CHAPTER

38

MYCOPLASMA AND RICKETTSIA

Helga Gerlach

The mycoplasmatales constitutes one order within the class Mollicutes that replicates mainly by binary fission. Strains that produce mycelia-like forms may propagate by dissociation of these "mycelia." Morphologically, colonies and single organisms can exist in multiple forms (coccoid, rods, ring-forms), depending on the physical properties of the media in which they are growing. In most cases, morphology is unsuitable for species differentiation. In contrast to bacteria, mycoplasmatales have no cell wall and are bound by a three-layer membrane. Thus, they are resistant to antibiotics that inhibit cell wall development (eg, penicillins, cephalosporins, bacitracin) and sulfonamides. Mycoplasmatales are fastidious and must obtain most of the nutrient requirements from the growth media because of their relatively small genome. They grow on agar media in small, fried egg-shaped colonies, which in many instances, can be recognized only under the microscope. Generally, specialized laboratories are necessary for isolation and identification. The lack of a cell wall makes the organism sensitive to inactivation outside the host (it survives only hours on dry surfaces, two to four days in water); therefore, transport media are necessary for shipping infected tissues intended for isolation attempts. Mycoplasmatales that are free in the environment are susceptible to all commonly used disinfectants. Organisms within host excretions are protected from contact with the disinfectant. Secretions and excretions must be removed before disinfecting procedures are effective.

The mycoplasmatales consist of three genera, which can be distinguished roughly by the following properties:

Mycoplasma need cholesterol for growth (production of the cellular membrane).

Acholeplasma do not need cholesterol for growth, but many strains can be inhibited by the thallium acetate that is commonly used for inhibiting gram-negative bacteria in media used for the isolation of mycoplasma.

Ureaplasma were formerly called T-strains because of their tiny colony sizes. They require urea for their energy metabolism and also cholesterol for growth.

Many isolates from companion and aviary birds have not yet been fully identified and have no valid name. In addition, the pathogenicity and epizootiology of these strains have not been defined to date.

Mycoplasmatales are distributed worldwide in connection with the poultry industry. There is little information on the prevalence of mycoplasmatales in captive or free-ranging Psittaciformes or other groups of birds. Isolations have been rare, and the importance of the majority of the strains is unknown. With intensified aviculture, increased farm sizes and population densities on these farms, more problems with mycoplasmatales can be expected.

FIG 38.1 High density, confined, indoor breeding operations increase the exposure of individual birds to a mixture of microorganisms that may include *Mycoplasma* spp. Damage to the respiratory tract caused by increased dust, dry-heated air and respiratory viral infections predispose birds to mycoplasma infections. Most infectious diseases are less of a problem in birds maintained in low density outdoor breeding facilities (reprinted with permission *J Assoc Avian Vet*).

Mycoplasmatales

The host spectrum of the mycoplasmatales is rather narrow (see Table 38.1), with the exception of *Mycoplasma cloacale* and the genus *Acholeplasma*. Reports suggesting isolates of well known species from unusual hosts should be met with skepticism. The various mycoplasmatales have similar biochemical properties and serologically cross-react with other species of the order, creating a high number of false-positive results (low specificity). The reason for these cross reactions is that the lack of a cell wall diminishes the antigenicity, which is probably governed by enzymes within the microorganisms. Because these enzymes are phylogenetically old and highly conserved, they do not vary much between genera. Physical methods such as electrophoresis (combined with blot methods) are more reliable than serologic methods for differentiating between species or strains.[28]

Transmission

Mycoplasmatales are relatively low in infectivity. Close contact between individuals is necessary for transmission, and infections are most common in dense populations (Figure 38.1). The respiratory and genital tracts are the primary portals of entrance. The organism is spread by respiratory excretions and by the gonads of both sexes as well as hematologically through the body. Infected air sacs can lead to contact transmission of the ovary (and developing follicle). Transovarian transmission is epornitically important, although in clinically healthy breeders, the egg transmission rate is low (between 0.1 and 1.0 %); however, there are some exceptions. The egg transmission rate of *M. meleagridis* in turkeys can be as high as 25%. This species causes predominantly a venereal disease. Infected breeders may be asymptomatic. Close contact is the primary mode of transmission in neonates. Offspring feeding on contaminated crop regurgitations (eg, crop milk in pigeons) may also become infected.

