CHAPTER

34

CHLAMYDIA

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The genus *Chlamydia* currently consists of two antigenically related species, *C. trachomatis* and *C. pneumoniae*,[22] which are restricted to humans, and *C. psittaci*, which has a wide host spectrum among birds (most Psittaciformes and at least 130 non-Psittaciformes), mammals (horse, cattle, sheep, roebuck, domesticated cat, guinea pig, dog) and humans.[4] *C. psittaci* can be highly contagious and induces a disease called psittacosis in parrots and ornithosis (by legal definition) in all other animals and man. Because the same agent is involved, the use of the term chlamydiosis to describe infections caused by this organism should be encouraged. Chlamydiosis is a reportable disease in many countries.

C. psittaci is an obligate intracellular bacterial parasite that contains DNA and RNA and has a rudimentary cellular wall that does not contain muraic acid or peptidoglycan.[43] This organism is capable of autonomic synthesis of species-specific enzymes, but depends on the host cell for energy (by means of adenosine triphosphate and nicotinamide adenosine diphosphate) and probably some amino acids, particularly tryptophan.[57] These requirements prevent chlamydia from growing on cell-free media.[19,23,27]

Chlamydia Replication Cycle

Figure 34.1 illustrates steps in the replication cycle of the chlamydial organism.

Step 1. The first step in replication is the attachment to and penetration of a target cell (mainly columnar epithelial cells of mucous membranes and mononuclear macrophages) by the infectious-toxic but nonpropagating elementary bodies (approximately 0.3 μm). The process is comparable to receptor-mediated endocytosis.[57] The chlamydia is enveloped in an endocytoplasmic vesicle where it remains throughout the replication cycle. By remaining in an endosome, the chlamydia is protected from host-derived lysozymes. The development of a phagolysosome (which would destroy the engulfed organism) is inhibited by chlamydial-derived proteins.[24]

Step 2. The second phase of replication is the transition of the metabolically inert elementary body into the large (0.5 to 1.5 μm), fragile, low density, metabolically active reticulate body. This phase of the replication cycle probably begins with the reduction of the disulfide bond that cross links the outer membrane proteins. The developing reticulate bodies possess several surface projections that are assumed to protrude through the endosomatic membrane to enable the uptake of nutrients from the host cytoplasm.[57] In other respects, the reticulate bodies resemble bacterial L-forms (which have defective cell walls), because they can persist in spite of circulating antibodies and therapeutics designed to inhibit cellular wall formation.

Step 3. The growth and binary fission of the reticulate bodies result in the production of many progeny and micro-colonies containing from 100 to 500 chlamydial organisms per cell. Multiple micro-colonies, also called "inclusions" (Levinthal-Cole-Lillie = LCL bodies) can occur in an infected cell. By the end of the replication cycle, enzymes produced by the intracellular parasite may induce lysis of the host cell (48 hours after the initial infection). These enzymes are susceptible to antibiotics. Endotoxicosis may occur in the host cell when lysosomes are destroyed and endosomatic enzymes are released into the cytoplasm.

Step 4. Maturation of the noninfectious reticulate bodies into infectious elementary bodies involves the restoration of the surface membrane and its associated toxicity. Chlamydia-specific lipopolysaccharide is brought to the host cell surface concomitantly with the growth of chlamydial organisms.[46] This glycolipid is assumed to reduce the fluidity of the plasma membrane, thereby protecting the chlamydial-infected cells from cytotoxic T-cells.[57]

Step 5. Newly formed elementary bodies are released, not always by lysis of the host cell.

Serovars

C. psittaci strains vary considerably in terms of virulence and antigenicity. In addition, five serovars have been distinguished among the avian strains by using monoclonal antibodies: psittacine, pigeon I, duck, turkey and pigeon II.[54]

Whether or not the parrot serovar and turkey serovar are really of particular importance as zoonotic agents[2] is a matter of controversy. It has been found that the different serovars do not only occcur in the named avian species but also in a variety of other host species. Virulent strains replicate more rapidly and enjoy a wider host spectrum. Chlamydia has a genus-specific lipoglycoprotein with an acid polysaccharide as the antigenic determinant (thermostable at 100°C for 30 minutes). Several proteinaceous antigens, including the major outer membrane protein, can show subspecies or even strain-specific variability.[23] Antibodies generated against most of the antigens are not correlated with protective immunity.[23]

Pathogenicity

The pathogenicity of chlamydia cannot be fully explained by the direct damage to the host cells. The most important virulence factor is a toxin, which occurs with various degrees of intensity in the different strains and is closely bound to the outer membrane of the elementary bodies. During chlamydial growth in a particular avian host, metabolic and structural changes occur that can alter its pathogenicity and antigenicity. The degree of change is governed by the number of passages in a particular species. The surface of the elementary bodies that are formed during the replication cycle contains heterologous "new" antigens, which are assumed to be host-specific.[1] Interspecies transfer (eg, in quarantine stations, breeding farms, multispecies aviaries, pet shops) of chlamydia can change the physicochemical properties and, therefore, the antigenic composition, the toxic components and, in terms of virulence, the host spectrum of the agent.[27] However, the newly acquired characteristics are not truly stable.

The outcome of an infection is dependent on the ratio of elementary bodies to macrophages. Lethal lytic reactions occur in phagocytes infected with high

