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Chlamydia psittaci infection in Polish walk-through commercial aviary

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Avian chlamydiosis in parrots is predominantly caused by genotype A of *Chlamydia (C.) psittaci* and has been described in different regions of the world. *C. psittaci* infections in birds may often transmit to humans. This report describes the unique case of *C. psittaci* occurrence in the multispecies walk-through commercial aviary situated in nonzoological garden conditions in the northern Poland, where birds had direct and constant contact with visitors. Furthermore, the effectiveness of the treatment was evaluated. Cloacal swabs were collected from parrots belonging to 3 bird families and 30 species at five time points: first survey (T_0; n=32), in two-week intervals during the treatment (T_1; n=42, T_2; n=42, T_3; n=53) and follow-up testing, six months after doxycycline administration (T_4; n=69). Based on 23S rRNA and *inc*A real-time PCR assays and sequencing of *omp*A gene, chlamydial agent was identified as genotype A of *C. psittaci* with overall percentage of parrots harbouring this pathogen at the first sampling at the level of 68.75. Monitoring survey in the aviary performed during six-week long doxycycline therapy and after administration of antibiotic confirmed the lack of pathogen shedding and proved effectiveness of the treatment. Described outbreak, to the best of authors knowledge, did not result in transmission to humans.

KEY WORDS: avian chlamydiosis / *C. psittaci* / doxycycline / parrots / treatment scheme

European colonization in the 18th century resulted in increased movement of animals including birds between continents. A large number of non-migratory parrot species were imported from Australia to private collections in United Kingdom, other European countries and the Americas. This was followed by reports of pneumonia

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cases in humans acquired from parrots [Vanrompay *et al.* 1995, Pospischil 2009]. *Chlamydia* as a causing agent of the disease, was described in 1930 after one of the most virulent outbreaks of chlamydiosis noted both in Europe and North America [Pospischil 2009]. Avian chlamydiosis had been reported in a variety of bird species including parrots in different parts of the world [Kaleta and Taday 2003]. Different *Chlamydia* species have been detected in Psittaciformes but the most common *Chlamydia* (*C.*) *psittaci* is currently classified into nine *omp*A genotypes and some provisional ones still growing in numbers. Each one has a certain host predilection: genotype A for parrots, B for pigeons, C for ducks and geese, D for turkeys, E for pigeons, ducks and other species, F for parakeets and E/B for ducks, turkeys and pigeons [Andersen 1991, Andersen 1997, Geens *et al*. 2005, Vanrompay *et al.* 1993]. Genotypes A is further divided into three subgroups: A-VS1, A-6BC and A-8455 [Beckmann *et al.* 2014].

Although only rare cases of *C. psittaci* have been recorded in humans in Poland in recent years [Anon. 2018], it could not be ruled out that human chlamydiosis is underdiagnosed or not reported properly. There is only one scientific report on shedding of *C. psittaci* in parrots from private aviaries and zoological shops in Poland [Piasecki *et al.* 2012]. The popularity of exotic parrot walk-through exhibitions has been increasing in recent years in the country. Worth noting is the fact that this kind of activity represents a new entertainment trend, completely independent from zoological gardens activity, thus not undergoing such a strict control. Taking into account that four chlamydia species, including *C. psittaci*, are known as threat for humans, supervising of avian breeding for presence of this zoonotic agent is important [Branley *et al.* 2016]. This study reports a case of *C. psittaci* infection in parrots from private walk-through commercial aviary in Poland. Research included molecular analysis which was performed to acquire data on *C. psittaci* strains causing the infection and designate genotype affiliation. Moreover, implemented treatment plan as well as assessment of its effectiveness is described.