Pathogenesis

Primary pathogenic strains, ie, strains that can damage epithelial cells and cause disease without additional factors, have to be distinguished from secondary pathogenic strains that need predamaged epithelium, and from strains that are assumed to be apathogenic. Mycoplasmatales preferably colonize the mucosa of the respiratory and the genital tracts. Strains capable of inducing systemic infections can be found in the brain and joints. Infections start with the adsorption of the organism to the surface of host cells (including erythrocytes with hemagglutinating strains). Multiplication takes place on the cell surface, and both the membrane integrity as well as the function of the host cell can be altered. Because the agent may be hidden in the recesses of the host cell membrane, it can remain rather inaccessible by therapeutics and the host defense mechanisms. As a consequence, only negligible amounts of humoral antibodies, if any, are produced. *M. gallisepticum* (and

TABLE 38.1 Avian Host Spectrum of Mycoplasmatales[13,17]

Species	Host Spectrum	Signs of Disease
M. gallisepticum	Chicken, turkey, guineafowl, peafowl, pheasants, partridge, rock partridge, Red-legged Partridge, Japanese Quail, Bobwhite Quail, House Sparrow, domestic duck and goose,[5] Canada Goose[5]	Rhinitis, sinusitis, tracheitis, air sacculitis, pneumonia, arthritis, encephalitis, ophthalmitis
M. gallinarum	Chicken, pheasant, Chinese Bamboo Partridge, House Sparrow, Demoiselle Crane, Domestic Goose, Bewick's Swan	Mild respiratory signs in geese also infected with parvovirus
M. pullorum	Chicken, Turkey, Pheasant, Partridge	Asymptomatic
M. gallinaceum	Chicken, Turkey, Pheasant, Hoopoe	Asymptomatic: complicated with PMV1
M. iners	Chicken, Turkey, Domestic Goose,[2] Golden Pheasant[31]	Asymptomatic
M. gallopavonis	Turkey, (Chicken?)	Mild respiratory signs only in turkeys
M. meleagridis	Turkey	Sinusitis, air sacculitis, infection of the genital tract
M. iowae	Chicken, Turkey, Yellow-crowned Amazon Parrot	Mild air sacculitis, in turkeys veneral transmission and reduced hatchability
M. columbinasale	Pigeon	Rhinitis, pharyngitis
M. columborale	Pigeon (Chicken ?)	Rhinitis, pharyngitis
M. columbinum	Pigeon	Asymptomatic
M. synoviae	Chicken, Turkey,[23] Guineafowl, Red-legged Partridge, Japanese Quail,[2] House Sparrow,[31] Tree Sparrow,[31] Domestic Duck and Goose	Sinusitis, synovitis, air sacculitis, hepatitis, splenomegaly
M. anatis	Domestic Duck, Greater Scaup, Common Teal and other Teals,[31] Domestic Goose,[2] Coot,[31] Common Shoveler[31]	Rhinitis, sinusitis, air sacculitis only if triggered by influenza virus
M. glycophilum	Chicken	
M. lipofaciens	Chicken	
M. cloacale	Turkey,[2] Domestic Duck and Goose,[2] Tufted Duck, European Pochard, Muscovy Duck, Skylark, Starling, Cockatiel, Lesser Spotted Woodpecker[4]	
M. anseris	Domestic Goose	Together with *M. cloacale* lesions in geese
M. spp. *n.n.* (7 different types)	Domestic Duck	Mild respiratory signs
M. sp. *n.n.*	Budgerigar	Air sacculitis
A. laidlawii B (*var. inocuum*)	Chicken, Pigeon, Greater Adjutant Stork, Night Heron	Asymptomatic
A. laidlawii A	Domestic Duck and Goose	Air sacculitis, conjunctivitis, cloacitis
A. axanthum	Domestic Goose Domestic Duck	Embryonal death, peritonitis, salpingitis, air sacculitis Conjunctivitis, cloacitis
A. equifetale	Chicken	
A. spp. *n.n.* (2 different types)	Pigeon	Rhinitis, pharyngitis
U. gallorale	Chicken, Red Junglefowl	Air sacculitis, pneumonia
U. spp. *n.n.*	Turkey, Jungle Bush Quail	Respiratory signs
Unidentified (3 different types)	Severe Macaw, Cockatoo spp., Cockatiel, Canary	Chronic conjunctivitis, rhinitis, sneezing, sinusitis, dyspnea, arthritis
Several types ?	Saker Falcon, Peregrine Falcon, Prairie Falcon, Rough-legged Buzzard, Common Buzzard, Griffon Vulture,[31] Common Kestrel	Synovitis, air sacculitis, catarrhal tracheitis, serofibrinous pneumonia, sitting on paralyzed hocks,
One type	Phasianinae	See text
Several types ?	Black-headed Gull, Brown-eared Bulbul, Phasianinae, White-fronted Goose	Asymptomatic

probably other strains of avian mycoplasmatales) has a special organelle for attaching to the host cell.

Depending on the virulence of the strain in question, cellular damage may be caused at the site of colonization. The host reacts with a serofibrinous inflammation and activation of the cell-mediated defense system. The excessive response of the latter (which is genetically determined) governs the type and magnitude of pathologic changes.

Many mycoplasmatales cause transformation of the host lymphoblasts (mainly T-cells) by excreting a mutagenic substance. Affected cells function improperly and there is a severe proliferation of immature lymphocytes in local lymph follicles with invasion of the lymphoid cells into the infected area. These altered lymph follicles can appear similar to those described for lymphoma. Other pathogenicity factors are cytotoxins (exotoxins, H_2O_2) and polysaccharides. Triggering factors for mycoplasmatales are immature epithelial membranes, environmentally induced dyspnea (heat, dry air) and damage to the respiratory epithelium (excess NH_3, paramyxovirus, reovirus, adenovirus, infectious bronchitis virus and *E. coli*). The involvement of several different factors in a flock outbreak creates a high variability in clinical and pathologic changes.