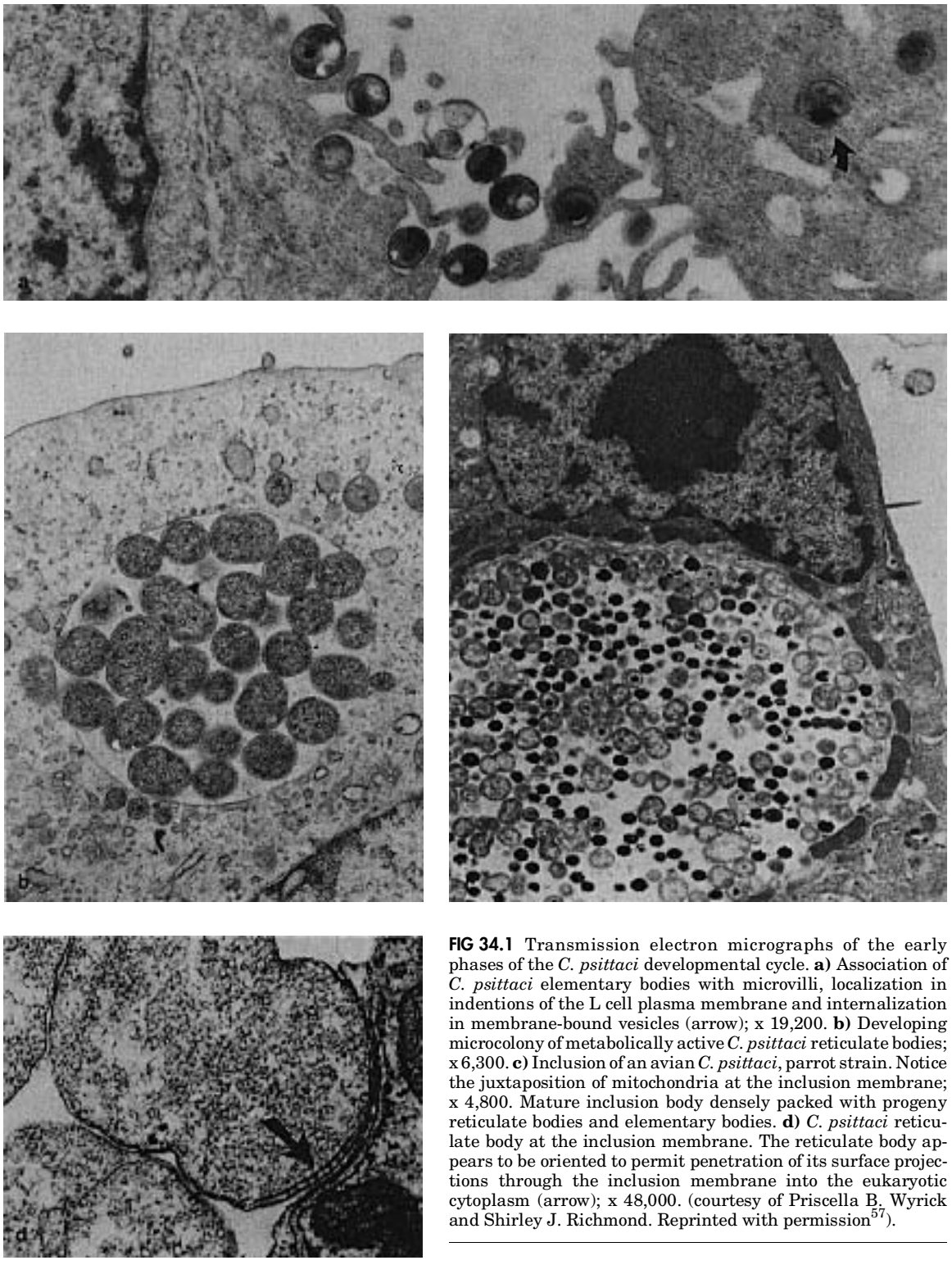

FIG 34.1 Transmission electron micrographs of the early phases of the *C. psittaci* developmental cycle. **a)** Association of *C. psittaci* elementary bodies with microvilli, localization in indentions of the L cell plasma membrane and internalization in membrane-bound vesicles (arrow); x 19,200. **b)** Developing microcolony of metabolically active *C. psittaci* reticulate bodies; x 6,300. **c)** Inclusion of an avian *C. psittaci*, parrot strain. Notice the juxtaposition of mitochondria at the inclusion membrane; x 4,800. Mature inclusion body densely packed with progeny reticulate bodies and elementary bodies. **d)** *C. psittaci* reticulate body at the inclusion membrane. The reticulate body appears to be oriented to permit penetration of its surface projections through the inclusion membrane into the eukaryotic cytoplasm (arrow); x 48,000. (courtesy of Priscella B. Wyrick and Shirley J. Richmond. Reprinted with permission[57]).

numbers of virulent chlamydial particles. Low doses of a virulent strain are rapidly inactivated by mononuclear and polymorphonuclear phagocytes. If the macrophage is damaged, the chances of the chlamydial organism to survive are reduced. Low doses of a nonvirulent strain do not stimulate an appropriate lytic reaction, resulting in macrophages that are converted into long-lived epithelioid cells that remain chronically infected (see Chapter 5). The life span of these epithelioid cells should govern the duration of antibiotic treatment. However, nothing is known about the longevity of these transformed cells in birds.[19] It is a high probability that infected macrophages transfer their "inclusions" during mitosis in the bone marrow onto the progeny cells.[57] Life-long carriers may be the result. Incomplete autosterilization and phagocytosis into "new" macrophages favor the selection of strains with low virulence for the species in question. These chronic infections favor shedding of large numbers of chlamydia that might be highly virulent for other avian species.[19]

Stability of Chlamydia

The infectious elementary bodies, which can be stained as described by Giemsa, Gimenez, Stamp, Macchiavello or Castaneda, can survive outside the host (protected by proteinaceous material) and inside host cells for several weeks (see Color 10). Bacterial-induced destruction of tissues and the presence of feces rapidly inactivate the organism. "Free" elementary bodies are relatively unstable and can be inactivated in the environment within days. Chlamydia is particularly sensitive to heat and one percent formalin if the temperature is above 20°C. Quaternary ammonium compounds and lipid solvents are poor choices for inactivating chlamydia. Infectivity has been shown to be destroyed within minutes by benzalkonium chloride.[23] In Europe, Orbivet™ and hydrogen peroxide have been shown to be effective against chlamydia.

