

## New insight on chlamydiae

Monika Szymańska-Czerwińska<sup>1,2,✉</sup>, Kinga Zaręba-Marchewka<sup>1</sup>,  
Krzysztof Niemczuk<sup>1,2</sup>

<sup>1</sup>Department of Cattle and Sheep Diseases, <sup>2</sup>Laboratory of Serological Diagnosis,  
National Veterinary Research Institute, 24-100 Puławy, Poland  
monika.szymanska@piwet.pulawy.pl

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### Abstract

This article provides an overview of the current knowledge on chlamydiae, which are intracellular bacteria belonging to the *Chlamydiaceae* family. Whole-genome sequencing leads to great increases in the available data about *Chlamydia* spp. Recently, novel chlamydial taxons in various hosts living in different environments have been recognised. New species and taxons with *Candidatus* status have been recorded mainly in birds and reptiles. *Chlamydia gallinacea* is an emerging infectious agent in poultry with indirectly confirmed zoonotic potential. Recently, a new group of avian *C. abortus* strains with worldwide distribution in various wild bird families has been described. The definition of *C. abortus* species became outdated with the discovery of these strains and has been amended. It now includes two subgroups, mammalian and avian, the latter including all isolates hitherto referred to as atypical *C. psittaci* or *C. psittaci/C. abortus* intermediates.

**Keywords:** chlamydiae, novel taxons, chlamydiosis, zoonosis.

### Introduction

Chlamydiae (in the Chlamydiales order, *Chlamydiaceae* family, *Chlamydia* and *Chlamydiifrater* genera) are a group of obligate intracellular bacteria distinguished by a biphasic developmental cycle comprising extracellular and intracellular stages. *Chlamydiaceae* representatives have relatively small genomes of about 1 Mbp, implying the absence of essential cellular pathways and consequential reliance on host cells for nutrients including amino acids, nucleotides and lipids. In the course of evolution, chlamydial genomes have undergone vast condensation, which was proven to derive from genome streamlining rather than degradation (39).

Chlamydiae are reported in various hosts living in different environments, including livestock and companion animals as well as wild species (3, 22). Chlamydial species with their main hosts are presented in Table 1. It is commonly known that most chlamydial taxons are highly capable of host switching. The advent of molecular techniques has enabled the detection of novel chlamydial species in a variety of host species; therefore, the host barriers for known *Chlamydiaceae*

members may be less impenetrable than previously thought. Avian and mammalian subgroups should now be included (54) in an expanded version of the species definition of *C. abortus* given by Everett *et al.* (8), because a monitoring survey of wild birds including isolation and whole-genome sequencing (WGS) of avian *C. abortus* strains indicated this wider host range. Shedders of chlamydiae are widespread among wild animals, although the bacteria mostly inhabit those animals as commensal species without any negative impact on the host or sympatric species (44). However, *Chlamydiaceae* cause a number of important diseases both in animals and humans. Chlamydiosis can cause economic losses in livestock including ruminants (*e.g.* cattle or sheep) and poultry. *Chlamydia gallinacea*, as an emerging chlamydial agent, impacts poultry production parameters negatively, was also detected in cattle in China, and poses a risk to human health, which attracts the interest of researchers (10, 11, 12, 20, 28, 42, 43). Chlamydial infection is a potential risk to public health in wildlife hosts in addition to being one in farmed animals; the ongoing global process of habitat destruction as well as environmental and climate changes demand that urgent surveillance of chlamydial

infection in these hosts be undertaken to better understand this risk.

Animals that are often latently infected or shed chlamydiae intermittently over a longer period of time are particularly dangerous to human health. The zoonotic potential of some chlamydial species is well documented in the literature (e.g. *C. psittaci*, *C. abortus* or *C. pneumoniae*), while for novel species the potential is still unclear or not fully confirmed (6, 29). The occurrence of chlamydiosis in humans depends on multiple factors including the intensity of exposure, microbial factors and route of transmission (34). Human infections are underdiagnosed and many are unreported because there is a lack of specific serological tools for detection of novel species and there are limited appropriate specimens available for real-time PCR testing.

