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Diversity of Chlamydia-Induced Diseases

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History of Disease Diversity of Chlamydial Infections

The pathogenic role of chlamydiae of the species *Chlamydia* (C) *psittaci* in several diseases of domestic animals and man is well established.[1] Infections with agents now known to be chlamydiae were first identified as the cause of atypical pneumonia as well as several epidemics of severe pneumonia in human subjects late in the last and the early part of this century.[2] Psittacosis was the term given to these human infections because it was recognized that the infection was contracted from parrots.[3] These observations directed attention to chlamydial agents as a cause of respiratory disease in birds and domestic poultry.[4] The first naturally occurring pneumonic chlamydial infection in mammals was identified in cats[5] and in laboratory mice in 1942.[6,7] Today pneumonia is a recognized manifestation of respiratory chlamydial infection in domestic animals. McNutt[8] described in 1940 a sporadically occurring encephalomyelitis in cattle. He isolated the causative agent in guinea pigs, and it was later classified as a chlamydial agent. Stamp and coworkers proved in 1950 that placental lesions and abortions in sheep were associated with chlamydial infections.[9] The disease was called enzootic abortion of ewes. Conceptually, a novel pathogenic potential of chlamydial infections in animals was thus identified. Years later it was found that chlamydiae are transmitted in semen of bulls and rams affected with seminal vesiculitis and epididymitis and that these infections are involved in forms of reproductive failure other than abortions.[10–13] Furthermore, chlamydiae were detected by culture as well as by DNA amplification through polymerase chain reactions (PCR) in milk samples from cows with mastitis.[14,15] Mastitis was induced in cows following intracisternal inoculation of chlamydial isolates from

ovine abortions,[16] sporadic bovine encephalomyelitis,[17] and intestinal infections.[18]

Detection of intestinal chlamydial infections of calves in 1951 represents another milestone.[19] This discovery initiated work on the importance of the intestinal habitat in perpetuating chlamydial infections in cattle, sheep, goats and swine, as well as in poultry and pet birds where this infection often is clinically inapparent and therefore of high epidemiological significance.[20] The arthropathogenic potential of specific chlamydial strains from sheep was recognized in the late fifties by Mendlowski and Segre.[21] Polyarthritis was seen particularly in sheep but also in calves.[22] While trachoma, a distinct disease in human subjects caused by chlamydial infections, had been recognized for centuries,[2] it was not until 1960 and later that chlamydial infections as a cause of conjunctivitis and other ocular diseases were differentiated in animals.[23] The first chlamydia-induced conjunctivitis was described in cats[23] and later in other domestic animal species including guinea pigs.[24] A severe form of keratoconjunctivitis associated with chlamydial infections was described in Australian koalas.[25] Records indicate that severe eye conditions associated with blindness and mortality of koalas leading to major die-offs were observed and recorded as long ago as 1887.[25] Additionally, koalas involved in the recent epidemics had genital infections leading to reproductive failure.[26] Epizootic die-offs among African clawed frogs in commercial operations were proven to be caused by chlamydial infections.[27] These ectothermic vertebrates had pyelogranulomatous inflammations leading to myocarditis, hepatitis, nephritis and lung lesions.[28]

Intestinal Infections and Enteritis

Epidemiology

Intestinal chlamydial infections may be found as persistent, clinically inapparent infection or as a cause of diarrhea in young animals; but they also function in initial events in the pathogenesis of other chlamydia-induced diseases.[1,20] They represent an epidemiological factor in perpetuating and spreading this infection in animal populations. Chlamydiae were first isolated from fecal and diarrhea fluid samples of calves in New York state in 1951. Calves as young as 2 days and adult cattle of various ages were found to excrete chlamydiae. Reports from Europe, Asia, Africa and Australia verified that intestinal chlamydial infections of cattle, sheep, and goats are worldwide.[20] Clinically normal goats and sheep harbored chlamydial agents in the intestines and excreted these organisms in

readily detectable amounts. Chlamydiae were also isolated from feces of pigs in Austria and Germany.[29,30] Evidently, the intestinal chlamydial infection of mammals is comparable to the chlamydial infection of acutely, as well as inapparently infected, birds, which are known to excrete chlamydiae in feces or diarrhea fluids over long periods of time.[4] The infectious chain involving intestinal infections of animals is tightly linked to fecal shedding of infectious chlamydiae. Susceptible hosts may acquire the infection by ingesting contaminated fomites or by inhaling chlamydia-laden dust.

Properties of Chlamydiae from Intestinal Infections

Chlamydiae isolated from feces or intestinal samples of different animal species have antigenic properties of serovar 1, which also includes isolates from abortions of ewes and cows, and of serovars 2, 3, 4 and 9,[31] which are now classified as *C. pecorum*.[102] The strain S-45 of serovar 5 isolated from feces of pigs has properties of *C. trachomatis*.[32] Detection and differentiation by PCR involving the genes of the major outer membrane protein (MOMP) place intestinal chlamydial isolates into the psittacosis, polyarthritis and trachoma groups. The DNAs of MOMP genes of mammalian serovar 1 chlamydiae have a restriction fragment length polymorphism (RFLP) of avian strains[33] but differences in antigens and the molecular mass of MOMP exist between the B577 prototype of serovar 1 and some avian isolates.

Clinical Signs

The effect of oral inoculation of calves with chlamydiae depends on the age at the time of exposure.[34] Newborn calves in herds infected with serotype 1 chlamydiae may have episodes of watery to mucoid diarrhea, slight fever and nasal discharge. Calves with serovar 2 infections had yellow, watery feces and became dehydrated, anorectic and depressed. They also developed fever and leukocytosis. The majority of these calves died within 17 days of exposure.[34] Sheep and goats with intestinal chlamydial infections usually had normal feces, but this infection has not been studied sufficiently in the young of these species or in swine.[20]

Cytopathic Functions in Pathogenesis

The events of chlamydial infections leading to enteritis and diarrhea were studied in newborn calves.[34] Chlamydiae were isolated from mucosal scrapings of the abomasum, duodenum, jejunum, ileum, caecum and colon at various times after oral inoculation. Fluorescent

antibodies revealed that the epithelial cells on the tips of the villi and in the intervillous zones as well as some cells in the crypts of Lieberkühn and the transition zones were infected.[35] Infection of intestinal cells occurred predominantly through the brush border. The host cell range included absorptive epithelial cells at the tips of the villi and the intervillous zones (Fig. 20.1), follicle-associated M cells, enterochro-

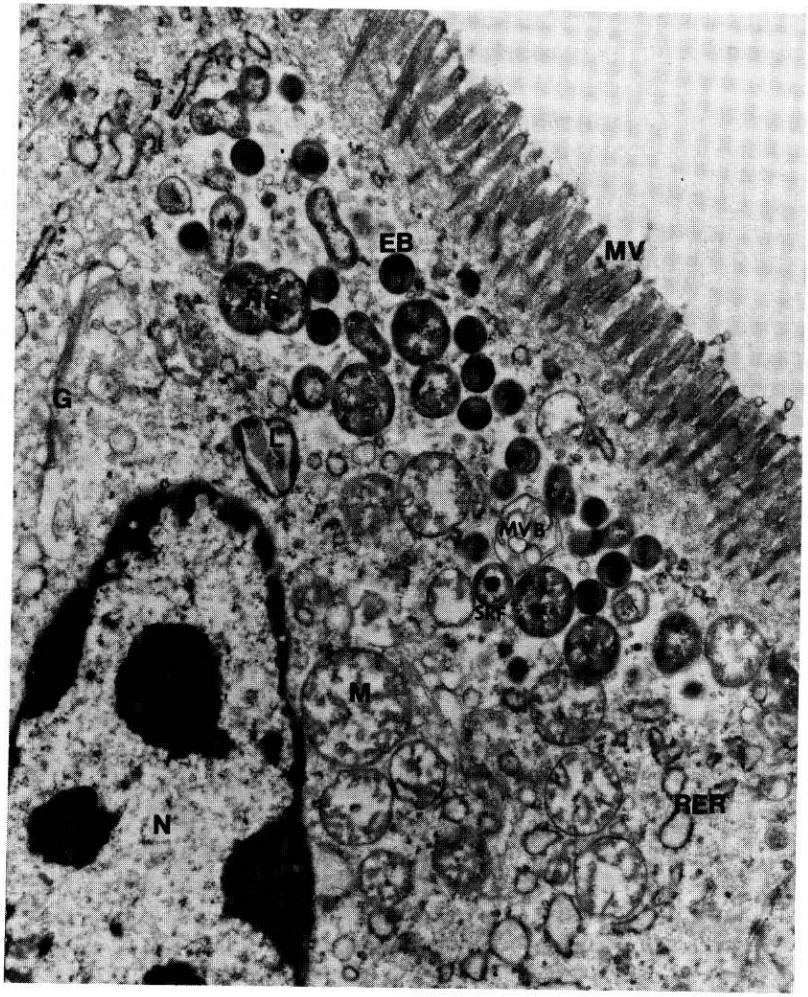

FIG. 20.1. Absorptive intestinal epithelial cell at a late stage of infection. Elementary bodies (EB) are more numerous than reticulate bodies (RB) and secondary reorganized or condensing forms (SRF). Mitochondria (M) are damaged, the rough endoplasmic reticulum (RER) and Golgi elements (G) are vesiculated, and the nucleus (N) is pycnotic. Lysosome-like structures (L) are present and microvilli (MV) are shortened. Uranyl acetate-lead citrate stain, ×5,600.[36]

maffin and goblet cells of the villi, and undifferentiated cells in the crypts, as well as macrophages and fibroblasts in the lamina propria and endothelial cells in the central lacteals.[36,37] Lysis of productively infected endothelial cells in the central lacteals released infectious chlamydiae into the circulating lymph and blood stream. Neutrophilic leukocytes rarely contained chlamydiae, but leukocytes pavement the vicinity of chlamydia-infected epithelial cells.[38,39]

When sequential stages of chlamydial development were correlated with ultrastructural lesions in infected intestinal cells, the earliest ultrastructural change was the space-occupying presence of the endosome containing the chlamydial dispersing form within deeper regions of the cytoplasm or in the Golgi region.[38] The rough endoplasmic reticulum remained near the inclusion, but the lysosomes appeared to migrate away and accumulated close to the cytoplasmic periphery. The number and prominence of free ribosomes and polysomes in the cytosol diminished in all types of cells infected. The mucus droplets became depleted in goblet cells. Chlamydial inclusions containing arrested reticulate bodies were located at the secreting face of the Golgi stacks and little mucus was formed. The specific granules of infected enterochromaffin cells, which supported productive chlamydial multiplication, decreased in number and electron density.[37–39]

The appearance of condensing forms and elementary bodies in infections of all cell types coincided with the beginning of severe degenerative changes characterized by cytoplasmic vesiculation, fragmentation of membranes and lysis of cellular organelles. The network of the smooth and rough endoplasmic reticulum dilated, became ill-defined and vacuolated. Golgi complexes became dilated and vesiculated. The nuclei were pyknotic or karyolytic after an initial loss of chromatin pattern and nucleoli, and separation of the membranes of the nuclear envelope. The chlamydial inclusion membranes ultimately ruptured, the plasmalemma lyzed, and chlamydiae were released.