Incubation Period

Incubation periods for *M. gallisepticum* are 6-21 days in chickens and 7-10 days in turkeys.[43] In other avian species and with other mycoplasmatales, long latency periods, egg transmission and the involvement of environmental factors make the determination of an incubation period difficult.

Clinical Disease and Pathology

Red Junglefowl

U. gallorale has been isolated from the pharynx of this species. Although the strain is serologically identical with isolates from the chicken, experimental infections did not cause disease in chickens.

Phasianinae

The Common Pheasant and its subspecies, *Crossoptilon* spp., the Golden Pheasant and probably other pheasants are susceptible. The main host is the Common Pheasant, which is typically maintained in large flocks. The strains of mycoplasma that are infectious to pheasants have been incompletely studied and documentations in the literature provide conflicting information.[13] The author's experience suggests that the Phasianinae have host-adapted strains, one that is apathogenic and another that experimentally reproduces typically defined signs of the disease. Clinical signs are most common in large groups of chicks at the age of two to eight weeks. Adults are rarely affected. A seasonal peak can be observed between June and August. The disease spreads slowly and not all aviaries are always affected. Morbidity is high. Mortality depends on secondary factors and can range from 30 to 90%. Blinking the eyes and scratching at the eyelids are the first clinical signs. Deterioration of the general condition, photophobia and swelling of the eyelids are followed by exudation, blepharoconjunctivitis and sometimes keratitis; approximately 25% of the corneal surface is affected. Death can be caused by cachexia as a result of blindness. Voluminous expansion of the infraorbital sinus, which contains only a small amount of exudate, may be observed. Birds are frequently dyspneic, particularly when agitated. At postmortem, the air sacs may be mildly inflamed or grossly normal.[13]

Japanese Quail

Experimental infections indicate that Japanese Quail are less susceptible to *M. gallisepticum* than are chickens. Isolation of the organism is possible from the trachea, lung and brain for weeks post-infection. The course is subclinical. Infections derived from contact with infected chickens or egg transmission have been documented.

Bobwhite Quail

This species is raised in large numbers in the southern parts of the United States. Dyspnea and anorexia have been observed. An isolate assumed to be *M. gallisepticum* from Bobwhite Quail caused typical lesions in turkey poults. However, a strain of *M. gallisepticum* experimentally given to Bobwhite Quail chicks did not result in lesions, and no antibodies were produced.

CLINICAL APPLICATIONS

- Mycoplasmatales are most important in dense populations of birds where direct transmission can easily occur. Control can be enhanced through sound hygiene.
- Mycoplasma are resistant to antibiotics that inhibit cell wall development (eg, penicillins, cephalosporins, bacitracin and sulfonamides).
- Mycoplasmatales that are free in the environment are susceptible to all commonly used disinfectants. Organisms within host excretions are protected from contact with the disinfectant. Secretions and excretions must be removed before disinfecting procedures are effective.
- With intensified aviculture, increased farm sizes and population densities on these farms, more problems with mycoplasmatales are to be expected.

Partridge

It is assumed that the same strains as in Phasianinae cause disease in the partridge, although this has not been proven. Affected birds develop infections that are similar to those seen in pheasants, but there is no defined seasonal peak. Birds up to 11 weeks of age show a swelling of the infraorbital sinuses which, in contrast to pheasants, are filled with a fibrinous, cheesy exudate (Figure 38.2). Free-ranging Red-legged Partridge usually develop clinical disease in August to December. Isolates are assumed to be identical to strains removed from pheasants and other partridges.

Rock Partridge

Disease has been described only in chicks and not in the respective breeding flock. Emaciation and swollen sinuses are the main clinical signs. Isolates assumed to be *M. gallisepticum* were experimentally apathogenic for chickens and turkeys.

Peafowl

Affected birds are lethargic, shake their heads to remove sticky nasal exudates, have swollen infraorbital sinuses and make gurgling respiratory sounds. Latent infections are thought to occur.

FIG 38.2 A young partridge was presented with a five-day history of progressive ocular irritation, photophobia, dyspnea and periocular swelling. Large, bilateral, periocular masses were noted on physical examination. Large quantities of necrotic debris were surgically removed from both intraorbital sinuses. *Mycoplasma* sp. was isolated from culture samples taken from the sinus cavities. The bird responded to postsurgical therapy with tylosin (courtesy of Helga Gerlach).

Guineafowl

An outbreak of infectious synovitis caused by *M. synoviae* in guineafowl could not be distinguished clinically or pathologically from the lesions that occur in chickens and turkeys.[26] In contrast to chickens and turkeys, affected guineafowl (including experimentally infected birds) developed severe amyloidosis and did not develop sinusitis.[29] Strains isolated from guineafowl are more virulent for guineafowl than for chickens.

