Reprinted from the AMERICAN JOURNAL OF VETERINARY RESEARCH, Vol. 53, No. 9, Pages 1482-1487.
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Biological properties and genetic analysis of the *ompA* locus in chlamydiae isolated from swine

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SUMMARY

Eight strains of *Chlamydia psittaci* isolated from swine with pneumonia, pleuritis, pericarditis, and enteritis were characterized through analysis of the major outer membrane protein gene *ompA* by a two-step polymerase chain reaction, by their interactions with cells in culture, and by the morphologic features and ultrastructure of intracellular inclusions. Amplified chlamydial *ompA* DNA fragments were differentiated by restriction endonuclease digestion. Chlamydial isolates were separated into 2 types on the basis of *ompA* restriction fragment length polymorphism. Strains of type L71 had finely granular inclusions, whereas those of type 1710S contained pleomorphic reticulate bodies (RB) in the inclusions, which are characteristic of aberrant chlamydial developmental forms. Chlamydial types L71 and 1710S required centrifuge-assisted inoculation for efficient infection of cell cultures. Cultivation in cell culture medium containing cycloheximide increased the numbers of chlamydial inclusions about 1.5-fold. These strains formed few elementary bodies in yolk sac cells of chicken embryos. Ultrastructurally, unique doublet RB were observed, particularly in strains of the *ompA* type L71. These doublets consisted of 2 RB, bounded by a cytoplasmic membrane, contained within a common cell wall and an extended periplasmic space. Ultrastructural examination of strains of the *ompA* type 1710S confirmed the aberrant chlamydial developmental forms, but evidence of viral infection of the RB as a cause of these aberrant forms was not found. The strain S45 isolated from intestinal sites of swine was a trachoma restriction fragment length polymorphism type. With the mouse biotype, it represented the second isolate from animals of *Chlamydia trachomatis*.

Chlamydiae are procaryotic pathogens that infect a wide range of homeotherms and poikilotherms.[1] They cause trachoma and respiratory tract infections in human beings, and represent a common agent of sexually transmitted disease in the human population.[2] In animals, they cause numerous economically important conditions, including abortion caused by placentitis and fetal infection, urogenital and respiratory tract infections, conjunctivitis, enteritis, polyarthritis, encephalomyelitis, and mastitis.[3]

Chlamydiae parasitize eucaryotic host cells,[1] and are characterized by distinct extracellular and intracellular forms. The infective elementary body (EB) is metabolically inactive. It reorganizes to the fragile reticulate body (RB) after entering the host cell. These RB are the metabolically active forms of chlamydiae, and divide by binary fission within phagocytotic vesicles. Condensation to EB and the subsequent lysis of the host cell completes the chlamydial developmental cycle.

The family Chlamydiaceae currently is divided into 3 species: *Chlamydia trachomatis*, *C pneumoniae*, and *C psittaci*. Although the first 2 species are almost exclusively human pathogens, the latter species has been isolated from a multitude of animal hosts, and sometimes from human beings. Distinct variants of the heterogeneous species *C psittaci* have been associated with specific hosts and disease syndromes.[4-7]

Chlamydial involvement in diseases of swine was explored in a number of investigations. In 1955, Willigan and Beamer[8] first associated chlamydia-like organisms with polyserositis and polyarthritis in swine. Antibodies against chlamydiae were found by capillary agglutination testing in 23% of sera from swine in Great Britain.[9] Chlamydiae have been isolated from as many as 35% of lungs from swine with bronchopneumonia in Eastern European countries and Russia.[10] High prevalence of chlamydial infection also was found in swine affected with polyarthritis and orchitis, and in sows that aborted. Isolation of chlamydiae from feces in 46% of healthy and sick swine was reported from Germany.[11] In Austria, numerous chlamydial strains were isolated from 62% of swine with polyarthritis and polyserositis; chlamydiae also were isolated from swine with pneumonia, conjunctivitis, inapparent intestinal tract infection, and enteritis, and from sows that aborted.[12,13]

Diseases induced by chlamydiae were reproduced experimentally in swine.[8,10,13,14] Pan and Cordy[15] demonstrated that a chlamydial isolate from pneumonia in sheep could elicit bronchopneumonia in swine. More recently, transmission electron microscopic evidence that chlamydial infection was associated with a severe form of pseudomembranous enteritis in swine experimentally infected with *Salmonella typhimurium* was reported.[16]