C. psittaci is endemic worldwide, where it is distributed liberally among free-ranging birds. As a rule, the organism is well adapted to avian hosts and causes few, if any, clinical signs or pathologic lesions. Clinical disease is precipitated mainly by human-induced conditions and procedures. Systematic testing using modern laboratory methods (various versions of the ELISA) has not been performed on free-ranging birds to determine the incidence or prevalence of infections. However, surveys of imported and domestically bred Psittaciformes as well as free-ranging and captive raptors and owls from Germany indicate that between 30 and 70% of the birds tested are infected.[12,13]

Transmission

Elementary bodies present in feather dust and dried feces are primarily dispersed through air circulation. Ingestion of elementary bodies results in infection of the intestinal epithelial cells. Vertical transmission through the egg has been documented in domesticated ducks,[32,47] Black-headed Gulls[32] and budgerigars,[44] and has been suggested in turkeys. Chlamydia can usually be detected in the feces ten days prior to the onset of clinical signs. High numbers of chlamydia can be found regularly or intermittently in the feces (up to 10^5 infectious units per gram of feces), urine, lacrimal fluid, nasal discharge, mucous from the oral and pharyngeal cavities and "crop milk" (pigeons) of infected birds. Insufficient information is available to establish the periods during which birds with clinical disease or carriers can transmit the organism.

Cockatiels are frequent carriers of chlamydia and can shed the agent in the feces for more than one year following an active infection. Infected ducks have been shown to shed chlamydia in the feces for 100 days, and harbor the organism on the nasal mucosa for 170 days. It has been suggested that birds may become subclinical carriers and cease shedding within 30 to 50 days of the initial infection; however, this theory cannot be substantiated using improved methods of chlamydial detection.[13] In any case, carriers may begin to shed the organism following a stressful event. Assumed spontaneous self-elimination of infections within a flock during a four- to five-month period cannot be confirmed. Transmission by hemophagous insects or mites is possible.

Some species like dogs, cats, horses, swine and man develop infections that do not seem to be transmissible to other members of the same species. In contrast, infected birds, cattle, sheep and goats readily transmit chlamydia to other members of the same species. A newly imported Amazon parrot with chlamydiosis was thought to have infected a cat that was restricted to the house (Harrison GJ, unpublished).

Pathogenesis

There are considerable differences between the susceptibility of various host species to chlamydia. The same strain can cause disease in different avian species, which can be distinguished by the number and

type of affected tissues and replication rate as determined by the period necessary for elementary bodies to appear. Similar differences are described with varying chlamydial strains in the same host species.[33] Young birds are generally more susceptible to infections than adults. Macaws and Amazon parrots appear to be more susceptible than Psittaciformes from South Asia, Australia and related islands (eg, cockatoos, lories, King Parrots). The African parrots are even less susceptible than the Asian Psittaciformes. These are generalizations with many exceptions, and the condition of a host is probably more important than any species-specific susceptibility (Figure 34.2). *C. psittaci* can cause a totally asymptomatic infection in mature hosts or acute, systemic, often fatal illness in young birds or with nonhost-adapted chlamydial strains. The precondition for such an adaptation is a latent infection of some time period. The virulence and toxicity of host-adapted strains can be most dramatic when they infect a different species.[19,28]

The virulence, antigenicity and biologic properties of chlamydial strains vary. The surface of the elementary bodies contains hepatotoxic and nephrotoxic components that disappear once the organism enters the host cell. The toxins are once again a factor following replication and release of progeny elementary bodies from the host cell. These toxins present on the elementary bodies induce the production of antibodies that neutralize the toxins and destroy infectivity. These toxins have not been isolated and characterized, but they are believed to be related to the few proteinaceous-specific membrane antigens of the intact elementary body. These proteins have low antigenicity.[28]

The outcome of an infection is determined by the mononuclear macrophages. If an elementary body is phagocytized and is not coated with opsonins, the organism can survive and replicate within the macrophage.[31] Lymphokines secreted by activated lymphocytes inhibit the replication of chlamydia found in phagosomes. In order to maintain inhibitory concentrations, the lymphokines must be continuously secreted. During persistent infection, chlamydia remain within a membrane-bound compartment and release infectious progeny and antigens via exocytosis. It is therefore difficult for the host to remove the microorganisms from an infected cell.[5] In addition, exocytosed antigens released from the cells may not be processed in a way that can be recognized by class I-restricted cytotoxic T-lymphocytes. This allows infection, and probably reinfection, to occur and be maintained in the presence of high levels of humoral antibodies.[57]

These interactions of the host immune system and the intracellular parasite cause the varied incubation times, clinical signs and pathology noted with chlamydial infections.[33] When virulent strains of chlamydia are inhaled, primary propagation occurs within the epithelial cells of the lung and air sacs. Direct spread of the organism from the air sacs to adjacent serosal membranes can lead to polyserositis, including pericarditis.[38]

If chlamydia organisms are ingested, they are believed to initially replicate mainly within the intestinal tract. Given the high number of birds with antibodies to chlamydia, most primary infections must occur without the development of obvious clinical signs. Birds may be fully susceptible following survival of a clinical disease. The amount of antitoxic antibodies seems too low to induce some immunity. It is uncertain if a latent infection prevents another chlamydial strain from entering the host.

FIG 34.2 A five-year-old African Grey Parrot was presented with a 12-day history of progressive upper respiratory disease, polyuria (biliverdinuria), diarrhea and anorexia. On presentation, the bird had a severe rhinitis, conjunctivitis, severe dyspnea and emaciation (275 g). Clinical pathology findings included WBC=35,000, AST=1800, CPK=550 and LDH=1400. Chlamydia antigen was detected in the feces and on a pharyngeal swab by antigen-capture ELISA. The client had an upper respiratory disease and flu-like symptoms. The bird improved the day after receiving an IV injection of Vibravenös and was switched to oral doxycycline. African Grey Parrots are generally considered resistant to chlamydiosis, but as indicated by this case, under some conditions they can become sick.

Incubation Period

Incubation periods for chlamydia are difficult to determine because of differences in strain virulence, varying clinical responses of a wide avian host range and the lifelong infections that can occur in some hosts. The minimum incubation period for naturally infected Psittaciformes is 42 days.[42] An incubation period of seven years was suggested for budgerigars.[48,51]

Clinical Disease and Pathology

The clinical progression of infections varies with the virulence of the infecting strain and the host species. Asymptomatic infections are characteristic in adult birds exposed to moderate numbers of a moderately virulent strain of chlamydia. These infected birds may shed the organism for several months while remaining asymptomatic. Extreme environmental changes or concurrent infections may activate persistent infections, resulting in the occurrence of clinical disease. Epizooitologically, outbreaks in offspring from asymptomatically infected parents and young birds to which they are exposed are common.

