

FROM MANY *CHLAMYDIAE* TO ONE HEALTH

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Introduction

The order *Chlamydiales* harbours members known for their relevance as human and animal pathogens. Until the 1990s it was thought that the *Chlamydiaceae*, represented as the only family of the order *Chlamydiales*, contained only one genus *Chlamydia* comprised of nine species. Research over the last 20 years, however, has resulted in the recognition and description or proposal of thirteen additional families of genetically related organisms collectively referred to as ‘*Chlamydia*-related bacterias’ (CRBs)(1). New research has also revealed that expanded taxonomic diversity even exists within the well described genus *Chlamydia* (2-4).

Many of the previously classified and newly described *Chlamydiae* are well recognized pathogens of humans and animals. While the zoonotic implications of many of these classified species is well known, recent research has uncovered new examples of their zoonotic potential either from existing known reservoirs or indeed new infection sources. Examples of this include the recent observation of zoonotic *Chlamydia caviae* presenting as severe community-acquired pneumonia (5) and the detection of *Chlamydia suis* in humans (6,7) exemplify the emergence of new risks and point to novel virulence properties for these microorganisms. Similarly the identification of novel *Chlamydia abortus* strains as zoonotic and abortive species in cattle and small ruminants (8) and the emerging role of *Chlamydia psittaci* in equine abortion (9), point both to the potential unrecognised impacts on animal health with significant potential for zoonotic risk. The zoonotic implications of novel members in several families of the order are presently largely unknown. The recent discovery of two new species (*Chlamydia gallinacea* and *Chlamydia avium*) in poultry and other birds but also in cattle (10), raises questions over their impact on animal health and the zoonotic risk they pose to humans given the well documented risk that other avian *Chlamydiae* (ie. *C. psittaci*) pose. The list of novel “environmental” species outside the *Chlamydiaceae* (1) continues to grow, with increasing evidence that the same species can be detected in both animals and humans. Together, these observations paint a picture that many chlamydial species are not restricted to one particular host, as was previously thought. Indeed, host species jump of *C. psittaci* has been suggested (11).

In this review, we highlight members of the phylum and discuss their zoonotic potential. Since the diversity and host range of known and novel species are expanding as well as their zoonotic potential, assessment of the exact risk nowadays obviously requires collaboration between veterinarians, human health clinicians as well as scientists in a ‘One Health’ setting. Here we will also describe the outline and results of such a platform.

Zoonotic infections caused by *Chlamydia abortus*

Zoonotic infections due to *C. abortus* and *C. psittaci* have been well-known for decades, causing abortion and psittacosis in humans, respectively. *C. abortus* is responsible for economic losses worldwide resulting from abortions, stillbirths and weak neonates in sheep

and goats and to a lesser extent in cattle, pigs, horses, wild ruminants and yaks (8,12). In pregnant ruminants, *C. abortus* initiates placental infection and inflammation resulting in abortion and massive shedding of the infectious organisms (13). Infectious elementary bodies are shed in the environment leading to massive contamination. This creates the potential for inhalation by humans exposed to *C. abortus*-infected animals. Pregnant women are particularly at risk with the potential to develop life-threatening *C. abortus* infections, resulting in septic abortion and stillbirth (14). Therefore, physicians and veterinarians should alert pregnant women to avoid exposure to small ruminants, particularly during the lambing and kidding period. Of note, *C. abortus*-induced abortions in women might remain undiagnosed as they are not routinely submitted to the laboratory for further investigations. Beyond the risk to pregnant women, a recent case of an atypical pneumonia due to *C. abortus* was reported in a laboratory worker involved in intranasal experimental *C. abortus* infections in sheep and in a farmer (15,16) highlighting the additional occupational risk posed by this bacteria.