Material and methods

Case presentation

The investigation was performed in the private walk-through commercial aviary localized in northern Poland. Altogether 106 birds were in the collection at the time of infection onset. Parrots belonging to 3 bird families and 30 species were tested during the study (Tab. 1). All birds were hand raised, had different country of origin (e. g. Germany, Czech, Poland) and were acquired in the age of 3-4 weeks. Each bird was quarantined at the time of the purchase and after general testing introduced to a group. Clinical investigation and interview with the owner did not reveal any indication of cause of the disease, as well as no clinical signs were observed and only death of five parrots was recorded. Therefore, birds were tested for presence of viral infection e.g. Borne Virus, alfaherpeswirus (PsHV) and Psittacine Beak and Feather Disease

Table 1 List of tested bird species

Cacatuidae	Psittaculidae
Cacatua	**Lorius**
Cacatua galerita	*Lorius chlorocercus*
Eolophus	*Lorius garrulus*
Eolophus roseicapillus	**Polytelis**
Nymphicus	*Polytelis swainsonii*
Nymphicus hollandicus	**Trichoglossus**
Probosciger	*Trichoglossus euteles*
Probosciger aterrimus	*Trichoglossus haematodus*
	Trichoglossus moluccanus
	Trichoglossus rubritorquis

Psittacidae	
Amazona	**Eclectus**
Amazona amazonica	*Eclectus roratus*
Amazona vinacea	**Pionus**
Ara	*Pionus chalcopterus*
Ara ararauna	**Poicephalus**
Ara chloroptera	*Poicephalus gulielmi*
Ara glaucogularis	**Primolius**
Ara macao	*Propyrrhura maracana*
Ara militaris	**Psittacula**
Ara severa	*Psittacula derbiana*
Aratinga	*Psittacula krameri*
Aratinga jandaya	**Psittacus**
Aratinga solstitialis	*Psittacus erithacus*
Cyanoliseus	**Pyrrhura**
Cyanoliseus patagonus	*Pyrrhura sp.*

Virus – (PBFDV) but all specific PCR's were negative. The tests for presence of virus infection were performed by commercial laboratory (personal communication with the owner). The investigation was continued and bacterial infection was suspected. Performed analysis led to diagnose of avian chlamydiosis.

Treatment protocol

The treatment with doxycycline has been implemented in all birds as follows: 100 mg/L doxycycline (Doxycycline 20% in powder, Interhemie, Netherlands) into drinking water was administrated for six weeks to most of the birds whereas hand fed ones were given medication dose with food daily. Doxycycline-medicated water was protected from light and calcium supplementation was withdrawn. Moreover, the biggest species of parrots were treated with intramuscular injections of doxycycline (Doxycycline hyclate IM) in dose of 100 mg/kg body weight every 5 days for the same period. Six months after the first treatment, preventive administration of doxycycline with drinking water was repeated.

Sample collection

Cloacal swabs were collected at five time points: screening survey (T_0; n=32), in two-week intervals during the treatment (T_1; n=42, T_2; n=42, T_3; n=53), follow-

up testing six months after antibiotic administration (T_4; n=69) and subjected to laboratory tests at the Reference Laboratory (RL) for chlamydiosis localized in the National Veterinary Research Institute in Pulawy, Poland. Twelve out of 32 birds tested at T_0 were chosen and individually analysed in every time point in order to assess the level of *C. psittaci* shedding (Table 2). At each time point, remaining swabs were sampled from randomly selected parrots present in the aviary. Additionally, the dead birds (n=5) were tested at the beginning of the investigation whereas T_4 sampling included all birds which were positive for chlamydia or might have contact with infected ones. Moreover, during six months following the first treatment, swabs from all new birds purchased for the aviary and random parrots from the current collection were subjected to *Chlamydiaceae* testing.