Clinical Signs

Young birds exposed to high doses of a virulent strain develop acute systemic infections frequently resulting in death. Clinical signs can include rough plumage, low body temperature, tremor, lethargy, conjunctivitis, dyspnea, rales, coryza (pigeons) and sinusitis (budgerigars). Emaciation, dehydration, yellowish-to-greenish droppings (suggesting liver involvement), or grayish, watery droppings may also be noted (see Color 8). Death ensues within 8 to 14 days. Spontaneous recovery is rare. Survivors may have poorly formed feathers. Table 34.1 lists the typical clinical pathology changes associated with a symptomatic chlamydial infection.

Subacute or protracted diseases are typical for all avian species with a reduced susceptibility or for those infected with a moderately virulent strain. Progressive emaciation, greenish diarrhea, occasional conjunctivitis and high levels of urates in the droppings are common. Clinical signs may be subtle and overlooked. Psittaciformes occasionally develop CNS signs, including paroxysmal or continuous clonic-tonic convulsions, tremors and opisthotonos. Untreated birds die within a few weeks. In the cockatiel and the Houbara Bustard, incapacitating flaccid paresis and paralysis have been described.

TABLE 34.1 Clinical Pathology Findings Associated with Chlamydiosis[41]

Parameter	Change
WBC	Elevated (2 to 3 times normal)
Hct	Decreased (20 to 30%)
Heterophils	Normal
Lymphocytes	Decreased to normal
Monocytes	Normal
Eosinophils	Normal
Basophils	Normal
SGOT	Elevated (> 3 times normal)
CPK	Elevated (> 2 times normal)
LDH	Elevated (> 2 times normal)
AST	Elevated (> 3 times normal)
Total protein	Slight increase
Uric acid	Normal
Bile acids	Elevated (> 2 times normal)

A distinct, sometimes recurrent, keratoconjunctivitis with no other, or only subtle, signs has been described for small Australian parakeets (especially in the genus *Neophema*), pigeons, ducks, and European finches (Figure 34.3). Diseases in *Neophema* spp. are frequently refractory to therapy. Conjunctivitis and nasal discharge are characteristic of chlamydiosis in domestic pigeons. Mortality rates of the ophthalmic form are about 10%, but can reach 100% if untreated.[19,27] Conjunctivitis may be the predominant clinical sign in infected domestic ducks and geese. Mortality, particularly in ducklings, can range between 10 to 80%.[52]

Chlamydiosis in ratites can cause clinical and pathologic lesions of a rather nonspecific type. High mortality has been reported in ostrich chicks infected with *C. psittaci*.[23,45] The chronic course is clinically inconspicuous, although anemia is common and LDH and AST levels may be increased five to ten times. Birds with persistent infections may not be recognized until they infect other animals or their caretakers. The documentation of infections in nestlings from an apparently healthy breeding pair is also suggestive of latently infected adults.

Gross Lesions

Gross lesions can vary as widely as the clinical disease. Acute lesions are characterized by hepatomegaly, fibrinous peritonitis, air sacculitis, perihepatitis, pericarditis, bronchopneumonia, enteritis and nephrosis. Miliary necrosis of parenchymal organs is common, probably due to the effects of chlamydial toxins. Splenomegaly is frequently discussed

FIG 34.3 A two-year-old cockatiel was presented for severe epiphora and conjunctivitis of two days' duration. A conjunctival scraping revealed a mixed population of gram-positive cocci and a few gram-negative rods. Gimenez staining was negative for chlamydia. The patient responded to treatment with tetracycline ophthalmic solution. Chlamydia is frequently implicated in conjunctivitis in cockatiels.

as a common finding in chlamydiosis (Figure 34.4). However, fibrinous air sacculitis is more indicative of chlamydiosis in Psittaciformes and pigeons (see Figure 12.52).

Splenomegaly may not occur with chlamydiosis at all. In sexually active males, chlamydial-induced orchitis or epididymitis results in permanent infertility. Oophoritis is rare.

Subacute to chronic lesions are characterized by anemia caused by a panmyelopathy in the bone marrow and tissue deficiencies of heterophils and macrophages.[19,27] The pathogenesis of the panmyelopathy is undetermined. Chronic cases are characterized by proliferation of connective tissue (up to cirrhosis) in the liver and kidney. Pancreatic necrosis has been described particularly in budgerigars and pigeons.

Histopathology

Histopathologic findings are mostly nonspecific except for the presence of LCL bodies, which are pathognomonic. LCL bodies can occur in many organs but are especially common in serosal membranes. Typical of more acute disease is the intrasinusoidal proliferation of Kupffer's star cells (pearl string-like appearance) in the liver. Proliferation of monocytes and activation of the RES may occur in parenchymal organs, particularly the spleen, liver and kidney. Epithelioid cell granulomas in the liver and pneumonia with proliferations of epithelial cells in the air capillaries are common with chronic cases. Swollen epithelial cells may be vacuolated, and immigration of lymphocytes into the damaged tissue can be seen. CNS lesions consist of nonpurulent meningitis. Secondary bacterial, fungal or viral infections may alter lesions and confuse chlamydial changes.[19,20,27]

Differential Diagnosis

The clinical and pathologic presentation of chlamydiosis is so variable that it can normally be ruled out only with laboratory investigations. The more common rule-outs include infections with herpesvirus, paramyxovirus, influenza A virus and Enterobacteriaceae, particularly salmonellosis. The CNS signs should be differentiated from Newcastle disease and salmonellosis, and the conjunctivitis in ducklings and goslings from influenza A infections and mycoplasmosis.

Diagnosis of Chlamydiosis

Diagnostic Methods

Cytology

Conjunctival smears of birds with conjunctivitis can be stained for LCL bodies (see Color 10). As a rule, smears contain heterophils, some lymphocytes, some plasma cells and occasionally macrophage-like cells containing intracytoplasmic LCL bodies. Preparations containing numerous cells provide the greatest likelihood of a positive diagnosis. Since LCL bodies are difficult to detect, a positive test is confirmatory while a negative smear does not rule out chlamydiosis. Immunofluorescent methods using commercially available conjugates[a] are more sensitive. Every veterinary hospital should be able to perform cytologic evaluation of imprint slides including postmortem samples of the liver, spleen and air sacs (see Chapter 10). Other diagnostic methods require a specialized laboratory.