Zoonotic infections caused by *Chlamydia psittaci*

Wild birds, pet birds and poultry are the major reservoir of *C. psittaci*. *C. psittaci* can be sub-clinical or result in respiratory, enteric and ocular signs. The outcome of infection is predicted to be influenced by the virulence of the infecting strains, the species, age and immune status of the bird hosts. Zoonotic transmission occurs through inhalation of an infectious aerosol or direct contact with contaminated feces or feathers. Humans at risk include bird owners, veterinarians, bird breeders and caretakers, pet shop staff, poultry, slaughterhouse and laboratory workers (17). Clinical signs in humans vary from inapparent infection to severe systemic disease with pneumonia, myocarditis and encephalitis. Human-to-human transmission is rare but has been reported recently in Sweden (18). Multidisciplinary approaches are needed to manage zoonotic events such as psittacosis cases in humans (19). A recent meta-analysis estimated that 1% of incident community-acquired pneumonia (CAP) cases in humans are caused by *C. psittaci* (20). In reality, diagnostic tests for *C. psittaci* are rarely performed when patients present with CAP and the common presumptive treatment for CAP often includes antibiotics which are not effective against *C. psittaci*. These factors impede the correct diagnosis of a *C. psittaci* infection in the patient and renders it impossible to trace the source of a human psittacosis case (20).

More recently, increased awareness of the zoonotic risk posed by this pathogen has arisen due to recent reports of *C. psittaci*-induced abortion cases in horses and zoonotic infections in New South Wales, Australia (21,22). An outbreak of five cases of human psittacosis in veterinary students and staff exposed to equine fetal membranes was reported in 2014. Involvement of a highly virulent *C. psittaci* strain of the 6BC lineage (genotype A), identical to lineages detected in Australian parrots, was demonstrated (23). Transmission via indirect contact, presumably via fecal environmental contamination from *C. psittaci*-infected parrots was suspected as infection source (21). Molecular typing of a *C. psittaci* strain obtained from another Australian equine abortion case showed close relationship to a strain detected in a dove (genotype B) and belonging to an evolutionary lineage of *C. psittaci* strains typically associated with infections in pigeons, globally (24). These recent findings highlight the existence of potential novel but unknown zoonotic sources, in this case *C. psittaci*-infected equine placental membranes. Students, veterinarians or stud staff have significant exposure to parturient horses and reproductive products (e.g. placental membranes) during their routine work. With the emergence of *C. psittaci* as an abortifacient agent in horses, a joint human and animal surveillance program in a sentinel horse-breeding region in Australia was initiated (9).

Zoonotic infections of *Chlamydia caviae*

C. caviae is an agent of infectious conjunctivitis in guinea pigs but its DNA has also been detected in swab samples of dogs, horses and rabbits (reviewed in (8)). Besides ocular signs, guinea pigs can also present with rhinitis or other respiratory signs and abortion. A previous case report in a guinea pig collection suggested *C. caviae* interspecies transmission (dog, cat) and zoonotic infection (owner with mild conjunctivitis) (25). More recently, three unrelated cases of zoonotic *C. caviae*-induced community-acquired pneumonia in otherwise healthy adults in their early 30s were described (5). All three patients suffered of severe respiratory failure after exposure to ill guinea pigs. They recovered after being treated with doxycycline. In one case, the transmission between the guinea pig and its owner was confirmed by analyzing sequences which were identical in the broncho-alveolar fluid from the patient and her guinea pig. Altogether, these recent case reports imply the transmission of *C. caviae* from guinea pigs to humans resulting in severe CAP. Physicians and veterinarians should be aware of the zoonotic potential of *C. caviae*, particularly as guinea pigs are very popular pets with frequent contact to children. This awareness is also important for researchers using *C. caviae* ocular and genital infection models to study human diseases.

Zoonotic infections of *Chlamydia felis*

C. felis is the agent of infectious conjunctivitis in cats and endemic worldwide. Prophylactic live-attenuated vaccines to protect cats against *C. felis* infections are available. The first case of a zoonotic infection was reported in 1969 (26). The cat owner presented with a follicular keratoconjunctivitis, his cat had a recent history of rhinitis and conjunctivitis. Cats might also remain persistently infected over a longer time period, maintaining an asymptomatic carrier status. Prolonged vaginal and rectal shedding in cats with conjunctivitis after experimental *C. felis* infection indicated that the genital and intestinal tract might be a reservoir for persistent infection (27). Such persistent infections might also represent a source of zoonotic transmission. Recent reports of zoonotic transmission from *C. felis*-infected cats are not prevalent in the literature but awareness of such infections is highly important in view of the recent case report in guinea pigs, the popularity of cats as pets and the occurrence of uncontrolled, non-vaccinated stray cat populations.