Domestic Duck

A variety of *Mycoplasma* and *Acholeplasma* strains can be isolated from domestic ducks. In the few isolates that have been evaluated experimentally, pathogenicity is limited to mild respiratory lesions, conjunctivitis and cloacitis. *M. anatis* may cause enzootic rhinitis, sinusitis, conjunctivitis and lacrimation in association with concommitant influenzavirus A infections. Morbidity may be as high as 50-80%, but mortality remains below 5%. As a rule, affected ducks recover spontaneously without therapy. Experimentally, the clinical disease can be produced using *M. anatis* and influenzavirus A,[32] although both infectious agents alone have been proven to be apathogenic. The role of *M. cloacale* as the cause of a cloacitis in ducks has not been fully studied. Most of the *Mycoplasma* and *Acholeplasma* strains described are capable of causing increased embryonic mortality.

Domestic Goose

Geese suffering from cloacitis and necrosis of the phallus were found to be infected with mixed cultures of *M.* spp. (mostly *M. cloacale*, but also *M. anseris* and strain 1220). Phallus lesions are characterized by serofibrinous inflammation of the mucous membrane of the lymph sinus, the glandular part of the phallus, and occasionally the cloaca and the peritoneum. Necrosis of the affected phallus can be severe if secondary pathogens are present. Mortality is less than 1%. *M.* spp. can be isolated from the phallic lymph secretion as well as the spleen, testes, air sacs, peritoneum and liver. The incidence of affected ganders in some flocks can be as high as 40-100%. High numbers of infertile eggs and a high incidence of embryonic death are common in affected flocks.[36,40]

A. axanthum may cause embryonic mortality (up to 60%) around the 13th day of incubation. The organism can be isolated from the respiratory tract and feces of breeding birds showing embryonic mortality. Infected adults develop fibrinous peritonitis, salpingitis and air sacculitis. Goslings from infected flocks and experimentally infected neonates can suffer from

mild to severe air sacculitis (depending on the virulence of the strain in question).[37] The pathogenicity of *A. axanthum* can be potentiated by concommitant infection with parvovirus, even if antibody titers are high enough to prevent the parvovirus from causing clinical disease.[22]

Domestic Pigeon

At least six different species of mycoplasmatales have been isolated from domestic pigeons.[13,15] All of them apparently are incapable of causing primary disease. Following natural or experimental infection with all except *M. columbinum* and *A. laidlawii* (which have not been tested), the agents can be isolated from various organs including brain, eye and joints of asymptomatic birds. The frequent colonization of the pharyngeal mucosa is epizootiologically important because pigeons feed their offspring crop milk. During the act of regurgitation the crop milk passes over the infected mucosa and may be contaminated. Although egg transmission has been proven, this means of transmission might play the most important role.

Clinical signs of rhinitis, sinusitis, tracheitis and conjunctivitis are generally chronic in nature and vary with secondary factors such as concomittant infections with *Salmonella* spp. or *Chlamydia psittaci*. Under these conditions, mycoplasmatales can be isolated from the lower third of the trachea, air sacs and occasionally lung, and birds frequently have persistent respiratory sounds and serofibrinous inflammation of these organs. The association between the colonization of the meninges and synovial structures by mycoplasmatales and the frequency of arthritis and meningoencephalitis caused by salmonellosis has not been determined. Further evidence for the apathogenicity of uncomplicated mycoplasmatal infections in pigeons is the fact that humoral antibodies only occasionally develop following natural or experimental infections. In contrast to some older reports, experimental infection of chickens with pigeon mycoplasma strains does not lead to clinical disease. *M. gallisepticum* does not affect pigeons. *M. columborale* was recovered from a pigeon flock with respiratory signs that responded to treatment with tylosin. Experimentally infected three-week-old chicks developed mild to severe air sacculitis, but were clinically asymptomatic. There is no report of natural infection in chickens with *M. columorale*.[25]

Other Pigeons

In addition to domestic pigeons, infections with mycoplasmatales have also been described in the Wood Pigeon,[20] Collared Dove[14] and Crowned Pigeon.[13] The isolation of *M. columbinum* and *M. columborale* has also been recovered from healthy "feral pigeons."[21]

Saker Falcon

Mycoplasma was isolated from the trachea of a Saker Falcon with insufflation of the soft tissues around the eye and between the rami mandibulares following each expiration. Similar respiratory signs occured in two contact birds.[10] A definitive connection between the clinical signs and the *M.* isolate was not established. Mycoplasma-induced synovitis has also been described in this species.[19]

Peregrine Falcon

A *Mycoplasma* sp. was isolated from the trachea of two Peregrine Falcons with anorexia, vomiting, respiratory sounds and tachypnea (60-70 beats per minute).[10] The animals responded to treatment with tylosin.