The purpose of this article is to review the most important novel developments in the field of animal chlamydiae research.

## Novel taxons

Access to molecular methods and whole-genome sequencing greatly increases the amount of data on record about chlamydiae. Bioinformatics tools order this

data and facilitate direct comparisons and evaluations of evolutionary relationships between different taxons and genotypes. As a result, the taxonomy of the *Chlamydiaceae* family has been constantly evolving. Since 1999 *Chlamydiaceae* has undergone a number of taxonomic reclassifications at the genus and species levels. The nomenclature has been under constant change in recent years. The genus *Chlamydia* was split into two genera, *Chlamydia* and *Chlamydophila*, based on analysis of the 16S and 23S rRNA genes (8). This change had been controversial for many years, until finally the chlamydial research community decided to restore the single genus, *Chlamydia*. Until recently, the *Chlamydiaceae* family was composed of this single genus, but it has now been extended to include a novel one (26, 36). Vorimore *et al.* (50) proved a new genus' affiliation based on ultrastructural analysis, comparison of 16S and 23S rRNA gene sequences, and whole-genome analysis of new strains isolated from flamingos. The researchers made the case for *Chlamydiifrater* gen. nov. belonging to the *Chlamydiaceae* family besides the *Chlamydia* genus proposed two new species, *Chlamydiifrater phoenicopteri* sp. nov. and *Chlamydiifrater volucris* sp. nov. New taxons were detected in the gastrointestinal tracts of flamingos in good health without clinical signs, and therefore they are considered commensal bacteria (50).

**Table 1.** The members of the family *Chlamydiaceae* and their main hosts

Family	Genus	Species	Host	Zoonotic potential
<i>Chlamydiaceae</i>	<i>Chlamydia</i>	mammalian <i>C. abortus</i>	cattle, small ruminants, human	proven (29, 32, 52, 53)
		avian <i>C. abortus</i>	wild birds	lack of evidence
		<i>C. avium</i>	pigeon, parrot	lack of evidence
		<i>C. buteonis</i>	raptor	lack of evidence
		<i>C. caviae</i>	guinea pig	limited evidence (33)
		<i>C. crocodili</i>	crocodile	lack of evidence
		<i>C. felis</i>	cat	limited evidence (4)
		<i>C. gallinacea</i>	poultry	indirect confirmation (24)
		<i>C. muridarum</i>	mouse, hamster	lack of evidence
		<i>C. pecorum</i>	cattle, small ruminants, pig, koala	lack of evidence
		<i>C. pneumoniae</i>	snake, frog, koala, horse, human	there is no direct evidence of zoonotic transmission (7, 16)
		<i>C. poikiloterma</i>	snake	lack of evidence
		<i>C. psittaci</i>	pigeon, parrot, human	proven – psittacosis (34)
		<i>C. serpentis</i>	snake	lack of evidence
		<i>C. suis</i>	pig	proven (47)
		<i>C. trachomatis</i>	human	lack of evidence
		<i>Cand. C. ibidis*</i>	sacred ibis	lack of evidence
		<i>Cand. C. corallus*</i>	snake	lack of evidence
		<i>Cand. C. sanzina*</i>	reptiles	lack of evidence
	<i>Cand. C. testudinis*</i>	tortoise	lack of evidence	
<i>Chlamydiifrater</i>	<i>Chlamydiifrater phoenicopteri</i>	flamingo	lack of evidence	
	<i>Chlamydiifrater volucris</i>	flamingo	lack of evidence	

