Curr Clin Micro Rpt (2015) 2:10–21
DOI 10.1007/s40588-014-0010-y

BACTERIOLOGY (N BOREL, SECTION EDITOR)

Avian Chlamydiosis

Konrad Sachse · Karine Laroucau · Daisy Vanrompay

Published online: 17 January 2015

Abstract Recent findings in research on avian chlamydiosis include an increase in the reported prevalence of *Chlamydia* (*C.*) *psittaci* in poultry flocks, detailed descriptions of molecular processes governing the course of infection in vivo, as well as the discovery of new chlamydial species. Here we review the major advances of the last 6 years. In particular, we suggest that the observed re-emergence of *C. psittaci* infections in domestic poultry are due to a reduction in the use of antibiotics and better diagnostic assays. Cellular and animal models have significantly contributed to improving our understanding of the pathogenesis, including the events leading to systemic disease. The elucidation of host–pathogen interactions revealed the efficiency of *C. psittaci* in proliferating and disseminating despite the action of pro-inflammatory mediators and other factors during host immune response. Finally, the recent introduction of *C. avium* and *C. gallinacea* sheds new light on the epidemiology and aetiopathology of avian chlamydiosis.

This article is part of the Topical Collection on *Bacteriology*

K. Sachse (✉)
Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Naumburger Str. 96a, Jena 07743, Germany
e-mail: konrad.sachse@fli.bund.de

K. Laroucau
ANSES, Animal Health Laboratory, Bacterial Zoonoses Unit, Paris-Est University, 23 avenue du général de Gaulle, 94706 Maisons-Alfort, France
e-mail: Karine.Laroucau@anses.fr

D. Vanrompay
Department of Molecular Biotechnology and Immunology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
e-mail: Daisy.Vanrompay@UGent.be

Keywords Avian chlamydiosis · Pathogenesis · Epidemiology · Aetiology · *Chlamydia psittaci* · *Chlamydia avium* · *Chlamydia gallinacea*

Introduction

Avian chlamydiosis, sometimes also referred to as psittacosis or ornithosis, is an important infectious disease of companion birds, especially psittacines, domestic poultry and wild birds. The infection usually becomes systemic and is occasionally fatal. Its main causative agent is the obligate intracellular Gram-negative bacterium *Chlamydia* (*C.*) *psittaci*. The infection is widespread and, due to carcass condemnation at slaughter, decrease in egg production, mortality and the expense of antibiotic treatment, represents a major factor of economic loss in birds commercially raised for meat and egg production [1], as well as posing a permanent risk for zoonotic transmission to man [2••].

Concerning the taxonomic classification of the family *Chlamydiaceae*, which now includes three members associated with avian hosts, it is important to note the recent return to the single genus *Chlamydia*. Based on clustering analyses of the *16S* and *23S* ribosomal RNA (rRNA) genes, Everett and colleagues [3] had proposed a subdivision of the former single genus *Chlamydia* into two genera: *Chlamydia* and *Chlamydophila*. However, this taxonomic separation proved consistent with neither the natural history of the organisms as revealed by genome comparisons nor with the largely similar morphology of all family members. Later on, comparative genome and proteome analysis of chlamydial species suggested that host-divergent strains of *Chlamydiaceae* are biologically and ecologically closely related. Apart from this, the

two-genus nomenclature was not widely used by the chlamydia research community. Formal efforts to reunite the members of *Chlamydiaceae* into a single genus *Chlamydia* began about 6 years ago [4, 5] and were finalised recently [6].

Aetiology and Epidemiology

The Causative Agent

As with any organism of the family *Chlamydiaceae*, *C. psittaci* undergoes a characteristic biphasic developmental cycle, during which it passes through three morphologically distinct forms termed elementary body (EB), reticulate body (RB) and intermediate body (IB) [7]. The EB is a small, electron-dense, spherical body, about 0.2–0.3 μm in diameter, which rivals mycoplasmas for the smallest of the prokaryotes. The electron-dense EB is the infectious form of the organism as it attaches to the target epithelial cell and gains entry. Inside the host cell, the EB expands in size to form the RB, i.e. the intracellular and metabolically active form. It is larger than the EB, measuring approximately 0.5–2.0 μm in diameter. The RBs divide by binary fission and thereafter re-transform into EBs. During this maturation, morphologically intermediate forms (IB) of 0.3–1.0 μm diameter with a central electron-dense core and radially arranged individual nucleoid fibres can be observed inside the host cell. An electron microscopy micrograph illustrating the morphology of chlamydial bodies is shown in Fig. 1.

In genetic and phenotypic terms, *C. psittaci* is a rather heterogeneous species. To reflect the variations among strains in host preference and virulence, genotypes based on outer membrane gene A (*ompA*) gene sequences are being used. Originally, nine *ompA* genotypes designated A to F, E/B, M56, and WC were introduced (reviewed in Harkinezhad et al. [8]). Later on, six additional genotypes found in psittacines and wild birds and designated 1V, 6N, Mat116, R54, YP84 and CPX0308 were proposed [9]. The classical genotypes A to F are known to naturally infect birds and are distinct from those isolated from chlamydiosis in mammals. Some avian genotypes appear to occur more often in a specific order of birds. Genotype A is endemic among psittacine birds and is considered to be highly virulent. Genotype B is endemic in pigeons and usually less virulent. Waterfowl most frequently seem to be infected with genotype C and E/B strains, while turkeys can harbour genotypes D and C [10]. In contrast, genotype E, also known as Cal-10, MP or MN, was first isolated during an outbreak of pneumonia in humans in the 1930s. Later on, genotype E isolates were obtained from a variety of bird species, including ducks, pigeons, ostriches and rheas. Genotype F is represented by the psittacine isolates VS225, Prk Daruma, 84/2334 and 10433-MA, but has also been isolated from turkeys [11]. The mammalian M56 and

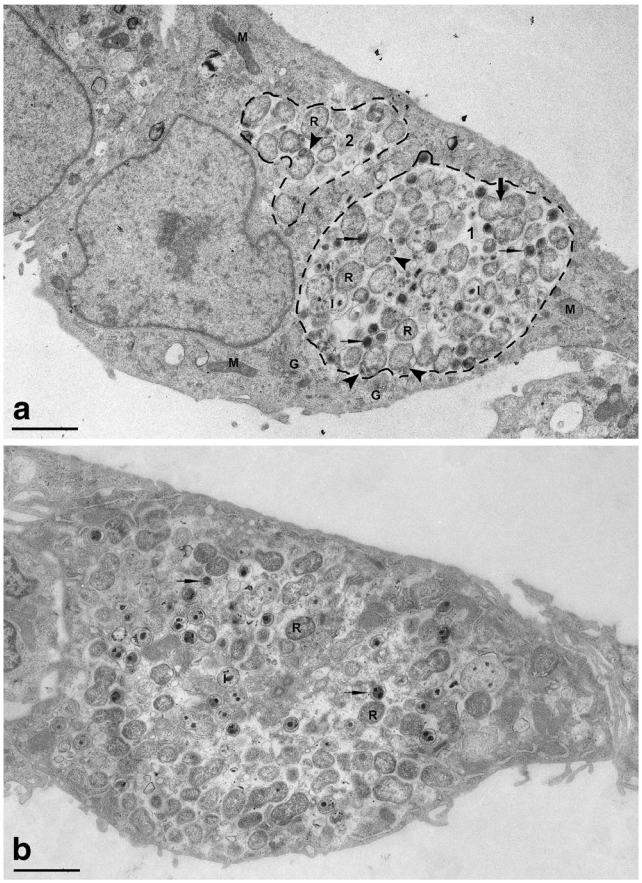

Fig. 1 Electron microscopic images of BGM cell culture infected with *Chlamydia* (*C.*) *avium* strain 10DC88^T (**a**) and *C. gallinacea* strain 08-1274/3^T (**b**). *Bar*=1.5 μm. **a** Two inclusions are depicted (1 and 2, indicated by *dashed lines*). Inclusion 1 contains a mixture of reticulate bodies (RBs) (R), elementary bodies (EBs) (*thin arrows*) and a few intermediate bodies (I). Inclusion 2 consists predominantly of RBs. Binary fission and budding events are marked with a *thick arrow* and *arrowheads*, respectively. Mitochondria (M) and stacks of Golgi membranes (G) are closely associated with the inclusion membrane. **b** The large inclusion contains predominantly RBs (R), many intermediate forms (I) and few EBs (*thin arrows*). Courtesy of Elsevier Ltd. http://dx.doi.org/10.1016/j.syapm.2013.12.004

WC genotypes were isolated from an outbreak in muskrats and hares, and an outbreak of enteritis in cattle, respectively. All genotypes should be considered to be readily transmissible to humans. The state of *C. psittaci* whole-genome analysis was discussed in a recent review [12••].

