

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

Papers in Veterinary and Biomedical Science

Veterinary and Biomedical Sciences,  
Department of

---

September 1996

## Intestinal lesions caused by two swine chlamydial isolates 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*

Follow this and additional works at: <https://digitalcommons.unl.edu/vetscipapers>



Part of the [Veterinary Medicine Commons](#)

---

Rogers, Douglas G. and Andersen, Arthur A., "Intestinal lesions caused by two swine chlamydial isolates in gnotobiotic pigs" (1996). *Papers in Veterinary and Biomedical Science*. 31.

<https://digitalcommons.unl.edu/vetscipapers/31>

This Article is brought to you for free and open access by the Veterinary and Biomedical Sciences, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Veterinary and Biomedical Science by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

## Intestinal lesions caused by two swine chlamydial isolates in gnotobiotic pigs

Douglas G. Rogers, Arthur A. Andersen

**Abstract.** The objective of this study was to determine whether 2 distinct chlamydial isolates recovered from the intestines and feces of diarrheic nursery pigs could cause intestinal lesions in gnotobiotic pigs. Both isolates share biological characteristics with *Chlamydia trachomatis*. Chlamydial isolates R27 and R19 were propagated in Vero cells or embryonated eggs, respectively, and suspended in sucrose-phosphate-glutamine buffer with 10% fetal bovine serum for inoculation. Sham inocula were prepared from uninfected cell culture lysates and from uninfected eggs. Each piglet was fed 1 ml of inoculum or sham inoculum at 3-4 days of age. Ten piglets were each fed  $10^9$  inclusion-forming-units (IFU) and 14 piglets were each fed  $10^6$  IFU of isolate R27; 5 control piglets were fed sham inoculum. Twenty piglets were each fed  $10^5$  IFU R19; 5 control piglets were fed sham inoculum. All infected piglets developed diarrhea 4-5 days postinfection (DPI). Most piglets fed  $10^9$  IFU R27 became anorexic, dehydrated, and weak and were necropsied 4-7 DPI. Piglets fed  $10^6$  IFU R27 or  $10^5$  IFU R19 were necropsied 4, 7, 10, 14, and 18 DPI. Diarrhea, although never profuse, persisted in the piglets fed  $10^6$  IFU R27 or  $10^5$  IFU R19 through 12 DPI. At necropsy, all diarrheic piglets had watery colonic contents with flecks of undigested curd. In small intestine, histologic lesions were seen most consistently in distal jejunum and ileum. Distal jejunum and ileum from piglets fed  $10^9$  IFU R27 and necropsied 4-5 DPI were characterized by villus atrophy and multifocal necrosis of villi; necrosis was limited to the tips or apical one half of villi. Mild to severe villus atrophy, lymphangitis, and perilymphangitis were seen in the distal jejunum and ileum from all infected piglets 7 and 10 DPI. Colon from 1 infected piglet necropsied 10 DPI had mild focal serositis; significant colonic lesions were not seen in the other infected piglets. Immunostaining done on sections of distal jejunum and ileum revealed chlamydial antigen in villus enterocytes, occasional goblet cells, and occasional cryptal enterocytes and in foci of lymphangitis and perilymphangitis; the amount of detectable chlamydial antigen decreased after 4 DPI. In colon, sparse positive staining was seen in surface enterocytes and cryptal enterocytes. Ultrastructural examination of ileal villus enterocytes revealed chlamydiae, often together with glycogen particles, in vacuoles or occasionally free in the cytoplasm. The results indicated that the swine chlamydial isolates used in this study are intestinal pathogens in gnotobiotic pigs.

Although chlamydiae have been isolated from or detected in the intestines of diarrheic swine,<sup>8,10,12</sup> the role of these organisms as significant intestinal pathogens is unknown. The fact that chlamydiae have been isolated from the feces and intestines of apparently healthy swine<sup>5,7</sup> has led to speculation that these organisms probably are not significant pathogens.<sup>8,10,12,16</sup>

We recovered 2 distinct chlamydial isolates from the feces and intestines of diarrheic nursery pigs. Originally, it was believed that both isolates shared characteristics with *Chlamydia psittaci*.<sup>1</sup> However, recent polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses indicate that both isolates are *C. trachomatis* or a closely related species (Andersen et al., unpublished data). The objectives of this study were to determine whether these

2 chlamydial isolates could cause enterocolitis in gnotobiotic pigs and if so to characterize the lesions by immunohistochemistry and light and transmission electron microscopy.

### Materials and methods

**Isolation of chlamydiae.** Fecal swab and intestinal specimens collected at necropsy from 2 6-wk-old diarrheic pigs were processed for the isolation of chlamydiae as previously described.<sup>13,15</sup> Chlamydial isolates were analyzed by PCR amplification of the major outer membrane protein genome using the primers and basic techniques previously described.<sup>3</sup> 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. Two distinct chlamydial isolates were identified and designated R27 and R19, respectively.