\* *Candidatus* species – strain isolation not yet achieved

There are currently nine well-known species (*C. trachomatis*, *C. suis*, *C. muridarum*, *C. pneumoniae*, *C. abortus*, *C. caviae*, *C. felis*, *C. pecorum* and *C. psittaci*) and six new ones have been discovered in recent years. These new species have been recorded mainly in birds (*C. avium*, *C. gallinacea* and *C. buteonis*) and in snakes (*C. serpentis* and *C. poikilotherma* (previously named *C. poikilothermis*), one having been discovered in crocodiles (*C. crocodili*) (5, 19, 38, 40). Moreover, there are four new taxons with *Candidatus* status: *Cand. C. corallus*, and *Cand. C. sanzinia* were described in snakes, *Cand. C. testudinis* was detected in tortoises, and *Cand. C. ibidis* was reported in avian hosts (17, 25, 46, 51).

### Avian chlamydiosis

Discovering new chlamydial agents makes avian chlamydiosis (AC) much more complex than has been believed for decades. It transpires that not only *C. psittaci*, may be involved in the aetiology of AC in birds, overturning what was previously thought. Besides new avian taxons (*C. avium*, *C. gallinacea*, *C. buteonis* and *Cand. C. ibidis*), other well-known species primarily harboured by non-avian hosts (such as *C. abortus*, *C. pecorum*, *C. trachomatis*, *C. suis* and *C. muridarum*), might also be involved in AC (20). Recent research suggests that wild birds are common hosts for not only *C. psittaci* but also new avian taxons (e.g. *C. gallinacea*, *C. avium* or *C. buteonis*) (19, 38). Unfortunately, the impact of infection with these species on host health and condition remains unknown.

Recent reports on *C. gallinacea* shed new light on chlamydiosis in poultry and led to rejection of the hypothesis of the domination of a well-known species – *C. psittaci* – in poultry flocks. It has been proved that *C. gallinacea* is an emerging agent widespread in domestic and industrial poultry. Its predominant shedding in poultry was reported in Argentina, China, the Netherlands, Poland, the USA, Australia, Italy and Mexico (10, 13, 21, 28, 42, 45). Interestingly, it was also found in wild birds: the crimson rosella (*Platycercus elegans*) and galah (*Eolophus roseicapillus*) parrot species common in Australia (41, 42) and the woodcock (*Scolopax rusticola*) in South Korea (15). However, knowledge of the epidemiology of *C. gallinacea* in Psittaciformes is still limited. *C. gallinacea* is predominantly found in asymptomatic chickens but substantially lower body weight gains in experimentally infected SPF broilers and low mortality in embryonated eggs after yolk sac inoculation with *C. gallinacea* were noticed (10, 12, 43).

A phylogenetic intermediate position between *C. psittaci* and *C. abortus* is observed in another new taxon – *C. buteonis* – harboured by raptor birds and first isolated from a red-shouldered hawk (*Buteo lineatus*) in North America. *Chlamydia buteonis* was recovered from tissue of the carrier's conjunctiva and content of the

cloaca. This bacterium can cause conjunctivitis and/or respiratory signs or be a contributing factor to these clinical manifestations (19).

*Chlamydia psittaci*, a well-known causative agent of avian chlamydiosis and human psittacosis, is a genetically heterogeneous species. Sachse *et al.* (37) recently provided novel insights into the genetic diversity within this species. Based on analysis of whole-genome sequences of *C. psittaci* strains, the existence of four major clades (1–4) within this species was revealed. The authors reported that genomic divergence is connected with past host change and covers deletions in the plasticity zone, 3D structural variations in immunogenic domains of the outer membrane protein *ompA*, and various protein repertoires belonging to the Pmp and Inc families considered to include virulence factors. *Chlamydia psittaci* strains of clade 1 have limited genetic divergence and have been isolated both from avian (90% of all isolates being psittacine strains) and non-avian hosts, in the latter case as strains which were previously acquired from birds. This clade includes typical *C. psittaci* strains which are more virulent than strains of other clades. Clade 2 comprises only one psittacine strain, Mat116, and three strains of non-psittacine origin. Members of clade 3 present the most genetic divergence and have been isolated both from mammalian (such as sheep, cattle and human) and non-psittacine avian (duck strain) just as clade 4 does (having mainly pigeon strains). Both clades are differentiated by the loss of membrane attack complex/Perforin (MACPF) in the plasticity zone, as well as aberrant repertoires of Incs and Pmps (37).

