January 1999

Conjunctivitis caused by a swine *Chlamydia trachomatis*-like organism in gnotobiotic pigs

Douglas G. Rogers
University of Nebraska - Lincoln, drogers1@unl.edu

Arthur A. Andersen
National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA

J Vet Diagn Invest 11:341–344 (1999)

Conjunctivitis caused by a swine *Chlamydia trachomatis*-like organism in gnotobiotic pigs

Douglas G. Rogers, Arthur A. Andersen

Abstract. The objective of this study was to determine whether a chlamydial strain recovered from growing and finishing swine with conjunctivitis or keratoconjunctivitis could cause the same infections in gnotobiotic pigs. The strain shares biological characteristics with *Chlamydia trachomatis*. After propagation in Vero cells and preparation of the inoculum (10^7 inclusion-forming units/ml), chlamydial strain H7 was instilled into the ventral conjunctival sac (0.15 ml/sac) of 12 anesthetized 3-day-old gnotobiotic piglets. Four age-matched gnotobiotic piglets were anesthetized and sham infected with uninfected cell culture lysates. None of the principal piglets developed clinical symptoms of conjunctivitis or keratoconjunctivitis. Principal piglets necropsied 7 days postinfection (DPI) had histologic lesions of mild or moderate conjunctivitis; immunohistochemical evaluation revealed chlamydial antigen in conjunctival epithelium. A majority of principal piglets necropsied at 14–28 DPI had histologic lesions of mild conjunctivitis, but chlamydial antigen was not detected by immunohistochemistry. The results indicated that chlamydial strain H7 can cause mild or occasionally moderate conjunctivitis in gnotobiotic pigs, but the conjunctival infection is asymptomatic.

Chlamydiae have been isolated from conjunctival swab specimens[1,8] and detected in conjunctival epithelium[8] from swine with conjunctivitis or keratoconjunctivitis, but the role of these organisms as significant corneal and/or conjunctival pathogens is unknown. Chlamydiae have been identified by an enzyme-linked immunosorbent assay in conjunctival swab specimens from clinically normal swine, leading to speculation that these organisms are not pathogens.[2]

The objective of the present study was to determine whether a chlamydial strain recovered from growing and finishing swine with conjunctivitis or keratoconjunctivitis could cause the same infections in gnotobiotic pigs. This strain is resistant to sodium sulfadiazine but forms intracytoplasmic inclusions filled with glycogen in cell monolayers. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses indicate that the strain is a *Chlamydia trachomatis*-like organism and is very similar to but distinct from the *C. trachomatis* strains that cause conjunctivitis and sexually transmitted diseases in humans (Andersen et al., unpublished data).

Materials and methods

Isolation of chlamydiae. A midwestern producer reported high prevalence of severe conjunctivitis, keratoconjunctivitis, and chemosis during the month of August in a herd of growing and finishing swine housed in relatively dust-free modified open-front units. Cotton-tip conjunctival swab specimens collected from 25 affected pigs were processed for the isolation of chlamydiae as previously described.[9,10] Chlamydiae were analyzed by PCR amplification of the major outer membrane protein genome using the primers and basic techniques reported previously.[3] The PCR product was verified by electrophoresis in 1.5% agarose. The product was then digested with the *Alu* I restriction endonuclease for RFLP analysis. Resulting fragments were electrophoresed on a 4% low-melting-point agarose gel and stained with ethidium bromide. One chlamydial strain was recovered from 6 affected pigs and was designated H7.

Preparation of the inoculum. Inoculum containing strain H7 was prepared by inoculating confluent Vero cell monolayers.[9] After titration in 24-hr Vero cell monolayers grown in 96-well multiwell dishes, the inoculum was diluted in sucrose–phosphate–glutamine buffer[10] to contain approximately 10^7 inclusion-forming units/ml. Sham inoculum was prepared in an identical manner from uninfected cell culture lysates.

Gnotobiotic pigs and inoculation. Sixteen gnotobiotic piglets obtained by closed hysterotomy[5] were housed and maintained as reported previously.[9] Fecal swab specimens were collected from each piglet prior to experimental inoculation and inoculated onto sheep blood agar for aerobic and anaerobic[a] bacteriologic culture and onto tergitol-7 agar for aerobic culture. Piglets were anesthetized with tiletamine HCl and zolazepam HCl[b] by intramuscular injection (0.088 ml/kg)[12] prior to experimental inoculation. Twelve 3-day-old piglets were inoculated by instillation of 0.15 ml of chlamydial inoculum into each ventral conjunctival sac using a sterile 2.54-cm 22-gauge bent ball-tip rodent gavage needle with an attached sterile tuberculin syringe. Four piglets were inoculated with sham inoculum in an identical manner.