**Preparation of the inocula.** Inoculum containing isolate R27 was prepared by inoculating confluent Vero cell monolayers as previously described.<sup>13</sup> After titration in 24-hr Vero cell monolayers grown in 96-well multiwell dishes, the final

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 October 23, 1995.

dosage of inoculum was determined to be approximately  $10^9$  inclusion-forming units (IFU)/ml. Sham inoculum was prepared in an identical manner from uninfected cell culture lysates. After piglets fed inoculum developed severe clinical symptoms 5-7 days postinfection (DPI), diluted R27 inoculum was used in the remainder of the study. Immediately prior to the inoculation of piglets, frozen stock inoculum ( $10^9$  IFU/ml) was allowed to thaw, and serial dilutions were made in sterile phosphate-buffered saline. The final dosage of diluted inoculum used to inoculate piglets was approximately  $10^6$  IFU/ml.

Isolate R19 initially was passed through embryonated eggs because of uncertainty as to the ability of the Vero cell monolayers to support growth. After 2 passages in Vero cell monolayers, a 1:10 dilution of cell culture harvest was used to inoculate 7-day embryonated eggs by the yolk sac route. A third egg passage was used to prepare stock culture. Infected yolk sacs from dead embryos were harvested, ground in cell culture medium, and centrifuged at 600 g for 10 min. The supernatant was diluted 1:8 in sucrose-phosphate-glutamine buffer with 10% fetal bovine serum<sup>4</sup> and frozen at  $-70$  C until used to prepare the inoculum. Prior to the inoculation of piglets, the preparation was tested for bacterial contamination by inoculation onto sheep blood agar, and the titer of Chlamydiae was determined by titration in Vero cell monolayers in multiwell plates. The dosage of inoculum was determined to be approximately  $10^5$  IFU/ml. Sham inoculum was prepared in an identical manner from uninfected embryonated eggs.

*Gnotobiotic pigs.* Fifty-four gnotobiotic piglets obtained by closed hysterotomy<sup>9</sup> were housed and maintained as previously described.<sup>13</sup> Fecal swab specimens were collected from each piglet prior to experimental inoculation and inoculated onto sheep blood agar for aerobic and anaerobic<sup>a</sup> bacteriologic culture and onto tergitol-seven agar for aerobic culture.

*Inoculation.* All piglets were fed 1 ml of inoculum or sham inoculum mixed with sterile milk replacer<sup>b</sup> when they were 3-4 days old. Ten piglets were each fed  $10^9$  IFU, and 14 piglets were each fed  $10^6$  IFU of isolate R27; 5 control piglets were fed sham inoculum. Twenty piglets were each fed the R19 inocula ( $10^5$  IFU), and 5 control piglets were fed sham inoculum. All piglets drank allotted milk replacer and in-ocula.

*Necropsy, histopathology, and transmission electron microscopy.* Piglets were monitored for diarrhea, dehydration, anorexia, and lethargy twice daily. Rectal temperatures were recorded once daily. Necropsies were done when principal piglets displayed prominent clinical symptoms or at predetermined intervals postinfection. Piglets were anesthetized with tiletamine HCl and zolazepam HCl<sup>c</sup> by the intramuscular route ( $0.09$  ml/kg)<sup>16</sup> prior to euthanasia by electrocution.

Tissue specimens from the following anatomic sites were collected from all piglets for histopathology (number of samples/site): duodenum (2), proximal jejunum (4), midjejunum (4), distal jejunum (4), ileum (2), proximal spiral colon (1), and distal spiral colon (1). Specimens were fixed in neutral-buffered 10% formalin, routinely processed, embedded in paraffin, sectioned at  $4$   $\mu$ m, stained with hematoxylin and eosin (HE), and examined by light microscopy. Specimens

of ileum from piglets fed both chlamydial isolates and necropsied at 4 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 ( $1$   $\mu$ m) 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.<sup>d</sup>

*Reisolation of chlamydiae, bacteriology, and virology.* Fecal swab specimens and specimens of duodenum, jejunum, ileum, colon, liver, and mesenteric lymph node from all piglets and lung from 1 of the infected piglets were processed for the isolation of chlamydiae as previously described.<sup>13</sup> Lung, liver, jejunum, ileum, and colon from all piglets were processed for aerobic and anaerobic<sup>b</sup> bacteriologic culture as previously described.<sup>13</sup> Specimens of jejunum and ileum from all piglets were examined for rotavirus (RV) and transmissible gastroenteritis virus (TGEV) by fluorescent antibody techniques, and feces from all piglets were examined for viruses by negative contrast electron microscopy. Lung, tonsil, spleen, thymus, and serum were collected from each piglet, and appropriate specimens were processed for the isolation of pseudorabies virus, parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV) as previously described.<sup>13</sup>

*Immunohistochemistry.* Sections cut at  $4$   $\mu$ m from selected blocks of paraffin-embedded distal jejunum, ileum, and colon were stained by an immunohistochemical procedure that has been previously described<sup>13</sup> but with the following modification. After deparaffinization and rehydration, sections were treated with 20% glacial acetic acid for 2 min at  $4$  C to quench endogenous alkaline phosphatase activity. Consequently, levamisole was not used. This treatment of sections with glacial acetic acid was longer than that recommended in the instructions included with the staining kit.<sup>c</sup> After treatment, sections were washed 3 times in Tris-Tween buffer as previously described<sup>13</sup> and then placed in Tris buffer containing 0.05% protease.<sup>f</sup> After staining was completed, sections were placed in an automatic stainer<sup>g</sup> and stained with hematoxylin<sup>h</sup> (5.5 min), washed, clarified<sup>h</sup> (1.5 min), washed, exposed to a bluing reagent<sup>h</sup> (2 min), dehydrated in graded alcohols,<sup>h</sup> and cleared in xylene. Coverslips were mounted with water-insoluble medium, and sections were examined by light microscopy. Sections of distal jejunum, ileum, and colon from age-matched, sham-infected piglets were used as negative tissue controls during each staining procedure. Other performance controls incorporated into each staining procedure were as previously described.<sup>13</sup>