In earlier investigations, 7 isolates from Austrian stud-

Received for publication July 18, 1991.
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The authors thank Drs. Harold R. Payne and William J. Todd for assistance with transmission electron microscopy; Dr. Lee A. Campbell, University of Washington, Seattle, for providing *Chlamydia pneumoniae* AR388 DNA; and Mamie Burrell for technical assistance.
Supported by a Veterinary Research Graduate Fellowship of the School of Veterinary Medicine, Louisiana State University; by grants from the Bundeskammer der Tieraerzte Oesterreichs, Austria, Agrarservice Ges mbH, Austria, and W. Schaumann Ges mbH, Germany; and by grants from Fromm Laboratories Inc, Grafton, Wisc, and Bayer AG, Leverkusen, Germany; by Special Research Grant 84-CRSR-2-2445, USDA and by Research Grant AI-08420 from the National Institute of Allergy and Infectious Diseases, Bethesda, Md.

ies had been examined for their biological properties, seroreactivities, and major outer membrane protein gene (*omp*A) DNA sequences.[4-6] The purpose of the study reported here was to characterize 8 additional Austrian chlamydial isolates from swine through restriction fragment length polymorphism (RFLP) analysis of the *omp*A locus, through interaction with cultured cells, and through examination of light microscopic and ultrastructural morphologic features.

Materials and Methods

Chlamydial strains—Eight strains of *C psittaci*, isolated from swine in Austria,[12,13] were analyzed in this study (strains L40, L39, L17, HsLuRZ, 1920BRZ, L1, R106, and 1886). These strains had been identified as chlamydiae through possession of the genus-specific lipopolysaccharide antigen.[13] All strains were propagated in developing chicken embryos as described previously.[5] Stock cultures were made by grinding freshly harvested yolk sacs with 2 ml of sucrose phosphate buffer solution, and were stored in aliquots at −70 C. For DNA isolation from chlamydial EB, these cultures were centrifuged at 200 × g for 10 minutes at 4 C; the interphase between buoyant lipid and cellular sediment was centrifuged at 14,000 × g for 30 minutes, and the sediment was subjected to DNA extraction as described previously.[17] The interphase also was used as inoculum for cell cultures after 1:100 dilution in cell-culture growth medium.

Amplification and analysis of chlamydial ompA loci—A polymerase chain reaction (PCR) method for amplification of chlamydial *omp*A DNA fragments, described by Kaltenboeck et al,[17] was used to characterize the *omp*A locus of the isolates. Briefly, a DNA fragment of approximately 1,120 base pairs (bp) was amplified from DNA extracted from stock cultures of porcine chlamydiae in a primary genus-specific PCR. This DNA fragment encompassed approximately 80% of the chlamydial *omp*A locus. Portions of this reaction were amplified in secondary genus-specific and group-specific PCR, using primers homologous to internal sequences of the primary DNA fragment. The DNA fragments were resolved by 1.5% agarose gel electrophoresis and ethidium bromide staining.

The secondary genus-specific PCR amplifies a DNA fragment, common to all chlamydiae, of approximately 930 bp, whereas the group-specific PCR yields DNA fragments of different lengths dependent on the chlamydial *omp*A type. This method distinguishes 4 groups of *omp*A types—those found in psittacosis, polyarthritis, pneumonia, and trachoma. Prototypes of these groups analyzed as controls in this investigation were strains B577 (psittacosis group); 66P130, LW613, L71, and 1710S (polyarthritis group); AR388 (pneumonia group); and S45 (trachoma group). The RFLP type of the *omp*A prototypes from these groups was determined by restriction of the secondary group-specific (polyarthritis, pneumonia, and trachoma) and genus-specific (psittacosis) DNA fragments, respectively. Restriction endonucleases used were *Hae* III (psittacosis), *Fnu* 4HI (pneumonia), *Pvu* II (trachoma), and *Bst* UI and *Bst* YI (polyarthritis).[17]

The *omp*A RFLP type of the 8 strains was determined by restriction of the secondary group-specific amplification product with the appropriate restriction endonucleases *Bst* UI and *Bst* YI. Ten to 20 µl of secondary PCR products were mixed with restriction buffer solution and incubated with 10 units each of *Bst* UI and *Bst* YI. The DNA fragments were resolved by 4% agarose gel electrophoresis (1:3 ratio of DNA-grade agarose to molecular-sieve agarose) and ethidium bromide staining.