Prevalence

All over the world, at least 465 avian species were found to be infected with this zoonotic agent [13]. Among pet birds, *C. psittaci* is highly prevalent in *Psittacidae*, such as cockatoos, parrots, parakeets and lories (16–81 %), as well as in *Columbiformes* (12.5–95 %).

Studies on turkey farms, where *C. psittaci* is nearly endemic, indicate a pathogenic interplay between this agent and *Ornithobacterium* (*O.*) *rhinotracheale* [11]. However,

explosive and devastating outbreaks such as occurred in first half of the twentieth century are rare nowadays. Instead, reduced feed intake and respiratory signs with or without low mortality characterise current outbreaks. Chlamydiosis in domestic ducks has been reported to be of economic importance and represent a public health hazard in Europe, China and Australia [14–18]. Incidental to studies of chlamydiosis in ducks, several investigators observed *C. psittaci* antibodies and/or clinical signs in geese and isolated the agent from diseased tissues. Recently, *C. psittaci* was repeatedly detected in chickens, where genotypes B, C, D, F and E/B predominated [19–22]. It is possible that the reduction of antibiotic use in the chicken industry has contributed to this new development. Yin and co-workers [20], demonstrated the pathogenicity of chicken-derived genotype B and D strains for specific pathogen-free (SPF) chickens. As in turkeys, *C. psittaci* was often found in conjunction with *O. rhinotracheale*. Chlamydiosis has been reported in farmed quail, peacocks and partridges [10]. Clinical signs and lesions were similar to those seen in other birds. Morbidity and mortality can be very high, especially in young birds.

The organism also infects wild birds, such as feral pigeons. Thirty-eight studies on the seroprevalence of *C. psittaci* in feral pigeons conducted from 1966 to 2005 revealed a seropositivity ranging from 12.5 to 95.6 %. More recent studies performed in feral pigeons in Italy, Bosnia and Herzegovina, and Macedonia revealed a seropositivity of 48.5, 26.5 and 19.2 %, respectively (reviewed in Magnino et al. [23]). Free-living pigeons are distributed worldwide in urban and rural areas, where close contact with humans in public places is common. Known reservoirs of *C. psittaci* also include Canada geese [24], seagulls, ducks, herons, egrets, pigeons, blackbirds, grackles, house sparrows and killdeer, all of which may excrete the pathogen without being visibly affected [25–27].

Disease and Transmission Pathways in Birds

Depending on many factors on both the pathogen and host side, avian chlamydiosis can take markedly different courses, i.e. severe in the acute phase, sub-clinical or inapparent, and also chronic [10]. Clinical signs in affected birds are usually non-specific and include respiratory symptoms, conjunctivitis, coryza, mucopurulent discharge from nose and eyes, cough, dyspnoea or greenish to greyish faeces. In addition, apathy, weariness, sudden death, stunting, anorexia and cachexia can indicate an ongoing *C. psittaci* infection [13]. Latent infection is more frequent than acute cases and outbreaks, but the full significance of this state is still poorly understood. There are data from cattle showing that the subclinical carrier status can lead to recurrent clinical disease and chronicity, and, consequently, retarded development of infected animals [28]. In this context, intermittent shedding of carriers represents an important reservoir of infection for birds and humans.

For treatment of clinically ill birds, various tetracyclins and fluoroquinolones are used. There is no commercially available vaccine.

Transmission pathways and mechanisms have been reviewed by Harkinezhad and colleagues [8]. Bird-to-bird transmission of *C. psittaci* usually occurs when dried faecal droppings or eye and nostril secretions containing the organisms are aerosolised and inhaled by a susceptible host. Transmission of *C. psittaci* in the nest is possible. In many species, such as *Columbiformes*, cormorants, egrets and herons, transmission from parent to young may occur through feeding, by regurgitation, while contamination of the nesting site with infective exudates or faeces may be important in other species, such as snow geese, gulls and shorebirds. Furthermore, *C. psittaci* can be transmitted from bird to bird by blood-sucking ectoparasites such as lice, mites and flies or, less commonly, through bites or wounds. Transmission of *C. psittaci* by arthropod vectors would be facilitated in the nest environment. Mites from turkey nests can contain chlamydiae, and simulid flies were suspected as possible vectors of transfer during an epidemic in turkeys in South Carolina.

Vertical transmission has been demonstrated in turkeys, chickens, ducks, parakeets, seagulls and snow geese [29, 30] and could serve as a route to introduce chlamydiae into a poultry flock. In addition, *C. psittaci* can be carried into flocks by wild birds. As contaminated feed or equipment can also be a source of infection, feed should be protected from wild birds. Careful cleaning of equipment used in several barns during the same production round is extremely important because *C. psittaci* can survive in faeces and bedding for up to 30 days.

Transmission to Humans

The first description of a psittacosis outbreak was published in 1879 by Ritter [31], who associated the human disease with an ongoing outbreak in pet parrots and finches. Pandemic outbreaks of human psittacosis in Europe and North America were regularly reported until the 1930s, and all of them could be traced back to import shipments of infected psittacine birds from South America.

More recently, cases of human psittacosis due to contact with psittacines, wild birds, ducks, turkeys, chickens and meat pigeons were reported in several countries [17, 22, 32, 2••, 33].

Transmission of *C. psittaci* predominantly occurs through inhalation of contaminated aerosols from respiratory and ocular secretions or dried faeces from a diseased animal or asymptomatic carrier after petting infected companion birds, handling infected avian tissues in the slaughterhouse or as a result of exposure to *C. psittaci* in excretions, e.g. from cage bedding. Handling the plumage of infected birds as well as

mouth-to-beak contact or biting represent a zoonotic risk. In addition, activities such as gardening and mowing or trimming lawns without a grass catcher have been associated with cases of human psittacosis (reviewed in Beeckman and Vanrompay [2••]). Human-to-human transmission is rare, but recently a new case has been described in Sweden [34].

Populations most at risk include bird owners, pet shop employees, taxidermists, veterinarians and poultry workers [35]. The course of psittacosis may vary from asymptomatic to a flu-like syndrome and involvement of the respiratory tract. Most infected people show mild symptoms, while immunocompromised people are at highest risk of developing clinical signs. Severe complications such as myocarditis, endocarditis, pericarditis, encephalitis, hepatitis, reactive arthritis, multi-organ failure, renal insufficiency, premature birth or foetal death are rare. Typically, from 2001 onwards, about 400 cases were reported annually in Europe, with a few mortalities per year. However, these numbers are likely an underestimation of the true prevalence due to incomplete laboratory diagnosis or unreported cases.

Molecular Pathogenesis of *Chlamydia psittaci* Infections

The Early Stage of Infection

The ability of *C. psittaci* to cause systemic infection in different host organisms is certainly related to its capability of entering almost any cell type, from epithelial cells, fibroblasts and macrophages, to dendritic cells (DCs), etc., which is known from many in vitro studies. This versatility also suggests that chlamydiae probably have a variety of different mechanisms for host cell entry at their disposal.

Molecular processes underlying chlamydial entry and uptake are still poorly understood. It is thought that EBs of *C. psittaci* infect their target cells through attachment to the base of cell surface microvilli [36], where they are engulfed by endocytic or phagocytic vesicles [37]. Initial attachment is mediated by electrostatic interactions, and the host protein disulfide isomerase has been identified as being essential for both *C. psittaci* attachment and entry into cells [38]. Various hypotheses are based on microfilament-dependent phagocytosis, receptor-/clathrin-mediated endocytosis, or the use of cholesterol-rich lipid raft domains [39]. The participation of actin and tubulin seems to be required for optimal intracellular proliferation of chlamydiae [40].