Budgerigar

A *Mycoplasma* sp. was isolated from a budgerigar with air sacculitis.[1] The serum of five contact birds revealed humoral antibodies against the homologous strain with titers between 1:160 and 1:640. Antibodies were not detected against *M. gallisepticum* and *M. meleagridis*. The budgerigar strain propagated in the embryonated chicken egg and showed no embryonal pathogenicity. Budgerigars experimentally infected with *M. gallisepticum*[6] and *M. synoviae*[3] developed clinical signs. Budgerigar are not considered to be a natural host of *M. gallisepticum* and *M. synoviae*.

Cockatiel

It has been assumed that conjunctivitis in cockatiels can be caused by mycoplasmatales, (see Color 26) as wet sneezes and sinusitis are common in those birds. Although mycoplasmatales can be isolated from some of these cases, their importance in the disease process has not been determined. From the clinical course and response to treatment it can be concluded that chlamydiosis and infections with polyomavirus are the main pathogens in these conditions.[9,12] Many cockatiels in Florida with symptoms of mycoplasmosis respond to tylosin (as an eyewash) or lincocinspectinomycin (Harrison GJ, unpublished).

Severe Macaw

An epornitic of mycoplasma was described in Severe Macaws with clinical and pathologic lesions in the respiratory tract. Although mycoplasmas were iso-

lated, no causal relationship between the agent and the disease could be established.[11]

Yellow-crowned Amazon

A flock of Yellow-crowned Amazon Parrots experienced high mortality (200 of 1100 birds) with an upper respiratory tract disease. Lesions were complicated by the presence of many bacteria and also some fungi. A mycoplasma strain (assumed to be *M. gallisepticum*) was isolated and used for experimental infections in budgerigars and chickens. Mild air sac lesions were induced in budgerigars, but the strain was apathogenic for chickens. The serologic evidence for *M. gallisepticum* was questionable.[3]

Cockatoo

An unidentified strain of mycoplasma was isolated from a group of cockatoos with severe air sacculitis.[12]

Canary

An unidentified strain of mycoplasma was recovered from a flock of canaries with a high incidence of wheezing and "tail-bobbing." The affected flock had suffered from canarypox.

Pathology

At necropsy, lesions caused by various mycoplasmatales in respective hosts vary in degree but not in presentation. Serous to serofibrinous conjunctivitis, rhinitis, sinusitis, tracheitis, air sacculitis and focal bronchopneumonia have all been described.[18] The nasal cavity and the infraorbital sinus frequently display a unilateral, seromucoid (later fibrinous) exudate that also fills the choanal fissure. In ducks and turkeys, the exudate is often semigelatinous, fibrinous or caseous, and leads to distension of the infraorbital sinus. The mucous membranes are swollen and may show petechiation. The tenacious exudate can be mixed with fibrinous debris.

Histopathologically, the disease is initially characterized by severe distension of the mucous glands, the swollen cells of which have particularly large nuclei. Subsequent proliferation of epithelial cells leads to multilayered, glandular epithelium, pressure on the glands themselves and mucoid degeneration. The superficial mucosal epithelia lose their cilia, proliferate to 10-15 cellular layers and finally show vacuolization and karyorrhexis. In contrast to various viral diseases, desquamation and necrosis of the epithelium are mild.

From the second week after infection, an infiltration of the lamina propria with lymphocytes and histiocytes is seen. Round-to-oval, up to 400 μm in diameter nodules that consist mainly of lymphocytes appear in the submucosa. The proliferation of the lymph follicles persists in the lower part of the trachea and the syrinx from the 2nd to the 12th week after infection. This is generally a longer course of reactions than are noted with other infections of the respiratory tract. As healing occurs, there is a proliferation of connective tissue in the submucosa. The histopathomorphologic changes vary depending on the presence of secondary bacterial or viral infections. Secondary fungal infections are rather rare.

Lesions of the air sacs start with edema between the inner and outer epithelial layers. The cellular reaction consists initially of subepithelial infiltrates of heterophils. The capillaries are engorged. Progression is marked by increased edema and growing numbers of heterophils followed by lymphocytes, macrophages and plasma cells. The normally flat epithelium becomes cubic, loses its cilia and is finally desquamated. Inflammatory exudate, mainly in the form of fibrin, appears on both sides of the air sac membrane. The host responds with proliferation of the endodermal epithelial layer and necrotic foci of epithelial cells and proliferation of fibrocytes and mononuclear cells (80% can be lymphocytes). A granulomatous reaction characterized by the formation of multinucleated giant cells occurs by the third week post-infection. Lymph follicles and diffuse mononuclear infiltrations govern the pathologic picture. Pneumonia is a rare complication in avian mycoplasmosis, and in most instances is caused by secondary infections with *E. coli*.