Chlamydial propagation in cell culture—The interaction of chlamydiae with cells in culture was analyzed in a modification of the biotyping procedure described by Spears and Storz.[4] Mouse fibroblast cell clone L 5b cells were grown as monolayers in medium 199, supplemented with 5% fetal bovine serum (FBS) and containing 12 m*M* sodium bicarbonate, 200 µg of streptomycin, and 75 µg of vancomycin/ml. Cells were trypsinized, suspended in growth medium, planted in 12-mm round coverslip vials (300,000 cells/vial in 1 ml of medium), and inoculated within 24 hours of planting.

Cells were rinsed 3 times with Dulbecco's phosphate buffer solution prior to inoculation. They were infected by adsorption of 0.5 ml of inoculum for 2 hours at 37 C and 5% CO_2 in an incubator (stationary inoculation) or by centrifugation at 1,500 × g for 30 minutes at 37 C (centrifugal inoculation). After inoculation, cells were rinsed and incubated with or without cycloheximide (1 µg/ml of growth medium). All experiments were performed in duplicate.

Coverslips were removed in 12-hour intervals, beginning 18 hours after inoculation. After fixation in Bouin's solution and Giemsa staining, coverslips were mounted on microscope slides and examined by bright-field microscopy at 450× magnification. The percentage of cells with inclusions was determined from at least 400 cells counted in 25 randomly chosen areas of the coverslips. Inoculated monolayers also were examined 30 hours after inoculation for glycogen in the inclusions.[18]

Transmission electron microscopy—Developing inclusions of 7 chlamydial strains (L40, L39, L17, HsLuRZ, 1920BRZ, L1, and R106) and late inclusions of 2 chlamydial strains (L40 and R106) were examined by transmission electron microscopy. The L 5b cells (n = 5,000,000) in 60-mm round petri dishes were inoculated with 5 ml of cell-culture inoculum by centrifugation at 37 C. At 24 or 42 hours after inoculation, the cultures were rinsed 3 times with Dulbecco's phosphate buffer solution, then fixed in situ for 1 hour at 4 C in 0.1*M* sodium cacodylate-hydrochloride buffer solution (pH 7.4) containing 0.1*M* sucrose and 2% glutaraldehyde.[19] The specimens were rinsed 3 times with 0.1*M* cacodylate buffer solution (pH 7.4) containing 0.25*M* sucrose, and were treated for 45 minutes with 1% osmium tetroxide in cacodylate buffer solution. After rinsing with water and staining for 45 minutes with 2% uranyl acetate in 0.1*M* sodium acetate buffer solution (pH 3.5), the monolayers were dehydrated in ethanol and propylenoxide, and embedded in Epon-Araldite resin. Silver-to-gold ultramicrotome sections were stained with uranyl acetate and lead citrate prior to electron microscopy.

Results

Genetic analysis of chlamydiae—Primary and secondary genus-specific amplification yielded single DNA

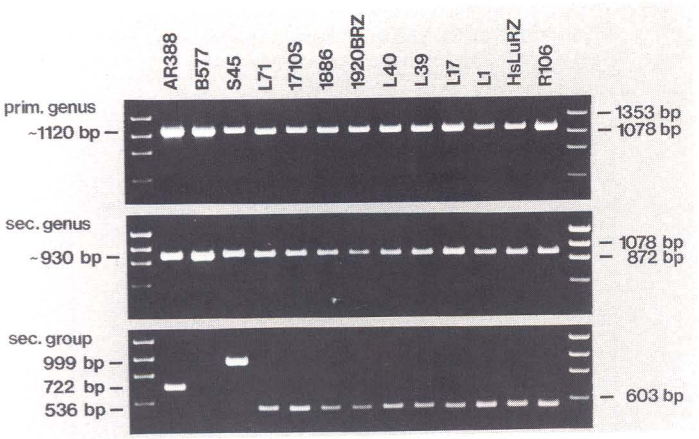

Figure 1—Agarose gel electrophoretogram of results of polymerase chain reaction (PCR) analysis for group determination of major outer membrane protein genotypes (*omp*A types) of porcine chlamydiae. Primary amplification products can be observed in the upper panel, secondary genus-specific PCR products in the middle panel, and secondary group-specific fragments in the lower panel. Strain AR388 represents the pneumonia group of *omp*A types, B577 the psittacosis group, S45 the trachoma group, and L71 and 1710S the polyarthritis group; all others are test strains. bp = base pair.

