Prevalence and Zoonotic Potential of Avian Chlamydia in Croatia

Pojavnost i zoonotski potencijal klamidija ptica u Hrvatskoj

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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.

Keywords:
Chlamydia sp. avian zoonosis Croatia

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 klinicama. 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 sistemskе ili kronične infekcije.

Materijali i metode: Prisutnost C. psittaci u pticama 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 uzorka 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. Nažalost klamidije kod različitih ptićih vrsta ukazuju na važnost pravilne provedbe zaštitnih mjera, uzimajući u obzir zoonotski potencijal klamidije koja potječe od ptica i peradi.
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 [7-10], Laroucau et al. [11] 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 pathoanatomical perspective, hepato- and splenomegaly are present, with fibrinous air sacculitis and peritonitis, while secondary infections aggravate clinical signs as well as pathoanatomical 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 Mini-prep 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% examined for the presence of *Chlamydiaceae* (Table 1).

**Table 1. Number of samples and the finding of *Chlamydiaceae* and *C. psittaci* in the period 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 Chlamydia originating from birds and poultry. Pathogen isolation 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 or other species than C. psittaci, whose pathogenicity in humans is rarely demonstrated. 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.

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