Specialized organelles of infected cells such as microvilli and terminal webs of enterocytes were also affected (Fig. 20.2). The microvilli lost their uniform, regular spacing and became shorter, irregularly shaped, and vesiculated. Their rootlets were lost and the terminal web became ill-defined. Lateral junctional complexes were displaced and fragmented. The desmosomes persisted, and converging fibrils remained in their vicinity. Diarrhea resulted from the enterocytes' loss of absorptive and digestive functions, interference with cellular transport and energy generation, accelerated death of infected intestinal epithelial cells, edema and cellular infiltration of the lamina propria mucosae.[35,39]

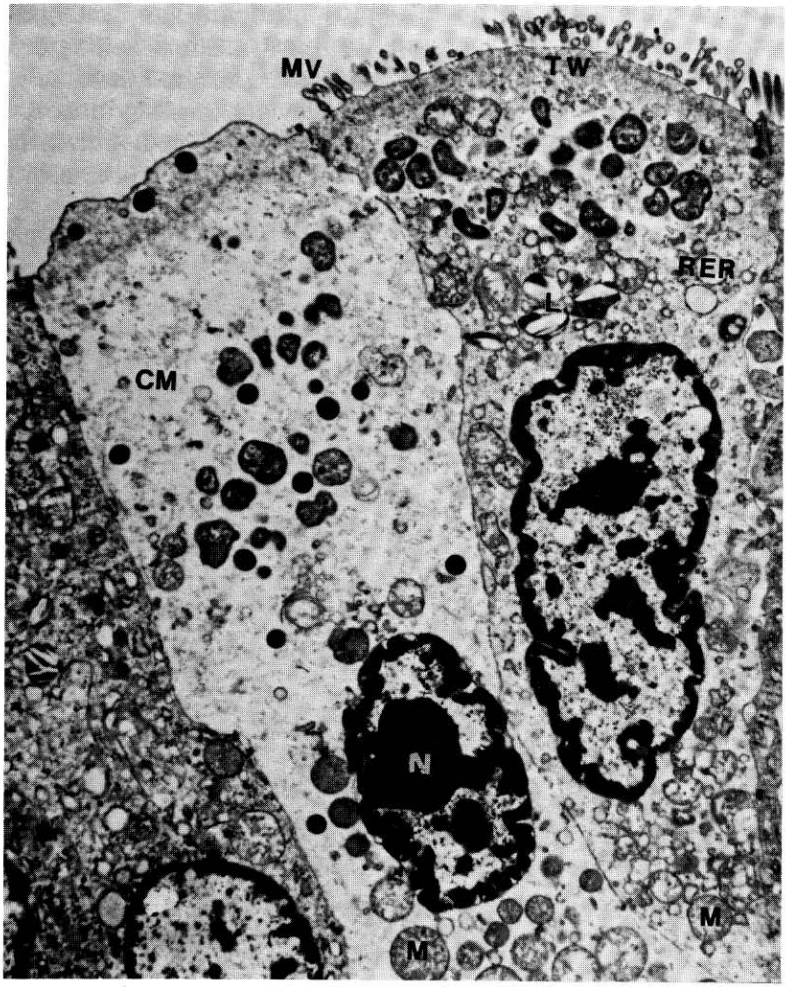

FIG. 20.2. Two absorptive intestinal epithelial cells in late stages of chlamydial infection. Nuclei (N) are pyknotic, the cytoplasmic matrix (CM) is dissolved, the terminal web (TW) is swollen lacking the rootlets of microvilli (MV). Uranyl acetate, lead citrate stain, $\times 11{,}520$.[36]

Pathological Lesions

The serosal surfaces of the abomasum and small intestine of calves were dull and granular. Paint-brush hemorrhages were present in the duodenal and cecal serosa. The mucosa of the entire small intestine was congested and petechiated. The jejunal wall was thickened by edema. The terminal parts of the ileum were more severely affected than other gastrointestinal regions. The ileocecal valve was congested

and often had numerous petechiae, while the cecal mucosa was hyperemic. Rugae of the colon were congested, and their free edges were slightly eroded. The mesenteric lymph nodes were enlarged, and the lymphatics of the mesentery were abnormally dilated.[40]

Histologically, the intestinal villi had a bulbous appearance caused by dilation of capillaries and central lacteals, edema and infiltration of mononuclear cells.[40] The inflammatory process spanned from the mucosal to the serosal surfaces in some instances. The epithelial cells desquamated extensively at many levels of the small intestine. Numerous crypts of Lieberkühn were distended by inflammatory leukocytes and sloughed, degenerated epithelial cells. The remaining epithelial cells lining such crypts were cuboidal rather than columnar. The Peyer's patches had lost their follicular architecture and contained necrotic centers, a feature also seen in the tonsils.

Diagnosis and Differentiation

The specific diagnosis must be established by demonstration of chlamydiae or chlamydial antigens or DNA in feces, diarrhea fluid or intestinal mucosal samples. The isolation of chlamydiae from fecal samples by the cell culture method is more sensitive and faster than the previously used propagation in yolk sacs of developing chicken embryos.[41] Demonstration of chlamydiae in intestinal sections after immunofluorescent staining provides a specific and rapid test.[35] Examination of diarrhea fluid by electron microscopic methods or ELISA for detecting chlamydiae has not been explored sufficiently, but these methods reveal most of the enteropathogenic viruses that must be considered for differential diagnosis. A sensitive tool to detect intestinal chlamydial infections will be PCR amplification of DNA of chlamydial MOMP genes with genus-specific oligonucleotide primers.[33]

The antibody response of newborn calves is weak following intestinal chlamydial infections. Newborn calves surviving the intestinal infection developed antibodies when they became 43–57 days old.[42] About 70% of clinically normal sheep that excreted chlamydiae in the feces did not have significant titers of antibodies, and the rest had low titers.[43]

Respiratory Infections and Pneumonia

Epidemiology

Chlamydia-induced pneumonia of different animal species was found to occur worldwide. A clinically inapparent respiratory chlamydial infection that can be activated to clinical pneumonia was first detected in mice.[6,7] The etiologic role of chlamydiae as a cause of

pneumonia is established in cats, sheep, goats, cattle, swine, and domestic and wild rabbits.[1,5,44–46] Some horses affected with respiratory disease in Australia and in England had chlamydial infections.[47,48] The respiratory disease of calves from which chlamydiae were isolated has been recognized in the United States and Canada, in Germany and in England.[20] Similarly, enzootic pneumonia of swine was associated with chlamydial infections in Russia and Balkan countries.[46] Chlamydial pneumonia of cats, often associated with conjunctivitis and rhinitis, was described in the United States, Canada, England, Switzerland, and Australia.[1,20]

Complicating infections with *Pasteurella, Hemophilus,* or *Mycoplasma* spp, as well as viruses, were reported to lead to more serious clinical signs and lesions. The stress of shipping or crowding, abrupt changes in the weather, or adjustment to a new environment after weaning creates conditions favorable for chlamydial pneumonia.[20] Respiratory chlamydial infections of calves, sheep, and goats is linked closely to shedding of chlamydiae by carriers with clinically inapparent intestinal infections. Coughing by affected animals creates infectious aerosols, and chances for close contact with inhalation of chlamydiae-laden dust particles are important in transmission of the infection. Serial pulmonary passages probably enhance the virulence of the agent.

Airborne transmission and direct contact spread chlamydial infection from cat to cat. Droplet infection is facilitated by frequent coughing and sneezing of diseased cats, because the discharges from eyes and nose are highly infectious. Rapid airborne spread to cage mates established epizootics in animal hospitals and catteries. Queens infect their newborn kittens. Infected cats are a danger to human companions, who may contract ocular infections and conjunctivitis from chlamydia-infected cats.

Properties of Respiratory Chlamydiae

Few isolates from respiratory infections were characterized. The feline pneumonia strain falls into the separate serovar 7 which appears to be host specific.[31] The RFLP of the MOMP gene DNA confirmed this further.[32] An isolate from bovine pneumonia together with some porcine chlamydiae were typed as a serovar 6. The Nigg strain of mouse pneumonitis is the murine biotype of *C. trachomatis*.[49]

Clinical Signs

Calves with pneumonia and positive chlamydial cultures from respiratory tract samples have serous, mucous or mucopurulent nasal

discharge. They usually are febrile and appear depressed, while a dry cough may be associated with dyspnea. Transient diarrhea is also observed. Often, pneumonic lesions are found incidentally at slaughter.

