MINI REVIEW

New and emerging chlamydial infections of creatures great and small

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Abstract

Until recently, our knowledge of the host range and diversity of members of the *Chlamydiaceae*, obligate intracellular bacterial pathogens of humans and animals, was thought to be nearly complete. Aided by advances in molecular diagnostics, a new picture is emerging, however, that the host barriers may be looser than previously thought for many chlamydial species. While cross-host transmission of chlamydial species is a concern for animal health, new reports highlight an emerging zoonotic risk for several species associated with intensification of farming and the widespread popularity of companion animals. The description of an expanded cohort of new species within this family from avian and reptilian hosts has also highlighted how much we still have to learn about the biology and pathogenicity of the *Chlamydiaceae* as a whole. Reports emerging about these relatives of the traditional chlamydial pathogens are matched by the continued identification of novel *Chlamydia*-related bacteria in the phylum *Chlamydiae,* providing evidence that many may be pathogenic to humans or animals and pose a zoonotic or vector-borne risk. The review examines the new hosts described for well-characterized chlamydial veterinary pathogens, emerging novel chlamydial species and the potential for these to cause disease in their respective hosts.

Keywords: *Chlamydia*, *Chlamydia*-related bacteria, chlamydiosis, disease, epidemiology, infection, pathogen, zoonosis
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Introduction

Bacteria in the family *Chlamydiaceae* are globally significant human and animal pathogens. Until recently, the *Chlamydiaceae,* the best-characterized family in the phylum *Chlamydiae,* comprised nine taxonomically recognized, well-defined species belonging to the genus *Chlamydia: C. trachomatis, C. muridarum, C. suis, C. psittaci, C. abortus, C. caviae, C. felis, C. pneumoniae* and *C. pecorum* [1]. This view has begun to rapidly change with the discovery and description of two novel chlamydial species and two *Candidatus* species in avian and reptile hosts: *C. avium, C. gallinacea* [2] and *Candidatus C. ibidis* [3] from domestic and wild birds and *Ca. C. sanzinia* from a captive snake [4]. Outside of this well-described family, wider sampling and advances in molecular methods have revealed a breadth of novel families within the phylum [5], collectively referred to as *Chlamydia*-related bacteria (CRBs) because of their phenotypic and genetic similarities but phylogenetic separation from the *Chlamydiaceae.*

The detection of emerging infectious diseases has been steadily increasing over the last 70 years and predominantly comprises zoonoses from wildlife [6], with wildlife species richness being a predictor for the emergence of zoonotic diseases with a wildlife origin [6]. The intensification of farming and widespread popularity of companion animals also present ongoing opportunities for emerging zoonoses [7]. Whilst improvements in surveillance and diagnostics have contributed to a wider recognition of the emergence of these pathogens [7], anthropogenic factors such as antimicrobial use, agricultural practices and human population density are drivers of emerging infectious diseases in general [6].

This review examines the new animal hosts described for well-characterized chlamydial veterinary pathogens, emerging novel chlamydial species and their potential to cause disease in humans, animals or both. We have limited our review to the