Zoonotic infections caused by *Chlamydia suis*

C. suis infects pigs and such infections have been associated with conjunctivitis, respiratory infections, reproductive disorders and enteritis (28). Under field conditions, however, chlamydial infections in pigs are commonly found in the intestine and are mostly associated with endemic subclinical infections. More importantly, *C. suis* is the only chlamydial species known to have naturally acquired genes coding for tetracycline resistance and it has been shown recently, that selective pressure appears to further promote resistance in fattening pig herds (29). Moreover, *C. suis* has a zoonotic potential with *C. suis* DNA detected in conjunctival swabs of Nepalese trachoma patients (30) and Belgian slaughterhouse workers (6,7). Isolation of *C. suis* from nasal, pharyngeal and stool samples taken from Belgian farmers was also reported recently (7), demonstrating the presence of replicating organisms and supporting the hypothesis of zoonotic transmission from pigs to humans. Further studies are needed to elucidate the role and pathogenic potential of such infections in humans, in particular in view of tetracycline-resistance gene transfer.

Zoonotic infections caused by *Chlamydia pneumoniae*

In humans, *C. pneumoniae*, an agent of respiratory infection, is nearly ubiquitous with high seropositivity rates in older populations. In animals, *C. pneumoniae* has a broad host range

with infections reported in horses, koalas and other Australian marsupials, frogs and reptiles. Associated diseases range from respiratory signs to granulomatous inflammation in inner organs (8). Phylogenetic comparisons between human and animal strains showed that animal isolates were more diverse than isolates of human origin, e.g. genes decayed in human isolates were intact in their corresponding animal homologues (31-33). Such findings might help to understand the zoonotic potential and evolutionary history of *C. pneumoniae*. While current reports describing confirmed zoonotic infections are lacking, the aforementioned observation led to the suggestion that *C. pneumoniae* animal strains are ancestral to human strains and that cross-host transmission from animals to humans may have occurred several times in the evolutionary history of this pathogen (33). We should also carefully observe the potential of zoonotic transmission of novel chlamydial isolates closely related to *C. pneumoniae* and recently detected in a variety of reptilian hosts such as snakes, turtles and tortoises (3,34-36).

Zoonotic potential of *Chlamydia avium* and *Chlamydia gallinacea*

C. avium and *C. gallinacea* are recently described avian *Chlamydiae* reported in European and Asian countries (2). Both novel species were also detected alongside *C. psittaci* in the same flock and even in the same bird. *C. avium* has been found in pigeons and psittacines which were either asymptomatic or had respiratory signs and/or diarrhea (2,37). The zoonotic potential of *C. avium* is unknown to date.

C. gallinacea has been detected in domestic poultry, guinea fowl, turkeys and ducks in European countries, China, Argentina and recently North America (38). It is thought to be an endemic organism in chicken with the ability to persist over time (39). There is a substantial level of genetic diversity among *C. gallinacea* strains (40). *C. gallinacea* is highly prevalent in chickens: a Dutch study (41) found *C. gallinacea* in 71 out of 151 layer farms whereas neither *C. psittaci* nor *C. avium* was present, 15% of backyard chicken in Italy were positive for *C. gallinacea* (42) and 12.4% of backyard poultry in Alabama, USA were also positive (38). Zoonotic potential of *C. gallinacea* has been suggested after an outbreak of atypical pneumonia in French slaughterhouse workers (43). In conclusion, *C. gallinacea* should be considered as a widespread chlamydial agent in poultry with zoonotic potential. Assessment of its pathogenicity, potential hosts and transmission efficiency warrants further investigations.

Zoonotic potential of *Chlamydia* related bacteria (CRB)

CRB or Chlamydia-like organisms (CLO) have been recently identified, isolated and described in hosts covering the whole animal kingdom (44). CRB play a pathogenic role in ruminant abortion (family *Waddliaceae* and *Parachlamydiaceae*, (45) as well as in epitheliocystis in fish, a common gill disease of aquaculture species (46). A zoonotic potential has been considered for *Waddlia chondrophila* and *Parachlamydia acanthamoebae*, as they have been found in placentas of women with adverse pregnancy outcomes such as miscarriage (47). CRB have also been linked to pneumonia and bronchiolitis in children and patients with community-acquired pneumonia possibly also zoonotically-acquired (48,49).

One Health

Human and animal health is closely interconnected by each other. Well-being in general is increasingly affected by global challenges such as malnutrition, emerging and endemic zoonotic diseases, antimicrobial resistance and climate change. A 'One Health' approach has been proposed to tackle the challenges through realizing and accepting that their complexity requires an inter-disciplinary approach. The term 'One Health' is used in many different contexts and by people with varying backgrounds. Here, but without claiming to present the one and only true interpretation, we summarize the term in the 'One Health Umbrella' (50).