Lambs responded with a rise in temperature 24–48 h after intranasal or intratracheal inoculation with the chlamydial agent of ovine pneumonia. The fever remained high for about 2 days and then subsided to normal levels by the 6th day. During the febrile period, the lambs were depressed and anorectic and displayed signs of respiratory distress, with an occasionally dry cough. The uncomplicated experimental lung infection induced relatively mild symptoms. The disease was often observed after feeder lambs were gathered from different herds for fattening in feed lots or irrigated pastures.[45,51]

Goats with chlamydial pneumonia had symptoms similar to those of sheep. Clinical signs of pigs with respiratory chlamydial infections were typical of enzootic pneumonia.[46]

Cats affected with chlamydia-induced pneumonia sneeze, cough, have fever and are depressed and anorectic.[52] Mucopurulent discharge and excessive fluid flow from the eyes and nostrils as a result of conjunctivitis, rhinitis and pharyngitis. The cats recover in 2–4 weeks after infection, but they frequently remain asymptomatic carriers. The disease usually is not fatal, but very young or elderly cats may die of severe pneumonia. Sequelae to feline pneumonia are chronic conjunctivitis, sinusitis, bronchitis and tracheitis.[5]

Pathogenetic Events

The sequence of events in the genesis of chlamydial pneumonia was studied in sheep and in calves. Pulmonary infections of lambs with chlamydiae most likely are initiated in the bronchiolus and spread from there to the lung parenchyma.[50,51] Lung involvement was most severe on the 5th day after inoculation, then signs of regression became evident, and lungs were virtually normal after 30 days. Chlamydia-induced pneumonia of sheep was classified as an interstitial bronchopneumonia. The pulmonary lesions were also considered as hyperplastic pneumonia because of alveolar epithelialization.

Pathological Changes

Consolidations of the anterior lobes and in the hilus region of lungs are observed in naturally occurring cases of chlamydial-induced

pneumonia of sheep and goats. Mature lesions consist of irregular but sharply defined areas of consolidation with a dull, grey–pink color. The lungs are heavy and fail to collapse. The consolidated areas are slightly elevated, lumpy and solid on palpation, and slightly opaque mucus appears in opened bronchioli. The mediastinal lymph nodes are usually enlarged. The lung lesions have streaky, irregular, dark-red bands during the evolving and resolving stages. These consist mostly of atelectatic regions that can not be distinguished from normal lung tissue by palpation alone.[50]

There was an early acute inflammatory response in terminal bronchioles and adjacent alveoli, followed by proliferation of alveolar cells and accumulation of macrophages within alveoli. Extensive alveolar epithelialization was present at the height of the pulmonary reaction 5 days after experimental injection. Reticulin stains revealed well-preserved alveolar architecture in densely consolidated regions which accounted, at least in part, for the ease with which resolution occurred.[50]

Diagnosis and Differentiation of Chlamydial Pneumonia

Clinical signs or the macroscopic pathologic changes are not specific enough to diagnose chlamydial pneumonia in animals and to differentiate them from viral or mycoplasma pneumonia. Impression smears from conjunctival and respiratory tract scrapings or from the margins of the pneumonic lesions provide a diagnosis if these preparations contain chlamydial elementary bodies or chlamydial inclusions in monocytes or conjunctival and respiratory epithelial cells. The use of fluorescent antibodies increases the diagnostic value of exfoliative cytologic findings.

Most reports on chlamydial pneumonia were based on an etiologic diagnosis with isolation and identification of the causative chlamydiae.[20,41] Chicken embryos were used as indicator hosts in the past, but cell culture methods are more sensitive and give earlier answers.[41] Samples from the reactive margins of the lung lesions contain viable chlamydial agent during the acute stage of the disease. Chlamydiae were isolated from nasal washings in bovine and equine pneumonia. Parallel testing of specimens for respiratory viruses on cultured animal cells and careful cultivating for bacterial and mycoplasmal species furnish the evidence needed for etiologic differentiation of the respiratory disease under study. Chlamydial pneumonia was diagnosed in calves from 4 out of 20 natural epizootics of pneumonia through a four-fold rise in the titer of genus-specific antibodies.[53]

Polyarthritis–Polyserositis

Epidemiology

Chlamydial polyarthritis of lambs is an acute febrile disease that occurs in epizootic proportions in the western and southern sheep-raising regions of the United States. The cause of the disease, which also is referred to as stiff lamb disease, was first identified by Mendlowski and Segre in 1960 as a chlamydial infection of the synovial tissues, with inflammation of most diarthrodial joints of the limbs, leading to stiffness and lameness.[21] A similar disease of calves was also recognized in the western United States.[54] Chlamydiae were isolated from the joints of calves affected with this disease.[22] Transmissible serositis of calves had previously been described in Australia.[55] Chlamydiae were isolated from synovial samples of swine suffering from polyarthritis in Austria.[29,56] The arthropathogenic potential was convincingly proven as yet another manifestation of chlamydial infections.

Chlamydial polyarthritis was observed in lambs out on ranges, as well as in lambs from farm flocks and feedlots; and morbidity ranged from 2 to 75%. Lambs that weighed 55–105 pounds appeared to be affected more often than smaller, younger lambs. The highest incidence of the disease among sheep on ranges was observed in July, August and September; while in feedlot lambs the disease is most prevalent in October, November and December.[21,54,57] The youngest calf that had naturally occurring chlamydial polyarthritis was 4 days old when the first signs of polyarthritis were detected. More commonly the calves are several weeks old. This condition is seen sporadically in individual calves in contrast to the disease in lambs. The agent is shed in feces, urine and also in conjunctival secretions.[22,58]

Properties of Arthropathogenic Chlamydiae

Isolates from joints of lambs, calves and swine as well as isolates from cattle with sporadic bovine encephalomyelitis belong to serovar 2 classified as *C. pecorum*.[31,102] The DNA of representative strains had less than 70% homology with DNA of other chlamydiae.[59–61] A multiple step PCR with primary genus specific and secondary group specific primers for the DNA of the MOMP gene also distinguished these strains as a distinct group of chlamydiae.[33] Infection with these strains is typically enhanced by cytoactive treatments of the cultured cells.[62] The effect on the cytoskeleton of infected cells distinguishes the serovar 2 strains as unique biovars.[62]

Several stable characteristics were thus identified that characterize the arthropathogenic chlamydiae as a separate chlamydial species.[102]

Clinical Signs

Rectal temperatures of affected lambs ranged from 39–42°C. The fever appeared to depend on the stage and acuteness of the disease. The lambs had varying degrees of stiffness, lameness and anorexia; and some had conjunctivitis (Fig. 20.3). Affected lambs were gaunt, depressed and reluctant to move or stand or bear weight on one or more limbs, and lingered behind the rest of the band; and many would lie down.[21,54,57]

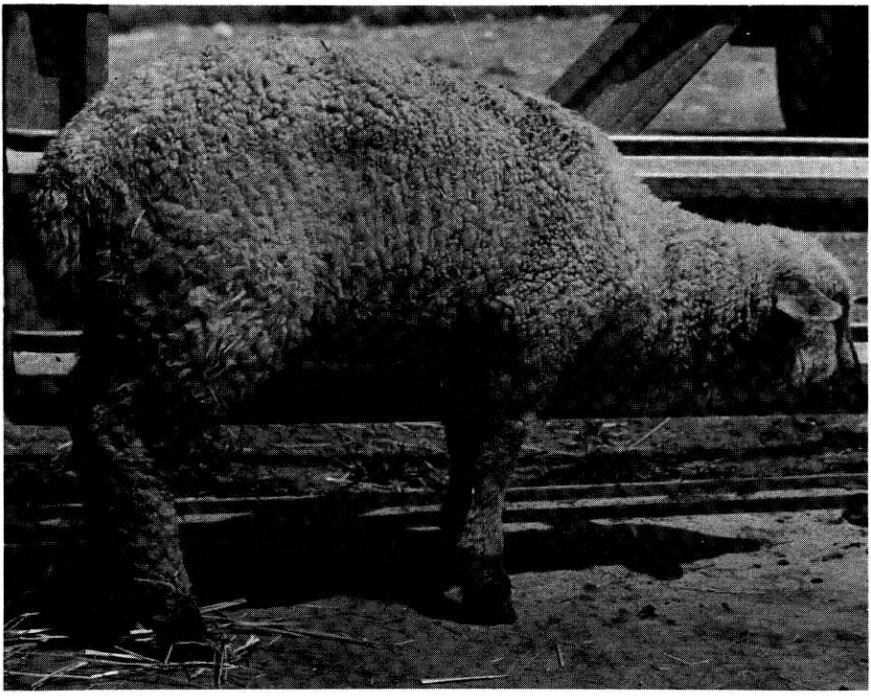

FIG. 20.3. Severe case of chlamydial polyarthritis in a lamb that is depressed, anorectic, gaunt, and reluctant to move.

Clinically affected calves are reluctant to move and they always have diarrhea. They usually are febrile, but they remain alert and nurse if carried to the dam and supported while they are suckling. Weight loss becomes evident within a few days. As the disease progresses the calves become depressed, favor their legs, and assume a hunched position while standing. When recumbent they extend

their legs passively, and the joints and tendons of the limbs are painful when palpated. Umbilical lesions or signs of nervous disorders have not been associated with this syndrome. Younger calves usually die a week or two after appearance of clinical signs.[22,58]

Pathogenic Events

Chlamydiae were isolated from the sites of the major lesions, the affected joints of naturally occurring cases of chlamydial polyarthritis of lambs and calves. These agents were also isolated from different organs and body excretions that included synovial tissues and fluids; lungs, liver, spleen, kidneys, brain and lymph nodes; conjunctival scrapings; composite samples of mucosa and content from abomasum, duodenum, jejunum, ilium and caecum; and samples of blood, cerebrospinal fluid, urine and feces.[58,64]

A chlamydemic phase precedes infection of the synovial tissues in the pathogenesis of chlamydial polyarthritis.[58,64–66] Polyarthritis can readily be reproduced experimentally by inoculating lambs or calves via the oral, subcutaneous, intramuscular, intravenous, or intra-articular routes. Three days after intra-articular inoculation of lambs, the directly inoculated joint was severely inflamed and had high chlamydial infectivity. Lesions were not seen in other joints at this time, but chlamydiae were recovered from the blood and uninoculated joints. Three days after intramuscular or intravenous inoculation of lambs, chlamydial agents were isolated from several joints, although gross lesions were minimal at this time. Irrespective of the routes of inoculation, hip, knee, shoulder, and elbow joints of lambs examined on the 7th day of the experiment had moderate to severe inflammatory changes, and chlamydiae were isolated from the affected joints. Involvement of most joints of the experimental lambs remained evident 21 days after inoculation, and chlamydiae were cultured from the majority of the affected joints tested. Maximum chlamydial infectivity was present in the joints 7 and 14 days after inoculation of the lambs and decreased progressively thereafter to become negative after the 28th day.[64]

Calves inoculated orally or parenterally with serovar 2 isolates from joints developed polyarthritis and polyserositis, and chlamydiae were recovered from the affected joints, internal organs and intestinal tracts.[62] Oral infection is expected to occur under field conditions because the intestine was found to be infected in sheep and calves with naturally occurring chlamydial polyarthritis.[22,34,64] Infection and multiplication occurred in the mucosa of the small and large intestines, leading to primary diarrhea following oral inoculation of calves with chlamydial strains of polyarthritis. The intestinal barrier

was breached by infection of endothelial cells of the central lacteals and release of infectious chlamydiae into the lymph flow.[38] Chlamydemia followed intestinal chlamydial multiplication and determined the further course of the disease.[65,66] Lesions were also evident in tonsils and Peyer's patches.