All samples were stored at -20˚C prior to DNA extraction. QIAamp DNA Mini Kit (Qiagen, Germany) was used for the DNA extraction according to the manufacturer's instructions. *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA gene fragment, as previously described by Ehricht *et al.* [2006], and *incA*-based *C. psittaci* real-time PCR by Menard *et al.* [2006] were used for diagnosis purposes. Panel of controls were included in each run. An analytical cut-off value of 38.5 for both tests was used. Sequencing of *ompA* gene from DNA samples with high amount and quality of DNA was performed with primer set CTU 5'-AT GAA AAA ACT CTT GAA ATC GG-3'/CTL5'- AA GAT TTT CTA GA(T/C) TTC AT(C/T) TTG-3' [Madani and Peighambari 2013]. PCR products were sent to Genomed (Poland) for sequencing and the data were analysed using Geneious Pro 8.0 software (Biomatters, New Zealand). Sequences were subjected to BLAST analysis against the GenBank database (NCBI). Dendrogram was constructed using Neighbor Joining (NJ) with the robustness of the clusters assessed by bootstrapping 1000 replicates. Sequences were deposited to the GenBank (NCBI) database under accession numbers MH507063 - MH507067.

Results and discussion

Avian chlamydiosis is a notifiable disease in Poland in accordance to national regulations [Anon. 2004]. Also human chlamydiosis cases should be reported in Poland and in the most other European countries as well as in the USA and Australia [Harkinezhad *et al*. 2009a]. There is no monitoring survey assessing the epidemiological situation of chlamydiosis in ornamental birds, including parrots, in Europe. There are no legal requirements for *C. pittaci* testing neither in birds kept in zoological gardens or aviaries nor in the ones imported to the country [Anon. 1997]. Thus, zoos that have a Balai number, test birds according to good veterinary practice. Furthermore, private holders of large aviaries perform the testing to prevent loses. Growing number of companion bird owners are also aware that testing for *C. psittaci* is important and it is usually done on post-purchase check up in the veterinary clinic. It should be noted that our recent reports on *Chlamydiaceae* prevalence revealed that *C. psittaci* is commonly occurring pathogen in wild birds in Poland [Szymańska-

Czerwińska *et al.* 2017a] and is also present in poultry [Szymańska-Czerwińska *et al.* 2017b]. However, data about chlamydiosis in our country, both in humans and ornamental birds, is very limited with only one scientific report considering *C. psittaci* shedding in asymptomatic parrots [Piasecki *et al.* 2012].

The outbreak of chlamydiosis in the aviary was confirmed by specific real-time PCR. *Chlamydiaceae* was found in 19/32 (59.38%) cloacal swabs from randomly selected parrots in the first sampling (T_0) with variable level of cloacal shedding ranging from Ct value of 21.61 to 38.01. All *Chlamydiaceae* positive samples were identified as *C. psittaci* (with Ct value 20.74 to 37.07). Further three samples, which gave Ct value above 38.5 (initially identified as negative result) in *Chlamydiaceae* real-time PCR, were positive in *inc*A assay which can be caused by higher specificity of the method. Therefore, final percentage of parrots harbouring *C. psittaci* at T_0 was 68.75% (22/32). Positive birds belonged to 3 families and 11 species: *Amazona* (*A.*) *amazonica*, *Ara* (*A.*) *ararauna*, *A. chloroptera*, *A. militaris*, *A. macao*, *A. glaucogularis*, *Cacatua galerita*, *Trichoglossus* (*T.*) *haematodus*, *T. euteles*, *T. moluccanus* and *Nymphicus* (*N.*) *hollandicus*. The cloacal swabs from dead birds (*Amazona* sp, *Poicephalus* (*P.*) *gulielmi*, *N. hollandicus*) were also *C. psittaci* positive with high level of shedding. After the first two weeks of doxycycline administration (T_1) only three parrots (out of 12 included in monitoring survey of *C. psittaci* shedding level) harboured *C. psittaci* and in two of them, *A. ararauna* and *N. hollandicus*, shedding was significantly lower than before, while in *A. amazonica* stayed on similar level (Tab. 2). The testing of cloacal swabs from the next time points (T_2, T_3 and T_4) did not show the presence of pathogens DNA. Moreover, ongoing survey also confirmed that *C. psittaci* infection has been eliminated in the aviary.