Notably, the shut-down of chlamydial protein synthesis apparently has no effect on *C. psittaci* uptake, which means that it does not require protein factors synthesised by the bacterial cell [41]. However, the internalisation process depends on the functioning of the type III secretion system (T3SS) or injectisome. This sophisticated macromolecular apparatus enables the microorganism to export effector proteins to the inclusion membrane and into the cytosol, where they modulate certain host cell functions through interaction with host proteins [42, 43]. It appears that newly formed EBs carry a pre-loaded T3SS in order to ensure rapid entry and subversion of new host cells. While the chlamydial T3SS remains active throughout the intracellular stage [37] or possibly the whole infection cycle [44], interactions of chlamydial effectors with host proteins seem to play a role from adhesion and internalisation of EBs to their release from the host cell [42]. The macromolecular protein complex of the T3SS also enables translocation into the host cell of bacterial proteins from an extracellular location across the bacterial cell envelope [45], as well as secretion of pre-synthesised proteins from the cell-attached EBs [46, 47].

In a comprehensive study, Beeckman and colleagues showed that the T3SS of *C. psittaci* participates in creating an optimal environment for intracellular bacterial growth [36]. Their structural investigations demonstrated the association of the essential structural T3SS protein SctW with the bacterium and the inclusion membrane, as well as the localisation of SctC and SctN proteins at the bacterium itself. Monitoring messenger RNA (mRNA) expression revealed that structural protein-encoding genes are transcribed from mid-cycle onwards (12–18 hpi), whereas the genes encoding effector proteins and putative T3SS-related proteins are expressed early (1.5–8 hpi) or late (>24 hpi) in the developmental cycle. These new insights are essential in improving our understanding of molecular mechanisms during *Chlamydia* spp. infection. It seems certain that T3SS effectors are a promising subject for researchers in order to elucidate crucial phenomena, such as host cell damage inflicted by the pathogen and evasion of the host immune system.

Chlamydial Proteins Involved in Host–Pathogen Interaction

The molecular processes underlying the intracellular survival of *C. psittaci* are still the subject of intensive research [12••]. Among the key players that are involved in targeting vital cellular pathways of the host cell are the Inc proteins [48, 49]. Type III secretion of both IncA and IncB and their incorporation in the inclusion membrane during *C. psittaci* infection has been experimentally demonstrated [50]. As their hydrophilic domain protrudes into the cytoplasma and interacts with host proteins, Inc proteins could be regarded as central regulators of pathogen–host interactions [51]. Indeed, several eukaryotic proteins have been identified as interaction partners for Inc proteins. Two recent studies identified host cell protein G3BP1 and components of the dynein complex (dynein motor proteins) as cellular interaction partners of IncA and IncB, respectively, in *C. psittaci* infection [52, 53]. The authors concluded that the interaction of chlamydial IncA and host G3BP1 affects c-myc expression and results in suppression of

cellular proliferation and host cell apoptosis [52]. The IncB-protein of *C. psittaci* was suggested to recruit dynein motor proteins [54] in order to control intracellular transport and perinuclear localisation of inclusions, which would enhance bacterial growth in infected cells [53]. The recent data of Böcker and colleagues [53] also revealed that the host protein Snapin forms a hetero-oligomeric complex with IncB and dynein, which enables it to physically connect *C. psittaci* inclusions with the microtubule network in infected cells.

Recruitment of mitochondria seems to be a characteristic and possibly unique feature of *C. psittaci* infection [55, 56]. Such a close association, which has not been observed with *C. trachomatis* or *C. pneumoniae* [57], can be expected to enhance acquisition of eukaryotic adenosine triphosphate (ATP) and, therefore, influence chlamydial proliferation. *C. psittaci* also produces the translocated actin-recruiting phosphoprotein (Tarp) protein, another T3SS effector involved in entry and intracellular survival of chlamydiae. Expression of the gene occurs late in the developmental cycle [36]. Subsequently, released EBs transport pre-synthesised Tarp into another host cell, where it takes part in actin remodelling [58].

Intracellular Persistence

Chlamydiae are able to enter a reversible persistent stage in their infection cycle, where they remain viable but non-cultivable. The morphology of this state is characterised by inclusions of reduced size that are filled with the so-called aberrant bodies, i.e. enlarged RBs. The somewhat indiscriminate use of the term persistence by certain chlamydiologists recently prompted statements of clarification in the literature. To avoid misunderstandings, the term "aberrant RB phenotype" rather than "persistence" has been proposed to refer to the phenomenon in vitro [59, 60].

Interestingly, the first experimental investigations on this subject were conducted with *C. psittaci* in the 1980s, but the vast majority of the more recent molecular studies focused on *C. trachomatis*. A number of physiologically relevant cell culture models were characterised in detail, for instance based on interferon (IFN)-γ-induced persistence (tryptophan depletion) [61], amino acid deficiency [62], iron depletion [63], exposure to antibiotics [64] and phage infection [65]. Even in the absence of inducers, spontaneous formation of aberrant inclusions and chlamydial bodies was also observed during long-term continuous infection of HEp-2 cells with *C. pneumoniae* [66]. Borel and colleagues described an in vitro model of dual infection with porcine epidemic diarrhoea virus (PEDV) and *C. abortus* or *C. pecorum* [67], in which they observed an ongoing chlamydial infection being arrested and accompanied by formation of aberrant bodies after inoculation with cell culture-adapted PEDV. The effect was most pronounced in the case of *C. pecorum* infection.

In vitro persistence of *C. psittaci* was studied in three different cell culture models, i.e. iron depletion, antibiotic treatment and IFN-γ exposure [68]. As expected, the phenotypical characteristics were the same as in *C. trachomatis* and *C. pneumoniae*, i.e. aberrant morphology of RBs, loss of cultivability and rescue of infectivity upon removal of inducers. In contrast, the response of *C. psittaci* to induced persistence at the transcriptional level was remarkably different. One of the general features observed was a consistent down-regulation of genes encoding membrane proteins, transcription regulators, cell division factors and EB–RB differentiation factors from 24 hpi onwards. Other genes showed variations in mRNA expression patterns depending on the induction mechanism, which implies that there is no persistence model per se. Compared with *C. trachomatis*, late shutdown of essential genes in *C. psittaci* was much more comprehensive with IFN-γ-induced persistence, which can be explained by the absence of a functional tryptophan synthesis operon in the latter [68]. Another distinctive feature of the IFN-γ model was the observed down-regulation of the chlamydia protein associating with death domains (*CADD*) gene at 48 hpi in *C. psittaci*. In *C. trachomatis*, the same gene was up-regulated at 48 hpi [69]. The CADD protein shares homology with the death domains of tumour necrosis factor family receptors and is known to induce apoptosis [70]. However, it has yet to be established whether these in vitro findings actually relate to latent, persistent or chronic infections in humans and animals.

In addition to numerous papers on in vitro persistence of chlamydiae, a few reports from in vivo studies also showed enlarged chlamydial bodies of *C. muridarum* [71], *C. suis* [72] and *C. pneumoniae* [73] in infected tissue. However, it is not clear whether these observations are due to induced persistence or merely illustrate that chlamydiae were stressed during infection. No observations reminding of aberrant morphology were made in the *C. psittaci* animal infection models discussed below. In any case, a cause–effect relationship between host response to infection and aberrant chlamydial bodies has yet to be demonstrated.

New Insights into Host Immune Response to Chlamydial Infection

C. psittaci seems to be particularly efficient in escaping from the innate immune response of the host. This conclusion was drawn from the data of an experimental study by Braukmann and colleagues [74] comparing *C. psittaci* and *C. abortus* infection in embryonated chicken eggs (see also the "Lessons Learned from Animal Models" section). When confronted with the release of pro-inflammatory mediators during early host response, the pathogen was shown to react with up-regulation of essential genes [74]. This included elevated mRNA expression rates of chlamydial IncA

(involved in stabilisation of the inclusion), *ftsW* (regulating binary fission of RBs), *groEL* (chaperone associating with macrophages) and *cpaf* (involved in processing of host proteins controlling the integrity of the inclusion). This particular response, which may include further chlamydial factors, probably enables *C. psittaci* to establish the infection and disseminate in the host organism. In contrast, the closely related *C. abortus* was unable to up-regulate the genes mentioned in the same experimental setting and, consequently, proliferated less intensely and disseminated to a lesser extent in the host organism than *C. psittaci*. These findings have been confirmed in analogous comparative infection experiments in young chicks [75].

The complement system is considered to be one of the crucial factors of innate immunity. This panel of approximately 40 serum factors is activated by surface components of the pathogen and is involved in modulation of the inflammatory response and protection from extracellular agents [76]. A recent study using a mouse model of pulmonary *C. psittaci* infection revealed early, high and long-lasting activation of the complement system [77]. Further experiments in C3aR-deficient mice suggested that the protective function of the complement cascade against *C. psittaci* was dependent on the anaphylatoxic peptide C3a and its receptor C3a/C3aR [78].