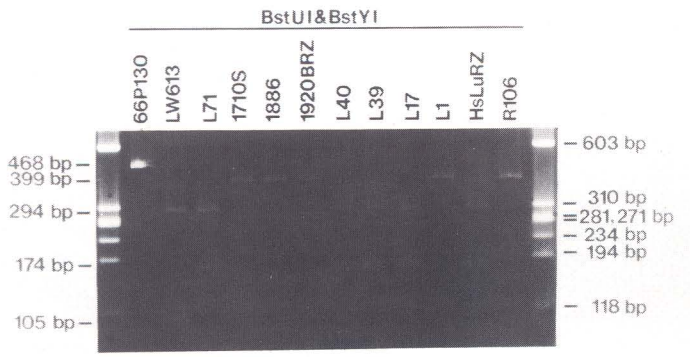

Figure 2—Agarose gel electrophoretogram of results of determination of the *omp*A RFLP type of isolates. The prototypes of the polyarthritis group of chlamydial *omp*A types and *omp*A RFLP types are strains 66P130, LW613, L71, and 1710S; all others are test strains. bp = base pair.

fragments of the expected sizes of approximately 1,120 and 930 bp, respectively (Fig 1), for all chlamydial *omp*A types. Secondary group-specific amplification yielded DNA fragments of 536 bp for all 8 strains and for polyarthritis group prototypes L71 and 1710S of porcine origin. Secondary group-specific PCR of the trachoma group prototype S45 of porcine origin resulted in an expected fragment of 999 bp, and of the pneumonia group prototype (strain *C pneumoniae* AR388), a fragment of 722 bp. Psittacosis group prototype B577 could not be amplified in secondary group-specific PCR, as anticipated, and was identified through amplification in the secondary genus-specific PCR.

The known *omp*A types of the polyarthritis group of chlamydiae yielded agarose gel-visualized restriction fragments of 468 bp (66P130), 294 and 105 bp (LW613), 294 and 174 bp (L71), and 399 bp (1710S), which defined the *omp*A RFLP types of this group (Fig 2). Strains 1886, 1920BRZ, L1, and R106 yielded *Bst* UI and *Bst* YI restriction fragments of 399 bp, and strains L40, L39, L17, and HsLuRZ yielded fragments of 294 and 174 bp.

Interaction of chlamydiae with cells in culture—Variables determined were number of infected L5b cells after stationary and centrifugal inoculation, morphologic features of early and mature inclusions, glycogen content of mature inclusions, and influence of cycloheximide on the number and morphologic features of the chlamydial inclusions.

All chlamydial strains had only minimal development of cytoplasmic inclusions (maximal prevalence, 0.7% of infected cells) 30 hours after stationary adsorption of the inoculum and cultivation without cycloheximide (Table 1). Treatment of cultures with cycloheximide did not substantially alter the number of inclusions, and the low number of cells containing inclusions precluded a meaningful analysis of the effect of cycloheximide after stationary inoculation.

Increases in the number of infected cells in all strains were observed after centrifugal inoculation (Table 1). Using the same quantity of chlamydiae as in stationary adsorption, 6.8 to 73.3% of cells were found to contain inclusions 30 hours after inoculation. At this time, lysis of the host cells was not evident, although chlamydial inclusions were generally large and easily detected. The addition of cycloheximide to the culture medium increased the percentage of chlamydia-infected cells as much as 2.6-fold, compared with values from centrifugal inoculation alone.

Early inclusions at 18 hours and mature inclusions at 30 and 42 hours after inoculation had regular margins with minimal indentations. Mature inclusions appeared as compact, rounded lobes with some irregular protrusions into the host-cell cytoplasm (Fig 3 and 4). Multiple inclusions per host cell frequently were observed, and evidence of fusion of inclusions was not found. Generally, inclusions of strains L40, L39, L17, and HsLuRZ developed more quickly than those of strains 1920BRZ, L1, and R106. Lysis of host cells infected with strains L40, L39, L17, and HsLuRZ occurred mostly between 30 and 42 hours after inoculation, whereas host cells infected with strains 1920BRZ, L1, and R106 had mostly small and only a few large inclusions 42 hours after inoculation, and were lysed even later.