Pathological Changes

The most striking tissue changes were articular and periarticular in naturally occurring and experimentally induced chlamydial polyarthritis of lambs and calves.[64,67] Radiologically, there was no discernible malalignment of the limbs or joints. Periarticular, subcutaneous edema and fluid-filled, fluctuating synovial sacs contributed to joint enlargement of affected calves. The larger, freely movable, weight-bearing joints—such as hip, stiffle, tarsal, atlanto-occipital, shoulder, elbow and the carpal joints of calves—contained excessive, grayish-yellow, turbid synovial fluid. Grayish-yellow fibrin flakes and plaques of different sizes and shapes were found in joints with advanced lesions. The fibrin plaque in the recesses of the affected joints often adhered firmly to the synovial membranes, and the joint capsules were thickened. Erosions or evidence of marginal compensatory changes could not be found on the articular cartilage. The tendon sheaths of severely affected lambs and calves were distended and contained creamy, grayish-yellow exudate. Surrounding muscles were hyperemic and edematous having petechiae in their associated fascial planes. Calves also had enlarged spleens and swollen lymph nodes.[67,68]

Consistent and striking histopathological changes involved the synovial membranes, joint capsule, tendon sheaths, ligaments, periarticular connective tissue and muscles.[67] The inflammatory reaction in the synovium, tendon sheaths and subsynovial tissues progressed from initial serous to fibrinopurulent inflammation to, finally, accumulation of lymphoid cells and fibroplasia (Fig. 20.4). Dimorphic inflammatory responses with some leukocytes along the synovial surface and mononuclear cell infiltration in the subsynovial strains were prominent. Areas of the synovial membranes under the fibrinopurulent exudate were necrotic and heavily infiltrated with neutrophils and lymphocytes during the early stages of the infection. Involvement of subsynovial connective tissue and muscle was limited to accumulation of mononuclear cells in perivascular areas and increasing fibrosis. The joint capsule and tendon sheath of advanced cases had undergone marked fibrotic thickening, which was associated with an extensive inflammatory response that consisted primarily of infiltration by plasma cells, monocytes, lymphocytes, macro-

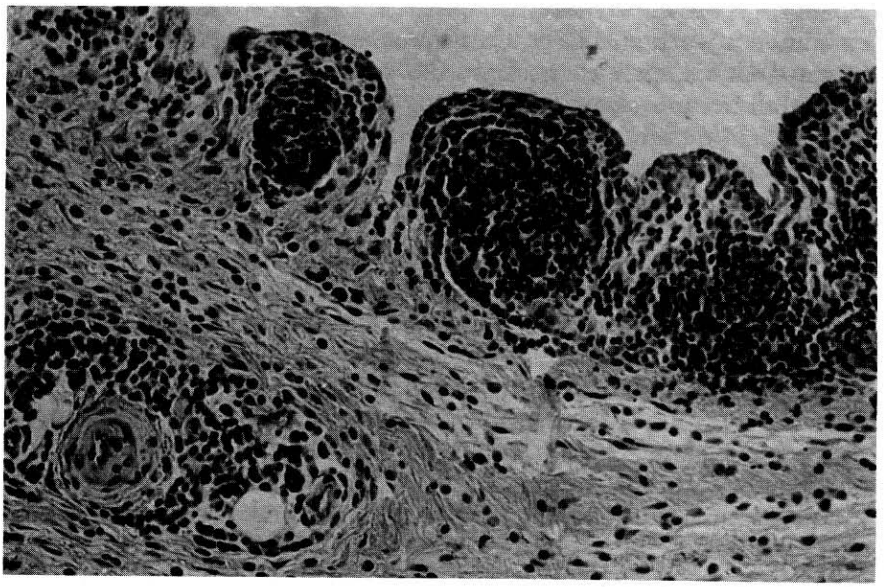

FIG. 20.4. Granulomatous lesions in synovial membrane of a lamb 21 days after intramuscular inoculation with an arthropathogenic chlamydial strain. Notice angiitis and perivascular infiltration. Hematoxylin and eosin stain, × 192.

phages and neutrophils. The synoviocytes became swollen and hyperplastic and assumed a pseudostratified appearance. The tendon sheaths had marked fibroblastic proliferation characterized by large, plump, round fibroblasts. Intra-articular masses of fibrin contained polymorphonuclear cells, monocytes and necrotic cellular debris. Chlamydial inclusions were found in fibroblasts, monocytes and endothelial and synovial cells in thin sections of affected joint tissues.[67]

Changes in muscles were periarticular and adjacent to tendinous insertions. These changes were best characterized as myositis because they consisted of infiltration with inflammatory cells and accumulation of edema fluid between individual muscle fibers and between muscle bundles.[67] Individual muscle fibers were intact and essentially normal, but hyperemia and hemorrhages were observed in muscles. The reactive processes in muscle tissues appeared to be dominated by fibroblastic proliferation suggestive of a musculotendofasciitis. Affected tendons and tendon sheaths contained edema fluid and were infiltrated by inflammatory cells.

Calves that were orally inoculated with chlamydial strains from polyarthritis harbored this infection in the kidneys and shed

chlamydiae in high titers in the urine.[34,69] The kidneys of calves examined a week and later after inoculation had multiple, grayish-white nodules visible through the capsule. Focal renal tubular dilatation became severe and cystic, involving the distal convoluted tubules. The epithelial cells of such tubules were flattened. The lumen contained epithelial cell casts. Focal and interstitial inflammatory reactions of lymphocytes and plasma cells were dispersed throughout the cortex.[69]

The lesions were most pronounced 6 weeks postinoculation. Sagittal sections displayed these lesions as wedges of various depths in the cortex. The tissue changes advanced to a chronic focal nonsupporative nephritis. The cellular reactions involved the medulla but more often the cortex. Fibrous tissue became more prominent around tubules. Glomeruli surrounded by inflammatory reactions became atrophic, and their Bowman capsules had various degrees of fibrosis. The glomerular tufts had proliferative changes involving the epithelial, endothelial and mesangial cells, with little encroachment on the patency of the blood capillaries but leading to obliteration of the urinary space of glomeruli.[69]

Diagnosis and Differentiation

Isolation of chlamydiae from the site of the major lesion, the affected joints, provides a definitive diagnosis. Cytologic investigations on synovial fluids or tissues may reveal the presence of chlamydial inclusions in cells of affected joints. Bacteriologic cultures of affected joints were negative in samples from sheep, but *Escherichia coli* or streptococci were occasionally cultured from joints of calves that were chlamydia-infected.[22,58]

The clinical and pathological features distinguish chlamydial polyarthritis from other conditions that cause stiffness and lameness in lambs. Lambs with mineral deficiency, osteomalacia, or white muscle disease usually do not have fever, unless the fever is caused by secondary bacterial infection. Sheep with chronic, advanced cases of arthritis caused by *Erysipelas insidiosa* usually have eburnations, with compensatory marginal lipping and osteophyte formation. Laminitis caused by bluetongue virus infection can be differentiated clinically, pathologically and etiologically from chlamydial polyarthritis. Detailed microbiologic investigations are needed to differentiate arthritis and serositis caused by myoplasma.[41]

Humoral Antibody Response

The serological test most widely used in the past to detect chlamydial antibodies in animal infections was the CF test.[20,41] Active

systemic chlamydial infections induce titers that have diagnostic significance if four-fold or higher antibody rises develop that are related to specific disease episodes. Since guinea pigs have naturally occurring chlamydial infections, guinea pig complement must be checked for freedom from chlamydial antibodies. Supplementation of guinea pig complement with fresh bovine serum free of chlamydial antibodies enhances the sensitivity of the CF test for bovine antibodies. The CF test has been virtually replaced by modern enzyme-linked immune assays.[41]

The antibody responses of cows experiencing chlamydial infections were recently analyzed with the enzyme linked immunosorbent assay (ELISA) and the indirect immunofluorescent antibody (IIFA) test.[42,70] The antibody responses were characteristic and diagnostically indicative. Partially purified elementary bodies were used to sensitize the test plates so that the predominant chlamydial antigens reactive in these tests were proteinaceous antigens of species-, type- and strain specificity. Heavy and light (H+L) chain specific conjugates were employed to detect all immunoglobulin isotypes. Interestingly, the chlamydia-specific antibody fraction of bovine serum contained high levels of noncomplement-binding IgG_2 when tested with the corresponding immunoglobulin subclass specific conjugates.[70–73] The ELISA can be set up with single serum dilutions of 1:100 if strictly quantitative evaluation and appropriate internal negative and positive controls are used.[71]

Treatment of Chlamydial Infections of Ruminants

The multiplication of chlamydiae in the cells of animals is inhibited or reduced by tetracyclines, chloramphenicol and erythromycin-like compounds, such as tylosin. To be effective the antibiotics must reach the intracellular sites where chlamydial multiplication occurs. When these antibiotics are used properly during the early stage of an infection, affected animals may respond favorably. The treatment usually does not eliminate chlamydial infections unless the antibiotics are given for an extended period.[19]

Dairy cattle of one closed breeding herd in which virtually all the animals had intestinal chlamydial infections, chlortetracycline (2 mg/kg body weight) was given to every animal orally for 7 days and then 1 mg/kg/day was given for the following 21 days. Though chlamydiae were not isolated from the feces of these cattle during the 2 weeks after treatment, a year later the cattle again shed chlamydiae in their feces even though the herd had remained closed. This was interpreted as a recurrence of the original infection.[74] There are no reports of specific studies of the effect of antibiotics on chlamydial

enteritis during the neonatal period or on pneumonia in calves. The previously mentioned antibiotics should be given in addition to supportive therapy when chlamydial infections are suspected.