### Extended definition of *C. abortus*

*Chlamydia abortus* has only been detected in mammals, and animals in this class can also be hosts to *C. psittaci*, although the latter bacterium was considered until recently to be the most common species recorded in avian hosts. In contrast to *C. psittaci*, the genome of *C. abortus* is considered to be relatively stable with very little diversity and to undergo little or no recombination. In recent years a number of *C. psittaci* strains that differ from the classical avian *C. psittaci* have been identified in various countries. Based on comparative sequence analysis and multi-locus sequence typing, it was suggested that these atypical *C. psittaci* strains are more closely related to *C. abortus* species (30, 48). Research on wild birds in Poland revealed the existence of avian isolates being *C. psittaci/C. abortus* intermediates with worldwide distribution in various wild bird families (*Anatidae*, *Corvidae* and *Rallidae*) (44). Genomic analysis performed by Zaręba-Marchewka *et al.* (54) in agreement with analyses conducted by Longbottom *et al.* (22) confirmed that strains demonstrating features characteristic of both *C. psittaci* and *C. abortus* are recent evolutionary ancestors of *C. abortus*. The effect of these findings was that avian isolates previously referred to as atypical

*C. psittaci* or *C. psittaci/C. abortus* intermediates were classified as a new group called avian *C. abortus*. Whole-genome sequencing and comparative genomic analyses indicate this new subgroup of *C. abortus* to comprise strains previously classified as belonging to genotype F of *C. psittaci*, including Prk/Daruma and 84/2334 strains (9, 49), as well as strains representing genotypes G1, G2 and 1V of avian *C. abortus* (23, 54). The taxonomic definition of *C. abortus* species became outdated in the light of this and was amended to include two subgroups, mammalian and avian (2). Avian *C. abortus* strains can be found in birds, mainly in waterfowl (genotypes G1 and G2), corvids (genotype 1V) and parrots (Prk/Daruma and 84/2334 strains) and can be isolated from the cloaca and oropharynx (44).

Avian *C. abortus* strains, in contrast to classical *C. abortus* isolates of mammal origin, carry a plasmid the structure of which is most similar to that of *C. psittaci* plasmids (23, 54). Further studies on avian *C. abortus* plasmids could influence the development of new vaccines against avian *C. abortus* and/or *C. psittaci* strains, as protein plasmids are being used in this field (31, 54). Contrary to the case of *C. psittaci*, there is no evidence of a relationship between shedding of avian *C. abortus* and clinical signs in avian hosts. A key region of chlamydial genomes associated with pathogenesis is the plasticity zone (PZ) (1, 27, 35). The size of the PZ ranges from 6 to 83 kilobase pairs depending on the chlamydial species (55). In most chlamydial species, the boundaries of this region are acetyl-CoA carboxylase (*accB*) and einosine-5'-monophosphate dehydrogenase (*guaB*) genes. However, avian *C. abortus* representatives as well as other *Chlamydia* species hosted by birds (*C. gallinacea*, *C. avium* and *C. buteonis*) have a lost or truncated *guaAB-add* operon (1, 27). The plasticity zone includes several virulence factors including cytotoxin genes, adherence factor, and the MACPF and phospholipase D enzymes. Considering the size, avian *C. abortus* strains have a rather reduced PZ in comparison to most *Chlamydia* spp., but a more extensive zone when compared to the classical *C. abortus* S26/3 with its structure most similar to *C. buteonis* RSHA and *C. psittaci* 6BC. Interestingly, avian *C. abortus* strains carry the cytotoxin gene which is absent in classical *C. abortus* S26/3. However, in contrast to *C. psittaci* 6BC and *C. buteonis* RSHA, the MACPF gene is absent from the avian *C. abortus* PZ, as it is from *C. abortus* S26/3 (54).