Necropsy and processing of specimens. Piglets were examined by flashlight for lacrimation, photophobia, conjunc-

From the Veterinary Diagnostic Center, Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE 68583-0907 (Rogers), and the National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA 50010 (Andersen).

Received for publication May 18, 1998.

tival hyperemia, conjunctival exudate, and corneal lesions twice daily. Prior to euthanasia and while still in the isolator units, piglets were anesthetized with tiletamine HC1 and zolazepam HC1 by intramuscular injection (0.088 ml/kg). Conjunctival and nasal swab specimens were collected at that time for the isolation of chlamydiae, for mycoplasmology, and for aerobic bacteriologic culture.[9]

Three principal piglets and 1 sham-infected piglet were euthanized at 7, 14, 21, and 28 days postinfection (DPI). At necropsy, globes together with attached palpebrae, conjunctival sacs, and nictating membranes were removed from each piglet. Specimens of cornea, palpebral conjunctiva, and nictitating membrane from all piglets and specimens of ileum from infected piglets that had developed diarrhea were fixed in neutral-buffered 10% formalin, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin (HE), and examined by light microscopy. Tissue sections also were stained by an immunohistochemical method to detect chlamydial antigen.[9] Specimens of palpebral conjunctiva from infected piglets necropsied 7 DPI were fixed in neutral phosphate-buffered 3% glutaraldehyde, postfixed in osmium tetroxide, dehydrated in graded ethanols, infiltrated with propylene oxide, and embedded in epoxy resin. Semithin (1 μm) sections were cut and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope.[c] Strips of palpebral conjunctival mucosa, feces, and specimens of ileum were collected from each piglet and processed for the isolation of chlamydiae.[9] Specimens of lung, liver, ileum, and colon from each piglet were processed for aerobic and anaerobic bacteriologic culture; tonsil and serum were processed for the isolation of porcine reproductive and respiratory syndrome virus (PRRSV).[9] Jejunum and ileum from diarrheic piglets were examined for rotavirus (RV) and transmissible gastroenteritis virus (TGEV) by fluorescent antibody techniques, and feces from these piglets were examined for viruses by negative contrast electron microscopy.

Results

Clinical signs. None of the principal piglets developed clinical signs of conjunctivitis or keratoconjunctivitis at any time during the study. One principal piglet necropsied at 7 DPI developed diarrhea just prior to euthanasia. Two additional principal piglets developed diarrhea at 8 and 9 DPI, respectively, and diarrhea persisted in these piglets until they were euthanized at 14 DPI. At necropsy, diarrheic piglets had watery colonic contents with flecks of undigested curd. Sham-infected piglets did not develop clinical signs during the study.

Histopathology, immunohistochemistry, electron microscopy, and reisolation of chlamydiae. Bilateral conjunctival lesions were seen histologically in the 3 principal piglets necropsied at 7 DPI. Palpebral conjunctivae were characterized by mild (1 piglet) or moderate (2 piglets) focally extensive conjunctivitis (Fig. 1); inflammation in the nictitating membranes was mild and multifocal. Inflammatory cells in the conjunctival propria-submucosa were predominantly lymphocytes, with smaller numbers of macrophages, plasma cells, neutrophils, and occasional eosinophils. Small numbers of inflammatory cells had infiltrated into overlying epithelium in foci. Minute coccobacilli, occasionally appearing as dense granular inclusions, were seen in cytoplasmic vacuoles in conjunctival epithelial cells, and infected epithelial cells occasionally had eccentrically displaced nuclei (Fig. 2). Immunohistochemistry confirmed the presence of chlamydial antigen in conjunctival epithelial cells (Fig. 3). Ultrastructurally, chlamydiae together with glycogen particles were seen in membrane-bound vacuoles in the cytoplasm of conjunctival epithelial cells (data not shown). There were no histologic lesions in corneal specimens from the 3 principal piglets necropsied at 7 DPI.