Early, as well as mature, inclusions of strains L40, L39, L17, and HsLuRZ had a regular, finely granular interior (Fig 3), but strains 1920BRZ, L1, and R106 had patchy, large, and irregular granules in early inclusions (Table 2). Some mature inclusions of strains R106 and L1 were finely granular, but most had a patchy appearance (Fig 4), and those of strain 1920BRZ had coarse and mainly

Table 1—Percentage of L5b cells with inclusions 30 hours after stationary and centrifugal inoculation with porcine chlamydial isolates and after cultivation with or without cycloheximide

Chlamydial strain	Stationary inoculation	Centrifugal inoculation	Stationary inoculation + cyclo-heximide	Centrifugal inoculation + cyclo-heximide
L40	0.7	73.3	0.8	94.0 (×1.3)*
L39	0.0	51.8	0.2	61.9 (×1.2)
L17	0.2	45.3	0.5	81.4 (×1.8)
HsLuRZ	0.0	52.4	0.0	78.4 (×1.5)
1920BRZ	0.5	17.5	0.6	42.5 (×2.4)
L1	0.1	53.0	0.1	52.0 (×1.0)
R106	0.0	6.8	1.5	18.0 (×2.6)

* Numbers in parentheses indicate comparison of values from centrifugal inoculation with cycloheximide and values from centrifugal inoculation without cycloheximide. Results are the means of duplicate determinations. Differences between duplicate samples were not > 17%.

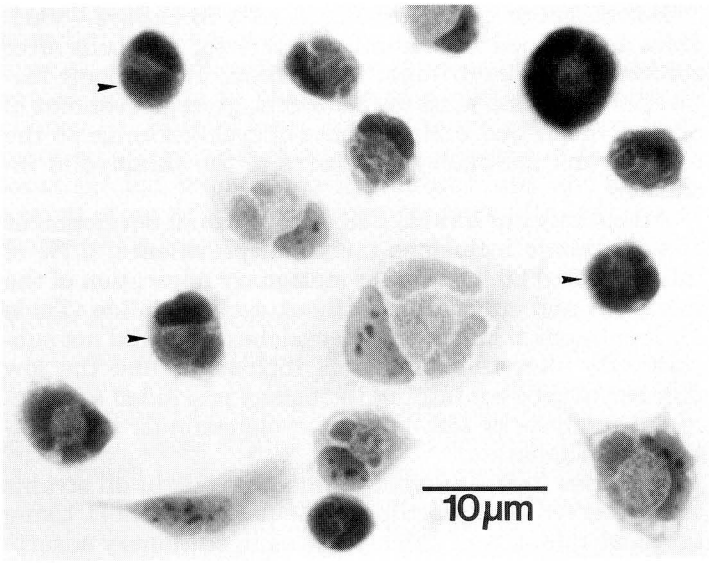

Figure 3—Photomicrograph of Giemsa-stained L 5b-cell monolayer 30 hours after inoculation with strain L40. Several cells contain multiple mature, finely granular inclusions with minimal indentations. The inclusions are densely filled with chlamydial developmental forms. Cells with late inclusions prior to lysis (arrowheads) have pyknotic nuclei and appear round.

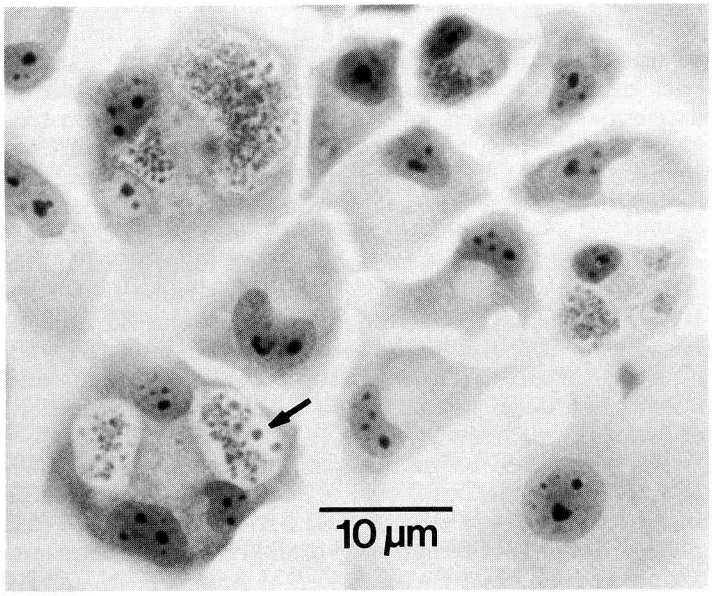

Figure 4—Photomicrograph of Giemsa-stained L 5b-cell monolayer 42 hours after inoculation with strain R106. Several cells contain multiple inclusions with patchy, irregular granules and regular margins. The inclusions are loosely filled with chlamydial developmental forms. The arrow marks a large, aberrant reticulate body.