Favorable responses were obtained when lambs affected naturally with chlamydial polyarthritis were treated with long-lasting penicillins, oxytetracycline or tylosin. The lambs were intramuscularly given 200 mg of tylosin or 250 mg of oxytetracycline for 2 days or 300,000 units of penicillin G once, as soon as clinical signs of chlamydial polyarthritis became evident. Chlortetracycline or tylosin was effective in the treatment of calves with polyarthritis–serositis and calves with chlamydial meningo-encephalomyelitis. High doses of these antibiotics were given as early as possible in the course of the disease. They were injected parenterally for at least 4–5 days. The body temperature of treated animals dropped significantly within 24 h after initiation of the treatment.

The most effective antibiotics for treating chlamydial infections are tetracyclines, but even these antibiotics suppress only chlamydial growth and multiplication and do not eliminate the infection without intact defense mechanisms of the host. Tetracyclines block protein synthesis by interfering with the transfer of amino acids from the activated transfer RNA to the growing peptide chain on the ribosome. Different strains of chlamydiae may differ in susceptibility to tetracyclines.[75]

Sporadic Bovine Encephalomyelitis

Epidemiology

Some cattle may develop pronounced signs of nervous disease and encephalomelitis following infection with serovar 2 chlamydiae.[1,8,76] The causal agent was isolated in 1940 by intraperitoneal inoculation of guinea pigs and later identified to have chlamydial properties.[8,76] Tissue reactions in the brain and associated adverse neurological signs may be induced in the course of systemic chlamydial infections of other animal species, as well as man. Popek identified 27 cases of human chlamydiosis with nervous signs associated with meningitis, encephalitis and myelitis.[77] Opossums with naturally occurring chlamydial infections developed paralysis of the hind legs and convulsions.[78] Meningeal and encephalitic lesions were found histologically in chickens, pigeons and turkeys with chlamydial infections.[4,79]

The clinical encephalitic manifestations of chlamydial infections were investigated mainly in cattle. Sporadic bovine encephalomyelitis was proven to occur in cattle of many parts of the United States

as well as in Japan, Czechoslovakia, Australia, Canada, Hungary and Germany.[1,20] Transmission by the oral–fecal route leads to this nervous disorder that affects cattle sporadically.[80] This disease is seen primarily in cattle younger than 2 years, although older animals may be affected. The morbidity is low, but the mortality of stricken cattle is 50–60%. Konrad and Bohac observed 100% mortality among SBE-affected cattle in Czechoslovakia.[81] The disease may remain enzootic in one herd, but, not infrequently, outbreaks follow introduction of susceptible cattle into established herds.

Clinical Signs

Depression and gradual loss of locomotor coordination are early clinical signs. The affected cattle become anorectic and develop a high fever approximately 2 weeks after exposure. Excessive salivation, dyspnea and mild diarrhea are signs in the early stages. Recovery may follow this stage, but in some instances the cattle develop neural involvement manifested by walking difficulties, a stiff gait and a tendency to stagger and circle, often falling over small obstacles. The limbs become progressively weaker, paralysis develops and the animals become recumbent and may exhibit opisthotonos. The course of the disease usually lasts from 10 to 14 days, but in some instances affected cattle may survive for a month.[8,80]

Pathogenesis and Pathological Changes

The dominant feature in the development of this disease is a generalized chlamydial infection. The chlamydial agent was isolated from blood, brain and cerebrospinal fluid and from liver, spleen, kidneys, lungs and peritoneal fluid.[76] Lesions in various organs are associated with vascular damage. The brain is the ultimate target organ, and the brain lesions usually are distributed through all parts of the brain, but they are regularly present in the medulla, pons and brain stem. The degree of brain involvement may well be the critical point that determines recovery or death. The arachnoid and ependymal tissues are favored sites of chlamydial multiplication. The factors that lead to the severe reaction in the brain are not well understood and could possibly involve immune-mediated injuries. Not all aspects of this disease have been reproduced experimentally; pathological changes of mild clinical cases have not been described, and fatal cases with no brain involvement are not on record. Although serositis was described, and fatal cases with no brain involvement are not on record. Although serositis was described often as a lesion in sporadic bovine encephalomyelitis, one should consider this nervous

disease as a distinct clinical entity, separate from polyarthritis–serositis of calves, until certain clinical, pathological and pathogenetic differences are resolved.

Macroscopic lesions when visible in the central nervous system are primarily hyperemia and edema. After an acute course of the sickness, few gross lesions are found, but the serous cavities may contain an increased amount of yellowish fluid. Some chronic cases have serofibrinous exudate in serous cavities with peritonitis, pleuritis or pericarditis. Microscopically, all parts of the brain and spinal cord have vasculitis with perivascular cuffing and parenchymal foci of inflammation composed of lymphoid and mononuclear cells. Similar inflammatory reactions may be seen in the leptomeninges. Small foci of granulomatous inflammation are found in kidneys, lungs and livers. Acute and chronic bronchiolitis with focal areas of pneumonia are observed. Fibrin on visceral surfaces of abdominal organs undergoes organization.[80]

Diagnosis

Etiologic confirmation of the cause of this disease can be achieved by isolating chlamydiae from brain tissues or fluids. Pathological lesions and other encephalitic diseases of cattle, including listeriosis, rabies, pseudorabies, malignant catarrhal fever and encephalitis caused by the virus of infectious bovine rhinotracheitis, must be differentiated by clinical, etiological and histological examination.[77] Rising chlamydial antibody titers are supporting a clinical diagnosis of chlamydial meningoencephalomyelitis.[39]

Conjunctivitis and Ocular Diseases

Epidemiology

While trachoma and inclusion conjunctivitis of man have a long history as specific and distinct clinical entities caused by *C. trachomatis*,[2] the study of ocular chlamydial infections of animals is of rather recent history.[1] Ocular diseases are now known to be caused by *C. psittaci* and *C. pecorum* in several mammalian species. Conjunctivitis and ocular involvement have long been recognized as clinical signs of chlamydiosis in pigeons, ducks and geese.[5]

Yerasimides was the first to isolate and identify a chlamydial agent from conjunctival exudate of a cat suffering from acute catarrhal conjunctivitis.[23] An infectious agent with chlamydial properties was

isolated from conjunctival samples of piglets affected with keratoconjunctivitis in Bulgaria.[82] Murray identified in 1964 inclusion conjunctivitis of guinea pigs as a prevalent chlamydial infection.[24]

The observation of chlamydial agents as the cause of contagious keratoconjunctivitis of sheep in England was based on cytologic examinations of conjunctival samples and on chemotherapeutic treatment of the infection.[83] Chlamydial agents were subsequently cultured in developing chicken embryos from conjunctival samples taken from sheep suffering from keratoconjunctivitis in the United States[84] and recently also from lambs in England.[85] Chlamydiae were also isolated from conjunctival scrapings of cattle afflicted with keratoconjunctivitis in Czechoslovakia and Germany.[20] Interestingly, Rowe and coworkers isolated chlamydiae in cultured cells from conjunctival samples of a calf from a free-living herd of African buffalos.[86] The studies of Cockram and Jackson are of unique interest because chlamydiae were isolated from eye samples of free-living koalas (*Phascolarctos cinereus*) severely affected with keratoconjunctivitis in Australia.[25]

Properties of Conjunctival Chlamydial Isolates

The isolates from cats belong to serovar 7 and those from guinea pigs are serovar 8. Serovar 2 isolates were recovered from conjunctivitis of lambs.[31,87] Serovar 7 and 8 infect L cells or other cultured cells readily while serovar 2 chlamydiae require infectivity enhancing conditions through treatment of cells with cytoactive compounds or centrifugation onto monolayers.[62,63]

Clinical Signs

Conjunctival chlamydial infections of different animal species probably lead to similar clinical signs. They have been studied in infections of cats, sheep and guinea pigs. The infection may become endemic in catteries, and cats may remain affected with conjunctivitis for as long as one year. Kittens born to affected or clinically recovered cats may have severe conjunctivitis at the time their eyelids normally open. Cats developed a severe conjunctivitis 3–5 days after conjunctival inoculation. On the 4th day, these cats invariably had fever, which lasted for 24 h. Chlamydial inclusion bodies were found in large numbers from the 3rd day after inoculation and persisted in decreasing numbers for 2 weeks. Many experimentally inoculated cats had clinical evidence of conjunctivitis for as long as 90 days.

Corneal involvement was not observed, but rhinitis and other signs of respiratory involvement were often seen.[52] Infections of the gastric mucosa was recently detected.[88,89] This site of infection appears to be an extension of the feline pneumonitis syndrome caused by chlamydiae. Mild gastritis was observed, but most cats with persistent gastric infection appeared clinically normal.

Early signs of chlamydial conjunctivitis of sheep consisted of chemosis and dilation of conjunctival vessels at the lid margins. Diffuse reddening was seen later in the lower fornices, where fine filaments of mucus were also present. The development of lymphoid follicles signaled intermediate stages of conjunctivitis involvement. Lymphoid follicles began as small, discrete, pale and elevated changes in the conjunctiva, enlarged to diameters of 3 mm and became confluent, forming delicate pink-to-red folds in the lower fornix and the 3rd eyelid. The bulbar surface of the 3rd eyelid had numerous follicles. Lymphoid follicles in the upper fornices developed into folds 2–4 mm high. Conjunctival hyperemia, edema and follicle development caused swelling of the periorbital tissues. Lacrimal drainage was compromised, epiphora was a common sign, and abundant seropurulent eye discharge was observed to seal the eye lids with a crust. Perilimbal edema of the cornea was associated with signs of severe inflammatory involvement of the bulbar conjunctiva and its extension into the cornea as keratitis (Fig. 20.5). Loops of blood vessels grew into the cornea at a rate of 1 mm per day. Neo-