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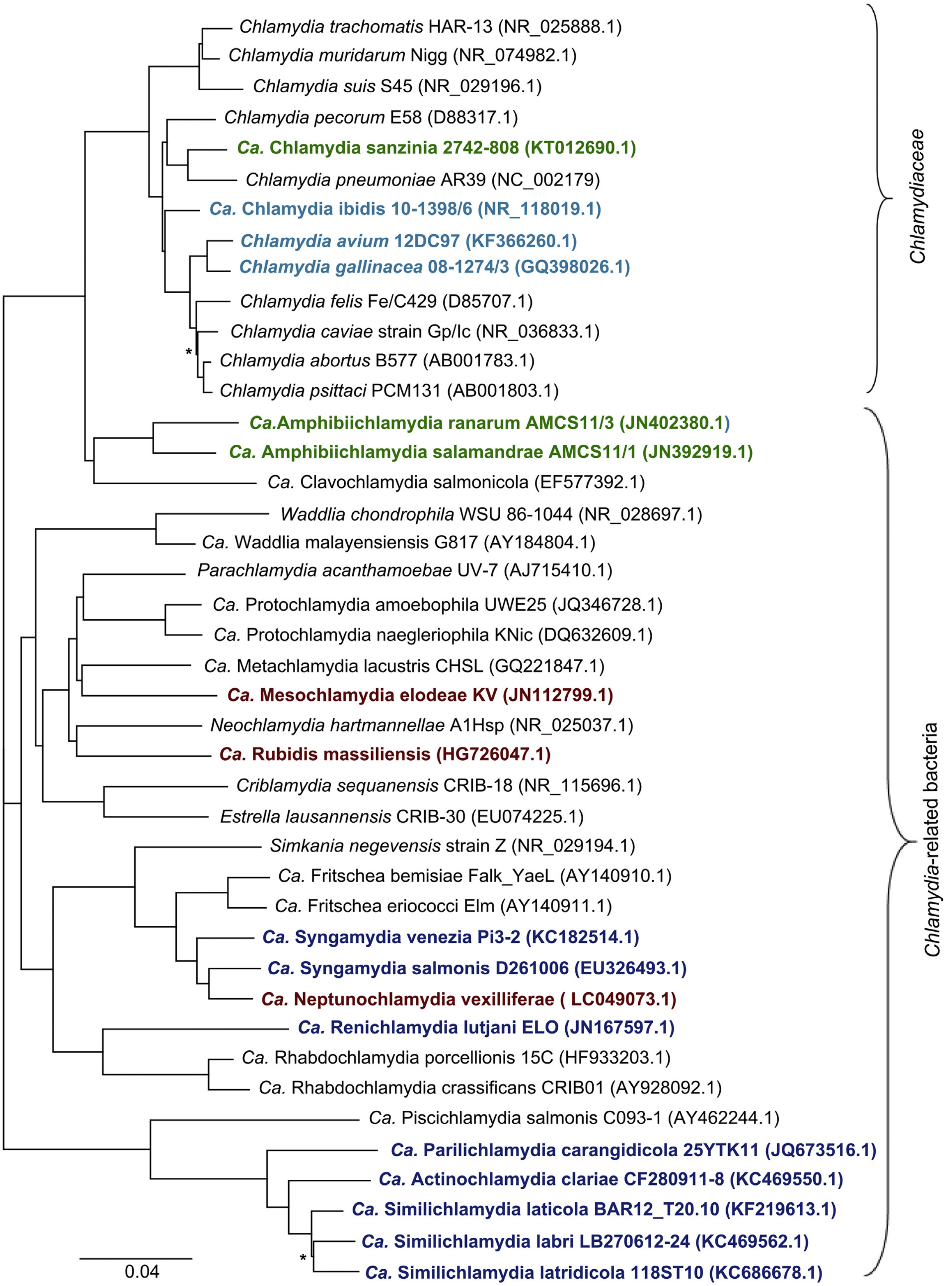
Chlamydia trachomatis HAR-13 (NR_025888.1)
Chlamydia muridarum Nigg (NR_074982.1)
Chlamydia suis S45 (NR_029196.1)
Chlamydia pecorum E58 (D88317.1)
***Ca.* Chlamydia sanzinia 2742-808 (KT012690.1)**
Chlamydia pneumoniae AR39 (NC_002179)
***Ca.* Chlamydia ibidis 10-1398/6 (NR_118019.1)**
***Chlamydia avium* 12DC97 (KF366260.1)**
***Chlamydia gallinacea* 08-1274/3 (GQ398026.1)**
Chlamydia felis Fe/C429 (D85707.1)
Chlamydia caviae strain Gp/Ic (NR_036833.1)
*
Chlamydia abortus B577 (AB001783.1)
Chlamydia psittaci PCM131 (AB001803.1)
Chlamydiaceae
***Ca.*Amphibiichlamydia ranarum AMCS11/3 (JN402380.1)**
***Ca.* Amphibiichlamydia salamandrae AMCS11/1 (JN392919.1)**
Ca. Clavochlamydia salmonicola (EF577392.1)
Waddlia chondrophila WSU 86-1044 (NR_028697.1)
Ca. Waddlia malayensiensis G817 (AY184804.1)
Parachlamydia acanthamoebae UV-7 (AJ715410.1)
Ca. Protochlamydia amoebophila UWE25 (JQ346728.1)
Ca. Protochlamydia naegleriophila KNic (DQ632609.1)
Ca. Metachlamydia lacustris CHSL (GQ221847.1)
***Ca.* Mesochlamydia elodeae KV (JN112799.1)**
Neochlamydia hartmannellae A1Hsp (NR_025037.1)
***Ca.* Rubidis massiliensis (HG726047.1)**
Criblamydia sequanensis CRIB-18 (NR_115696.1)
Estrella lausannensis CRIB-30 (EU074225.1)
Simkania negevensis strain Z (NR_029194.1)
Ca. Fritschea bemisiae Falk_YaeL (AY140910.1)
Ca. Fritschea eriococci Elm (AY140911.1)
***Ca.* Syngamydia venezia Pi3-2 (KC182514.1)**
***Ca.* Syngamydia salmonis D261006 (EU326493.1)**
***Ca.* Neptunochlamydia vexilliferae (LC049073.1)**
***Ca.* Renichlamydia lutjani ELO (JN167597.1)**
Ca. Rhabdochlamydia porcellionis 15C (HF933203.1)
Ca. Rhabdochlamydia crassificans CRIB01 (AY928092.1)
Ca. Piscichlamydia salmonis C093-1 (AY462244.1)
***Ca.* Parilichlamydia carangidicola 25YTK11 (JQ673516.1)**
***Ca.* Actinochlamydia clariae CF280911-8 (KC469550.1)**
***Ca.* Similichlamydia laticola BAR12_T20.10 (KF219613.1)**
***Ca.* Similichlamydia labri LB270612-24 (KC469562.1)**
*
***Ca.* Similichlamydia latridicola 118ST10 (KC686678.1)**
Chlamydia-related bacteria
0.04