Mycoplasma colonization of the mucosa of the urogenital tract can cause pathologic lesions, although colonization of the phallus may be inconspicuous. Histologically, lesions are mainly seen in the part of the mucosa where the majority of the glands are situated (species-specific differences). Submucosal proliferation of lymph follicles and disseminated infiltration of lymphocytes into the tissue are the main lesions. In ganders, the phallus is enlarged and covered with fibrinous exudate, and may finally become necrotic if secondary infections occur. Synovial membrane lesions have been rarely reported in companion and aviary birds.[23]

Differential Diagnosis

The rule-out list includes many viral, bacterial and fungal diseases. In Psittaciformes, pigeons, ducks and geese, chlamydiosis is the main rule-out. The genital tract can be infected by other microorganisms as well. Embryopathologic lesions and embryonal death are suggestive. With mycoplasmatales, infected embryos generally die late in incubation. Embroyos that die after pipping frequently have air sacculitis of the left thoracic air sac group (exceptions are geese that have air sacculitis bilaterally). After hatching, chronic lymphofollicular proliferation can be so severe that lymphoma must be considered in the rule-out list.

Diagnosis

A tentative diagnosis can be made by histopathologic examination. Isolation of the agent is necessary for identification and biologic assays. Because of the fastidious nature of this organism and the difficulties in identifying the agent, specialized laboratories are necessary to isolate *Mycoplasma*. In addition, the mycoplasmatales need to be differentiated from bacterial L-forms. Swabs from the upper respiratory tract, or the phallus in males, can be taken from live birds. Endoscopic biopsies of affected air sacs are useful diagnostic aids. Samples from air sacs, salpinx, lungs and spleen should be collected for postmortem evaluation. Transport media are necessary for shipping samples. They should contain heart infusion broth, mycoplasma broth or another similar medium with penicillin (2000 IU/ml). Because the organisms are primarily to be protected from drying during transport and are not supposed to grow, the pH is not of particular importance (however, it should be around 7 or slightly above). Penicillin does not affect acholeplasma, but thallium-acetate does; therefore, mycoplasma broth that contains thallium-acetate should not be used for material from geese.

Indirect diagnosis of mycoplasmatales by serology is hampered by false-positive (cross-reactions) and false-negative tests. The presence of a mycoplasmatales on a mucosal surface usually does not stimulate production of humoral antibodies. Antigen-recognizing cells become active only after the mucosa has been penetrated. The most frequently used tests are: serum slide agglutination (SSA), HI test (for the hemagglutinating species), growth- or metabolic-inhibition test, immunodiffusion test, immunofluorescence test, ELISA, and recently the polymerase chain reaction.

Treatment

Clinical infections can be treated with tylosin, spiramycin and erythromycin or spectinomycin in combination with clindamycin or pleuromutilin. The efficacy of tetracyclines against avian mycoplasmatales has yet to be proven. However, in Psittaciformes, the tetracyclines are recommended because of the clinical similarities between mycoplasmosis and chlamydiosis. The pigeon strains are highly resistant to erythromycin and, to a lesser extent, tylosin. Only pleuromutilin was able to inhibit 57 of 65 strains recovered from pigeons.[15] The LD_{50} for pleuromutilin in pigeons is 440 mg/kg, considerably less than for chickens and turkeys. This drug must be carefully used when treating pigeons that are feeding offspring or if used in the water during hot weather.

Spiramycin given parenterally to Ploceidae, Estrildae and even canaries may lead to sudden death from unknown causes. The same dose given via drinking water is well tolerated. Spiramycin is one of the macrolid antibiotics and is given at a dose of 100 mg/kg body weight IM, or 100-200 mg/kg body weight orally. Since the primary patent has expired, several manufacturers produce it. Enrofloxacin has been used to treat mycoplasmosis in poultry. There have been no reports of success in treating mycoplasmosis with enrofloxacin in other birds. Treatment is designed to allow clinically affected birds to recover. The organism is difficult to eliminate.

Control

Recovery from mycoplasmosis results in the production of a low antibody level and a persistent infection. *In vitro*, mycoplasmatales can propagate in the presence of homologous antibodies, indicating that humoral antibodies are not correlated with immunity. The disease is governed by the excessive reactions of the cell-mediated immune system. Therefore, vaccinations might sensitize a bird to the organism and cause a severe reaction to a field exposure. Theoretically, vaccines that prevent mycoplasmatales from attaching to the mucus cells might give protection.

Rickettsia

Little information is available on rickettsial infections in birds. Rickettsia form a group of microorganisms, the taxonomy of which has still not been fully determined. They are obligatory cellular parasites, and can be differentiated from chlamydia by the absence of a developmental cycle and the capacity to synthesize energy-rich compounds (ATP). Rickettsia are small rods or coccoids with an average size of 0.3 to 0.5 μm in diameter and 0.8 to 2.0 μm in length. They may also be pleomorphic, and are generally nonmotile. Multiplication takes place by binary fission. The organism parasitizes reticuloendothelial cells, vascular endothelial cells or erythrocytes. Infections may occur in arthropods, which can serve as vectors or as primary hosts. The mutualistic forms in insects are considered to be essential for development and reproduction of the host.[47] Rickettsia may cause disease in humans, many vertebrates and insects. The organisms can be cultured in embryonated chicken eggs or metazoan cells. The chlamydial staining procedures may be used, although some changes are made particularly in fixation.

The rickettsia have historically been divided into three families:[47] Rickettsiaceae, Bartonellaceae and Anaplasmataceae. The latter two families are no longer considered to be Rickettsia and are "phylogenically unaffiliated bacteria" (Gothe, unpublished).