## Results

*Clinical symptoms and necropsy.* The necropsy schedule is shown in Table 1. Rectal temperatures of sham-infected piglets were  $38.5$ - $39.0$  C throughout the study, and these piglets did not exhibit any clinical symptoms. All principal piglets developed diarrhea 4-5 DPI, regardless of the isolate or dosage of chlamydiae. The 4-day-old piglets fed  $10^9$  IFU R27 were active prior to necropsy, but the 5- and 7-day-old piglets fed this dosage were euthanized after they became an-

**Table 1.** Necropsy schedule for piglets with 2 chlamydial isolates.

Inoculum	Dosage (IFU*)	Days postinfection	No. principals	No. controls
R27	10 <sup>9</sup>	4	4	1
		5	1	...
		7	5	...
R27	10 <sup>6</sup>	4	1	...
		7	1	1
		10	5	1
		14	4	1
		18	3	1
R19	10 <sup>6</sup>	4	4	1
		7	4	1
		10	4	1
		14	4	1
		18	4	1

\* Inclusion-forming units.

orexic, dehydrated, and recumbent or stood hunched in the corners of the isolator units. Piglets fed 10<sup>6</sup> IFU R27 or 10<sup>5</sup> IFU R19 had diarrhea, and some became dehydrated through 12 DPI. Three piglets fed 10<sup>6</sup> IFU R27 or 10<sup>5</sup> IFU R19 became lethargic and transiently anorexic 6-7 DPI, but the remaining 31 piglets fed these respective isolates and dosages remained active and continued to drink their allotted milk replacer throughout the study. Diarrhea was never profuse in any of the infected piglets during observation, regardless of the isolate or dosage fed, but occasionally it did become projectile when handling the piglets. Rectal temperatures of the infected piglets throughout the course of diarrhea were 38.9-39.9 C.

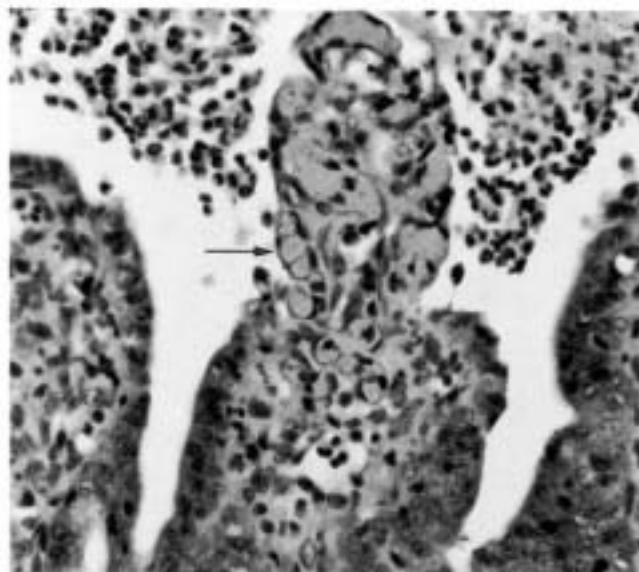
At necropsy, mild hyperemia of the jejunum and ileum was seen in piglets fed 10<sup>9</sup> IFU R27. The presence of chyle in mesenteric lymphatics draining jejunum and ileum was variable in diarrheic piglets that did not become anorexic, regardless of the isolate or dosage of chlamydiae. All diarrheic piglets had watery yellow-white, yellow-brown, or brown-green colonic contents, with flecks of undigested curd. Although diarrhea was not seen immediately prior to necropsy at 14 DPI, 2 piglets fed 10<sup>6</sup> IFU R27 and 2 piglets fed 10<sup>5</sup> IFU R 19 had watery colonic contents with flecks of undigested curd. Remaining 14-day-old and all of the 1 S-day-old piglets fed 10<sup>6</sup> IFU R27 or 10<sup>5</sup> IFU R 19 had colonic contents that were similar in appearance to those of the sham-infected piglets. One 10-day-old piglet fed 10<sup>6</sup> IFU R27 also had bilateral antero-ventral consolidation of the lungs. There were no gross lesions in the sham-infected piglets.

