detection of *Chlamydia psittaci* and *C. pecorum* by high-sensitivity real-time PCR reveals high prevalence of vaginal infection in Cattle. J Clin Microbiol 2003; 41: 1726-1729.

[4] Beeckman, DSA, Vanrompay DCG. Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. Clin Microbiol Inf, 2009; 15: 11-17.

[5] Sachse K, Laroucau K, Riege K, et al. Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. Nov. And *Chlamydia gallinacea* sp. Nov. Syst Appl Microbiol, 2014; 37:79–88.

[6] Burt, SA, Röring RE, Heijne M. *Chlamydia psittaci* and *C. avium* in feral pigeon (*Columba livia domestica*) droppings in two cities in the Netherlands. Vet Q, 2018; 38: 63-66.

[7] Guo W, LI J, Kaltenboeck B, Gong J, Fan W, Wang C. *Chlamydia gallinacea*, not *C. psittaci*, is the endemic chlamydial species in chicken (*Gallus gallus*). Sci Rep, 2016; 18: 19638.

[8] Hulin V, Oger S, Vorimore F, et al. Host preference and zoonotic potential of *Chlamydia psittaci* and *Chlamydia gallinacea* in poultry. Pathog Dis, 2015; 73: 1-11.

[9] Li L, Luther M, Macklin K, et al. *Chlamydia gallinacea*: a widespread emerging Chlamydia agent with zoonotic potential in backyard poultry. Epidemiol Infect, 2017; 145: 2701-2703.

[10] Heijne M, Van Der Goot JA, Fijten H, et al. A cross sectional study on Dutch layer farms to investigate the prevalence and potential risk factors for different Chlamydia species. Plos One, 2018; 13 (1), e0190774.

[11] Sachse K, Laroucau K. Two more species of *Chlamydia*–does it make a difference? Path Dis, 2015: 73: 1–3.

[12] Laroucau K, Vorimore F, Aaziz R, Berndt A, Schubert E, Sachse K. Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. Inf Gen Evol, 2009; 9:1240-1247.

[13] Pantchev A, Sting R, Bauerfeind R, Tyczka J, Sachse K. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. Vet J, 2009; 181: 145-150.

[14] Sachse K, Kuehlewind S, Ruettger A, Schubert, E Rohde G. More than classical *Chlamydia psittaci* in urban pigeons. Vet. Microbiol, 2012; 157: 476-480.

[15] Guo W, Li J, Kaltenboeck B, Gong J, Fan W, Wang C. *Chlamydia gallinacea*, not *C. psittaci*, is the endemic chlamydial species in chicken (*Gallus gallus*). Sci Rep, 2016; 18: 19638.

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

[17] Harkinezhad T, Geens T, Vanrompay D. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. Vet Microbiol, 2009; 135: 68–77.

[18] Vanrompay D. Avian Chlamydiosis. In: Swayne D. Diseases of Poultry. 13th edition. Ames, Wiley- Blackwell Publishing, USA; 2013; 1055-1073.

[19] Heddema ER, Van Hannen EJ, Duim B, Vandenbroucke-Grauls CMJE, Pannekoek Y. Genotyping of *Chlamydophila psittaci* in Human Samples. Emerg Infect Dis, 2006; 12: 1989–1990

[20] Vanrompay D, Harkinezhad T, Van De Walle M, et al. *Chlamydophila psittaci* Transmission from Pet Birds to Humans. Emerg Infect Dis, 2007; 13: 1108–1110.

[21] Jelocnik M, Jenkins C, O'Rourke B, Barnwell J, Polkinghorne A. Molecular evidence to suggest pigeon-type *Chlamydia psittaci* in association with an equine foal loss. Transbound. Emerg Dis, 2018; 65:911-915.

[22] Jenkins C, Jelocnik M, Micallef Ml, et al. An epizootic of *Chlamydia psittaci* equine reproductive loss associated with suspected spillover from native Australian parrots. Emerg Microbes Infect, 2018; 16: 88.

[23] Taylor KA, Durrheim D, Heller J, et al. Equine chlamydiosis — An emerging infectious disease requiring a one health surveillance approach. Zoon Pub Health, 2018; 65: 218–221.

24] Shivaprasad HL. Avian chlamydiosis. In: Brugere-Picoux J, Vaillancourt J-P. Manual of Poultry Diseases. Toppan Printing Leefung; 2015: 273-278.

[25] Avian Chlamydiosis. Section 2.3. Chapter 2.3.1. OIE Terrestrial Manual, 2018. Available at: http://www.oie.int/standard-setting/terrestrial-manual/access-online/

[26] Quilicot AMM, Horvatek Tomić D, Gottstein Ž, Lukač M, Prukner-Radovčić E. Comparison of sampling procedures for the detection of Chlamydiaceae in free-range chicken flocks. In: Book of Abstracts Veterinary Science and Profession (Zagreb). Croatia: Faculty of Veterinary Medicine University of Zagreb, 2017: 58.

[27] Dixon L, Diaz-Cano SJ, Schulte K-M. Psittacosis infection and tracheobronchomalacia in a patient undergoing thyroidectomy. BMJ Case Rep. 2011;2011:bcr0720114516.

[28] Aigelsreiter A, Gerlza T, Deutsch, AJA, et al. *Chlamydia psittaci* infection in nongastrointestinal MALT lymphomas and their precursor lesions. Am J Clin Pathol, 2011; 136: 480.

[29] Stinco G, Fabris M, Pasini E, et al. Detection of DNA of *Chlamydophila psittaci* in subjects with psoriasis: a casual or a causal link? Br J Dermatol, 2012; 67:926-928.

[30] Paul L, Comstock J, Edes K, Schlaberg R. Gestational Psittacosis Resulting in Neonatal Death Identified by Next-Generation RNA Sequencing of Postmortem, Formalin-Fixed Lung Tissue. Open Forum Infect Dis, 2018;5(8):ofy172.

[31] Chan J, Doyle B, Branley J, et al. An outbreak of psittacosis at a veterinary school demonstrating a novel source of infection. One Health, 2017; 3: 29-33.

[32] Mair-Jenkins J, Lamming T, Dziadosz A, et al. A Psittacosis Outbreak among English Office Workers with Little or No Contact with Birds. PLOS Currents Outbreaks, 2018;10:ecurrents.outbreaks.b646c3bb2b4f0e3397183f31823bbca6.

[33] Wallensten A, Fredlund H, Runehagen A. Multiple human-to-human transmission from a severe case of psittacosis, Sweden, January–February 2013. Euro Surveill, 2014; 19: 20937

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

[35] Ménard A, Clerc M, Subtil A, Mégraud F, Bébéar C, De Barbeyrac B. Development of a real-time PCR for the detection of Chlamydia psittaci. J Med Microbiol, 2006; 55: 471-473.

[36] Laroucau K, Aaziz R, Meurice L, et al. Outbreak of psittacosis in a group of women exposed to Chlamydia psittaci-infected chickens. Euro Surveill, 2015; 20: 21155.

[37] Quilicot AM, Prukner-Radovčić E. *Chlamydia gallinacea* in chickens. In: Book of Abstracts VI Mediterranean Poultry Summit (Torino). Italy: WPSA, 2018:96

[38] Petrovay F, Balla E. Two fatal cases of psittacosis caused by *Chlamydophilla psittaci*. J Med Microbiol, 2008; 57: 1296-1298.