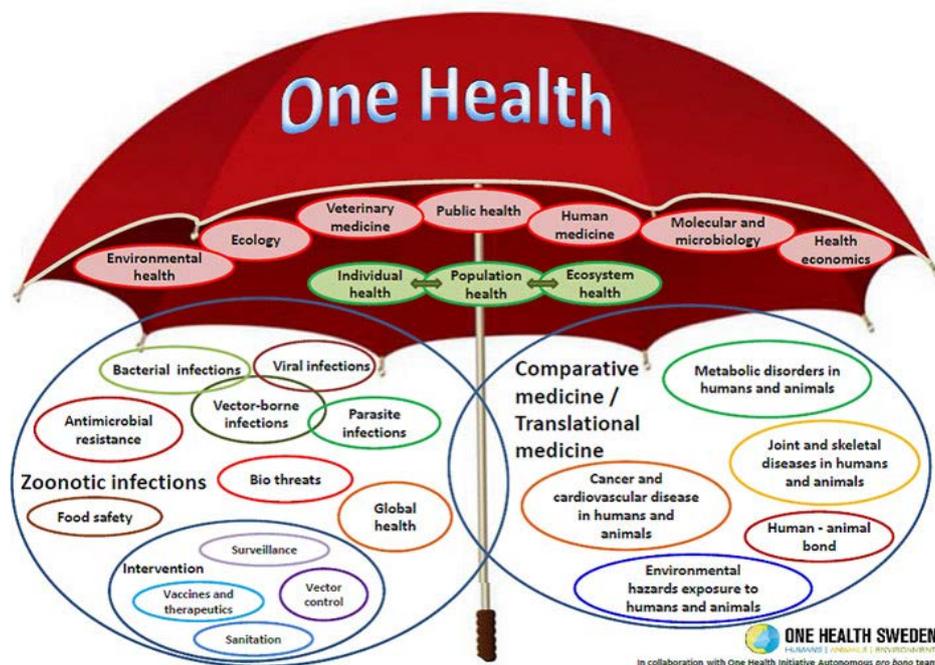


Figure 1. The ‘One Health Umbrella’ to illustrate the scope of the One Health concept. www.onehealthinitiative.com and (50)

How can the One Health approach serve to reduce disease burden caused by members of the phylum *Chlamydiae*?

In the Netherlands, psittacosis is a notifiable disease. The annual number of Dutch notified human psittacosis cases varies (n= 25-85) but it is generally accepted that this number is an underestimation. As already discussed, this is likely due to the fact that only in very severe cases of pneumonia diagnostics are carried out and even when patients are hospitalized, in 85% of the cases the causative agent of the pneumonia is not specified (51). Recent studies estimated approximately 750 annual human cases of psittacosis of which approximately 500 cases result in hospitalization and that ~1% of the cases the total number of 30,000 hospitalized cases of nationwide of community acquired pneumonia (CAP) are caused by *C. psittaci*. (20,52). The contribution of different bird species to human disease in the Netherlands is unknown. As the Dutch poultry sector represents about 100 million birds and high prevalence is mentioned in the literature, it is of importance to investigate the zoonotic potential of these livestock hosts (53).

Illustrating the potential seriousness of zoonotic outbreaks to public health, two large zoonotic outbreaks recently occurred (Avian Influenza and Q fever (54,55)) in the Netherlands. It was then exemplified that an adequate response to such outbreaks was hampered because systematic exchange of interdisciplinary data between human and veterinary domains was lacking (56). Given the zoonotic potential of the broader chlamydial diversity in the phylum *Chlamydiae* discussed in this review, and taking into account that the ‘cases’ published in the literature represent only the tip of the iceberg, it can be concluded that for a timely response to

zoonotic outbreaks caused by *Chlamydiae*, exchange of epidemiological, clinical and laboratory data between human and veterinary health chains is essential. Here we will highlight the approach used in the Netherlands to reduce the disease burden caused by *C. psittaci*.

Plat4m-2Bt-psittacosis: an integrated human-veterinary approach.