patchy granules. Addition of cycloheximide to the culture medium resulted in slightly irregular and coarse granules of early, but not mature, inclusions of strain L40, and did not alter finely granular, early, and mature inclusions of strains L39, L17, and HsLuRZ. Strains 1920BRZ, L1, and R106 incubated with cycloheximide had uniformly patchy mature inclusions (Fig 4). With cycloheximide, chlamydial inclusions generally were larger than those formed without cycloheximide, and lysis of heavily infected L5b cell monolayers at later times (42 and 54 hours after inoculation) was complete. Glycogen was not detected in inclusions of the strains analyzed in this study.

Table 2—Results of *ompA* restriction fragment length polymorphism analysis and examination of inclusions in chlamydial strains isolated from clinically ill swine

Chlamydial strain	*ompA* type	Inclusion type	Host clinical condition
L40	L71	Finely granular	Pneumonia
L39	L71	Finely granular	Pneumonia, pleuritis, pericarditis
L17	L71	Finely granular	Pneumonia
HsLuRZ	L71	Finely granular	Pneumonia
1920BRZ	1710S	Aberrant	Pneumonia
L1	1710S	Aberrant	Pneumonia
R106	1710S	Aberrant	Pneumonia, pleuritis, enteritis
1886	1710S	ND	

ND = Not determined.

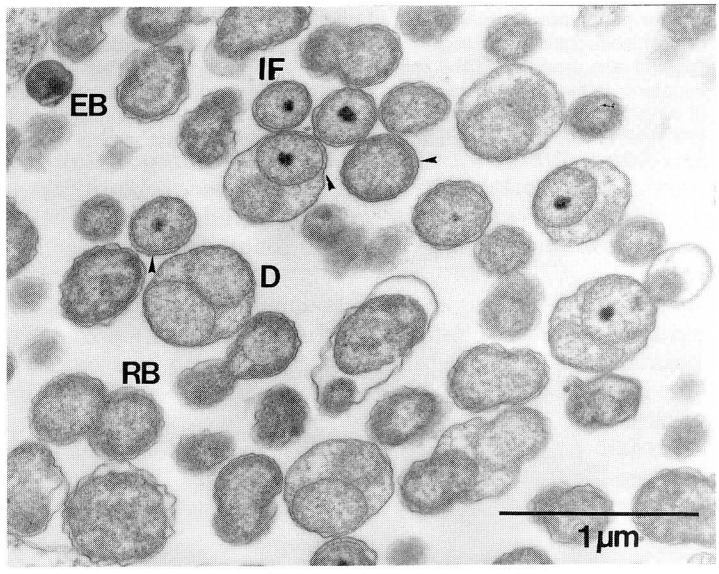

Figure 5—Transmission electron micrograph of a chlamydial inclusion of strain L40 in an L 5b-monolayer cell 24 hours after inoculation. The predominant form in the inclusion is the reticulate body (RB), but some intermediate forms (IF) and 1 elementary body (EB) also are visible. Several RB doublets (D) with a large cytoplasmic space can be observed. The periplasmic space is filled with electron-translucent, amorphous, and fibrillar material. Some of the RB and doublets inside the inclusion have an apparently rigidified double-trilaminar arrangement of parts of the cytoplasmic and outer membranes (arrowheads). These regions appear to be divided by subtle structures perpendicular to the membranes.

Infected host cells had a slightly swollen appearance at later stages of the chlamydial developmental cycle. They contained pyknotic nuclei and appeared round before lysis. As in infected yolk sacs, only few EB were found, even in heavily infected cell cultures at late stages of the infection and after lysis of the host cells.

