Morphometric Analysis of Enteric Lesions in C3H/HeN Mice Inoculated with *Serpulina hyodysenteriae* Serotypes 2 and 4 with or without Oral Streptomycin Pretreatment

Jagannatha V. Mysore  
*University of Nebraska - Lincoln*

Gerald Duhamel  
*University of Nebraska - Lincoln*, gduhamel1@unl.edu

Follow this and additional works at: [http://digitalcommons.unl.edu/vetscipapers](http://digitalcommons.unl.edu/vetscipapers)

Part of the Biochemistry, Biophysics, and Structural Biology Commons, Cell and Developmental Biology Commons, Immunology and Infectious Disease Commons, Medical Sciences Commons, Veterinary Microbiology and Immunobiology Commons, and the Veterinary Pathology and Pathobiology Commons

Mysore, Jagannatha V. and Duhamel, Gerald, "Morphometric Analysis of Enteric Lesions in C3H/HeN Mice Inoculated with *Serpulina hyodysenteriae* Serotypes 2 and 4 with or without Oral Streptomycin Pretreatment" (1994). Papers in Veterinary and Biomedical Science. 267.  
[http://digitalcommons.unl.edu/vetscipapers/267](http://digitalcommons.unl.edu/vetscipapers/267)

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.
Morphometric Analysis of Enteric Lesions in C3H/HeN Mice Inoculated with *Serpulina hyodysenteriae* Serotypes 2 and 4 with or without Oral Streptomycin Pretreatment

Jagannatha V. Mysore and Gerald E. Duhamel

**ABSTRACT**

The segmental distribution and sequential progression and the role of the indigenous bacterial flora in the development of enteric lesions associated with *Serpulina hyodysenteriae* infection in laboratory mice have not been defined. We examined the distribution and sequential morphometric changes in the large intestine of mice orally inoculated with *S. hyodysenteriae* serotypes 2 and 4. To determine the role of colonization resistance conferred by the indigenous bacterial flora, 40 female C3H/HeN mice were administered water alone or water containing 5 mg/mL streptomycin sulfate *ad libitum* for seven days prior to orogastric inoculation either with *S. hyodysenteriae* or sterile trypticase soy broth (TSB). Clinical signs were monitored daily and three mice per group were necropsied on postinoculation days (PID) 7 and 14 for pathological assessment of the cecum, proximal colon, transverse colon, and descending colon, and bacteriological culture of the cecum for *S. hyodysenteriae*. Weekly pooled fecal samples were collected from each group for determination of total numbers of anaerobe bacteria. Gross examination revealed soft fecal pellets on PID 7 and 14 and catarrhal typhlitis on PID 14, irrespective of streptomycin pretreatment. The recovery rates of *S. hyodysenteriae* from the ceca of serotype 2- and serotype 4-inoculated mice was 100 and 91.7%, respectively. Statistically significant differences in morphometric changes between TSB- and *S. hyodysenteriae*-inoculated mice were present on PID 7 and 14 and were restricted to the cecum. Although oral administration of streptomycin for seven days prior to *S. hyodysenteriae* inoculation resulted in a significant reduction in the numbers of fecal anaerobes, it did not affect the colonization, distribution, severity, or progression of cecal lesions.