In this context, it is relevant to note that C3a/C3aR can also activate DCs, which would facilitate their migration to draining lymph nodes and enhance presentation of chlamydial antigens to $CD4^+$ and $CD8^+$ T cells [56].

In a recent study in a mouse model, *C. psittaci*-infected murine DCs were shown to use autophagosomal and endovacuolar processing for degradation of bacterial compartments, as well as proteolytic production of chlamydial peptide antigens [79]. It has been suggested that these findings could be used for the design of vaccines based on DC-targeting antigens [12••]. A more detailed discussion of recent advances in the elucidation of host immune response to *C. psittaci* infection can be found in the review by Knittler and colleagues [56].

Lessons Learned from Animal Models

While in vitro models of infection can be very helpful in identifying individual factors and elucidating their involvement in pathogenesis, the complexity of multiple interactions between host and pathogen, as encountered in the natural infection, can be better emulated in animal models. Therefore, experimental studies in animals have the potential to generate novel insights and improve our understanding of the processes occurring in the natural host or, in the case of zoonoses, in humans.

Recently, the SPF chicken and the chicken embryo models were used to study the pathogenicity of different *C. psittaci* strains and compare *C. psittaci* and *C. abortus* infections [74, 20, 80]. Both models represent versatile tools for characterising chlamydial strains and species in terms of invasiveness, virulence and elicited immune response (see also the "New Insights into Host Immune Response to Chlamydial Infection" section).

The in ovo model has a far greater potential than serving as culture medium for intracellular bacteria and viruses. An experimental protocol starting with inoculation of *Chlamydia* spp. onto the chorioallantoic membrane (CAM) resembles natural infection across epithelial layers. As shown in a recent study, closely monitoring the course of infection allows both investigation of the innate immune response to the chlamydial challenge and identification of molecular processes on the chlamydial side. Braukmann and colleagues [74] highlighted several aspects of host–pathogen interaction in a comparative study on *C. psittaci* and *C. abortus* infection.

Kalmar and colleagues comparatively investigated pathology and host immune response, as well as systemic dissemination and expression of essential chlamydial genes in experimental aerogeneous infection with *C. psittaci* and *C. abortus*, in SPF chicks [75]. They observed that clinical symptoms appeared sooner and were more severe in the *C. psittaci*-infected group. *C. psittaci* disseminated more efficiently in the host organism than *C. abortus*, which was in line with higher and faster infiltration of immune cells by the former, as well as more macroscopic lesions and epithelial pathology. Monitoring mRNA expression rates of immunologically relevant factors in thoracic air sac tissue revealed that IFN-γ, interleukin (IL)-1β, IL-6, IL-17, IL-22, lipopolysaccharide-induced tumour necrosis factor (LITAF) and inducible nitric oxide synthase (iNOS) were significantly stronger up-regulated in *C. psittaci*- infected birds between 3 and 14 days post-infection. At the same time, transcription rates of the chlamydial genes *groEL*, *cpaf* and *ftsW* were consistently higher in *C. psittaci* during the acute phase. These findings were in accordance with the data from the in ovo study [74] and confirm the capacity of *C. psittaci* to evade the immune response of the avian host more efficiently than other *Chlamydia* spp.

A number of studies in a calf model have significantly improved our understanding of the course of systemic *C. psittaci* infection and the pathology in the host organism. Especially with the human infection in mind, the bovine host as a model offers a number of advantages over mice. For instance, the segmental anatomy and lack of collateral airways in the bovine lung facilitate the study of pathophysiological mechanisms of pulmonary dysfunctions. Validated non-invasive lung function tests are available, and the body size of calves allows repeated sampling and thorough monitoring of clinical and immunological parameters [80]. In a series of infection trials, Reinhold and colleagues were able to demonstrate four

essential characteristics of *C. psittaci* infection in its various manifestations [81–84]:

1. The severity of clinical signs during the acute phase of infection directly depended on the inoculation dose. Administration of 10^6 inclusion-forming units (ifu) of *C. psittaci* strain DC15 per calf caused mild respiratory and clinical signs, while doses of 10^7 to 10^8 ifu led to a moderate and 10^9 ifu to a severe course. This correlation of dose and response proved reproducible in extent and quality of lung lesions and also corresponded to deteriorations of respiratory functions [81, 84].
2. The bovine model reflected characteristic features of natural chlamydial infections in animals and humans. A typical course included acute clinical illness in the initial phase (2–3 dpi), which subsided considerably, but not completely, until 10 dpi [82]. The next stage was characterised by a protracted clinically silent course, which included intermittent mild symptoms, faecal pathogen excretion, transient chlamydaemia and slightly elevated levels of monocytes and lipopolysaccharide-binding protein in blood. Interestingly, these features were also observed in sentinel calves that socialised with clinically diseased animals and naturally acquired the infection.
3. The humoral immune response was generally weak. Only two-thirds of the calves experimentally challenged with a high dose developed specific antibodies against *C. psittaci*, which became detectable between 7 and 14 dpi. This supports the notion that the cellular rather than humoral immune response plays a central role in controlling anti-chlamydial immunity in infected hosts.
4. In the acute phase of respiratory disease, inflammatory cells were recruited to the site of *C. psittaci* infection. Damage of the alveolar–capillary barrier caused by pulmonary inflammation manifested itself by altered cytology, as well as elevated concentrations of eicosanoids and total protein in broncho-alveolar lavage fluid [81]. The inflammation ultimately caused ventilatory disorders and inhibited pulmonary gas exchange [83, 84].

Implications of the Discovery of *C. avium, C. gallinacea* and *C. ibidis*: New Agents of Avian Chlamydiosis?

More Avian Chlamydia *spp. Defined*

Until very recently, *C. psittaci* was considered to be the sole causative agent of the disease. In the light of new evidence suggesting that avian chlamydiosis may involve more chlamydial agents, this paradigm is likely to change. In the past decade, diagnostic investigations of *Chlamydia* spp. infection in birds in Germany, France and Italy produced a number of unclear findings, as the chlamydial agent appeared to be different from *C. psittaci* and the other eight established species of the family *Chlamydiaceae*. These atypical strains were identified in poultry, pigeons, ibis and psittacine birds. The use in routine diagnosis of broad-range diagnostic assays for *Chlamydiaceae* in combination with species-specific detection tools was an important prerequisite for these discoveries. The preconceived idea of avian chlamydiosis being due to *C. psittaci* alone is probably one of the reasons why the atypical strains were not discovered earlier. Another aspect is that these new chlamydiae can easily be missed in cell culture due to slow and often reluctant growth in comparison to *C. psittaci*.

In 2005, *Chlamydiaceae*-positive but *C. psittaci*-negative avian strains were identified in symptomless chickens of a contact flock involved in an outbreak of psittacosis in Germany [22]. Three years later, an epidemiological investigation in poultry breeder flocks in France, which had been prompted by cases of atypical pneumonia in poultry slaughterhouse workers, led to the isolation of non-classified chlamydial strains closely related to the German strains from seven different flocks, whereas *C. psittaci* was found only in one of the 25 flocks examined [85]. The agent, later defined as *C. gallinacea*, has since been found in several European countries and China [86, 87], as well as in Australia [88].

Retrospective analysis of strains isolated from urban pigeons in Italy in 2006 also identified genetically related non-classified strains of *Chlamydiaceae*. In 2009, two severe outbreaks in breeder flocks of psittacines in Germany were attributed to closely related chlamydial strains. Notably, no other potential pathogen was found in these parrot flocks. Furthermore, pigeons were found to be a major host of this agent, later designated *C. avium*, in surveys in France [89] and Germany [90]. In the same period, investigations conducted on a wild ibis population in France in 2010 led to the identification of different *Chlamydiaceae*-positive but *C. psittaci*-negative strains.

Finally, characterisation of selected atypical isolates from the above studies focused on 16S rRNA-based phylogenetic analysis, multi-locus sequence analysis, phenotypic characterisation, as well as whole-genome analysis of type strains 10DC88^T (*C. avium*), 08-1274/3^T (*C. gallinacea*) and 10-1398/6^T (*C. ibidis*). Based on comparative analysis with the other established species of the family *Chlamydiaceae*, *C. avium* and *C. gallinaceae* were proposed as new species [91•], and *C. ibidis* was given the *Candidatus* status [92•]. Basic characteristics of the three avian *Chlamydia* spp. are given in Table 1.