**Histopathology.** Sections of distal jejunum and ileum from the 4- and 5-day-old piglets fed 10<sup>9</sup> IFU R27 had patchy hyperemia and were characterized by moderate to severe multifocal villus atrophy with mul-

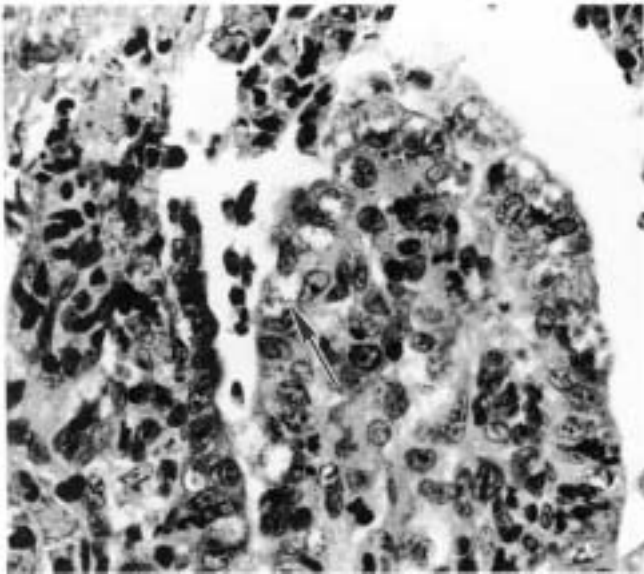


**Figure 1.** Ileum from piglet fed 10<sup>9</sup> inclusion-forming units of isolate R27; 4 days postinfection. Note atrophy and multifocal necrosis (arrowheads) of villi. Villus has lost apical epithelium but the lamina propria remains intact (arrows). Inflammatory cells and debris are in the intestinal lumen. HE.

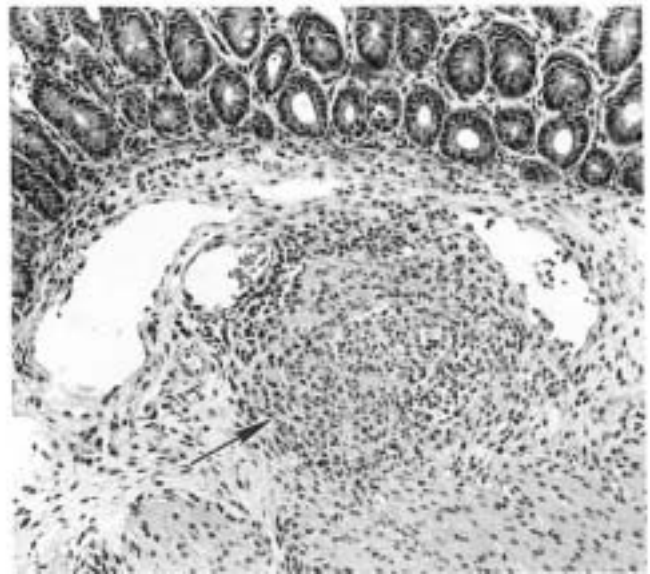
tifocal necrosis of villi; large numbers of neutrophils and abundant cellular debris were present in the lumen. Necrosis was limited to the tips or apical one half of atrophic villi (Fig. 1). Occasional villi had lost apical epithelium, but the lamina propria remained intact (Figs. 1, 2). Barely discernible coccobacilli (Fig. 3) or finely granular basophilic organisms were seen in cy-



**Figure 2.** Higher magnification of an ileal villus that has lost apical epithelium. The lamina propria is hyperemic but intact. Note basal lamina (arrow). HE.



**Figure 3.** Higher magnification of 2 villi shown in Fig. 1. Note small numbers of minute coccobacilli in vacuoles in villus enterocytes (arrow). Villus on the left has undergone necrosis. HE.



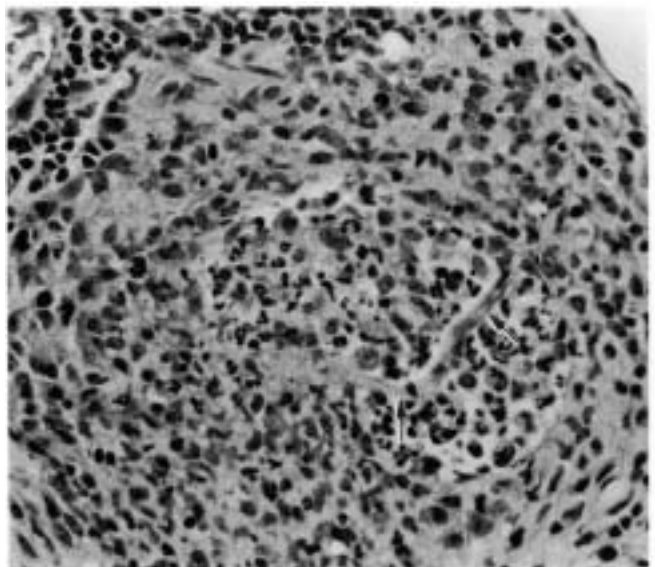
**Figure 4.** Distal jejunum from piglet fed  $10^9$  inclusion-forming units of isolate R27; 7 days postinfection. Note focus of inflammation (lymphangitis and perilymphangitis) in the submucosa (arrow). Venues are dilated. HE.

toplasmic vacuoles in lining enterocytes. Epithelium lining atrophic villi was low columnar, low cuboidal, or attenuated and often covered lamina propria with karyorrhectic debris. Small numbers of neutrophils with occasional macrophages and eosinophils were scattered in the lamina propria; occasional crypts were filled with neutrophils. Moderate multifocal villus atrophy with occasional fusion of villi was seen in mid-jejunum from 3 of the 4- and 5-day-old piglets fed  $10^9$  IFU R27.