Rocky Mountain Spotted Fever (RMSF)

RMSF is a mammalian disease caused by *Rickettsia rickettsii*. Prior to the availability of antibiotics (tetracyclines), infections were characterized by high mortality rates. The pathogenicity (for many other rickettsia as well) involves a toxin-like action that damages endothelial cells, inducing increased capillary permeability, plasma flow into the tissue, hemoconcentration and eventual circulatory collapse. Cytotoxicity is thought to be the mechanism by which rickettsia gain access to the cytoplasm of the host cells.[41]

RMSF is transmitted by ticks (mainly *Dermacentor* spp.) and rodents. Dogs and opossum are thought to be reservoirs. Birds, including chickens, several Columbiformes, pheasants, Falconiformes and the Magpie, are susceptible to experimental infection and may also serve as reservoirs. Pigeons may be particularly important reservoirs.[24] Clinical abnormalities have not been described in infected birds.

Q-Fever

Q-fever, caused by *Coxiella burnetti*, is an aerosol-borne disease in humans with worldwide distribution. Direct and indirect transmission (arthropods) can occur in humans and other host species. This agent differs considerably from other members of the Rickettsiaceae. The cells are smaller (0.2 to 0.4 μm by 0.4 to 1.0 μm) and replicate in vacuoles of the host cells. In contrast to chlamydia, *C. burnetti* infections result in the formation of phagolysosomes. Two different phase variations are distinguished. Phase I is more virulent, presumably because of the high amount of lipopolysaccharides, and occurs naturally. Phase II appears following repeated passages in the yolk sac of embryonated chickens. The occurrence of plasmids has been established. The replication cycle includes production of endospore-like bodies, which are probably the cause of the high tenacity of *C. burnetti*. This organism is resistant to chemical disinfectants and high temperatures that might kill other rickettsia. Metabolic activity in these endospore-like bodies is extremely low outside the host. Survival, which can be several years in the feces of ticks, is augmented by dryness. Chlorine-containing preparations are recommended for disinfection. The effects of phenol and formaldehyde products are debatable.[35]

The host spectrum of *C. burnetti* is wide, and includes arthropods (particularly ticks), birds and mammals. An infectious cycle in free-ranging animals, arthropods, birds and mammals is differentiated from an infectious cycle in domesticated animals (sheep, goats, cattle, dogs). Cross transmission, including in man, is possible from both cycles.[35]

Avian susceptibility to *C. burnetti* seems to be high, and this organism has been demonstrated in at least 49 avian species.[8,30,39] The type of feeding behavior, breeding areas and seasonal migrations are the main factors for the spread of the organism. Carrion-eating birds may be infected by ingesting the infected placenta of ruminants from endemic areas. The feeding location of granivores and insectivores is more important than their food preferences. Birds that live in close contact with humans (synanthrops) are exposed more frequently to *C. burnetti* than birds that avoid civilization (exanthrops). The susceptibility to infec-

tion in domesticated birds is variable.[8,38,39] Chickens are apparently most susceptible and may shed the agent in the feces for 7 to 40 days post-infection. Vertical transmission via the egg may also occur. Domesticated pigeons are next in susceptibility. Domesticated turkeys, ducks and geese are rarely infected. All the free-ranging urban pigeons that were examined (125) in the Netherlands showed CF titers of ≤1:20 against *C. burnetti*.[7] In experimentally infected domesticated pigeons, the agent could be isolated from the spleen and lung 58 days post-infection.[34] No clinical disease has been observed in any susceptible avian species.

C. burnetti can be identified in tissues using various staining methods (Giemsa, Macchiavello, Castañeda) as for chlamydia. The organism replicates in embryonated chicken eggs and various cell cultures. Antibodies can be demonstrated using the CF test or the ELISA. Not all exposed domestic pigeons have been found to produce CF antibodies.[34] Antibody production is not related to immunity.

Therapy with tetracyclines is effective for the clinical disease, but elimination of the organism is not possible. Treatment for infected birds is not encouraged because of the high immunosuppressive side effect of the tetracyclines.

Aegyptianella (Ae.)

Ae. pullorum is the causative agent of anemia and hepatitis in chickens and other birds. It is an erythrocytic parasite that produces endocytoplasmic inclusions, which stain using the Giemsa or Pappenheim procedures. The inclusions measure 0.3 by 4.0 μm, and each can contain up to 26 initial bodies (reproducing form up to 0.8 μm in diameter). In many instances, the inclusions are polymorphic (round, oval, ring- or horseshoe-shaped) and are separated from the plasma by a single-layered membrane. Infection of the cell starts with an endocytosis-like process followed by vesiculation in the erythrocyte. Exocytosis is one way in which the organism is released from the infected host cell. However, the host erythrocytes are usually damaged by the parasite, leading to lysis and release of the parasite into the plasma. Arthropods, mainly ticks of the genus *Argas*,[16] are essential for transmission.