last 4 years, since the isolation and description of *Ca. C. ibidis,* to highlight the recent and rapid changes in our understanding of the diversity of relationships between chlamydial species and their hosts.

Old *Chlamydia* Species, New Infections

A series of recent discoveries have cast doubts over our complete understanding of the natural host range and transmission networks of the established species described in the family *Chlamydiaceae*.

From a public health perspective, the most important of these new discoveries relate to *C. psittaci,* traditionally recognized as an avian pathogen and potential cause of zoonotic disease. Most human psittacosis cases, which manifest as atypical pneumonia, are confined to bird keepers, poultry workers and healthcare workers [8,9]. Human-to-human transmission appears possible but is exceptionally rare [10,11], while there is growing evidence for transmission from birds to other mammalian species such as livestock [12,13]. In some of these cases, farm environments possibly resulted in direct contact between birds and humans [13].

An interesting recent case of equine reproductive loss has placed a renewed spotlight on this pathogen [14]. In this case, the strain isolated from an abnormal horse placenta was most closely related to the *C. psittaci* sequence type 24/6BC clade, a clonally related and highly virulent cluster of strains thought to be shed primarily by infected parrots but also linked to indirect transmission to humans via environmental contamination [15]. Interestingly, while the human cases were not confirmed, contact with the infected reproductive material from the affected horse in this case was subsequently linked to a cluster of human psittacosis [14,15], highlighting a new potential route of transmission for this zoonotic agent. Historically, reports of *C. psittaci* infections in horses are incredibly rare, although there is some evidence that these infections may be an important underdiagnosed cause of equine reproductive disease [14]. These reports and others highlight the need for environmental monitoring to limit the exposure of people involved in agricultural practises and animal care and husbandry.

In terms of new zoonotic threats, there is also growing molecular evidence that *C. suis,* a chlamydial species endemic to pigs, can infect humans [12]. Recently, *C. suis* was detected in the air and on surfaces at a slaughterhouse in Belgium, where swabs from two human conjunctivae were also positive for this pathogen [16]. At the same time, a broad study of chlamydial agents in Nepal detected *C. suis* DNA in the eyes of patients with trachoma, a debilitating ocular disease traditionally linked to the human chlamydial pathogen *C. trachomatis* [17]. While the zoonotic potential of *C. suis* exposure is unclear, what is troubling is that *C. suis* is the only known chlamydial species to naturally harbour a tetracycline resistance gene cassette [18], which, in laboratory conditions, can be transferred from *C. suis* to *C. trachomatis* and *C. muridarum* by recombination [19], highlighting the potential threat that this veterinary pathogen may pose to human health.