Moderate to severe multifocal to diffuse villus atrophy with occasional fusion of villi and cryptal hyperplasia were seen in the distal jejunum and ileum of all 7-day-old piglets fed  $10^9$  IFU R27. The submucosa had variable edema; lymphatics and blood vessels were occasionally dilated. Scattered lymphatics in the submucosa and serosa were plugged with neutrophils, macrophages, and occasional fibrin deposits, and they were often surrounded by cuffs of neutrophils, macrophages, occasional lymphocytes (Figs. 4, 5), and occasional eosinophils. Mild multifocal villus atrophy was seen in duodenum, proximal jejunum, and mid-jejunum from 1 of these 7-day-old piglets fed  $10^9$  IFU R27.

Mild multifocal villus atrophy was seen in the ileum of 1 4-day-old piglet fed  $10^5$  IFU R19. There were no lesions in the other 4-day-old piglets fed  $10^6$  IFU R27 or  $10^5$  R19, even though some of these piglets had diarrhea prior to necropsy. Mild to severe multifocal to diffuse villus atrophy, together with lymphangitis and perilymphangitis in the submucosa and serosa, were seen in the distal jejunum and ileum from all 7-

and 10-day-old piglets fed  $10^6$  IFU R27 or  $10^5$  IFU R19 (Fig. 6). Epithelium lining atrophic villi was attenuated, and scattered crypts were hyperplastic (Fig. 6). The lamina propria of atrophic villi occasionally had karyorrhectic debris, and there was occasional fusion of villi. Mild inflammatory cell infiltrates were occasionally scattered in the lamina propria. Mild mul-



**Figure 5.** Higher magnification of the focus of inflammation shown in Fig. 4. Lymph vessel is plugged with and surrounded by neutrophils, macrophages, and lymphocytes. Note valve of lymph vessel (arrows). HE.



**Figure 6.** Ileum from piglet fed  $10^6$  inclusion-forming units of isolate R27; 10 days postinfection. Note severe villus atrophy. Epithelium lining atrophic villi is attenuated, and scattered crypts are hyperplastic. HE.

tifocal villus atrophy and lymphangitis and perilymphangitis in the submucosa and serosa were seen in ileum and distal jejunum from 2 14-day-old piglets fed  $10^6$  IFU R27 and from 2 14-day-old piglets fed  $10^5$  IFU R19. There were no lesions in remaining 14-day-old piglets fed  $10^6$  IFU R27 or  $10^5$  IFU R19. There were no lesions in the 18-day-old piglets fed either isolate, and there were no lesions in the small intestine from any of the sham-infected piglets.

One 10-day-old piglet fed  $10^6$  IFU R27 had mild focal inflammation in the serosa of the proximal colon, but there were no significant lesions in colons from the other infected piglets. Compared to the sham-infected piglets, colons from several infected piglets necropsied 7 DPI or later had increased numbers of lymphocytes and macrophages in the lamina propria.

**Immunohistochemistry.** Application of the immunohistochemical stain to sections of distal jejunum and ileum from all infected 4-day-old piglets revealed chlamydial antigen in villus enterocytes, occasional goblet cells, and occasional cryptal enterocytes. Residual alkaline phosphatase activity of the brush border was faint and was easily distinguished from the intensely stained chlamydial antigen. Antigen was most abundant in sections from the piglets fed  $10^9$  IFU R27, where it was clearly associated with villus atrophy and necrosis (Fig. 7). Although histologic lesions were not seen in a majority of the diarrheic 4-day-old piglets fed  $10^6$  IFU R27 or  $10^5$  IFU R19, chlamydial antigen was present in the sections.

Positive staining generally was sparse in sections of

distal jejunum and ileum from all infected 7- and 10-day-old piglets, regardless of the isolate or dosage fed. In addition to villus enterocytes, chlamydial antigen was occasionally seen in macrophages in the lamina propria and in foci of inflammation in the submucosa and serosa (Fig. 8).

Application of the immunohistochemical stain to sections of colon from all 4-, 7-, and 10-day-old piglets revealed chlamydial antigen in surface enterocytes and cryptal enterocytes and occasionally in macrophages in the lamina propria. Positive staining generally was sparse in colon from these piglets, regardless of the isolate or dosage fed.

**Transmission electron microscopy.** Ultrastructural examination of ileal specimens from selected 4-day-old piglets fed R27 or R19 inocula revealed chlamydiae in the cytoplasm of villus enterocytes and occasional goblet cells. Intracytoplasmic chlamydiae were most often in membrane-bound vacuoles, but occasionally they were free in the cytoplasm. Infected enterocytes often had few or no surface microvilli, intracytoplasmic lipid globules (Fig. 9), swollen mitochondria, dilated endoplasmic reticulum, membranous inclusions, and occasional autophagosomes. In addition to all morphologic forms of the chlamydial developmental cycle, intracytoplasmic vacuoles often contained lipid globules, membranous debris (Fig. 9), and glycogen particles (Figs. 9, 10). Dead or degenerate enterocytes with intracytoplasmic chlamydiae were present in the intestinal lumen.

**Microbiology.** Chlamydiae were reisolated from ileum, colon, and fecal swab specimens from infected piglets throughout the study. Chlamydiae were reisolated from duodenum, jejunum, mesenteric lymph node, and liver from a majority of infected piglets necropsied through 10 DPI and from mesenteric lymph node and liver from several piglets necropsied 14 and 18 DPI. In addition, chlamydiae were isolated from lung from the piglet that had pneumonia at necropsy 10 DPI. Chlamydiae were not isolated from the sham-infected piglets.