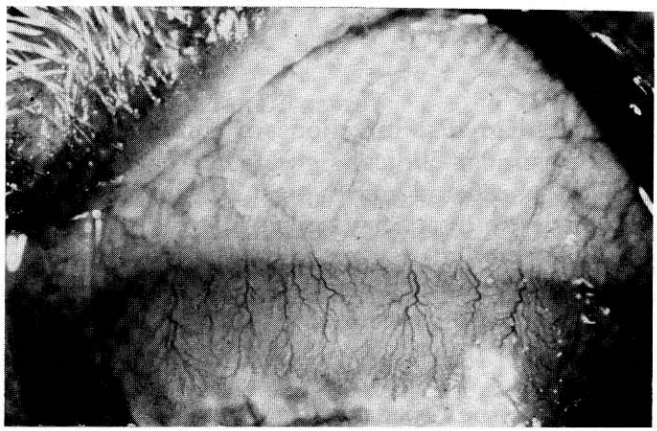

FIG. 20.5. Eye lesions of a lamb with chlamydial infection. Notice bulbar conjunctivitis, neovascularization and keratitis. Third eye lid is to the left.

vascularization of the cornea occurred in the upper limbus, often extended into the cornea for distances of 5 mm, and became completely perilimbal. Both eyes were found to be equally affected in 80% of the lambs. The disease was self-limiting in uncomplicated cases because full resolution of lesions was observed. Complications consisted of opacity of the entire cornea and corneal ulceration.[20,84,90]

Ten to eighty-five per cent of the lambs with conjunctivitis studied in the western United States had polyarthritis, and virtually all lambs with polyarthritis had conjunctivitis. Chlamydiae of serovar 2 were isolated from conjunctiva, blood and joint samples of affected lambs.[54,84] Goats experiencing chlamydial abortions also had keratoconjunctivitis.[91]

Chlamydial inclusion conjunctivitis affects many herds of guinea pigs and is predominantly found in 4–8 week old animals. A special effort is necessary to detect the inflammatory conjunctival reaction with its light, yellowish-white discharge. An intense conjunctivitis arises 3–6 days after experimental infection of guinea pig eyes which induces a watery to mucopurulent discharge, chemosis and follicular hypertrophy in the palpebral conjunctiva. Localized subepithelial cellular infiltrates are frequently seen in the peripheral cornea. Pannus or micropannus form in most inoculated guinea pigs as early as 5 days after exposure. The keratoconjunctivitis is self-limiting and clears up within 3–4 weeks.[24]

Pathogenesis and Pathological Changes

The development of chlamydial inclusion bodies in epithelial and monocytic cells was associated with mononuclear and heterophilic cellular responses in cats, lambs, piglets, and guinea pigs (Fig. 20.6). Inclusion bodies with chlamydial features were found in the conjunctival epithelial cells that also evidenced cytopathic changes and lysis.[24,53,90] The epithelium and subepithelial tissues of the conjunctiva were infiltrated with polymorphonuclear leukocytes in the early stages, while subepithelial collections of macrophages and mononuclear cells were found in the peripheral limbus, and mononuclear cells dominated the histological changes in the advanced stages. Persistent antigenic stimulation provoking chronic inflammations in chlamydial infections is considered to be one pathogenic mechanism. The guinea pig model of inclusion conjunctivitis was used in an elegant series of experiments to identify chlamydial fractions involved in the deleterious immune responses.[92] Triton X100 extracts of chlamydial elementary bodies elicited ocular hypersensitivity in immunized guinea pigs. This response was genus

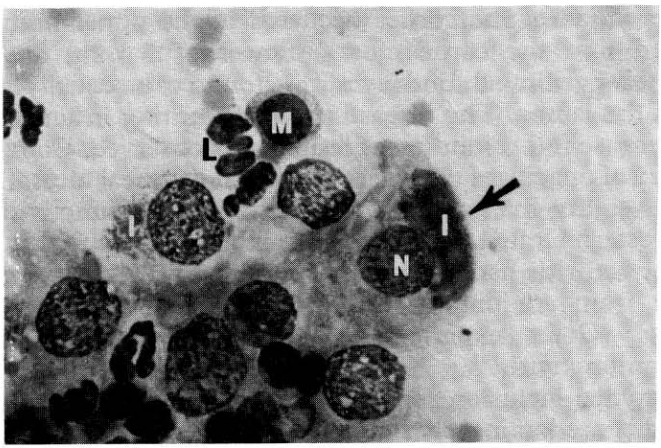

FIG. 20.6. Chlamydial inclusions (I) in conjunctival cells from lamb with conjunctivitis. Arrow points towards infected cells with nucleus (N). A monocyte (M) and numerous leukocytes (L) are present. Giemsa stain, × 600.

specific, but it was not elicited by purified LPS. A 57 kDa protein carried the function. This heat-stable antigen, associated with elementary and reticulate bodies of both chlamydial species, was identified as a member of stress–response proteins.[93]

Calves with systemic chlamydial infections leading to polyarthritis also developed conjunctivitis, and signs of blindness were observed in cattle with sporadic encephalomyelitis. Intraocular structures involved were the retina and the optic nerve. Optic papillitis consisted of mononuclear inflammatory reactions extending from the lamina cribosa to the head of the optic nerve. Histopathological alterations were present in the nerve fiber layer and the inner nuclear layer of the sensory retina. An inflammatory reaction extended towards the inner limiting membrane of the retina. Histiocytic cells, lymphocytes and a few polymorphonuclear leukocytes formed the inflammatory foci of granuloma-like appearance. Bilateral anterior uveitis was observed after intravenous inoculation of albino rabbits with a chlamydial strain isolated from muskrats. This uveitis disappeared about 10 days later, but iritis persisted longer, and mononuclear cells invaded the iris and the ciliary body.[94]

Diagnosis

Exfoliative cytologic examinations of conjunctival scrapings from subjects with conjunctivitis can give valuable diagnostic

information.[40] The demonstration of cytoplasmic chlamydial inclusions in conjunctival epithelial cells or monocytes is an unequivocal indication of chlamydial infection (Fig. 20.6). Extracellular chlamydial elementary bodies can be distinguished in the background of such smears. Unfortunately, typical inclusions are present only in the early stages of the ocular chlamydial infection. Application of fluorescent antibody technique facilitates the detection of chlamydial conjunctivitis in animals. Monoclonal antibodies improved detection of this infection in conjunctival samples from cats.[95] Care should be taken to collect conjunctival samples for cytologic studies by scraping the conjunctival surface to collect fresh cells. Conjunctival cells in smears from affected eyes occur singly or in 2- to 3-cell groups, while cells in samples from normal eyes maintain their intercellular bridges to appear as sheets.[90]

The sampling techniques described in conjunction with attempts to express follicular contents are optimal for isolating chlamydiae from conjunctival lesions. The samples should be collected in a small volume of diluent. The developing chicken embryo, inoculated by the yolk sac route, is a good indicator host in isolating chlamydiae from ocular infections of several animal species.[83] Chlamydiae were isolated from conjunctival scrapings of 42% of the lambs affected with eye lesions.[87] Cell culture methods probably are even more sensitive, and they give definitive results within days instead of weeks. Bacterial contamination can be controlled by decontamination through differential centrifugation and use of specific antibiotics. Secondary infections with different kinds of bacteria or mycoplasma often are present in chronic stages of the disease. Viral infections of the conjunctiva must be differentiated by corresponding etiologic investigation.[40] Some information can be gained in this respect by cytologic examination of conjunctival smears if viral agents that induce specific inclusions are involved.

Vaccines to Prevent Feline Conjunctivitis and Pneumonia

Research on live and killed vaccines to prevent chlamydial infections of cats dates back to 1952.[96] These efforts gave insight into the challenges in preventing these infections in animals and are of interest because of the live chlamydial vaccine that was developed.[97] The chlamydiae were propagated in the yolk sacs of developing chicken embryos or recently in feline cell cultures. The live vaccine given intramuscularly afforded the best clinical protection of cats that were challenge-inoculated by the conjunctival and nasal routes.[98–101] Vaccination induced antibodies detected in the indirect immunof-

luorescent test and a cell-mediated immune response which were assessed in the lymphocyte blastogenesis test.[99]

Assessment of clinical signs appeared to be the most reliable method for evaluation of protection afforded by this vaccine.[98–101] Vaccinated cats did not develop fever after challenge-inoculation but test-infected controls had high fever after inoculation. Other clinical signs involving the upper respiratory tract and the eyes were significantly reduced in vaccinated cats. However, chlamydiae were shed in eye and respiratory secretion by some vaccinated cats following challenge inoculation. Shedding lasted from 5 to 9 days in vaccinates and 4 to 17 days in the controls. Killed chlamydial vaccines induced virtually no, or only inferior, protection to challenge inoculation and adjuvants did not change this measurably.[96,99]

References

1. Storz, J. 1971. *Chlamydia and Chlamydia Induced Diseases*. Charles C. Thomas, Publ. Co. Springfield, Ill. 358 pages.
2. Schachter, J. and Dawson, C.R. 1978. *Human Chlamydial Infections*. PSG Publishing Company, Inc. Littleton, MA.
3. Morange, A. 1895. De la psittacose, ou infection spediale determinée par des perruches. Thése Paris, Académie de Paris.
4. Meyer, K.F. 1967. The host spectrum of psittacosis–lymphogranuloma venereum (PL) agents. *Am. J. Ophthal.* **63:** 1225/199–1246/220.
5. Baker, J.A. 1942. A virus from a pneumonia of cats and its possible relation to the cause of atypical pneumonia in man. *Science* **96:** 475–476.
6. Gönnert, R. 1941. Die Bronchopneumonie, eine neue Viruskrankheit der Maus. *Zentbl. Bakt. I. Orig.* **147:** 161–174.
7. Nigg, C. 1942. Unidentified virus which produces pneumonia and systemic infection in mice. *Science* **95:** 49–50.
8. McNutt, S.H. 1940. A preliminary report on an infectious encephalomyelitis of cattle. *Vet. Med.* **35:** 228–231.
9. Stamp, J.T., McEwen, A.D., Watt, J.A.A. and Nisbet, D.J. 1950. Enzootic abortion in ewes. I. Transmission of the disease. *Vet. Rec.* **62:** 251–254.
10. Storz, J., Carroll, E.J., Ball, L. and Faulkner, L.C. 1968. Isolation of a psittacosis agent (*Chlamydia*) from semen and epididymis of bulls with seminal vesiculitis syndrome. *Am. J. vet. Res.* **29:** 549–555.
11. Rodolakis, A. and Bernard, K. 1977. Isolation of *Chlamydia ovis* from genital organs of rams with epididymitis. *Bull. Acad. vet. Fr.* **50:** 65–70.
12. Bowen, R.A., Spears, P., Storz, J. and Seidel, G.E. Jr 1978. Mechanisms of infertility in genital tract infections due to *Chlamydia psittaci* transmitted through contaminated semen. *J. infect. Dis.* **138:** 95–98.
13. Barron, A.L., White, H.J., Rank, R.G. and Soloff, B.L. 1979. Target tissues associated with genital infection of female guinea pigs by the chlamydial agent of guinea pig inclusion conjunctivitis. *J. infect. Dis.* **139:** 60–68.
14. Blanco-Lozelier, A. 1969. Aislamiento de un agente del grupo psittacosis linfogranuloma venereo (PLV) en mastitis bovinas. *Patronato Biol. Anim.* **13:** 179–186.