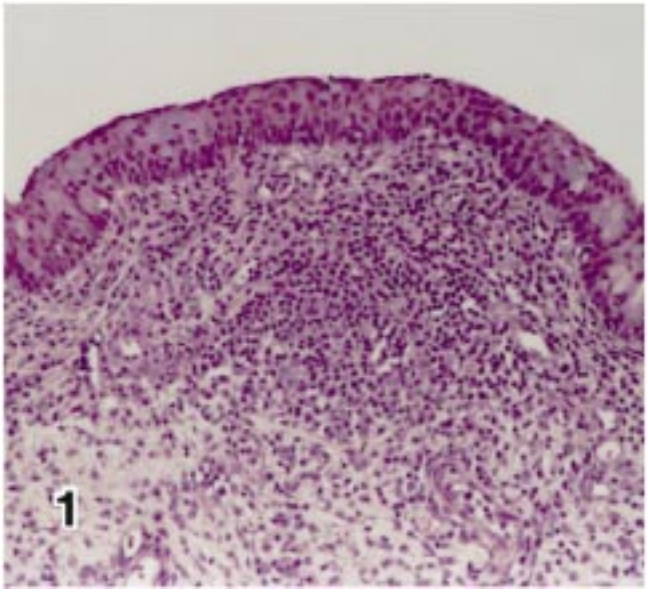

Figure 1. Palpebral conjunctiva; infected piglet necropsied at 7 days postinfection. Moderate focal conjunctivitis. Extensive distribution of the lesion is not evident. HE.

Bilateral conjunctival lesions also were seen histologically in the 3 principal piglets necropsied at 14 DPI. Palpebral conjunctivae and nictitating membranes were characterized by mild multifocal lymphoplasmacytic to lymphohistiocytic conjunctivitis (Fig. 4). Similar mild multifocal lesions were seen bilaterally in 2 principal piglets from each group necropsied at 21 and 28 DPI, respectively. The third principal piglet from each group necropsied at 21 and 28 DPI, respectively, did not have conjunctival lesions. Immunohistochemical evaluation did not reveal chlamydial antigen in conjunctival specimens from any of the principal piglets necropsied at 14, 21, and 28 DPI, and there were no histologic lesions in corneal specimens from these piglets. There were no histologic lesions in

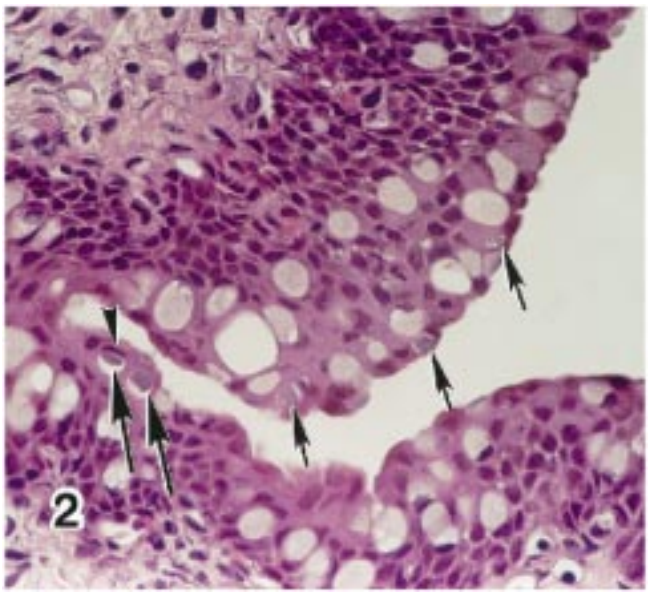

Figure 2. Conjunctival fornix; infected piglet necropsied at 7 days postinfection. Chlamydiae in conjunctival epithelial cells (arrows). Note dense chlamydial inclusions (large arrows) and eccentrically displaced nucleus of epithelial cell (arrowhead). HE.