Ultrastructural analysis of chlamydial inclusions—We examined 24-hour-old inclusions of strains L40, L39, L17, HsLuRZ, 1920BRZ, L1, and R106 by transmission electron microscopy. In an electron micrograph of the inclusions of strain L40 (Fig 5), RB were predominant, but intermediate forms and 1 EB also were observed. Many of the RB appeared as unique doublet forms, consisting of 2 RB or intermediate forms bounded by a chlamydial cytoplasmic membrane and enveloped by a single, loosely fitting cell wall. The periplasmic space was filled with

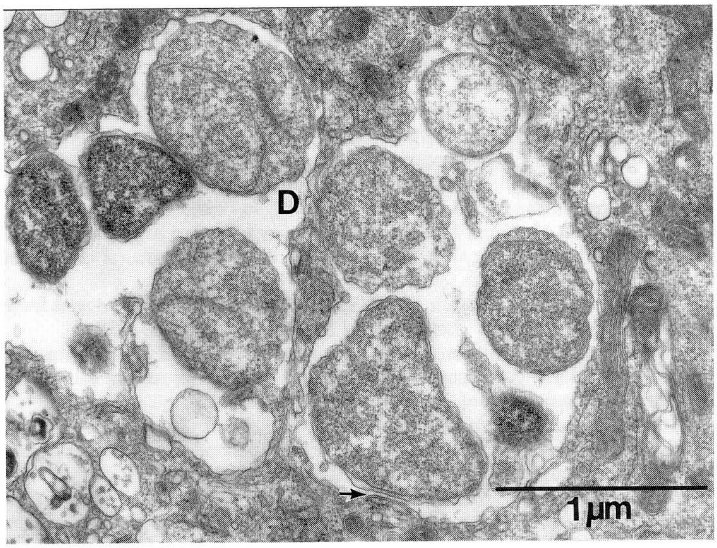

Figure 6—Transmission electron micrograph of a chlamydial inclusion of strain R106 in an L 5b-monolayer cell 24 hours after inoculation. Pleomorphic large RB filled with amorphous and fibrillar material are visible in these adjacent inclusions. One RB doublet (D) can be seen.. A double-trilaminar envelope structure can be observed in an RB in contact with the inclusion membrane (arrowhead).

electron-translucent, amorphous, and fibrillar material. Some of the RB inside the inclusions, as well as in contact with the inclusion membranes, had an apparently rigidified double-trilaminar arrangement of parts of the cytoplasmic and outer membranes. These regions appeared to be divided by subtle structures perpendicular to the membranes. The large and modified periplasmic space also was observed in many single RB. Mainly single RB, few doublet RB, and only rare EB of strain L40 were found 42 hours after inoculation in the few remaining inclusions, although infected host cells already were lysed or were in the process of disintegration.

Inclusions of strains L39, L17, and HsLuRZ resembled those of L40, but inclusions of strains 1920BRZ, L1, and R106 were strikingly different. Pleomorphic, large RB containing amorphous and fibrillar material of intermediate electron density were predominant in the transmission electron micrograph of 2 adjacent R106 inclusions 24 hours after inoculation (Fig 6). The apparently septate, double-trilaminar envelope structures also were found in some large RB inside the inclusion and in contact with the inclusion membrane. Doublet forms of the RB, similar to those in strain L40, were observed, but were less prominent than in the L40 electron micrograph (Fig 5). Only the large, aberrant chlamydial forms of strain R106, but no EB, were found in disintegrating infected cells at 42 hours after inoculation. Inclusions of strains 1920BRZ and L1 closely resembled those of strain R106.

Cultivation with cycloheximide did not visibly influence the ultrastructure of developmental forms of the chlamydial strains examined in this investigation. We found no ultrastructural evidence for viral infection of RB.[20]

Discussion

Chlamydiae isolated from swine possess unique properties that distinguish them from other chlamydiae. Three major clusters of strains can be discerned on the basis of *omp*A type, interaction with cells in culture, and morphologic features and ultrastructure of chlamydial developmental forms.

Results of the PCR-based genetic analysis unequivocally separate the chlamydiae in this investigation into 2 *omp*A, and *omp*A RFLP, types. Isolates L40, L39, L17, and HsLuRZ represented the L71 *omp*A RFLP type, whereas strains 1920 BRZ, L1, R106, and 1886 had *omp*A restriction fragments consistent with the 1710S type. Chlamydial *omp*A DNA sequences were found to be more conserved than was serovar reactivity.[6] We therefore consider strains of the *omp*A RFLP type L71 as mammalian serovar 4 of *C psittaci*, like strain L71,[5] and strains of the *omp*A RFLP 1710S as serovar 6, like strain 1710S. Earlier, a third porcine *omp*A type, strain S45, was considered mammalian serovar 5 of *C psittaci*.[5] Recently, this isolate was identified as a member of the species *C trachomatis* on the basis of glycogen found in mature inclusions[6] and inhibition of growth by sulfadiazine.[4] In our investigation, this strain was used as a prototype of the trachoma group of chlamydial *omp*A types.[17]