15. KALTENBOECK, B., KOUSOULAS, K.G. and STORZ. J. 1991. Detection and strain differentiation of *Chlamydia psittaci* mediated by a two-step polymerase chain reaction. *J. clin. Microbiol.* **29:** 1969–1975.
16. BOULANGER, P. and BANNISTER, G.L. 1959. Abortion produced experimentally in cattle with an agent of the psittacosis lymphogranuloma venereum group of virus. *Can. J. comp. med. vet. Sci.* **23:** 259–265.
17. ENRIGHT, J.B., SADLER, W.W. and ROBINSON, E.A. 1958. Sporadic bovine encephalomyelitis in California. *Proc. U.S. Livestock sanit. Ass.* **62:** 127–135.
18. RONSHOLT, L. and BASSE, A. 1981. Bovine mastitis induced by a common intestinal *Chlamydia psittaci* strain. *Acta vet. scand.* **22:** 9–22.
19. YORK, C.J. and BAKER, J.A. 1951. A new member of the psittacosis–lymphogranuloma group of viruses that causes infection in calves. *J. exp. med.* **93:** 587–604.
20. STORZ, J. and KRAUSS, H. 1985. Chlamydial infections and diseases of animals. In: *Handbook on Bacterial Infections in Animals,* Vol. V, p. 447–531. H. BLOBEL and T. SCHLEISSER (Eds), Fischer Verlag, Jena.
21. MENDLOWSKI, B. and SEGRE, D. 1960. Polyarthritis in sheep. I. Description of the disease and experimental transmission. *Am. J. vet. Res.* **21:** 68–73.
22. STORZ, J. SMART, R.A., MARRIOTT, M.E. and DAVIS, R.V. 1966. Polyarthritis in calves: Isolation of psittacosis agents from affected joints. *Am. J. vet. Res.* **27:** 633–641.
23. YERASIMIDES, T.G. 1960. Isolation of a new strain of feline pneumonitis virus from a domestic cat. J. infect. Dis. **106:** 290–296.
24. MURRAY, E.S. 1964. Guinea pig inclusion conjunctivitis virus. 1. Isolation and identification as a member of the psittacosis–lymphogranuloma–trachoma group. *J. infect. Dis.* **114:** 1–12.
25. COCKRAM, F.A. and JACKSON, A.R.B. 1981. Keratoconjunctivitis of the koala bear caused by chlamydial agents. *J. Wildlife Dis.* **17:** 497–504.
26. MCCOLL, K.A., MARTIN, R.W., GLEESON, L.J., HANDASYDE, K.A. and LEE, A.K. 1984. Chlamydia infection and infertility in the female koala *(Phascolarctos cinereus). Vet. Rec.* **115:** 655–657.
27. NEWCOMER, C.E., ANVER, M.R., SIMMONS, J.L. WILCKE, B.W. JR and NACE, G.W. 1982. Spontaneous and experimental infections of *Xenopus laevis* with *Chlamydia psittaci. Lab. Anim. Sci.* **32:** 680–686.
28. WILCKE, B.W. JR, NEWCOMER, C.E., ANVER, M.R., SIMMONS, J.L. and NACE, G.W. 1983. Isolation of *Chlamydia psittaci* from naturally infected African clawed frogs *(Xenopus laevis). Infect. Immun.* **41(2):** 789–794.
29. KÖLBL, O. 1969. Untersuchungen über das Vorkommen von Miyagawanellen beim Schwein. *Wiener Tieräztl. Monatsschr.* **56:** 332–335.
30. LEONHARD, I., WITTENBRINK, M.M. and BISPING, W. 1988. Nachweis von *Chlamydia psittaci* im Kot von Schweinen. *Berl. Münch. Tierärztl. Wschr.* **101:** 124–128.
31. PEREZ-MARTINEZ, J.A. and STORZ, J. 1985. Antigenic diversity of *Chlamydia psittaci* of mammalian origin determined by microimmunofluorescence. *Infect. Immun.* **50:** 905–910.
32. KALTENBOECK, B. and STORZ. J. 1992. Biological properties and genetic analysis of the ompA locus in chlamydiae isolated from swine. *Am. J. vet. Res.* **53:** 1482–1487.
33. KALTENBOECK, B., KOUSOULAS, K.G. and STORZ, J. 1992. Two-step polymerase chain reactions and restriction endonuclease analyses detect and differentiate ompA DNA of the genus *Chlamydia. J. clin. Microbiol.* **30:** 1098–1104.

34. Eugster, A.K. and Storz, J. 1971. Pathogenic events in intestinal chlamydial infections leading to polyarthritis in calves. *J. infect. Dis.* **123:** 41–50.
35. Eugster, A.K., Joyce, B.K. and Storz, J. 1970. Immunofluorescence studies on the pathogenesis of intestinal chlamydial infections in calves. *Infect. Immun.* **2:** 351–359.
36. Todd, W.J., Doughri, A.M. and Storz, J. 1976. Ultrastructural changes in host cellular organelles in the course of the chlamydial developmental cycle. *Zentlbl. Bakt. I. Orig.* **236:** 359–373.
37. Doughri, A.M., Altera, K.P. and Storz, J. 1973. Host cell range of chlamydial infection in the neonatal bovine gut. *J. comp. Path.* **83:** 107–114.
38. Doughri, A.M., Altera, K.P., Storz, J. and Eugster, A.K. 1973. Electron microscopic tracing of pathogenetic events in intestinal chlamydial infections of newborn calves. *Exp. molec. Path.* **18:** 10–17.
39. Doughri, A.M., Altera, K.P., Storz, J. and Eugster, A.K. 1973. Ultrastructural pathogenic changes in the chlamydia-infected ileal mucosa of newborn calves. *Vet. Path.* **10:** 114–123.
40. Doughri, A.M., Young, S. and Storz, J. 1974. Pathologic changes in intestinal chlamydial infections of newborn calves. *Am. J. vet. Res.* **35:** 939–944.
41. Storz, J. 1990. Rickettsia and chlamydia. In: *Diagnostic Procedures in Veterinary Bacteriology and Mycology*, pp. 309–331. Carter, G.R. and Cole, J.R. (Eds), Academic Press.
42. Perez-Martinez, J.A. and Storz, J. 1985. Chlamydial infections of cattle. *Modern Vet. Pract.* **66:** 517–522, 603–608.
43. Storz, J. and Thornley, W.R. 1966. Serologische und aetiologische Studien über die intestinale Psittakose-Lymphogranuloma-Infektion der Schafe. *Zentlbl. Vet.* **13:** 14–24.
44. Baker, J.A. 1944. Virus causing pneumonia in cats and producing elementary bodies. *J. exp. Med.* **79:** 159–172.
45. McKercher, D.G. 1952. A virus possibly related to the psittacosis–lymphogranuloma–pneumonitis group causing a pneumonia in sheep. *Science.* **115:** 543–544.
46. Tolybekow, A.S., Wischnajakowa, L.A. and Dobin. M.A. 1973. Die atiologische Bedeuteung eines Erregers aus der Bedsoniengruppe für die Enzootische Pneumonie der Schweine. *Monatsch. vet.* **28:** 339–344.
47. Moorthy, A.R.S. and Spradbrow, P.B. 1978. *Chlamydia psittaci* infection of horses with respiratory disease. *Equine vet. J.* **10:** 38–42.
48. Burrell, M.H., Chalmers, W.S.K. and Kewley, D.R. 1986. Isolation of *Chlamydia psittaci* from the respiratory tract and conjunctivae of thoroughbred horses. *Vet. Rec.* **119:** 302–303.
49. Moulder, J.W., Hatch, T.P., Juo, C.-C., Schachter, J. and Storz, J. 1984. Genus 1. Chlamydia Jones, Rake and Strearns 1945, pp. 729–739. In: Krieg, N.R. and Holt, J.G. (Eds), *Bergey's Manual of Systematic Bacteriology*. Vol. 1. The Williams and Wilkens Co., Baltimore.
50. Dungworth, D.L. and Cordy, D.R. 1962. The pathogenesis of ovine pneumonia. I. Isolation of a virus of the PL group. *J. comp. Path. Ther.* **72:** 49–76.
51. Dungworth, D.L. and Cordy, D.R. 1962. The pathogenesis of ovine pneumonia. II. Isolation of virus from faeces: Comparison of pneumonia caused by faecal, enzootic abortion and pneumonitis viruses. *J. comp. Path. Ther.* **72:** 71–79.
52. Cello, R.M. 1967. Ocular infections in animals wih PLT (Bedsonia) group agents. *Am. J. Ophthal.* **63:** 1270/224–1273/247.