The expansion of the host range of members of the *Chlamydiaceae* is not just limited to humans. The veterinary pathogen *C. pecorum* is linked to a range of diseases in cattle, sheep, pigs and goats, including polyarthritis, encephalomyelitis and conjunctivitis [20]. Rare cases of *C. pecorum*–associated abortion in ruminants have also been reported [21]. While this pathogen has been long recognized as a major threat to the survival of Australia's iconic native marsupial, the koala [22], recent molecular and serologic studies have shown other wildlife globally may harbour strains of this pathogen. These hosts include ibex, red deer, buffalo and wild boar [22]. The impact that these infections have is currently unclear, however [22]. Two studies from Argentina and Japan also suggest that *C. pecorum* may be present in low levels in passeriform, psittaform and columbrid birds [23,24], thus potentially representing one method by which *C. pecorum* is spread between such diverse hosts.

New *Chlamydia* Species, New Threats?

Advances in molecular methods, combined with improved sampling techniques and an expansion of animal studies, has continued to uncover novel diversity in the phylum *Chlamydiae,* including the identification of potential new chlamydial pathogens of humans and animals. Fig. 1 illustrates the novel species that have been identified in the last 4 years within the *Chlamydiaceae* family and more broadly across the phylum *Chlamydiae*.

Perhaps the biggest shakeup to the chlamydial field in the last few years was the discovery and description of three new taxa in avian hosts: *C. avium,* comprising strains from pigeons and psittacine birds [2]; *C. gallinacea,* comprising strains from poultry [2]; and *Ca. C. ibidis,* from the digestive tracts of feral

FIG. 1. 16S rRNA gene-based phylogenetic tree depicting evolutionary relationships of *Chlamydiales*. Species described in last 4 years are in bold type. Species descriptions during this time have been limited to reptilian and amphibian (green), avian (pale blue), piscine (dark blue) and protozoan (dark red) hosts. Sequences were downloaded from GenBank and aligned using MAFFT before tree construction using FastTree in Geneious v7.1.9 [36]. Asterisks denote branches with support values <50%.

ibises in France [3]. Although these novel species were only initially described recently in Europe, *C. avium* and *C. gallinacea* are now considered to be widespread in both European and Asian countries [25,26].

Interestingly, whilst endemic to poultry, *C. gallinacea* does not appear to be restricted to these hosts, with a recent study reporting its presence in 88.9% of *Chlamydia*-positive vaginal swabs from cattle throughout China [27]. The genotypes described were identical to poultry strains from a sympatric area, suggesting a potential cross-host transmission event and/or a genotype specific to this region. Adding to this list of novel avian chlamydial species, several novel unclassified chlamydial species were also reported in faecal specimens in wild seabirds such as penguins and gulls [28,29]. These taxa are closely related despite their geographically distinct locations.

Reptiles also appear to harbour an untapped diversity of chlamydia with the identification of a novel *Candidatus* species in a pet Madagascan tree boa in Switzerland (*Ca. C. sanzinia*) [4] closely related to *C. pneumoniae*. This sample originated from a broader study of chlamydial agents in captive snakes, with molecular evidence suggesting that additional novel *C. pneumoniae*–like strains may also be present [30]. In other studies, phylogenetic analysis of 16S rRNA and *ompA* sequences from lung, liver, spleen and brain tissues from crocodiles showed three genotypes within a novel clade related to *C. caviae* and *C. felis* [31]. A separate study identified a 55% prevalence of *Chlamydiaceae* in Australian crocodiles with conjunctivitis and/or pharyngitis syndrome, but no sequencing was conducted to further identify the agent or agents [32].

Lastly, a novel lineage has been described in female roe deer from France. The lineage comprises vaginal and faecal samples and branches close to *C. trachomatis* and *C. suis* [33]. The pathogenic potential is unknown, but these novel taxa highlight how little we know about emerging chlamydial infections in wild animals.

Beyond the family *Chlamydiaceae,* the identification of potentially pathogenic CRBs has been an ongoing trend for the last 20 years, since their first reports [5]. Probably the best studied of these is *Waddlia chondrophila,* which is considered to be an emerging threat to the reproductive health of humans and cattle [34]. *W. chondrophila* was first associated with pregnancy failure in a dairy herd by serology and was subsequently been detected in the vaginal swabs from cows that had aborted [34]. In the first step to conclusively evaluating the pathogenic potential of this agent in cattle, a recent study revealed that experimental infection with *W. chondrophila* failed to cause abortion in a similar way to the other chlamydial abortigenic agent, *C. abortus* [35]. While placental infection was observed, this only occurred in one of the animals challenged, with the conclusions that first, *W. chondrophila* infection is opportunistic, and second that a role in abortion remains to be confirmed. With questions over the role of this pathogen in animals, it is interesting to note that several studies have recently postulated that this agent may be zoonotic, with evidence of its presence in the placentas of women with adverse pregnancy outcomes [34].