We are not aware of other chlamydial strains forming RB doublets like strains of the *omp*A RFLP type L71, and to a lesser extent, like those of the 1710S type. The large periplasmic space in these doublets, also found in many single RB, resembled the ultrastructural morphologic features of *C pneumoniae*.[21] Recently, phylogenetic relationships among the genus *Chlamydia* were inferred on the basis of *omp*A DNA sequences.[6] The polyarthritis group of *omp*A types, which includes the porcine types L71 and 1710S, was related in this study to *C pneumoniae*.

Our finding of modified double-trilaminar membrane structures confirmed observations made in all 3 chlamydial species by Peterson and de la Maza[22]. Interestingly, we identified this apparently rigidified and septate arrangement of the envelope, not only in RB that were in contact with the inclusion membrane, but also in RB located in the interior of the inclusions. Understanding of the function of these structures will need further investigations.

All porcine chlamydiae required centrifugal inoculation for efficient infection of cell cultures. Cultivation with cycloheximide resulted in only marginal increases of inclusion numbers and did not separate these chlamydiae into distinct groups. However, light microscopic and ultrastructural features of the chlamydial inclusions allowed clear discrimination of 2 clusters of strains, concordant with *omp*A type and serovar. Strains L40, L39, L17, and HsLuRZ of the L71 *omp*A RFLP type represented 1 cluster, with finely granular inclusions containing uniformly sized chlamydial developmental forms. The other cluster comprised strains 1920BRZ, L1, and R106 of the 1710S *omp*A RFLP type, which formed aberrant inclusions with large, pleomorphic developmental forms.

The strains of the 1710S *omp*A RFLP type resembled mammalian serovar-strains of *C psittaci* in their biological and ultrastructural properties.[5,19,23] This serovar, represented by prototype strain LW613, is a member of the polyarthritis group of chlamydial *omp*A types and is phylogenetically closely related to the *omp*A types L71 and 1710S.[6] In a previous study,[19] inclusions of strain LW613, grown in L929 cells with minimal essential medium supplemented with 5% FBS, contained large RB as well as pyknotic chlamydial forms, and were highly pleo-

morphic.[19] Low yield of infective organisms was produced in these cultures. Cultivation of LW613-infected monolayers with the same medium, but containing 10% FBS and 0.5% glucose, resulted in finely granular inclusions and a high yield of infective organisms. High dependence on host cell-derived nutrients is believed to render strain LW613 sensitive to low nutrient supply of the host cell. In conditions of host-cell starvation, LW613 cannot compete efficiently for nutrients, resulting in retarded development, large aberrant RB, and low yield of infective organisms.

We did not find ultrastructural evidence in inclusions of porcine chlamydiae for another cause of aberrant RB morphologic features, such as the infection of RB by a virus.[20] High nutrient dependence therefore might have elicited the aberrant forms in our chlamydiae. Moreover, we consistently observed only few EB in yolk sac and cell cultures of porcine chlamydial isolates, suggesting that these organisms are characterized by high nutrient dependence.

Spears and Storz[4] observed only a single class of biotype-4 inclusions in porcine chlamydiae of the *ompA* RFLP types L71 and 1710S. They used a different culture system from that used in this investigation. Phenotypic expression of the porcine chlamydial genotype thus might be modulated by variations in host cells and culture medium; this observation parallels morphologic discrepancies in EB of *C pneumoniae* that were propagated in various culture systems.[21,24]

High nutrient requirements, particularly in *ompA* RFLP type 1710S, might have direct repercussions on chlamydial pathogenicity. Recently, a 57-kDa chlamydial homolog of the GroEL stress response protein of *Escherichia coli* was identified as hypersensitivity antigen in the *C psittaci* infection, guinea pig inclusion conjunctivitis.[25,26] High amounts of this protein were produced if the developmental cycle of *C psittaci* was blocked in vitro by penicillin. In these conditions, the RB reorganized inefficiently to EB, and large, aberrant RB were formed.[25] Thus, although the cytocidal effect of developmentally retarded chlamydiae might be reduced in infected swine, increased production of the 57-kDa stress-response protein might provoke a deleterious, delayed-type hypersensitivity response.

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