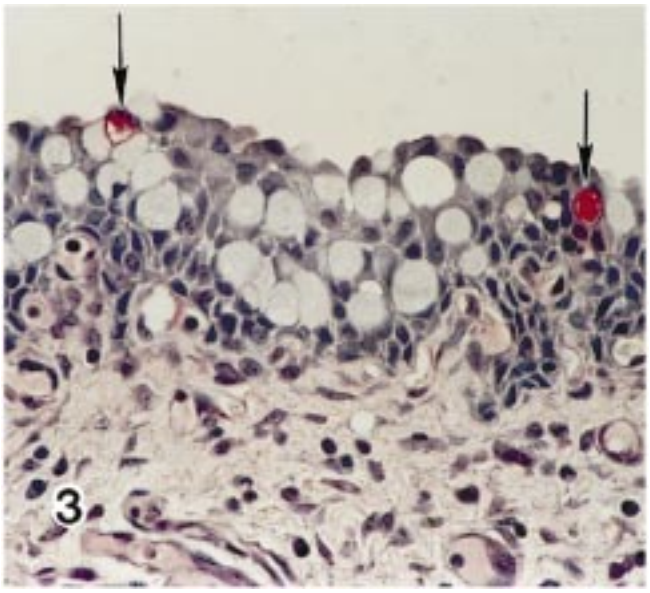

Figure 3. Palpebral conjunctiva; infected piglet necropsied at 7 days postinfection. Chlamydial antigen in conjunctival epithelium (arrows). Avidin–biotin–alkaline phosphatase stain, hematoxylin counterstain.

corneal or conjunctival specimens from the sham-infected piglets.

Chlamydiae were reisolated from conjunctival mucosae, conjunctival swab specimens, and nasal swab specimens from the 3 principal piglets necropsied at 7 DPI. Chlamydiae also were reisolated from nasal swab specimens from 1 principal piglet necropsied at 14 DPI and from 2 principal piglets necropsied at 21 DPI. In addition, chlamydiae were isolated from fecal and intestinal specimens from all of the principal piglets. Histologic lesions in the ileum from the 3 diarrheic piglets necropsied at 7 DPI (1 piglet) and 14 DPI (2 piglets) were characterized by moderate diffuse villus atrophy. One of the diarrheic piglets also had multifocal lymphangitis/perilymphangitis in the ileal submucosa. Immunohistochemistry revealed variable amounts of chlamydial antigen in the villus enterocytes of atrophic villi. Chlamydiae were not isolated from the sham-infected piglets.

Bacteriology, mycoplasmology, and virology. *Bacillus* sp. was isolated from intestinal and nasal swab specimens from several principal and sham-infected piglets necropsied at 14–28 DPI. However, there were no other bacteria, mycoplasmas, or PRRSV isolated from swab and/or tissue specimens from any of the piglets. Fluorescent antibody tests for RV and TGEV done on jejunum and ileum from the diarrheic piglets were negative, and virus particles were not seen in the feces from these piglets.

Discussion

The results of this study indicated that primary conjunctival infections with chlamydial strain H7 are subclinical. Microscopic conjunctival lesions in the principal piglets necropsied at 7 DPI were, however, similar to those seen in symptomatic nursery pigs with conjunctivitis and conjunctival *Chlamydia* infection.[8] Nursery pigs in the case study were housed in dusty rooms, and the conjunctivae from some of those pigs

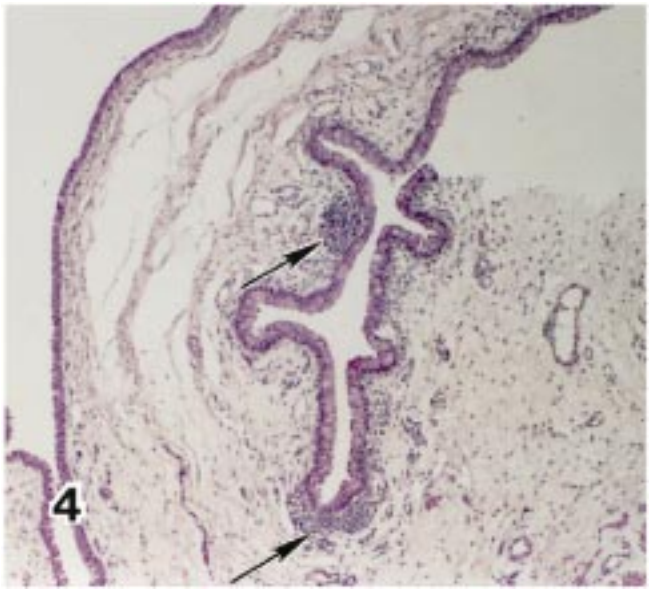

Figure 4. Nictitating membrane; infected piglet necropsied at 14 days postinfection. Mild multifocal conjunctivitis (arrows).

were concurrently infected with *Mycoplasma hyorhinis* and other bacterial organisms. Thus, additional factors might have contributed to the clinical symptoms seen in the nursery pigs. Nursery pigs in the case study also might have been infected with 1 or more chlamydial strains that are more virulent than strain H7. Chlamydiae originally isolated from the conjunctivae of nursery pigs in the case study did not grow well in vitro; thus, it was impossible to compare those isolates to strain H7 on the basis of virulence and PCR-RFLP analyses.