Culture

Culture of chlamydia is routinely performed in McCoy cell line, Buffalo Green Monkey cells or chicken embryo fibroblasts.[16] Cell culture is sensitive and able to detect small numbers of chlamydia within two to three passages. For isolation, parenchymal organs (liver, spleen, lungs, kidneys,) and feces should be shipped in transport medium (glucose 74.6 g/l,

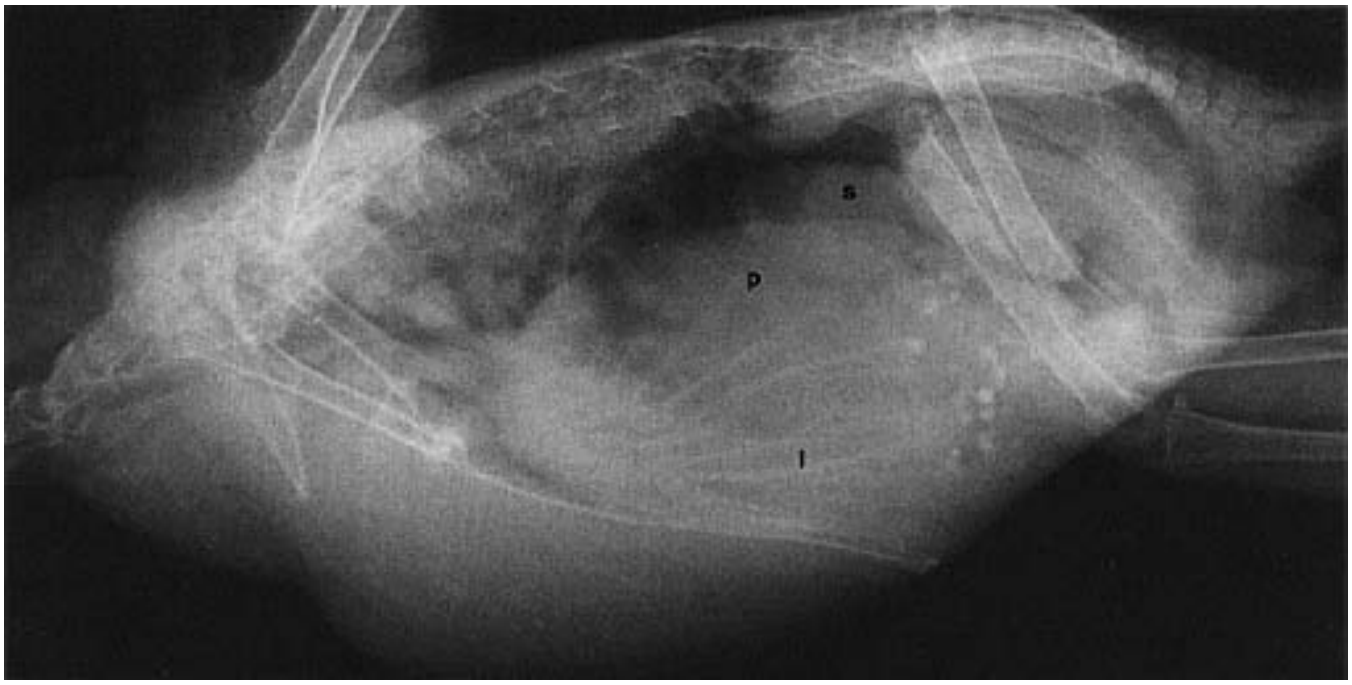

FIG 34.4 A mature Amazon parrot was presented with biliverdinuria, diarrhea, dyspnea and anorexia of four days' duration. The bird had been obtained from a "bird farm" about six weeks earlier. Radiographic findings included hepatomegaly (l) and splenomegaly (s). The enlarged liver is displacing the proventriculus (p) and the spleen dorsally. The bird responded to oral doxycycline and improved 12-16 hours after the initial dose.

K_2HPO_4 1.237 g/l, L glutamic acid 0.721 g/l, 10% fetal calf serum, vancomycin and streptomycin 100 μg/ml, gentamicin and nystatin 50 μg/ml).[50] Sucrose albumin phosphate solution (pH 7.2) cooled to 4°C is an effective storage and transport medium for feces. Fecal samples and tissue samples contaminated with feces are cleaned by labor-intensive centrifugation. The first passage takes up to six days, the second and third passages require three days each, so that three passages require approximately two weeks. Culture is the only way to directly demonstrate *Chlamydia psittaci*.

Antigen Detection Systems

Highly sensitive and specific ELISA test systems are available for detecting chlamydial antigen or anti-chlamydial antibodies (Table 34.2). An antigen test kit developed for human *C. trachomatis* has been used successfully for *Chlamydia psittaci*, which has the same group-specific antigens. Comparisons between this test kit and cell culture indicated that false-negative results occurred with ELISA when insufficient numbers of chlamydial particles (less than 2.5 ng[3,53] [600 elementary bodies]) were present in the sample. False-negative cell culture results occurred when chlamydial organisms were no longer viable.[14,15,34]

Culture results were poorest with fecal samples that were desiccated, subjected to bacterial deterioration or contaminated with litter or other "foreign" materials. The sensitivity was 84.2 % for ELISA and 80% for cell culture when a cloacal swab shipped in transport medium was used for testing instead of feces.[13] In evaluating 7,000 cloacal swabs for the diagnosis of chlamydiosis, it was determined that the antigen ELISA is sensitive, quick to perform (results in four hours) and can be made noninfectious for laboratory staff by heating at 100°C for 15 minutes. Cloacal