Table 1 Basic characteristics of avian *Chlamydia* (*C.*) spp.

Species	Major hosts	Pathogenicity	Type strain	Genome size (bp)	No. of predicted proteins	16S rDNA difference (%)[a]	Detection assays
C. psittaci	Birds, mammals	Systemic respiratory disease	6BC	1,171,660	975	0	rtPCR, DNA microarray, ELISA [100]
C. avium	Pigeons, parrots, probably wild birds	Respiratory disease	10 DC88	1,041,169	940	1.95	rtPCR [93], DNA microarray
C. gallinacea	Chickens, turkeys, guinea fowl, ducks, probably other poultry	To be investigated	08-1274/3	1,045,134	907	1.88	rtPCR [87], DNA microarray

[a] Compared with *C. psittaci*

rDNA ribosomal DNA, *rtPCR* real-time PCR

Epidemiology of *C. avium* and *C. gallinacea*

Since *C. avium* and *C. gallinacea* were introduced very recently, virtually all studies on avian chlamydiosis have focused on *C. psittaci* so far. While specific PCR assays for the detection of the new species are now available [87, 86, 93], specific serological tools are still missing. It is possible that some of the older papers on avian chlamydiosis dealt with *C. avium* or *C. gallinacea* instead of *C. psittaci*, especially those based on serological evidence.

From the limited data that are currently available, *C. avium* seems to frequently occur among pigeons, whereas *C. gallinacea* is probably widely disseminated among poultry. Recent data from urban pigeons in Germany [90] and France [89] identified *C. avium* in four of the 128 (3 %) and in ten of the 125 (8 %) *Chlamydiaceae*-positive samples, respectively. In German breeder pigeon flocks, *C. avium* was found in four of the 27 (14.8 %) flocks [94]. In psittacine birds, the general prevalence of *C. avium* cannot be assessed as the findings reported so far represent individual cases. Prevalence studies on *C. gallinacea* in chicken and turkey flocks of four European countries and China revealed that its prevalence could even be higher than that of *C. psittaci* [86]. *C. gallinacea* was detected in 95 of the 110 (86.5 %) chlamydia-positive samples, whereas *C. psittaci* was only detected in two samples. In a survey conducted over 1 year in a slaughterhouse, *C. gallinacea* was detected in 321 of the 401 (80.0 %) *Chlamydiaceae*-positive samples from 129 French poultry flocks [95]. In contrast, only *C. psittaci* was found in a similar survey conducted in 19 Belgian chicken farms [96]. In Australia, *C. gallinacea* was detected in two of the 27 (7.5 %) *Chlamydiaceae*-positive samples from chickens, whereas *C. psittaci* was identified in five other samples [88]. Results differ greatly from one study to another and from one country to another. It is probable that the environment and farming practices, including cleaning and disinfection procedures, have a strong impact on circulation and persistence of chlamydiae in farms.

It is likely that *C. avium* and *C. gallinacea* will not be the last chlamydial species to be discovered in birds. Recent studies have provided evidence on further non-classified *Chlamydiaceae* species in seabirds [97], pigeons [90] and ducks [95]. The diversity among *Chlamydiaceae* species is probably far greater than currently conceived.

Pathogenicity of *C. avium* and *C. gallinacea*

The pathogenicity of the newly introduced species has yet to be systematically investigated. In the surveys reported to date, no clinical signs have been observed in chickens carrying *C. gallinacea* [85], nor in most of the *C. avium* carriers among pigeons. However, it seems likely from currently available data that *C. avium* is able to cause respiratory disease in parrots and pigeons [91•]. In analogy to the established *Chlamydia* spp., the new chlamydiae could survive as commensals in the gastrointestinal tract for extended periods before eliciting cases of disease, as discussed in two recent reviews [98, 28]. However, co-infections with other *Chlamydia* spp., bacteria or viruses [99] could exacerbate the course of *C. avium* or *C. gallinacea* infections as already reported for *C. psittaci*-infected turkeys (see the "Aetiology and Epidemiology"section). While cases of co-infection between the new chlamydiae and *C. psittaci* have been reported in pigeons [94] and poultry [87], evidence on possible interaction, such as synergetic or competitive effects, in the course of co-infection is still lacking. It would be interesting to re-examine samples from previous outbreaks of avian chlamydiosis for the presence of the two new species.

The zoonotic potential is still unknown, although there is a possibility of *C. gallinacea* being involved in zoonotic transmission. Those cases of atypical pneumonia reported among slaughterhouse workers exposed to chickens infected with

C. gallinacea [85] can be taken as an indication, even though previous exposure of these workers to *C. psittaci* cannot be excluded. However, these cases could not be definitively clarified because species-specific serological tools are not available for chlamydiosis. This striking deficit should be addressed in future research.

Conclusion

The results of field surveys in Europe and elsewhere in the past decade indicate a rise in the prevalence of *Chlamydia* infections in poultry flocks. This increase has been partly attributed to improved diagnostics, but could also be due to reduced use of antibiotics in poultry. In addition, organic poultry production, where free-range facilities allow contact with feral birds and pathogen-containing faeces, might also have contributed to this increase.

Recent advances in research on the pathogenesis of avian chlamydiosis include the generation of comprehensive datasets on host–pathogen interaction obtained from in vitro, in ovo and in vivo infection models. Following rapid entry into host cells, which is controlled by specific surface proteins and T3SS effectors, *C. psittaci* was shown to efficiently disseminate within the animal host, causing systemic disease. The pathogen seems to be capable of evading the action of host pro-inflammatory mediators more efficiently than other chlamydiae. When facing the host immune response it was shown to up-regulate essential chlamydial genes. A number of new molecular factors that are important for intracellular proliferation and progression of the infection have been identified.

Following the discovery of two new avian chlamydial species, aetiopathology and epidemiology of avian chlamydiosis will have to be revised, since *C. psittaci* no longer seems to be the only chlamydial agent involved. Although it is too early for a final assessment of the importance of *C. gallinacea* and *C. avium*, veterinarians, physicians, diagnosticians and researchers should take the new developments into account and consider possible involvement of the new agents in cases of avian chlamydiosis.

Compliance with Ethics Guidelines

Conflict of Interest Dr Sachse, Dr Laroucau and Dr Vanrompay each declare they have no conflicts of interests.

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. European Commission, Health & Consumer Protection Directorate-General. (2002) Avian chlamydiosis as a zoonotic disease and risk reduction strategies. SCAHAW (Scientific Committee on Animal Health and Animal Welfare), SANCO/AH/R26/2002. http://ec.europa.eu/food/fs/sc/scah/out73_en.pdf
2. •• Beeckman DS, Vanrompay DC. Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. Clin Microbiol Infect. 2009;15(1):11–7. doi:10.1111/j.1469-0691.2008.02669.x. *Comprehensive review on epidemiology, transmission pathways, clinical course, diagnosis and possible treatment for psittacosis in humans. It is emphasised that the general awareness towards psittacosis among medical workers is low and methods used in routine diagnosis should be updated.*
3. Everett KD, Bush RM, Andersen AA. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol. 1999;49 Pt 2:415–40.
4. Stephens RS, Myers G, Eppinger M, Bavoil PM. Divergence without difference: phylogenetics and taxonomy of *Chlamydia* resolved. FEMS Immunol Med Microbiol. 2009;55(2):115–9. doi:10.1111/j.1574-695X.2008.00516.x.
5. Kuo C-C, Stephens RS, Bavoil PM, Kaltenboeck B. Genus *Chlamydia* Jones, Rake and Stearns 1945, 55. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman WB, editors. Bergey's Manual of Systematic Bacteriology, vol. 4. 2nd ed. Heidelberg: Springer; 2011. p. 846–65.
6. Sachse K, Bavoil PM, Kaltenboeck B, Stephens RS, Kuo C-C, Rosselló-Móra R, Horn M. Emendation of the family *Chlamydiaceae*: Proposal of a single genus, *Chlamydia*, to include all currently recognized species. Syst Appl Microbiol. 2015. doi:10.1016/j.syapm.2014.12.004.
7. Matsumoto A, Manire GP. Electron microscopic observations on the effects of penicillin on the morphology of *Chlamydia psittaci*. J Bacteriol. 1970;101(1):278–85.
8. Harkinezhad T, Geens T, Vanrompay D. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. Vet Microbiol. 2009;135(1–2):68–77. doi:10.1016/j.vetmic.2008.09.046.
9. Sachse K, Laroucau K, Hotzel H, Schubert E, Ehricht R, Slickers P. Genotyping of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of ompA genes. BMC Microbiol. 2008;8:63.
10. Vanrompay D (2013) Avian Chlamydiosis. In: Swayne D, editor. Diseases of Poultry, 13th edition 2013. Ames: Wiley- Blackwell Publishing, Section III: Bacterial Diseases, Chapter 24
11. Van Loock M, Geens T, De Smit L, Nauwynck H, Van Empel P, Naylor C, et al. Key role of *Chlamydophila psittaci* on Belgian turkey farms in association with other respiratory pathogens. Vet Microbiol. 2005;107(1–2):91–101. doi:10.1016/j.vetmic.2005.01.009.
12. •• Knittler MR, Sachse K. *Chlamydia psittaci*: update on an underestimated zoonotic agent (Minireview). FEMS Pathog Dis. 2014. doi:10.1093/femspd/ftu1007. *Review of current knowledge on C. psittaci as a pathogen. Recent advances in genomic*