*Bacillus* sp. was isolated from intestinal specimens from several principal and sham-infected piglets necropsied 14 and 18 DPI. Bacteria were not isolated from other tissue specimens collected from principal or sham-infected piglets. Fluorescent antibody tests for RV and TGEV done on jejunum and ileum from all piglets were negative, and viruses were not seen in feces by negative contrast electron microscopy. Pseudorabies virus, PPV, and PRRSV were not isolated from specimens from principal or sham-infected piglets.

## Discussion

The results of the present study indicated that swine chlamydial isolates R27 and R19 are intestinal patho-

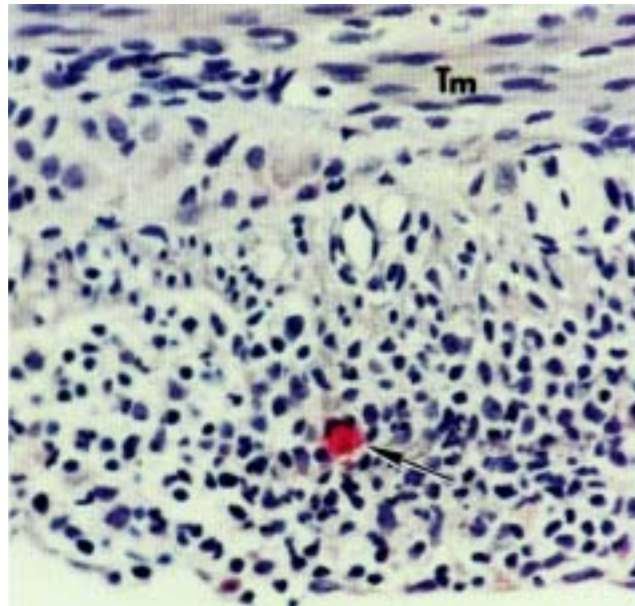
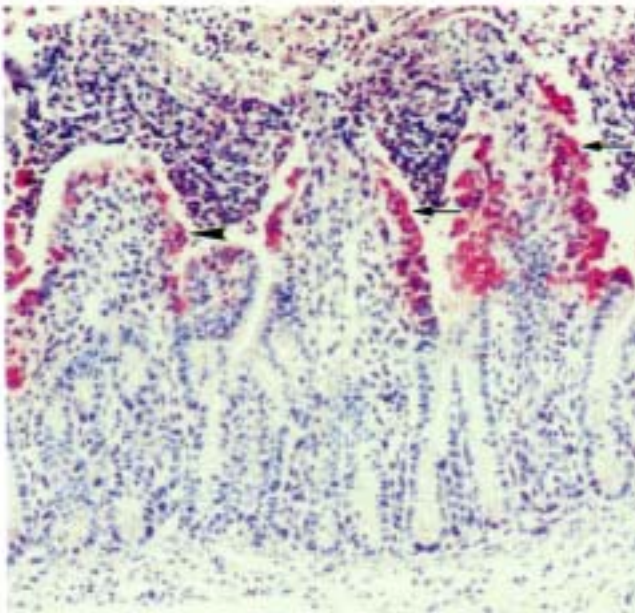


Figure 7. Ileum from piglet fed  $10^9$  inclusion-forming units of isolate R27; 4 days postinfection. Chlamydial antigen is present in epithelium of villi that have undergone necrosis (arrows) and in epithelium covering moderately atrophic villus (arrowhead). Avidin-biotin-alkaline phosphatase stain, hematoxylin counterstain.

Figure 8. Distal jejunum from piglet fed  $10^6$  inclusion-forming units of isolate R27; 10 days postinfection. Chlamydial antigen is present in focus of serosal inflammation (arrow). Note tunica muscularis (Tm). Avidin-biotin-alkaline phosphatase stain, hematoxylin counterstain.

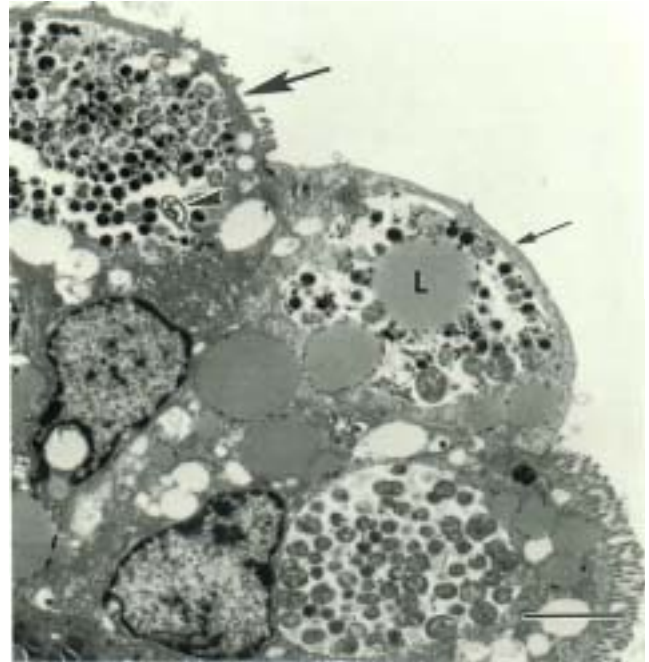


Figure 9. Transmission electron micrograph of ileal enterocytes from piglet fed isolate R19; 4 days postinfection. Enterocytes have intracytoplasmic vacuoles filled with chlamydiae, lipid (L), and membranous debris (arrowhead). Barely discernible, flocculent electron-dense material (glycogen) is present with chlamydiae in 2 of the vacuoles. The cytoplasm of enterocytes also has lipid and small clear vacuoles. Note the reduction in (large arrow) or absence of (small arrow) surface microvilli. Bar = 3.0  $\mu$ m.