Table 2. Results summary for individual birds selected to monitor health status of private collection. T_0, T_1, T_2 and T_3 swabs were collected in two-week intervals, whereas T_4 samples were taken six months after T_3

Species	Time point				
	T_0 (Ct)	T_1 (Ct)	T_2	T_3	T_4
Amazona amazonica (Orange-winged amazon)	-	-	-	-	-
Amazona amazonica (Orange-winged amazon)	+(31.98)	+(32.81)	-	-	-
Ara ararauna (Blue-and-yellow macaw)	+(33.04)	+38.03)	-	-	-
Ara ararauna (Blue-and-yellow macaw)	+(33.72)	-	-	-	-
Ara chloropterus (Red-and-green macaw)	+(34.98)	-	-	-	-
Ara militaris (Military macaw)	+(33.55)	-	-	-	-
Nymphicus hollandicus (Cockatiel	-	-	-	-	-
Nymphicus hollandicus (Cockatiel)	+(25.64)	+(33.14)	-	-	-
Nymphicus hollandicus (Cockatiel)	+(36.57)	-	-	-	-
Nymphicus hollandicus (Cockatiel)	-	-	-	-	-
Nymphicus hollandicus (Cockatiel)	-	-	-	-	-
Nymphicus hollandicus (Cockatiel)	+(20.74)	-	-	-	-

It should be highlighted that *C. psittaci* was found in 3 out of 9 swabs from cockatiels (*N. hollandicus*) sampled at T_0 with the highest Ct value noted (20.74) in one of the samples. Moreover, 3 out of 5 dead birds were also *N. hollandicus*. This high level of shedding and fatal outcome in 3 cockatiels support hypothesis that they could be the source of infection. It should be noted that substantial number of positive results in species-specific assay at T_0 was recorded among *Ara* genus (11/13, 84.62%). There were also a few cases of *C. psittaci* detection confirmed in new birds before introduction to the aviary but these have not been included into collection in order to prevent reintroduction of pathogen.

Taking into account increasing popularity of exotic animals exhibitions as well as parrots being one of the most popular pets this report may be very useful to shed a light on zoonotic threat posed by *C. psittaci*. Our data and the paper published by Piasecki *et al.* [2012] revealing overall prevalence of this pathogen in Polish psittacine populations at the level of 10.3% may suggest that *C. psittaci* can be quite common in private collections of exotic birds in the country. The phenomenon of asymptomatic shedding in course of avian chlamydiosis is typical and was noted both in our investigation and also by other researchers [Greco *et al.* 2005; Piasecki *et al.* 2012]. Asymptomatic infection may have different reasons starting with low virulence of *C. psittaci* strains and ending with resistance of some bird species. Infected birds may excrete high level of bacteria without clinical signs for a long time if the conditions in which the birds are kept reduce stressful situations. Whereas sudden change of environmental conditions can cause the onset of diseases [van Buuren *et al*. 1994, Harkinezhad *et al*. 2009a]. In the described case, parrots had been introduced into the new rooms followed by opening of the aviary for public. The stress might trigger the intensive intracellular multiplication of the pathogen resulting in the sudden death of a few of the parrots.

Analysis confirmed the presence of *C. psittaci* in all positive samples but further investigation was conducted to acquire information weather the infection was monoclonal or was caused by various strains from different sources. *Omp*A sequence analysis revealed that all obtained amplicons (3 from T_0, 2 from T_1) were identical (935 bp) no matter different time of sampling and host species. BLAST analysis allowed to assign the sequence to genotype A (Fig. 1). This genotype seems to be the most common and usually associated with psittacine birds [Vanrompay *et al.* 1993]. Thus, our results are in agreement with the global predominance of genotype A in parrots, which is also often identified in humans [Heddema *et al.* 2006a; Heddema *et al.* 2006b; Wreghitt and Taylor 1988]. It should be noted that none of the workers, nor their families, who have contact with *C. psittaci*-positive birds from the aviary, had respiratory symptoms or other clinical signs typically occurring in humans in course of chlamydia infection. Also the serological response (commercial testing of human samples) was not detected in these individuals.