analysis, as well as research on molecular host–pathogen interaction, innate and adaptive immune response in clinical, sub-clinical and persistent infection are discussed.

13. Kaleta EF, Taday EM. Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. Avian Pathol. 2003;32(5):435–61. doi:10.1080/0307945031000159361 3.
14. Cao J, Yang Q, Yang L, Liu Z, He C. Epidemic investigation of avian *Chlamydia psittaci* in Beijing and other provinces around. Vet Sci China. 2006;56:340–9.
15. Guérin JL, Ballot A, Sraka B, Léon O (2006) Portage de *Chlamydophila psittaci* dans la filiére canard mulard: évaluation du portage chez les reproducteurs et incidence sur le statut du caneton. In: Proceedings des 7èmes Journées de la recherche sur les palmipèdes à foie gras 18–19 October 2006, Arcachon, France: pp. 37–40
16. Haas WH, Swaan CM, Meijer A, Neve G, Buchholz U, Beer M, et al. A Dutch case of atypical pneumonia after culling of H5N1 positive ducks in Bavaria was found infected with *Chlamydophila psittaci*. Euro Surveill. 2007;12(11):E071129 071123.
17. Laroucau K, de Barbeyrac B, Vorimore F, Clerc M, Bertin C, Harkinezhad T, et al. Chlamydial infections in duck farms associated with human cases of psittacosis in France. Vet Microbiol. 2009;135(1–2):82–9. doi:10.1016/j.vetmic.2008.09.048.
18. Yin L, Kalmar ID, Lagae S, Vandendriessche S, Vanderhaeghen W, Butaye P, et al. Emerging *Chlamydia psittaci* infections in the chicken industry and pathology of *Chlamydia psittaci* genotype B and D strains in specific pathogen free chickens. Vet Microbiol. 2013;162(2–4):740–9. doi:10.1016/j.vetmic.2012.09.026.
19. Dickx V, Geens T, Deschuyffeleer T, Tyberghien L, Harkinezhad T, Beeckman DS, et al. *Chlamydophila psittaci* zoonotic risk assessment in a chicken and turkey slaughterhouse. J Clin Microbiol. 2010;48(9):3244–50. doi:10.1128/JCM. 00698-10.
20. Yin L, Lagae S, Kalmar I, Borel N, Pospischil A, Vanrompay D. Pathogenicity of low and highly virulent *Chlamydia psittaci* isolates for specific-pathogen-free chickens. Avian Dis. 2013;57(2): 242–7.
21. Zhang F, Li S, Yang J, Pang W, Yang L, He C. Isolation and characterization of *Chlamydophila psittaci* isolated from laying hens with cystic oviducts. Avian Dis. 2008;52(1):74–8.
22. Gaede W, Reckling KF, Dresenkamp B, Kenklies S, Schubert E, Noack U, et al. *Chlamydophila psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. Zoonoses Public Health. 2008;55(4):184–8. doi:10.1111/j.1863-2378.2008.01108.x.
23. Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovc A, Prukner-Radovcic E, et al. Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. Vet Microbiol. 2009;135(1–2):54–67. doi:10.1016/j.vetmic.2008.09.045.
24. Dickx V, Kalmar ID, Tavernier P, Vanrompay D. Prevalence and genotype distribution of *Chlamydia psittaci* in feral Canada geese (*Branta canadensis*) in Belgium. Vector Borne Zoonotic Dis. 2013;13(6):382–4. doi:10.1089/vbz.2012.1131.
25. Herrmann B, Persson H, Jensen JK, Joensen HD, Klint M, Olsen B. *Chlamydophila psittaci* in fulmars, the Faroe Islands. Emerg Infect Dis. 2006;12(2):330–2.
26. Kalmar ID, Dicxk V, Dossche L, Vanrompay D. Zoonotic infection with *Chlamydia psittaci* at an avian refuge centre. Vet J. 2014;199(2):300–2. doi:10.1016/j.tvjl.2013.10.034.
27. Blomqvist M, Christerson L, Waldenstrom J, Herrmann B, Olsen B. *Chlamydia psittaci* in Swedish wetland birds: a risk to zoonotic infection? Avian Dis. 2012;56(4):737–40.
28. Reinhold P, Sachse K, Kaltenboeck B. *Chlamydiaceae* in cattle: commensals, trigger organisms, or pathogens? Vet J. 2011;189(3): 257–67. doi:10.1016/j.tvjl.2010.09.003.
29. Wittenbrink MM, Mrozek M, Bisping W. Isolation of *Chlamydia psittaci* from a chicken egg: evidence of egg transmission. Zentralbl Veterinarmed B. 1993;40(6):451–2.
30. Lublin A, Shudari G, Mechani S, Weisman Y. Egg transmission of *Chlamydia psittaci* in turkeys. Vet Rec. 1996;139(12):300.
31. Ritter J. Über Pneumotyphus, eine Hausepidemie in Uster. Dtsch Arch klin Med. 1879;25:53.
32. Verminnen K, Duquenne B, De Keukeleire D, Duim B, Pannekoek Y, Braeckman L, et al. Evaluation of a *Chlamydophila psittaci* diagnostic platform for zoonotic risk assessment. J Clin Microbiol. 2008;46:281–5.
33. Rehn M, Ringberg H, Runehagen A, Herrmann B, Olsen B, Petersson AC, et al. Unusual increase of psittacosis in southern Sweden linked to wild bird exposure, January to April 2013. Euro Surveill. 2013;18(19):20478.
34. Wallensten A, Fredlund H, Runehagen A. Multiple human-to-human transmission from a severe case of psittacosis, Sweden, January-February 2013. Euro Surveill. 2014;19(42).
35. Deschuyffeleer TP, Tyberghien LF, Dickx VL, Geens T, Saelen JM, Vanrompay DC, et al. Risk assessment and management of *Chlamydia psittaci* in poultry processing plants. Ann Occup Hyg. 2012;56(3):340–9. doi:10.1093/annhyg/mer102.
36. Beeckman DS, Geens T, Timmermans JP, Van Oostveldt P, Vanrompay DC. Identification and characterization of a type III secretion system in *Chlamydophila psittaci*. Vet Res. 2008;39(3): 27. doi:10.1051/vetres:2008002.
37. Dautry-Varsat A, Balana ME, Wyplosz B. Chlamydia–host cell interactions: recent advances on bacterial entry and intracellular development. Traffic. 2004;5(8):561–70. doi:10.1111/j.1398-9219.2004.00207.x.
38. Abromaitis S, Stephens RS. Attachment and entry of *Chlamydia* have distinct requirements for host protein disulfide isomerase. PLoS Pathog. 2009;5(4):e1000357. doi:10.1371/journal.ppat.1000357.
39. Stuart ES, Webley WC, Norkin LC. Lipid rafts, caveolae, caveolin-1, and entry by Chlamydiae into host cells. Exp Cell Res. 2003;287(1):67–78.
40. Escalante-Ochoa C, Ducatelle R, Haesebrouck F. Optimal development of *Chlamydophila psittaci* in L929 fibroblast and BGM epithelial cells requires the participation of microfilaments and microtubule-motor proteins. Microb Pathog. 2000;28(6):321–33. doi:10.1006/mpat.2000.0352.
41. Jutras I, Subtil A, Wyplosz B, Dautry-Varsat A (2004) Chlamydia. Intracellular pathogens in membrane interactions and vacuole biogenesise (Molecular Biology Intelligence Unit). Gorvel J-P, editor. New York: Springer-Verlag New York, LLC. pp. 179–189
42. Scidmore MA. Recent advances in Chlamydia subversion of host cytoskeletal and membrane trafficking pathways. Microbes Infect. 2011;13(6):527–35. doi:10.1016/j.micinf.2011.02.001.
43. Hsia RC, Pannekoek Y, Ingerowski E, Bavoil PM. Type III secretion genes identify a putative virulence locus of Chlamydia. Mol Microbiol. 1997;25(2):351–9.
44. Saad N. The role of the type III secretion system of *Chlamydia psittaci* in human macrophages [PhD thesis]. Ghent: University of Ghent, Faculty of Bioscience Engineering (Academic year 2010–2011). 2011
45. Betts-Hampikian HJ, Fields KA. The Chlamydial TYPE III secretion mechanism: revealing cracks in a tough nut. Front Microbiol. 2010;1:114. doi:10.3389/fmicb.2010.00114.
46. Fields KA, Mead DJ, Dooley CA, Hackstadt T. *Chlamydia trachomatis* type III secretion: evidence for a functional apparatus during early-cycle development. Mol Microbiol. 2003;48(3): 671–83.
47. Jamison WP, Hackstadt T. Induction of type III secretion by cell-free Chlamydia trachomatis elementary bodies. Microb Pathog. 2008;45(5–6):435–40. doi:10.1016/j.micpath.2008.10.002.