53. HARBOURNE, J.F. 1966. Survey of bovine respiratory diseases with special references to the serological examination of paired serum samples. *Vet. Rec.* **78:** 749–752.
54. STORZ, J., SHUPE, J.L., JAMES, L.F. and SMART, R.A. 1963. Polyarthritis of sheep in the intermountain region caused by a psittacosis–lymphogranuloma agent. *Am. J. vet. Res.* **24:** 1201–1206.
55. LITTLEJOHNS, I.R., HARRIS, A.N.A. and HARDING, W.B. 1961. Sporadic bovine encephalomyelitis. *Aust. Vet. J.* **37:** 53–54.
56. KÖLBL, O., BURTSCHER, H. and HEBENSTREIT, J. 1970. Polyarthritis bei Schlachtschweinen. Microbiologische, histologische und fleisch-hygienische Untersuchungen und Aspekte. *Wien. Tierärztl. Monatsschr.* **57:** 355–361.
57. PIERSON, R.E. 1967. Polyarthritis in Colorado feedlot lambs. *J. Am. vet. Med. Ass.* **150:** 1487–1492.
58. STORZ, J., SHUPE, J.L, SMART, R.A. and THORNLEY, R.W. 1966. Polyarthritis of calves: Experimental induction by a psittacosis agent. *Am. J. vet. Res.* **27:** 987–995.
59. COX, R.L., KUO, C.-C., GRAYSTON, J.T. and CAMPBELL, L.A. 1988. Deoxyribonucleic acid relatedness of chlamydia sp. strain TWAR to *Chlamydia trachomatis* and *Chlamydia psittaci*. *Int. J. System. Bact.* **38(3):** 265–268.
60. TIMMS, P., EAVES, F.W., GIRJES, A.A. and LAVIN, M.F. 1988. Comparison of *Chlamydia psittaci* isolates by restriction endonuclease and DNA probe analyses. *Infect. Immun.* **56:** 287–290.
61. FUKUSHI, H. and HIRAI, K. 1989. Genetic diversity of avian and mammalian *Chlamydia psittaci* strains and relation to host origin. *J. Bact.* **171(5):** 2850–2855.
62. SPEARS, P. and STORZ, J. 1979. *Chlamydia psittaci*: Growth characteristics and enumeration of serotypes 1 and 2 in cultured cells. *J. infect. Dis.* **140:** 959–967.
63. SPEARS, P. and STORZ, J. 1979. Biotyping of *Chlamydia psittaci* based on inclusion morphology and response to DEAE-dextran and cycloheximide. *Infect. Immun.* **24:** 224–232.
64. STORZ, J., SHUPE, J.L., MARRIOT, M.E. and THORNLEY, W.R. 1965. Polyarthritis of lambs induced experimentally by a psittacosis agent. *J. infect. Dis.* **115:** 9–18.
65. STORZ, J., MARRIOTT, M.E. and THORNLEY, W.R. 1968. The dynamics of the blood infectious phase in psittacosis-induced abortions in animals. *J. infect. Dis.* **118:** 333–339.
66. ATA, F.A. and STORZ, J. 1974. Chlamydial blood clearance in convalescent sheep. *Cornell Vet.* **64:** 25–36.
67. SHUPE, J.L. and STORZ, J. 1964. Pathologic study of psittacosis lymphogranuloma polyarthritis of lambs. *Am. J. vet. Res.* **25:** 943–951.
68. CUTLIP, R.C. and RAMSEY, F.K. 1973. Ovine chlamydial polyarthritis: sequential development of articular lesions in lambs after intraarticular exposure. *Am. J. vet Res.* **34:** 71–75.
69. DOUGHRI, A.M., EUGSTER, A.K. and ALTERA, K.P. 1978. Pathological studies on chlamydial kidney infections in newborn calves. *Arab develop. Sci. Technol.* **1:** 10–18.
70. PEREZ-MARTINEZ, J., SCHMEER, N. and STORZ, J. 1986. Bovine chlamydial abortion: Serodiagnosis by modified complement fixation, indirect inclusion fluorescence, and enzyme-immunoassay. *Am. J. vet. Res.* **47:** 1501–1506.
71. SCHMEER, N., PEREZ-MARTINEZ, J.A., SCHNORR, K. and STORZ, J. 1987. Dominance of *Chlamydia psittaci*-specific IgG_2 isotypes in naturally and experimentally infected cattle. *Vet. Immun. Immunopath.* **15:** 311–322.

72. SCHMEER, N., SCHNORR, K.L., PEREZ-MARTINEZ, J., STORZ, J. and KRAUSS, H. 1987. Specific interaction of bovine IgG_1 and IgG_2 subclasses with different chlamydial antigens. *Zentlbl. Bakt. I. Orig.* **266:** 305–315.
73. BOROVIK, R.V., KURBANOV, I.A. and TERSKIKH, I.I. 1978. Specific activity of cattle immunoglobulins in experimental chlamydia infection. *Vop. Virusol.* **4:** 485–488.
74. PAGE, L.A., MATTHEWS, P.J. and SMITH, P.C. 1973. Natural intestinal infection with *Chlamydia psittaci* in a closed bovine herd: Serologic changes, incidence of shedding, antibiotic treatment of the herd, and biologic characteristics of the chlamydiae. *Am. J. vet. Res.* **34:** 611–614.
75. JAWETZ, E. 1969. Chemotherapy of chlamydial infections. *Adv. Pharmac. Chemother.* **7:** 253–282.
76. WENNER, H.A., HARSHFIELD, G.S., CHANG, T.W. and MENGES, R.W. 1953. Sporadic bovine encephalomyelitis. II. Studies on the etiology of the disease, isolation of nine strains of an infectious agent from naturally infected cattle. *Am. J. Hyg.* **57:** 15–29.
77. POPEK, K. 1964. Komplikationen des Nervensystems bei der Ornithose des Menschen. *Arch. exp. Vet.* **18:** 201–209.
78. ROCCA-GARCIA, M. 1949. Viruses of the lymphogranuloma–psittacosis group isolated from opossums in Columbia, opossum virus A. *J. infect. Dis.* **85:** 275–289.
79. PIERCE, K.R. and MOORE, R.W. 1965. Meningoencephalitis in turkeys experimentally infected with ornithosis. *Avian Dis.* **9:** 266–271.
80. HARSHFIELD, G.S. 1970. Sporadic bovine encephalomyelitis. *J. Am. vet. Med. Ass.* **156:** 466–477.
81. KONRAD, J. and BOHAC, J. 1959. Encephalomyelitis in cattle. *Vet. Casopis.* **8:** 228–238.
82. PAVLOV, P., MILANOV, M. and TSCHILEV, D. 1963. Recherches sur la rickettsiose keratoconjunctivale du porc en Bulgarie. *Ann. Inst. Pasteur.* **105:** 450–454.
83. DICKINSON, L. and COOPER, B.S. 1959. Contagious conjunctivokeratitis of sheep. *J. Path. Bact.* **78:** 257–266.
84. STORZ, J., PIERSON, R.E., MARRIOTT, M.E. and CHOW, T.L. 1967. Isolation of psttacosis agents from follicular conjunctivitis of sheep. *Proc. Soc. exp. Biol. Med.* **125:** 857–860.
85. WILSMORE, A.J., DAGNALL, G.J. and WOODLAND, R.M. 1990. Experimental conjunctival infection of lambs with a strain of *Chlamydia psittaci* isolated from the eyes of a sheep naturally affected with keratoconjunctivitis. *Vet. Rec.* **127:** 229–231.
86. ROWE, L.W.R., HEDGER, R.S. and SMALE, C. 1978. The isolation of a *Chlamydia psittaci*-like agent from a free-living African buffalo. *(Syncerus caffer). Vet. Rec.* **103:** 13–14.
87. STEPHENSON, E.H., STORZ, J. and HOPKINS, J.B. 1974. Properties and frequency of isolation of chlamydiae from eyes of lambs with conjunctivitis and polyarthritis. *Am. J. vet. Res.* **35:** 177–180.
88. HARGIS, A.M., PRIEUR, D.J. and GAILLARD, E.T. 1983. Chlamydial infection of the gastric mucosa in twelve cats. *Vet. Path.* **20:** 170–178.
89. GAILLARD, E.T., HARGIS, A.M., PRIEUR, D.J., EVERMANN, J.F. and DHILLON, A.S. 1984. Pathogenesis of feline gastric chlamydial infection. *Am. J. vet. Res.* **45:** 2314–2323.
90. HOPKINS, J.B., STEPHENSON, E.H., STORZ, J. and PIERSON, R.E. 1973. Clinical characteristics of eye lesions of lambs affected with chlamydial conjunctivitis and polyarthritis. *J. Am. vet. Med. Ass.* **163:** 510–513.

91. EUSTER, A.K., JONES, L.P. and GAYLE, L.G. 1977. Epizootics of chlamydial abortions and keratoconjunctivitis in goats. *Proc. Am. Ass. vet. Lab. Diag.* **20:** 59–78.
92. MORRISON, R.P., LYNG, K. and CALDWELL, H.D. 1989. Chlamydial disease pathogenesis. Ocular hypersensitivity elucidate by a genus-specific 57-kD protein. *J. exp. Med.* **169:** 663–675.
93. MORRISON, R.P., BELLAND, R.J., LYNG, K. and CALDWELL, H.D. 1989. Chlamydia disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J. exp. Med.* **170:** 1271–1283.
94. DOUGHRI, A.M. and STORZ, J. 1978. Ocular histopathologic lesions in chlamydial infection of newborn calves. *Arab. develop. J. Sci. Technol.* **1:** 5–9.
95. WILLS, J.M., MILLARD, W.G. and HOWARD, P.E. 1986. Evaluation of a monoclonal antibody based ELISA for detection of feline *Chlamydia psittaci*. *Vet. Res.* **119:** 418–420.
96. MCKERCHER, D.G. 1952. Feline pneumonitis. I. Immunization studies in kittens. *Am. J. vet. Res.* **13:** 557–561.
97. BURCH, G.R., YORK, C.J., JOHNSTON, R.V. and MAYER, K. 1958. Feline pneumonitis vaccine. *Allied Vet.* **29:** 4–7.
98. MITZEL, J.R. and STRATING, A. 1977. Vaccination against feline pneumonitis. *Am. J. vet. Res.* **38:** 1361–1363.
99. SHEWEN, P.E., POVEY, R.C. and WILSON, M.R. 1980. A comparison of the efficacy of a live and 4 inactivated vaccine preparations for the protection of cats against experimental challenge with *Chlamydia psittaci*. *Can. J. comp. Med.* **44:** 244–258.
100. WILLS, J.M., GRUFFYDD-JONES, T.J., RICHMOND, S.D., GASKALL, R.M. and BOURNE, F.J. 1987. Effect of vaccination on infection due to feline *Chlamydia psittaci*. *Infect. Immun.*
101. KOLAR, J.R. and RUDE, T.A. 1977. Clinical evaluation of a commercial feline pneumonitis vaccine. *Feline Pract.* **7:** 47–50.
102. FUKUSHI, H. and HIRAI, K. 1992. Proposal of *Chlamydia pecorum* sp. nov. for *Chlamydia* strains derived from ruminants. *Int. J. Syst. Bact.* **42:** 306–308.