The organism is most common in tropical and subtropical regions including the Mediterranean. The host spectrum is probably incomplete but certainly includes chickens, quail, Columbiformes, Strigidae, Falconidae, Accipitridae, crows, canaries, ostriches, ducks, geese and some Psittaciformes such as *Agapornis* spp.[16,17,33]

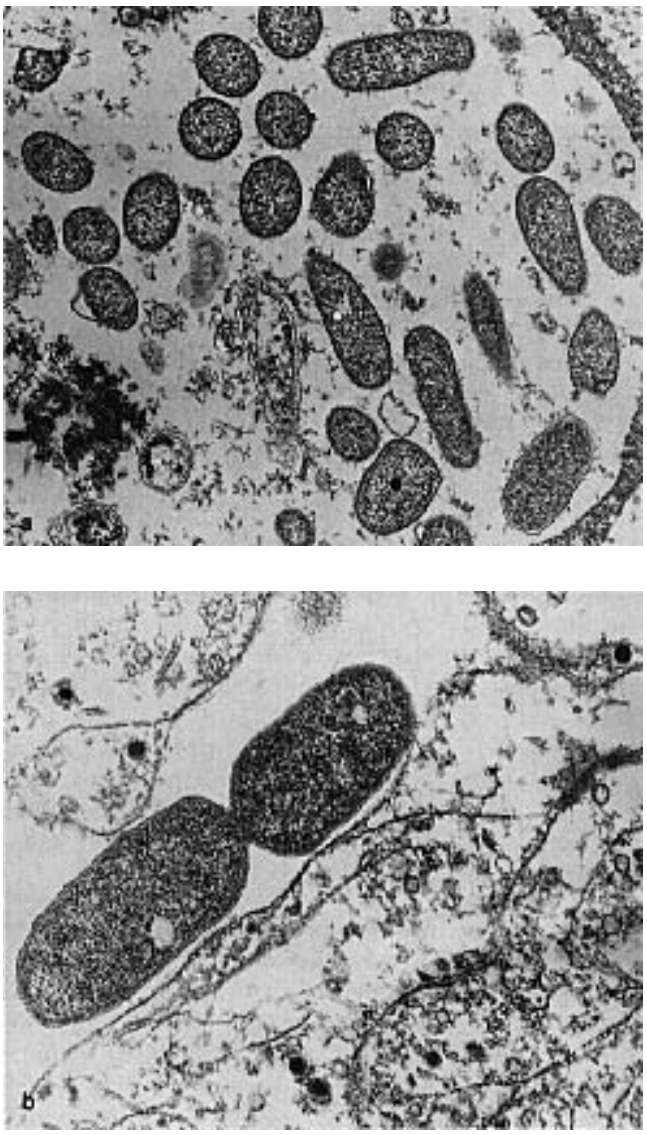

FIG 38.3 **a)** A group of Gouldian Finches died following an onset of clinical signs that included depression, dyspnea and coughing. Electron microscopy of tracheal epithelial cells revealed intracytoplasmic inclusion bodies with organisms morphologically suggestive of rickettsia. Magnification: x 22,250. **b)** Same cell type with rickettsia in binary fission; magnification: x 52,500; (courtesy E. Göbel).

Clinical signs in young birds are characterized by an acute onset of anemia, anorexia, weakness, weight loss, greenish diarrhea and death.[33] Chronic infections in older birds are characterized by icterus, which may not be clinically recognizable. The post-

mortem examination reveals anemia as well as a considerable enlargement of the liver and spleen.

Small inclusion bodies were demonstrated in the majority of the mature erythrocytes in two domestically raised Eclectus Parrot neonates (six weeks old) with heterophilia (toxic heterophils) and anemia. These intracellular parasites resembled those identified as *Aegyptianella* in several imported African Grey Parrots. Following a long-term course of doxycycline therapy, the parasites were no longer identifiable in the erythrocytes. The parents that produced these neonates were also positive for aegyptianella and responded to long-term doxycycline therapy.[33]

In gallinaceous birds, the age and condition of the host govern the pathogenesis and outcome of the infection. Up to 60% of the erythrocytes may be infected in one-day-old chicks, while by one year of age less than 1% of the erythrocytes may be infected.[16] Mortality is higher in chicks less than two days of age.

The rule-out list includes internal bleeding, chlamydiosis and chronic diseases of various etiology. For diagnosis, a blood smear stained according to Giemsa or Pappenheim shows the parasites in the erythrocytes. Tetracyclines are effective for treatment. Tick control is mandatory to prevent reinfection and epizootics.

Unclassified

There are indications that diseases caused by rickettsia other than aegyptianella may occur. Tracheal epithelial cells in Gouldian Finches with severe respiratory disease were filled with cytoplasmic "inclusions." Many of the affected birds died. Electron microscopy of the epithelial cells revealed particles that were morphologically compatible with rickettsia (Figure 38.3). Treatment with tetracyclines was successful. Isolation was not possible because the material had been prepared for histopathology.[27]

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CHAPTERS

VI

SECTION SIX

SURGERY

VI