48. Moore ER, Ouellette SP. Reconceptualizing the chlamydial inclusion as a pathogen-specified parasitic organelle: an expanded role for Inc proteins. Front Cell Infect Microbiol. 2014;4:157. doi:10.3389/fcimb.2014.00157.
49. Valdivia RH. Chlamydia effector proteins and new insights into chlamydial cellular microbiology. Curr Opin Microbiol. 2008;11(1):53–9. doi:10.1016/j.mib.2008.01.003.
50. Beeckman DS, Geens T, Timmermans JP, Van Oostveldt P, Vanrompay DC. Identification and characterization of a type III secretion system in *Chlamydophila psittaci*. Vet Res. 2008;39(3):27. doi:10.1051/vetres:2008002.
51. Mital J, Miller NJ, Dorward DW, Dooley CA, Hackstadt T. Role for chlamydial inclusion membrane proteins in inclusion membrane structure and biogenesis. PLoS One. 2013;8(5):e63426. doi:10.1371/journal.pone.0063426.
52. Borth N, Litsche K, Franke C, Sachse K, Saluz HP, Hanel F. Functional interaction between type III-secreted protein IncA of *Chlamydophila psittaci* and human G3BP1. PLoS One. 2011;6(1):e16692. doi:10.1371/journal.pone.0016692.
53. Böcker S, Heurich A, Franke C, Monajembashi S, Sachse K, Saluz HP, et al. *Chlamydia psittaci* inclusion membrane protein IncB associates with host protein Snapin. Int J Med Microbiol. 2014;304(5–6):542–53. doi:10.1016/j.ijmm.2014.03.005.
54. Roberts AJ, Kon T, Knight PJ, Sutoh K, Burgess SA. Functions and mechanics of dynein motor proteins. Nat Rev Mol Cell Biol. 2013;14(11):713–26. doi:10.1038/nrm3667.
55. Matsumoto A. Isolation and electron microscopic observations of intracytoplasmic inclusions containing *Chlamydia psittaci*. J Bacteriol. 1981;145(1):605–12.
56. Knittler MR, Berndt A, Bocker S, Dutow P, Hanel F, Heuer D, et al. *Chlamydia psittaci:* New insights into genomic diversity, clinical pathology, host-pathogen interaction and anti-bacterial immunity. Int J Med Microbiol. 2014;304:877–93. doi:10.1016/j.ijmm.2014.06.010.
57. Matsumoto A, Bessho H, Uehira K, Suda T. Morphological studies of the association of mitochondria with chlamydial inclusions and the fusion of chlamydial inclusions. J Electron Microsc (Tokyo). 1991;40(5):356–63.
58. Engel J. Tarp and Arp: how Chlamydia induces its own entry. Proc Natl Acad Sci U S A. 2004;101(27):9947–8. doi:10.1073/pnas.0403633101.
59. Wyrick PB. *Chlamydia trachomatis* persistence in vitro: an overview. J Infect Dis. 2010;201 Suppl 2:S88–95. doi:10.1086/652394.
60. Bavoil PM. What's in a word: the use, misuse, and abuse of the word "persistence" in *Chlamydia* biology. Front Cell Infect Microbiol. 2014;4:27. doi:10.3389/fcimb.2014.00027.
61. Beatty WL, Byrne GI, Morrison RP. Repeated and persistent infection with Chlamydia and the development of chronic inflammation and disease. Trends Microbiol. 1994;2(3):94–8.
62. Coles AM, Reynolds DJ, Harper A, Devitt A, Pearce JH. Low-nutrient induction of abnormal chlamydial development: a novel component of chlamydial pathogenesis? FEMS Microbiol Lett. 1993;106(2):193–200.
63. Raulston JE. Response of *Chlamydia trachomatis* serovar E to iron restriction in vitro and evidence for iron-regulated chlamydial proteins. Infect Immun. 1997;65(11):4539–47.
64. Pantoja LG, Miller RD, Ramirez JA, Molestina RE, Summersgill JT. Characterization of *Chlamydia pneumoniae* persistence in HEp-2 cells treated with gamma interferon. Infect Immun. 2001;69(12):7927–32. doi:10.1128/IAI. 69.12.7927-7932.2001.
65. Hsia R, Ohayon H, Gounon P, Dautry-Varsat A, Bavoil PM. Phage infection of the obligate intracellular bacterium, *Chlamydia psittaci* strain guinea pig inclusion conjunctivitis. Microbes Infect. 2000;2(7):761–72.
66. Kutlin A, Flegg C, Stenzel D, Reznik T, Roblin PM, Mathews S, et al. Ultrastructural study of *Chlamydia pneumoniae* in a continuous-infection model. J Clin Microbiol. 2001;39(10):3721–3. doi:10.1128/JCM. 39.10.3721-3723.2001.
67. Borel N, Dumrese C, Ziegler U, Schifferli A, Kaiser C, Pospischil A. Mixed infections with *Chlamydia* and porcine epidemic diarrhea virus - a new in vitro model of chlamydial persistence. BMC Microbiol. 2010;10:201. doi:10.1186/1471-2180-10-201.
68. Goellner S, Schubert E, Liebler-Tenorio E, Hotzel H, Saluz HP, Sachse K. Transcriptional response patterns of *Chlamydophila psittaci* in different in vitro models of persistent infection. Infect Immun. 2006;74(8):4801–8. doi:10.1128/IAI. 01487-05.
69. Belland RJ, Nelson DE, Virok D, Crane DD, Hogan D, Sturdevant D, et al. Transcriptome analysis of chlamydial growth during IFN-gamma-mediated persistence and reactivation. Proc Natl Acad Sci U S A. 2003;100(26):15971–6. doi:10.1073/pnas.2535394100.
70. Stenner-Liewen F, Liewen H, Zapata JM, Pawlowski K, Godzik A, Reed JC. CADD, a Chlamydia protein that interacts with death receptors. J Biol Chem. 2002;277(12):9633–6. doi:10.1074/jbc.C100693200.
71. Phillips Campbell R, Kintner J, Whittimore J, Schoborg RV. *Chlamydia muridarum* enters a viable but non-infectious state in amoxicillin-treated BALB/c mice. Microbes Infect. 2012;14(13):1177–85. doi:10.1016/j.micinf.2012.07.017.
72. Pospischil A, Borel N, Chowdhury EH, Guscetti F. Aberrant chlamydial developmental forms in the gastrointestinal tract of pigs spontaneously and experimentally infected with *Chlamydia suis*. Vet Microbiol. 2009;135(1–2):147–56. doi:10.1016/j.vetmic.2008.09.035.
73. Borel N, Summersgill JT, Mukhopadhyay S, Miller RD, Ramirez JA, Pospischil A. Evidence for persistent *Chlamydia pneumoniae* infection of human coronary atheromas. Atherosclerosis. 2008;199(1):154–61. doi:10.1016/j.atherosclerosis.2007.09.026.
74. Braukmann M, Sachse K, Jacobsen ID, Westermann M, Menge C, Saluz HP, et al. Distinct intensity of host-pathogen interactions in *Chlamydia psittaci*- and *Chlamydia abortus*-infected chicken embryos. Infect Immun. 2012;80(9):2976–88. doi:10.1128/IAI.00437-12.
75. Kalmar I, Berndt A, Yin L, Chiers K, Sachse K, Vanrompay D. Host-pathogen interactions in specific pathogen-free chickens following aerogenous infection with *Chlamydia psittaci* and *Chlamydia abortus*. Vet Immunol Immunopathol. 2015. doi:10.1016/j.vetimm.2014.12.014.
76. Klos A, Wende E, Wareham KJ, Monk PN. International Union of Basic and Clinical Pharmacology. [corrected]. LXXXVII. Complement peptide C5a, C4a, and C3a receptors. Pharmacol Rev. 2013;65(1):500–43.
77. Bode J, Dutow P, Sommer K, Janik K, Glage S, Tummler B, et al. A new role of the complement system: C3 provides protection in a mouse model of lung infection with intracellular *Chlamydia psittaci*. PLoS One. 2012;7(11):e50327. doi:10.1371/journal.pone.0050327.
78. Dutow P, Fehlhaber B, Bode J, Laudeley R, Rheinheimer C, Glage S, et al. The complement C3a receptor is critical in defense against *Chlamydia psittaci* in mouse lung infection and required for antibody and optimal T cell response. J Infect Dis. 2014;209(8):1269–78. doi:10.1093/infdis/jit640.
79. Fiegl D, Kagebein D, Liebler-Tenorio EM, Weisser T, Sens M, Gutjahr M, et al. Amphisomal route of MHC class I cross-presentation in bacteria-infected dendritic cells. J Immunol. 2013;190(6):2791–806. doi:10.4049/jimmunol.1202741.
80. Prohl A, Ostermann C, Lohr M, Reinhold P. The bovine lung in biomedical research: visually guided bronchoscopy, intrabronchial inoculation and in vivo sampling techniques. J Vis Exp. 2014;89. doi:10.3791/51557.