CLINICAL APPLICATIONS

- Interspecies transfer of chlamydia (in quarantine stations, breeding farms, multispecies aviaries, pet shops) can change the physicochemical properties, antigenic composition, toxic components and the host spectrum of the organism.
- Surveys indicate that between 30 and 70% of the birds tested have anti-chlamydial antibodies. Clinical disease is precipitated mainly by human-induced conditions and procedures.
- Chlamydia can usually be detected in the feces ten days prior to the onset of clinical signs.
- Carriers may begin to shed the organism following a stressful event.
- Antibody production with an active infection may be poor, and birds that survive infection are fully susceptible to disease.

swabs, not fecal samples, should be used for testing. The former does not require a centrifugation step and probably contains a higher concentration of chlamydial organisms (possibly in cloacal mucosa cells).[12]

Extremely high concentrations of avian *Staphylococcus aureus* (more than 10^8,[51] or more than 1.5×10^9/ml suspension[3]) can cause false-positive ELISA results.[53] *Actinobacillus salpingitidis*, which is rarely found in feces,[3] and *Acinetobacter calcoaceticum*[53] can also cause false-positive results. *Staphyloccus hyicus*, a non-avian staphylococcus, has also been implicated in false-positive reactions.[53] False-negative results with the antigen ELISA may occur because of the irregularity of antigen shedding in latently or persistently infected birds.[11,34,51] Administration of several antibiotics including chloramphenicol, tylosin, erythromycin and tetracyclines can also interfere with the detection of chlamydia in cell culture.

Comparison of Antigen Capture Tests

The reproducibility of some latex agglutination tests has been poor.[3] The latex agglutination test Clearview Chlamydia (CC)[b] used for the diagnosis of *Chlamydia trachomatis* by means of cervical epithelial cells can be easily performed in the veterinary hospital.[3] The CC test was found to be more sensitive (detected 130 elementary bodies/ml) than the IDEA[c] test (required 600 elementary bodies/ml of sample). However, the CC test is unsuitable for use with homogenized organs, fecal material or samples purified by centrifugation. When cloacal samples were collected and transported in tubes provided by the manufacturer, CC and IDEA agreed in 84.6% of the samples. Samples positive only with CC could not be confirmed positive by other methods (one exception). The CC test is more likely to have false positives as a result of bacterial contamination than is the IDEA test. Moderate to high numbers of a mixed bacterial flora, high numbers of *Staphylococcus aureus, Pasteurella multocida*, and *Sarcina* sp. can cause false-positive CC results.[3]

The Chlamydiazyme[d] test kit was compared with the IDEA for detection of chlamydial antigen.[39] Chlamydiazyme was found to be less sensitive, but was more likely to have false positives from nonspecific cross reactions (decreased specificity). These findings were confirmed by other testing, and the Chlamydiazyme test system was estimated to detect 312.5 pg (= 4,800 particles). False-positive results occurred with *A. calcoaceticus, S. aureus* and *S. hyicus, Legionella pneumophilus, Bartonella bacilliformis, Corynebacterium pyogenes, Pasteurella multocida* and *Enterococcus faecalis*.

The Surecell[e] ELISA test kit produced by Kodak is easy to use and can be performed in any hospital. Unfortunately, it, like other antigen detection tests that use antibodies, is plagued with false-positive results, probably due to cross-reacting bacteria. A recent study indicated that this test had a specificity of 80% and a sensitivity of 100% (compared to culture). Cross reactions were not found to occur with a variety of bacteria.[37] The minimum quantity of chlamydial material that the producer claims can be detected is 70 per gram. In some cases, birds may have chlamydiosis and are shedding insufficient numbers of organisms to be detected by an antigen capture system. The development and use of *C. psittaci*-specific DNA probes may prove to be the best method to detect birds that are actively shedding the organism.

TABLE 34.2 Antigen Detection Systems for Chlamydia

Diagnostic Test	Number of Elementary Bodies Detected	False positives (specificity)	False negatives (sensitivity)
IDEA[c]	600	+	+
Culture		None	
Clearview Chlamydia[b]	130	+++	+
Chlamydiazyme[d]	4800	+++	+
SureCell[e]	70	+	+

Specificity: Some bacteria will cross-react in antigen detection kits for chlamydia, creating false positives. ++ = some bacteria; +++ = many bacteria.
Sensitivity: The sensitivity of any chlamydia antigen test is affected by the number of elementary bodies present. + = high sensitivity; ++ = lower sensitivity.
Antigen detection systems are used to document shedding in clinically affected birds.

Antibody Tests

Detection of anti-chlamydial antibodies using complement fixation (CF) was proven to be unsuitable because birds produce mainly non-CF antibodies following a chlamydial infection.[26,49] The C1 of guinea pig complement, which is a critical component of the CF test, is incompatible with the serum of many avian species. A test that functions independent of the species in question was necessary for serologic diagnosis of chlamydiosis in the class Aves. An inhibitory ELISA (= BELISA) that recognizes four times more infected birds than CF has been developed.

The relationship between CF and BELISA indicates that high anti-chlamydial antibody titers detected by

CF and BELISA are indicative of a positive reaction; low titers are diagnostic only with BELISA.[34] Ten months following an experimental chlamydial infection, CF antibodies decrease considerably, while BELISA shows a continuous increase. This finding suggests that the composition of the antibodies detected varies[49] and that only those antibodies detected by BELISA are stimulated by the permanent intracellular presence of chlamydia.

A comparison of antigen excretion and antibody status showed that flocks with clinically affected birds had higher antibody titers and excreted chlamydia at a higher rate than non-clinically affected flocks. A small number of birds with an extinction just beneath the cutoff and no demonstrable antibodies gave the reasons for a final correction of the cutoff value. BELISA is suitable for identifying infected birds, whether they excrete the agent or not.[12] A commercially available test kit using the principles of BELISA has been developed (*C. psittaci*-AK-EIA).[f] The antigen and antibody ELISA tests have been compared with cell culture and CF for the detection of chlamydia in thousands of field cases.[8,10,12,13,34] False-negative results may occur with this test kit in fresh infections (no antibody production as yet), following treatment (inhibition of antibody production and no shedding of the agent), pre-test handling of the samples and cross reactions with bacteria.