In the project ‘Plat4m-2Bt-psittacosis: an integrated human-veterinary approach’, a ‘One Health’ web-based framework that facilitates the exchange of epidemiological, clinical and molecular data from both human and animal cases of psittacosis was constructed. This platform is used to improve *C. psittaci* source tracking by infectious disease professionals and aims to reduce the disease burden of psittacosis. This project started in 2014 and is financed by The Netherlands Organization for Health Research and Development (ZonMw). In total 10 partners are participating of which five are from the human health domain and five from the animal health domain. The project is outlined in Fig. 2.

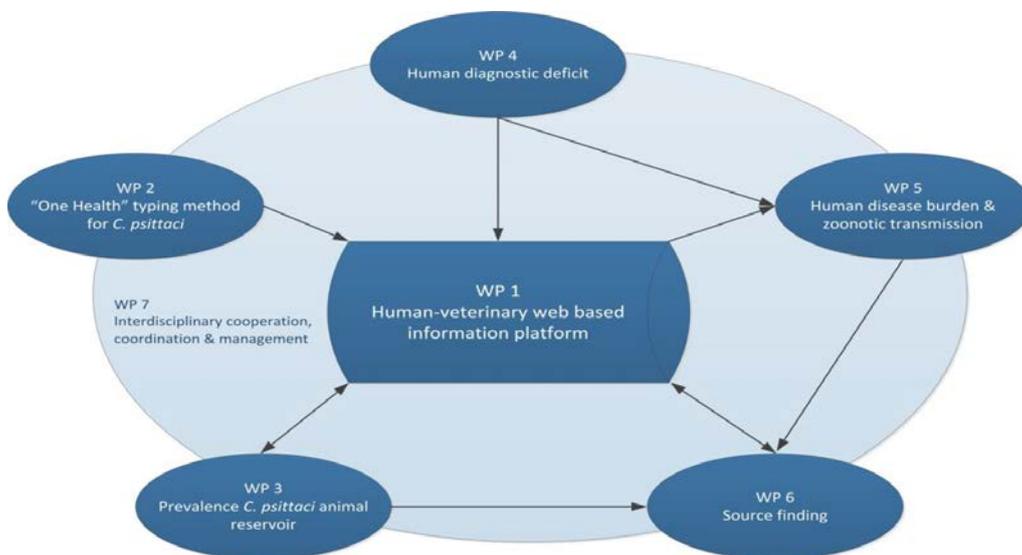


Figure 2. Organisation of ‘Plat4m-2Bt-psittacosis’. The project consists of seven work packages (WP). Each WP addresses a specific objective.

The core of the project is represented by the first objective in Working Package 1 (WP1): to develop an integrated human-veterinary web-based information platform for exchange of disease information and to facilitate location of infection sources. To facilitate the open exchange of data, an interdisciplinary cooperation between local and national infectious disease expert, clinicians, infectious disease control professionals and molecular biologists from the domains of human health and veterinary health had to be realized and disseminated (WP7). This process was very challenging because of the fact that personal data protection is much more advanced in the human medical field and the legal framework between human medical and veterinary domains. As a consequence of that, the completion of the consortium agreement took 3.5 years and hampered the project’s progress.

The second objective was to harmonise the *C. psittaci* molecular typing method for source-tracking. One single method for typing is now shared in both human and veterinary laboratories (WP2) (16). To support source tracking efforts, the prevalence of *C. psittaci* in

animal reservoirs was determined (WP3) (41) and a diagnostic kit for the detection of *C. psittaci* in human respiratory samples was distributed in medical microbiology laboratories (WP4). The human disease burden and zoonotic transmission are determined by mapping incidence and genotypes in humans and animals (WP5) (20,52,53). Lastly, a platform for source tracking was implemented (WP6).

As such, this 'One Health' approach has resulted in the harmonisation of molecular methods that enable sharing of data and facilitate source tracking between human and animal cases of psittacosis. In addition, this harmonised approach also enabled the detection of novel cases of zoonotic *C. caviae* pneumoniae and the first reports of *C. gallinacea* DNA on 71 farms in the Netherlands (5,41). Detailed research findings will be presented by H.J. Roest and M. Heijne in the session "Zoonosis and One Health" of this meeting.

Conclusions

The number of members of the phylum *Chlamydiae* is expanding, as well as our understanding of their zoonotic potential. Control of zoonoses caused by *Chlamydiae* requires an integrated interdisciplinary approach in which professionals from the domains of human health and veterinary health share methodology and data. The 'One Health' project 'Plat4m-2Bt-psittacosis' has shown to be a successful example of such an approach.

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