### Novel reptilian species

Beside birds, poikilothermic animals are also an ecological niche for *Chlamydiaceae*. Shedders of *Chlamydiaceae* have been observed among reptiles including tortoises belonging to the *Testudinidae* family. *Chlamydia pneumoniae* is presently considered the dominant species in reptiles and is reported in snakes and chameleons. Interestingly, the existence of a new

*Candidatus Chlamydia* species was recorded in tortoises in Italy, Poland and Germany (14, 25): a new chlamydial taxon closely related to *C. pecorum*. It could be widespread in tortoises showing clinical signs or free of signs; therefore, further investigations into its prevalence are needed. Three new taxons closely related to *C. pneumoniae* (*Cand. C. corallus*, *Cand. C. sanzina* and *C. serpentis*) and a new species related to *C. caviae* (*C. poikilotherma*) were recently recognised in snakes (40, 46). Another new species, *C. crocodili*, hosted by Siamese crocodiles (*Crocodylus siamensis*) is closely related to *C. poikilotherma* and *C. caviae*, and its prevalence in other crocodiles species cannot be excluded. Infections in crocodiles could be asymptomatic or lead to kyphoscoliosis in juveniles, conjunctivitis, pharyngitis, ascites, depression, anorexia and death (5). The zoonotic potential of these new reptilian species has not yet been evaluated.

### Zoonotic potential

Zoonotic transmission of a few chlamydial species (*C. psittaci*, *C. abortus* and *C. suis*) is well-proven, while for the others the evidence is limited (e.g. *C. felis* and *C. caviae*). Evidence is also lacking on the zoonotic potential of novel reptilian and avian species (excluding *C. gallinacea*) (Table 1). Regarding this species, its zoonotic potential was suspected for the first time after the occurrence of atypical pneumonia in poultry slaughterhouse workers in France; however, this potential has not been definitively confirmed in the years since that occurrence, mainly because no species-specific serological method has been developed for detection of the immune response in human samples (18). Recently, *C. gallinacea* was detected by Marchino *et al.* (24) in sputum samples from farm workers having contact with infected poultry. For the first time, at international level, the hypothesis of the transmission of *C. gallinacea* from birds to humans was partially confirmed as phenomenon not restricted to France. Contrasting results were published by Dutch scientists, who failed to detect *C. gallinacea* in throat swabs taken from humans exposed to *C. gallinacea*-excreting poultry (13). It should be highlighted that there is no relation between the detection of *C. gallinacea* DNA in sputum and the appearance of clinical signs in humans. Therefore, in order to confirm the possibility of zoonotic transmission, further research on and isolation of *C. gallinacea* strains directly from human specimens is needed.

*Chlamydia psittaci* is a well-documented zoonotic avian pathogen, but avian shedders might be a source of transmission also to other animal species. Transmission of *C. psittaci* from birds to horses has been documented. The most pathogenic *C. psittaci*, 6BC, can be transmitted from equines to humans, as reported in Australia as the cause of an outbreak of respiratory illness in veterinary school students who had been exposed to equine foetal membranes (3). The

mechanism of transmission between horses and humans is still not clear (34).

*Chlamydia pneumoniae*, a common chlamydial agent in humans, is also detected in animals (e.g. koalas); however, there are no reports of zoonotic cases. Genetic analyses of human *C. pneumoniae* isolates confirmed that they are likely to have evolved from animal strains. Therefore animal isolates might be zoonotic, but it also cannot be ruled out that humans might be able to infect animals. Taking into account the common prevalence of this bacterium in people, zoonotic transmission would be difficult to confirm. Transmission between animals and humans has not been documented for *C. muridarum* or *C. pecorum*.

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