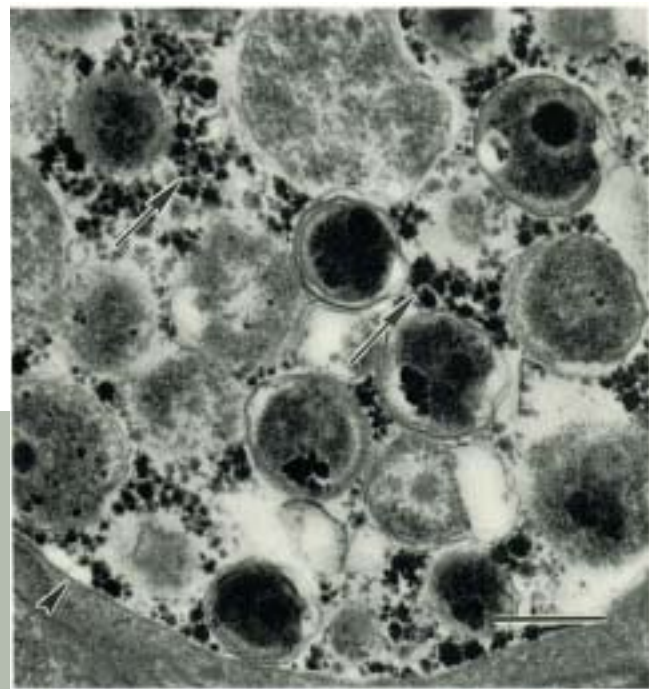


Figure 10. Ileal enterocyte from a piglet fed isolate R19; portion of an intracytoplasmic vacuole is filled with glycogen (arrows) and Chlamydiae. Note limiting membrane of the vacuole (arrowhead). Bar = 0.5  $\mu$ m.

gens in gnotobiotic pigs, and lesions are seen most consistently in distal jejunum and ileum. Other investigators have reported villus atrophy and necrosis in the small intestine of piglets naturally infected with chlamydiae.<sup>10</sup> Lesions described for the naturally infected piglets are similar to those seen in the 4- and 5-day-old piglets fed large numbers ( $10^9$  IFU) of isolate R27. Isolate R27 was extremely virulent when large numbers of organisms were fed to piglets early in the present study. Consequently, smaller numbers of organisms were fed to piglets during the remainder of the study.

The lesions in the gnotobiotic pigs also are similar to those described for conventional calves experimentally infected with chlamydiae.<sup>4</sup> Lymphangitis and perilymphangitis caused by translocation of chlamydiae across the intestine are similar to lesions seen in the submucosa and serosa of the experimentally infected calves. Colonic lesions were seen in the calves, but colonic lesions were not consistently seen in the piglets in the present study.

The histologic lesions in the 4- and 5-day-old piglets fed  $10^9$  IFU R27 suggested that loss of apical enterocytes lining villi is the initial event in the pathogenesis of the intestinal lesions. Apical enterocytes are in the G<sub>1</sub> phase of the cell cycle, a phase required for the replication of chlamydiae.<sup>2</sup> Loss of apical enterocytes is followed by necrosis of the lamina propria or replacement by absorptive cells.

Again, it is worth noting that a majority of the diarrheic 4-day-old piglets fed  $10^6$  IFU R27 or  $10^5$  IFU R19 did not have histologic lesions. It is possible that lesions were not found because of inadequate sampling. Conversely, infected enterocytes might have become functionally altered in the early stages of pathogenesis, causing diarrhea but no histologic lesions. The few swine chlamydial isolates studied thus far have high nutrient requirements,<sup>6</sup> which could adversely alter host cell function. Infected enterocytes from these and other 4-day-old piglets often had ultrastructural changes suggestive of altered cell function.

The isolates used in this study have not been classified with certainty, but PCR and RFLP analyses indicate that both isolates are *C. trachomatis* or a closely related species (Andersen et al., unpublished data). Both isolates are sensitive to sodium sulfadiazine and form inclusions filled with glycogen in cell monolayers (Andersen et al., unpublished data), which also are characteristics of *C. trachomatis*.<sup>11</sup> The fact that intracytoplasmic vacuoles filled with glycogen and chlamydiae were seen ultrastructurally in enterocytes from pigs in this study might further support this concept. Although the *C. trachomatis* classification traditionally has included human chlamydial isolates and 1 mouse isolate,<sup>11</sup> *C. trachomatis* recently has been associated with intestinal infections in swine.<sup>5,17</sup> More recently,

an isolate believed to be *C. trachomatis* has been shown to cause pneumonia in swine.<sup>13</sup> The R27 isolate used in the present study probably is a lung pathogen as well; it caused pneumonia in 1 of the piglets, presumably after aspiration of inoculated milk replacer.