81. Reinhold P, Ostermann C, Liebler-Tenorio E, Berndt A, Vogel A, Lambertz J, et al. A bovine model of respiratory *Chlamydia psittaci* infection: challenge dose titration. PLoS One. 2012;7(1): e30125. doi:10.1371/journal.pone.0030125.
82. Ostermann C, Ruttger A, Schubert E, Schrodl W, Sachse K, Reinhold P. Infection, disease, and transmission dynamics in calves after experimental and natural challenge with a Bovine Chlamydia psittaci isolate. PLoS One. 2013;8(5):e64066. doi:10.1371/journal.pone.0064066.
83. Ostermann C, Linde S, Siegling-Vlitakis C, Reinhold P. Evaluation of pulmonary dysfunctions and acid base imbalances induced by *Chlamydia psittaci* in a bovine model of respiratory infection. Multidiscip Respir Med. 2014;9(1):10. doi:10.1186/2049-6958-9-10.
84. Ostermann C, Schroedl W, Schubert E, Sachse K, Reinhold P. Dose-dependent effects of *Chlamydia psittaci* infection on pulmonary gas exchange, innate immunity and acute-phase reaction in a bovine respiratory model. Vet J. 2013;196(3):351–9. doi:10.1016/j.tvjl.2012.10.035.
85. Laroucau K, Vorimore F, Aaziz R, Berndt A, Schubert E, Sachse K. Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. Infect Genet Evol. 2009;9(6): 1240–7.
86. Zocevic A, Vorimore F, Marhold C, Horvatek D, Wang D, Slavec B, et al. Molecular characterization of atypical Chlamydia and evidence of their dissemination in different European and Asian chicken flocks by specific real-time PCR. Environ Microbiol. 2012;14(8):2212–22. doi:10.1111/j.1462-2920.2012.02800.x.
87. Laroucau K, Aaziz R, Meurice L, Servas V, Chossat I, Royer H, et al. (2014) Outbreak of psittacosis in a group of women exposed to *Chlamydia psittaci*-infected chickens. Euro Surveill. In press [EMID:ce1270d610605ffb]
88. Robertson T, Bibby S, O'Rourke D, Belfiore T, Agnew-Crumpton R, Noormohammadi AH. Identification of Chlamydial species in crocodiles and chickens by PCR-HRM curve analysis. Vet Microbiol. 2010;145(3–4):373–9. doi:10.1016/j.vetmic.2010.04.007.
89. Gasparini J, Erin N, Bertin C, Jacquin L, Vorimore F, Frantz A, et al. Impact of urban environment and host phenotype on the epidemiology of *Chlamydiaceae* in feral pigeons (*Columba livia*). Environ Microbiol. 2011;13(12):3186–93. doi:10.1111/j.1462-2920.2011.02575.x.
90. Sachse K, Kuehlewind S, Ruettger A, Schubert E, Rohde G. More than classical *Chlamydia psittaci* in urban pigeons. Vet Microbiol. 2012;157(3–4):476–80. doi:10.1016/j.vetmic.2012.01.002.
92.• Sachse K, Laroucau K, Riege K, Wehner S, Dilcher M, Creasy HH, et al. Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. Syst Appl Microbiol. 2014;37(2):79–88. doi:10.1016/j.syapm.2013.12.004. *Introduction and description of C. avium and C. gallinacea as new species within the recombined genus Chlamydia. Data on morphology, epidemiology and pathogenicity, as well as phylogenetic and genomic parameters are presented.*
93.• Vorimore F, Hsia RC, Huot-Creasy H, Bastian S, Deruyter L, Passet A, et al. Isolation of a new *Chlamydia* species from the Feral Sacred Ibis (*Threskiornis aethiopicus*): *Chlamydia ibidis*. PLoS One. 2013;8(9):e74823. doi:10.1371/journal.pone.0074823. *First description of this Candidatus species. Data from detailed genomic analysis, morphology and comparative phylogeny are presented and discussed.*
93. Zocevic A, Vorimore F, Vicari N, Gasparini J, Jacquin L, Sachse K, et al. A real-time PCR assay for the detection of atypical strains of *Chlamydiaceae* from pigeons. PLoS One. 2013;8(3):e58741. doi:10.1371/journal.pone.0058741.
94. Krautwald-Junghanns ME, Stolze J, Schmidt V, Bohme J, Sachse K, Cramer K. Efficacy of doxycycline for treatment of chlamydiosis in flocks of racing and fancy pigeons [in German]. Prax Ausg K Kleintiere Heimtiere. 2013;41(6):392–8.
95. Hulin V, Oger S, Vorimore F, Aaziz R, de Barbeyrac B, Berruchon J, et al. Host preference and zoonotic potential of *Chlamydia psittaci* and *Chlamydia gallinacea* in poultry. Pathog Dis. 2015 (in press).
96. Lagae S, Kalmar I, Laroucau K, Vorimore F, Vanrompay D. Emerging *Chlamydia psittaci* infections in chickens and examination of transmission to humans. J Med Microbiol. 2014;63(Pt 3): 399–407. doi:10.1099/jmm. 0.064675-0.
97. Christerson L, Blomqvist M, Grannas K, Thollesson M, Laroucau K, Waldenstrom J, et al. A novel *Chlamydiaceae*-like bacterium found in faecal specimens from sea birds from the Bering Sea. Environ Microbiol Rep. 2010;2(4):605–10. doi:10.1111/j.1758-2229.2010.00174.x.
98. Rank RG, Yeruva L. Hidden in plain sight: chlamydial gastrointestinal infection and its relevance to persistence in human genital infection. Infect Immun. 2014;82(4):1362–71. doi:10.1128/IAI.01244-13.
99. Loock MV, Loots K, Zande SV, Heerden MV, Nauwynck H, Goddeeris BM, et al. Pathogenic interactions between Chlamydophila psittaci and avian pneumovirus infections in turkeys. Vet Microbiol. 2006;112(1):53–63.
100. Sachse K, Vretou E, Livingstone M, Borel N, Pospischil A, Longbottom D. Recent developments in the laboratory diagnosis of chlamydial infections. Vet Microbiol. 2009;135:2–21.