Strain H7 was recovered from the conjunctivae of growing and finishing swine housed in a relatively dust-free environment. A large population of houseflies was present in the facilities, but whether or not flies or environmental factors contributed to the severe conjunctivitis and keratoconjunctivitis seen in this herd is unknown. Conjunctival swab specimens from affected pigs were not examined for mycoplasmas, bacteria, or viruses, but 1 or more of these agents may have played a role in the pathogenesis of the disease. Although strain H7 was recovered from only 6 of 25 pigs, the possibility that chlamydiae played some role in the pathogenesis cannot be ruled out because these organisms can be difficult to isolate under field conditions. There is no way of knowing whether affected pigs in this herd and pigs in the case study[8] were continually reinfected with chlamydiae. Current evidence suggests that the severe clinical symptoms and lesions seen in human trachoma are the result of repeated *C. trachomatis* infections and probably are immunologically mediated.[4,6,11]

Although strain H7 did not cause clinical signs of conjunctivitis or keratoconjunctivitis in gnotobiotic pigs in this study, it did cause diarrhea in several pigs, presumably after they swallowed conjunctivally instilled inoculum. The ileal lesions caused by strain H7 correlate with those seen in an earlier study in which gnotobiotic pigs were orally infected with 2 other swine *C. trachomatis*-like organisms.[7]

Acknowledgements

We thank Linda Hornung, Ginger Mills, Mavis Seelmeyer, and Dr. James Illg for assistance. This work was supported by funds from USDA Specific Cooperative Agreement 58-3630-4-125. This report is published as article 12210 of the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln.

Sources and manufacturers

a. BBL GasPak, Becton Dickinson and Co., Cockeysville, MD.
b. Telazol, Ft. Dodge Laboratories, Ft. Dodge, IA.
c. Phillips 201, Eindhoven, The Netherlands.

References

1. Cello RM: 1967, Ocular infection in animals with PLT (Bedsonia) group agents. Am J Ophthalmol 63:1270–1273.
2. Davidson HJ, Rogers DP, Yeary TJ, et al.: 1994, Conjunctival microbial flora of clinically normal pigs. Am J Vet Res 55:949–951.
3. Denamur F, Sayada C, Souriau A, et al.: 1991, Restriction pattern of the major outer-membrane protein gene provides evidence for a homogenous invasive group among ruminant isolates of *Chlamydia psittaci*. J Gen Microbiol 137:2525–2530.
4. Grayston JT, Wang SP, Yeh JL, Kuo CC: 1985, Importance of reinfection in the pathogenesis of trachoma. Rev Infect Dis 7: 717–725.
5. Miniats OP, Jol D: 1978, Gnotobiotic pigs—derivation and rearing. Can J Comp Med 42:428–437.
6. Morrison RP, Lyng K, Caldwell HD: 1989, Chlamydial disease pathogenesis. Ocular hypersensitivity elicited by a genus-specific 57 kD protein. J Exp Med 169:663–675.
7. Rogers DG, Andersen AA: 1996, Intestinal lesions caused by two swine chlamydial isolates in gnotobiotic pigs. J Vet Diagn Invest 8:433–440.
8. Rogers DG, Andersen AA, Hogg A, et al.: 1993, Conjunctivitis and keratoconjunctivitis associated with chlamydiae in swine. J Am Vet Med Assoc 203:1321–1323.
9. Rogers DG, Andersen AA, Hunsaker BD: 1996, Lung and nasal lesions caused by a swine chlamydial isolate in gnotobiotic pigs. J Vet Diagn Invest 8:45–55.
10. Spence WN, Johnson FWA: 1983, Simple transport medium for the isolation of *Chlamydia psittaci* from clinical material. Vet Rec 113:535–536.
11. Taylor HR, Johnson SL, Prendergast RA, et al.: 1982, An animal model of trachoma. II. The importance of repeated infection. Invest Ophthalmol Visual Sci 23:507–515.
12. Thurmon JC, Benson GJ, Tranquilli WJ, Olson WA: 1988, The anesthetic and analgesic effects of Telazol and xylazine in pigs: evaluating clinical trials. Vet Med 83:841–845.