From a diagnostic perspective, the lesions seen in piglets in this study are not pathognomonic for chlamydial infection. The lesions could suggest a viral etiology or coccidiosis, and arriving at a diagnosis of chlamydial infection in naturally infected pigs could be difficult. Chlamydiae can be very difficult to detect in histologic sections. The use of fluorescent antibody or immunohistochemical techniques might aid in the diagnosis if chlamydial antigen is present in any given section. The amount of detectable chlamydial antigen in intestinal specimens decreased considerably between 4 and 7 DPI in the present study, and thus it would be imperative to examine distal jejunum and ileum from naturally infected pigs early in the course of diarrhea. The isolation of chlamydiae from intestinal specimens or feces might be attempted, but this procedure has limitations. Many chlamydiae are difficult to grow in either embryonated eggs or in tissue culture. Under field conditions, the titers of chlamydiae are often low, and care must be used in handling the specimens for isolation. The use of commercial antigen capture tests could be advantageous because isolation of chlamydiae is not required; however, sensitivity of these tests can be inadequate for detecting small numbers of organisms. Also, some commercial tests might give false-positive results because of cross-reactions with gram-negative bacteria.<sup>14</sup> Currently, it is not known whether all intestinal chlamydiae are pathogens.

The diagnosis of swine enteric chlamydial infections is relatively rare, presumably because of a lack of knowledge regarding the role of chlamydiae as enteric pathogens and because of diagnostic procedural limitations. There also may be a tendency to incriminate known pathogens in certain diarrheal diseases of swine, thus ignoring a possible synergistic role for chlamydiae. Additional knowledge regarding the role of chlamydiae as intestinal pathogens will be generated from increased awareness, surveillance studies, further characterization of chlamydial isolates, and experimental infections.

#### Acknowledgements

We thank Linda Hornung, Wendy Hambly, Everine Breden, Tom Bargar, Robin Olmscheid, and Mavis Seelmeyer for assistance. This work was supported by funds from USDA Specific Cooperative Agreement No. 58-3630-4-125. This report is published as paper no. 11317, 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. SPF-Lac, Borden, Norfolk, VA.
- c. Telazol, Ft. Dodge Laboratories, Ft. Dodge, IA.
- d. Philips 201, Eindhoven, The Netherlands.
- e. Vectastain ABC-Alkaline Phosphatase, Vector Laboratories, Burlingame, CA.
- f. Protease XIV, Sigma Chemical Co., St. Louis, MO.
- g. Jung Autostainer XL, Leica Instruments, Nussloch, Germany.
- h. Richard-Allan Medical, Richland, MI.

### References

1. Andersen AA, Rogers DG: 1994, Characterization of *Chlamydia psittaci* isolates from swine. Proc Int Symp Hum Infect 8:578-581.
2. Barker IK, Van Dreumel AA, Palmer N: 1993, The alimentary system. In: Pathology of domestic animals, ed. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, p. 253. Academic Press, San Diego, CA.
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. Doughri AM, Young S, Storz J: 1974, Pathologic changes in intestinal Chlamydial infection of newborn calves. Am J Vet Res 35:939-944.
5. Kaltenboeck B, Kousoulas KG, Storz J: 1992, Two-step polymerase chain reactions and restriction endonuclease analyses detect and differentiate *ompA* DNA of *Chlamydia* spp. J Clin Microbiol 30:1098-1104.
6. Kaltenboeck B, Storz J: 1992, Biological properties and genetic analysis of the *ompA* locus in chlamydiae isolated from swine. Am J Vet Res 53:1482-1487.
7. Kölbl V: 1969, Untersuchungen über das vorkommen von miyagawanellen beim schwein. Wien Tierärztl Monatsschr 56: 332-335.
8. Koudela B, Vitovec J, Sterba J: 1990, Concurrent infection of enterocytes with *Eimeria scabra* and other enteropathogens in swine. Vet Parasitol 35:71-77.
9. Miniats OP, Jol D: 1978, Gnotobiotic pigs-derivation and rearing. Can J Comp Med 42:428-437.
10. Nietfeld JC, Janke BH, Leslie-Steen P, et al.: 1993, Small intestinal *Chlamydia* infection in piglets. J Vet Diagn Invest 5:114-117.
11. Page LA: 1968, Proposal for the recognition of two species in the genus *Chlamydia* Jones, Rake, and Steams, 1945. Int J Syst Bacteriol 18:51-66.
12. Pospischil A, Wood RL: 1987, Intestinal *Chlamydia* in pigs. Vet Pathol 24:568-570.
13. 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.
14. Sanderson TP, Andersen AA: 1990, Use of enzyme immunoassays for *Chlamydia trachomatis* to detect *Chlamydia psittaci* in animal specimens. Proc Comm Diagn Technol Anim Health Monit 1990:171-180.
15. Spence WN, Johnson FWA: 1983. Simple transport medium for the isolation of *Chlamydia psittaci* from clinical material. Vet Rec 113:535-536.
16. 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.
17. Zahn I, Szeredi L, Schiller I, et al.: 1995, Immunhistologicsher nachweis von *Chlamydia psittaci/pecorum* und *C. trachomatis* im ferkel-darm. J Vet Med (B) 42:266-276.