The high sensitivity of BELISA has shown that *C. psittaci* antibodies are more widely distributed than previously thought. Sustained detection of antibodies by BELISA suggests that chlamydia may cause a life-long persistent infection, which is difficult to eliminate with treatment.[7]

Treatment of Chlamydiosis

Therapeutic Agents

Many countries have instigated governmental regulations for treatment and control of chlamydiosis to prevent zoonotic infections. The following therapeutic considerations address only the scientific aspects of treating chlamydiosis, and the reader should be aware of local laws governing therapy. Several antibiotics have *in vitro* activity against chlamydia, but only the tetracyclines and enrofloxacin have been used successfully *in vivo*, the latter only in limited trials.[40]

The tetracyclines alter the replication of chlamydia by inhibiting the synthesis of enzymes, the growth and fission of the reticulate bodies and possibly the reorganization of the elementary bodies. Antimicrobial-induced damage that occurs to the reticulate and elementary bodies may be temporary, with the organism resuming normal replication within 5.5 days of ceasing therapy. The host defense mechanisms must be intact to remove damaged chlamydial elements before they can recover and begin replicating.[17] Providing the immune system with the time necessary to remove these damaged reticulate and elementary bodies is one reason for long-term anti-chlamydia therapy.

Tetracyclines are effective only against actively metabolizing microorganisms, ie, during growth or fission. This drug is not effective in treating latently or persistently infected birds in which the chlamydia is located inertly in macrophages. The hypothesis that chlamydia is eliminated by the natural replacement of infected host cells (if treatment is continued for such prolonged periods) has not been confirmed using currently available diagnostic techniques.

Strains of chlamydia that are resistant to tetracyclines are still rather rare (one strain from ducks > 75 µg tetracycline),[35] but strains with reduced sensitivity continue to be recognized.[18] It has been shown that there is no direct correlation between the blood level of tetracyclines and therapeutic efficacy. Thus, the suggested blood level of >1 µg/ml cannot be assumed to equate with successful treatment. In some situations, subtherapeutic blood levels (<1 µg/ml) may be successful[58] while in other cases, full therapeutic levels do not resolve an infection.[28] These clinical experiences have been supported by laboratory testing.

The *in vitro* MIC for 12 *C. psittaci* strains ranged from 0.01 to 0.08 µg/ml, and 0.2 µg/ml completely inhibited the production of elementary bodies of 62 strains in cell culture. Varying dosages of antibiotics in owls resulted in almost equal plasma concentrations but different time periods of shedding the agent following the discontinuation of the treatment (high dosages shedding 4.5 months, low dosages 9.5 months until the end of the trial).[11] Some chlamydial strains can develop resistance to tetracycline if exposed to sub-therapeutic levels for prolonged periods of time.

In acutely sick birds chlamydial organisms undergoing rapid metabolism, and treatment with tetracyclines leads to immediate cessation of shedding and

a clinical recovery in accordance with the severity of the parenchymatous lesions. In these birds, elimination of *Chlamydia psittaci* is possible; however, under practical conditions, not likely. Nevertheless, treatment reduces the infectious pressure in the environment and, therefore, minimizes the risk of infection for humans and other animals. Birds with severe lesions may die, even if the agent is completely inactivated.

Chlortetracycline

Chlortetracycline (CTC) for oral application can be administered in soft mixed feed (cooked grain with or without egg [yolk, albumen]), in commercial parrot pellets or on dehulled seeds covered with CTC. The latter is recommended (500 ppm) for budgerigars and small finches. Food containing 5,000 ppm of CTC is normally provided, although there are many avian species, particularly among the Psittaciformes, that reach effective blood levels with CTC concentrations of 2,000 to 2,500 ppm. Birds will generally consume more food when it contains a lower concentration of CTC. Birds that have been shown to do well with food containing lower concentrations of CTC are listed in Table 34.3.

Chlortetracycline is renally excreted and should be used cautiously in patients with kidney damage (see Chapter 17). Birds dislike eating medicated feed or pellets, and therapeutic blood levels are reached only within ten days. Because infected birds will continue to shed, the delayed induction of proper blood levels poses an additional risk for caretakers and for other birds. No other food components can be fed during the treatment period.

TABLE 34.3 Birds That Respond to Lower Food Concentrations of CTC[27,41]

Large macaws	Eastern Rosella
Genus *Agapornis*	Pale-headed Rosella
Grey-cheeked Parakeet	Red-fronted Parakeet
Canary-winged Parakeet	Turquoise Parakeet
Red-winged Parrot	Scarlet-crested Parrot
Mulga Parrot	Bourke's Parrot
Western Rosella	Cockatiel

Oxytetracycline

Intramuscular injections of oxytetracycline (OTC) at a dosage level of 100 mg/kg have been suggested. The birds listed in Table 34.3 should be given 75 mg/kg. Oxytetracycline (LA 200) produces a long-lasting blood level at a dose of 75-100 mg/kg body weight. Injections induce effective blood levels within hours, and the shedding of *Chlamydia psittaci* will stop 24 hours post-injection. This treatment regime also allows a bird to remain on its normal diet while being treated (see Chapter 18). OTC has the same side effects as CTC. In addition, severe muscle necrosis may occur at the site of injection.

Doxycycline

Doxycycline is a preparation that has been developed for intravenous administration in humans. The solvents are different in doxycycline products manufactured in the United States and Europe. Intravenous preparations available in the United States cause severe local necrosis of the muscles when given intramuscularly. European preparations may be safely given intramuscularly and induce blood levels of 1 µg/ml that last approximately seven days when administered at a dose of 75 to 100 mg/kg body weight. The quantity of drug to be injected is rather large, and several injection sites should be used.

During long-term treatment, which is still legally stipulated in many countries, the drug is increasingly eliminated from the blood so that injection intervals decrease. Some countries have regulations controlling the injection intervals, although these should vary according to the species. Doxycycline is excreted mainly extrarenally (feces, bile), and the metabolites are microbiologically almost inert. This treatment reduces the destruction of autogenous intestinal flora seen with other tetracyclines. A doxycycline-medicated food was found to provide >1 µg/ml plasma concentration in a group of psittacine birds (Table 34.4).