Chlamydiae are strictly intracellular bacteria and their eradication is usually difficult. Moreover, extracellular form can persist in the environment in feces, feathers,

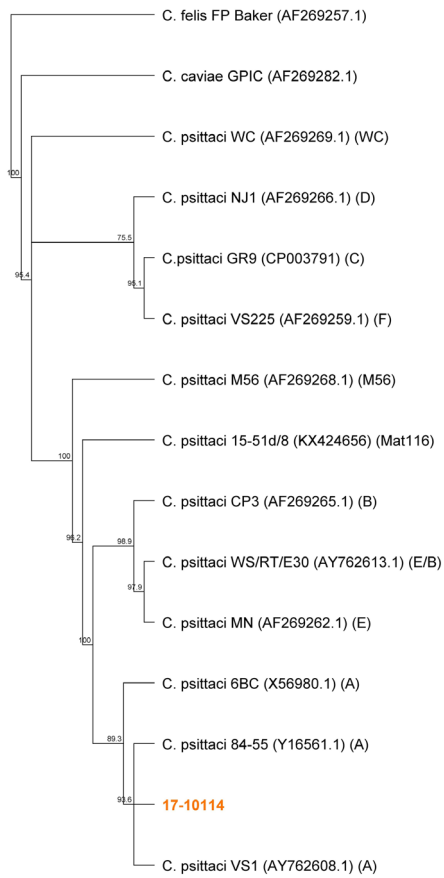

Fig 1. Dendrogram depicting position of *omp*A sequences obtained during the study (1013 bp alignment length). *C. felis* was used as an outgroup.

discharge from beaks and eyes causing re-infection [Harkinezhad *et al.* 2009a; Sillis and Longbottom 2011]. Tetracycline and doxycycline are usually drugs of choice with 45 days duration of the therapy to avoid relapses of the disease [Longbottom and Coulter 2003, Smith *et al.* 2005]. The percentage of *C. psittaci*-positive birds was 68.75 (T_0) at the diagnostic survey showing substantial spread in the collection. First 14 days of treatment significantly reduced the number of shedders. Nevertheless, detection of *C. psittaci* in three birds at T_1 confirms that short therapy is not effective enough. Thus, antibiotic administration was prolonged to 45 days as usually recommended. All tested birds were *Chlamydiaceae*-negative in consecutive samplings. Ongoing checks on randomly selected birds from the aviary during six months after outbreak were recommended as the precaution measure. Moreover, follow up two-week doxycycline therapy, 6 months after the main scheme, ended with all birds sampling. All obtained

samples gave consistent negative results confirming the effectiveness of treatment protocol used.

Presented data confirmed the case of avian chlamydiosis without clinical manifestation. Current report indicates that proper antimicrobial therapy and sufficient duration of the treatment allows high probability of pathogen eradication. Moreover, our results are important not only from the veterinary inspection point of view but mainly for breeders, pet owners and people having occasional contact with psittacine in households and during more and more popular walk-through exhibitions of exotic parrots. Many breeders and pet owners are still not aware of the threat posed by *C. psittaci* shedders. Furthermore, due to low availability of detection tools and difficult differential diagnosis, human chlamydiosis caused by this pathogen is still underdiagnosed [van Droogenbroeck *et al.* 2009, Harkinezhad *et al.* 2009b, Vanrompay *et al.* 2007]. Presence of genotype A, being one of the most virulent and often connected with human cases, reported in this paper confirms the need of overall monitoring survey implementation in aviaries, zoological gardens and private households.

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