The most convincing evidence for a pathogenic role in the CRBs comes from species in the recently described *Ca*. Parilichlamydiaceae family [37] (Fig. 1). These bacteria, as well as several other species in the families *Simkaniaceae, Ca*. Piscichlamydiaceae, and *Ca*. Clavochlamydiaceae, have been described in association with epitheliocystis, the common gill disease of wild and cultured fish [37]. Whilst this disease is by no means new, our understanding of the aetiologic agents has only been possible through advances in molecular methods. It may also be that the incidence of epitheliocystis may be on the rise as a result of increased aquaculture activity globally, including the introduction of new wild fish species into culture, to address the growing demand for sustainable food sources.

Recent studies in arthropods and other well-recognized vectors have offered a richness of emergent groups of chlamydial species. Ticks are prevalent vectors for other pathogens. Molecular studies in Europe, Africa and Australia have recently revealed that these arthropods also harbour CRBs [38–41]. Notably, the chlamydial species identified in these ticks primarily belonged to the *Ca*. Rhabdochlamydiaceae, *Parachlamydiaceae,* and *Simkaniaceae,* with *Ca*. Rhabdochlamydiaceae DNA being most abundant, suggesting that ticks are the natural hosts of the latter agents [41]. Although the pathogenic potential of these CRBs is unclear, a recent study examining skin biopsy samples from tick bites identified tick-related chlamydial sequences in the samples but not from samples taken from controls [39], highlighting the importance of studies to evaluate the role that these CRBs might play in human and animal health.

Along with arthropods, bats are well-characterized vectors for a plethora of human and animal pathogens. So far, two novel *Waddlia* species have been isolated from or detected in the urine [42] or tissue [43] of bats in Malaysia and Mexico, respectively. Building on a metagenomic study that identified *Chlamydiae* in bat faeces, a recent screening study reported both novel *Chlamydia* lineages as well as novel *Ca*. Rhabdochlamydiaceae members in bat droppings in Finland [44]. Interestingly, insects collected at feeding sites also harboured a range of *Chlamydiales,* but none were identical to the sequences from bats. The significance of these findings to human and animal health is still unclear.

Challenges and Future Directions

Reports describing the emergence of previously described chlamydial species in new hosts and novel chlamydial species have only been possible with advances in culture-independent molecular methods and diagnostics [45]. While these methods have played a significant role in expanding our understanding of chlamydial diversity and in detecting new and emerging chlamydial disease threats, the significance of detecting chlamydial DNA in specimens in the absence of other methods raises questions over whether these reports indicate exposure to these organisms rather than actual evidence of viable bacterial replication. Thus, discrepancies between detection methods must be addressed. In the absence of challenge studies, which are few and far between in recent years, serologic recognition of a specific chlamydial antigen or isolation of a viable organism would ideally enable differentiation between whether these detection events are representative of sporadic, endemic or epidemic infections and assist stakeholders in understanding the significance of these reports. Such studies are sorely needed but are also obviously challenging, given the range of confounding factors including the lack of commercially available reagents for nonmodel organisms and the logistical and economic challenges associated with the collection of the appropriate specimens for such studies.

On a positive note, while updating chlamydial taxonomy has not been without its challenges, the recent acceptance of a new taxonomic system for this group of intracellular pathogens [1], as well as the identification and use of an expanded gene set encompassing phylogenetic markers capable of differentiating taxa at the species, genus and family level [46], will support the efforts of researchers to describe new and emerging chlamydial pathogens. With the increasing ease and decreasing cost of culture-independent genome sequencing, it is anticipated that these methods will come into use more frequently for emerging chlamydial pathogens. However, culture of viable organisms and challenge experiments as that recently performed [2,3,36] will nevertheless remain the reference standard for assessing the true pathogenic potential of novel chlamydial infections.

Conflict of Interest

None declared.

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