TABLE 34.4 Doxycycline-medicated Food Diet*[41]

29% canned cooked kidney beans
29% canned whole corn
29% cooked white rice
13% dry oatmeal cereal (by weight)
1000 mg doxycycline hyclate (from capsules) per kg of feed

* Adapted from Flammer K, et al. Proc Assoc Avian Vet, 1991, pp 1-5. Medicated diets have been found to maintain acceptable plasma doxycycline concentrations in Goffin's Cockatoos, African Grey Parrots, Blue-fronted Amazon Parrots and Orange-winged Amazon Parrots.

An antimicrobial that can be added to the drinking water and effectively treat chlamydia in Psittaciformes remains elusive, but enrofloxacin has shown some potential. Birds in the USA with severe, acute chlamydiosis can be initially treated with an IV injection of Vibramycin, followed by oral doxycycline when the bird is stabilized (generally in 24 hours).

A micronized suspension of doxycycline has shown moderate promise in the treatment of chlamydia. In one study involving pigeons, IM administration of

micronized doxycycline (100 mg/kg body weight) three times at weekly intervals maintained a plasma level about 1 μg/ml for 43 days. (More research is planned to increase the doxycycline concentration from today's 66 mg/ml to 132 mg/ml and a prolonged plasma level accordingly.) There was no clinical evidence of pain or histologic lesions suggestive of necrosis associated with the injection site. Exercise would cause a sharp rise in the plasma doxycycline concentrations.[6]

Apart from specific treatment with tetracyclines, symptomatic therapy in acutely sick birds is frequently necessary. Birds should be kept isolated in warm rooms, and intravenous fluids, hepatoprotective therapy and paramunity inducers should be administered according to the clinical signs. Chicks should be fed frequently with small amounts of a liquid formula.

Enrofloxacin

Enrofloxacin inhibits the *in vitro* growth of *C. psittaci*, but only a few avian strains have been tested. The MIC of enrofloxacin for *C. psittaci* was found to be 0.125 mg/l; the minimum bactericidal concentration is much higher: 50 to 75 mg/l. Concentrations between 0.5 and 1.0 mg/l evoked irreversible damage to the majority of the chlamydia particles.[40]

Preliminary results indicate that treatment with enrofloxacin-medicated food for three weeks was effective in eliminating chlamydia from parakeets. Seven groups of experimentally infected budgerigars and other psittaciforme birds (Alexander Ring-necked Parakeet, Senegal Parrot, Canary-winged Parakeet) were effectively treated for 14 days with medicated food containing 500 ppm (budgerigars=250 ppm) enrofloxacin. From seven days after the beginning of treatment until four to five weeks after the end of treatment, no chlamydia could be isolated. Complete elimination of chlamydia from a quarantined group of 196 Senegal Parrots was reached only after substituting their normal mixed food with medicated corn containing 1000 ppm enrofloxacin.[40] A minimum blood level of 0.5 mg/l for enrofloxacin for at least 14 days was considered necessary to control chlamydiosis.[36,40]

Control

Persistent, probably life-long, infections require new ideas on control. Legal regulations should be reformulated and concentrate on clinically sick and seropositive birds. Seronegative birds should not be treated. During treatment and in clinically healthy but infected flocks, regular cleaning and disinfecting programs will minimize the chlamydial contamination in the environment and reduce the occurrence of reinfection or transmission. Birds that recover from chlamydiosis are fully susceptible to future infections. Ideally, breeding birds would be seronegative for chlamydia but, given the prevalence of the organism as detected by antibody titers in the companion bird population, it seems unlikely that a seronegative population could be established. Free-ranging birds that may transmit chlamydia should not have access to aviary birds.

Vaccination programs for the control of chlamydiosis remain elusive because chlamydia effectively inhibit the host defense system (see pathogenesis). Subunit vaccines designed to inhibit or block the host membrane receptors could damage normal epithelial cells.[53] Although the group-specific antigen is common to almost all chlamydial strains, it does not elicit a protective response. The antigenic variability among the avian strains is large, so that polyvalent vaccines might be necessary.[7] Because cell-mediated immunity plays an important role in the host defense to chlamydia, vaccines may sensitize the host and initiate excessive host reactions and disease.

Zoonotic Potential

C. psittaci strains from Psittaciformes, domesticated ducks (in Europe) and turkeys (in USA) appear to cause the most severe disease in humans. It appears that the host animal in which chlamydial passage occurs prior to the human infection influences the pathogenicity of the agent for humans. The only reported case of human chlamydiosis from free-ranging birds involved the Northern Fulmar on the Faroe Islands.[19] Pigeon strains of chlamydia are considered less virulent for humans.

Human infections are characterized by flu-like clinical signs including a high fever, severe headaches, chills, shortness of breath and general debilitation. If untreated, atypical pneumonia or CNS signs mainly caused by meningitis can develop, in addition to liver and kidney lesions due to the presence of toxicity.[52]

In rare cases, neuritis with severe pain is described. Chronic manifestations can be arteritis, cardiovascular insufficiencies and thrombophlebitis including insufficiency of the venal valves. Treatment with doxycycline is recommended for three weeks. A four-fold increase in titer should not be expected to occur in humans being treated with tetracyclines, and diag-

nosis requires culture or detection of antigen in sputum (antigen ELISA). As in birds, the CF test is not sensitive enough for accurate diagnosis in humans. Serologic determinations with the antibody ELISA have shown that humans can also be carriers following treatment, and recrudescence is possible when strong stressors activate the agent.

Chlamydiosis is a reportable disease in the United States because of its potential as a zoonotic agent. Current regulations dictate closing a business or aviary, a forced quarantine period and treatment of all exposed birds with chlortetracycline-medicated foods. These recommendations do not effectively address the problems associated with treating or controlling chlamydiosis and should be evaluated and modified accordingly.

Products Mentioned in the Text

a. Imagen Chlamydia Test kit, Röhm Pharma, Darmstadt, Germany
b. Clearview Chlamydia (CC), Abbott Unipath, Bedford UK
c. IDEA Chlamydia test kit, Röhm Pharma, Darmstadt, Germany
d. Chlamydiazyme, Fa. Abbot,
e. Surecell, Eastman Kodak, Rochester, NY
f. *Chlamydia psittaci*-AK-EIA, Röhm Pharma, Darmstadt, Germany

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