**RÉSUMÉ**

La distribution segmentaire, la progression séquentielle et le rôle de la flore bactérienne indigène dans le développement des lésions entériques associées à une infection par *Serpulina hyodysenteriae* chez des souris de laboratoire ne sont toujours pas connus. Nous avons étudié la distribution et les changements morphométriques séquentiels du gros intestin de souris inoculées oralement avec *S. hyodysenteriae* sérotypes 2 et 4. Afin d'évaluer le rôle de la flore bactérienne indigène dans la résistance à la colonisation, 40 souris femelles C3H/HeN ont reçu de l'eau uniquement ou de l'eau contenant 5 mg/mL de streptomycine *ad libitum* pendant sept jours avant d'être inoculées par voie orogastrique avec *S. hyodysenteriae* ou un bouillon stérile. Les signes cliniques ont été notés à chaque jour et trois souris de chacun des groupes ont été necropsiées aux jours 7 et 14 post-inoculation (PI) pour examen du caecum, du côlon proximal, du côlon transverse et du côlon descendant et pour culture bactérienne du cæcum. Des pools de fèces ont été obtenus à chaque semaine pour déterminer le nombre total de bactéries anaérobies. L'examen macroscopique a révélé la présence de fèces molles aux jours 7 et 14 PI et d'une typhlitis catarrhal au jour 14 PI, que les animaux aient reçu ou non de la streptomycine. *S. hyodysenteriae* sérotypes 2 et sérotypes 4 ont été résolés respectivement de 100 et 91,7 % des caeca des souris inoculées. Des différences statistiquement significatives de changements morphométriques ont été observées aux jours 7 et 14 PI entre les souris inoculées avec un bouillon stérile et celles ayant reçu *S. hyodysenteriae* et ce, uniquement dans le caecum. Bien que l'administration orale de streptomycine ait réduit de façon significative le nombre d'anaérobies fécaux, elle n'a pas affecté la colonisation, la distribution, la sévérité ou la progression des lésions. *(Traduit par Dre Christiane Girard)*

**INTRODUCTION**

Swine dysentery is a mucohemorrhagic diarrheal disease that affects growing-finishers pigs worldwide (1). The primary etiological agent of swine dysentery is an anaerobic, oxygen-tolerant, gram-negative spirochete bacterium, *Serpulina hyodysenteriae*, and nine different serotypes have been identified based on antigenic differences in lipopolysaccharide composition of the cell wall (1,2). House mice (*Mus*...
musculus) have been shown to act as biological vectors of S. hyodysenteriae in nature, and laboratory mice have been used as a model to evaluate the enteropathogenicity of swine intestinal spirochetes, and in studies aimed at elucidating the pathogenesis of Serpulina hyodysenteriae infection (3–8). Results from studies using conventional and germfree pigs and mice suggest a synergistic role for indigenous gram-negative anaerobes of the large intestine in the production of enteric lesions associated with S. hyodysenteriae infection (6.9–12). Oral administration of streptomycin has been shown to result in enhanced susceptibility of the host to certain enteric pathogens (13). The mechanism of enhanced susceptibility with streptomycin is attributed to alterations in the relative composition of the indigenous enteric bacterial flora; obligate gram-negative anaerobes are more resistant to aminoglycoside antibiotics than the facultative gram-negative anaerobes (14,15).

The purpose of this study was to determine the segmental distribution and characterize the sequential development of enteric lesions associated with S. hyodysenteriae infection in laboratory mice. Additionally, the role of the indigenous bacterial flora in the development of enteric lesions associated with S. hyodysenteriae infection was examined by determining the effect of oral streptomycin administration prior to inoculation with S. hyodysenteriae. Computer-assisted morphometry was used as a tool to quantitatively assess the segmental distribution and temporal progression of enteric lesions associated with S. hyodysenteriae infection (16).

**MATERIALS AND METHODS**

**MICE**

Forty inbred, eight-week-old, C3H/HeN female mice were obtained from a commercial supplier (Sasco Company, Omaha, Nebraska). The mice were housed and cared for in accordance with approved guidelines of the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. The mice were fed ad libitum a commercial rodent diet (Wayne Rodent Blox 8604, Teklad Premier Lab Diets, A Harlan Sprague Incorporated Company, Madison, Wisconsin) and aspen shavings were used as bedding (American Excelsior Company, Arlington, Texas). Tap water was given in nipple glass bottles. A 12/12 hour light/dark cycle was maintained in the animal facility.

**BACTERIA**

Mice were inoculated with either S. hyodysenteriae serotype 2, strain B204 or serotype 4, strain A1. Both strains of S. hyodysenteriae were propagated in prereduced anaerobically-sterilized trypticase soy broth medium as described (8).

**EXPERIMENTAL DESIGN**

Mice were randomly assigned to one of six experimental groups (A through F) with eight mice in groups A and B and six mice in each of the remaining groups. Prior to inoculation with S. hyodysenteriae, mice in groups B, D and F received streptomycin sulfate (Sigma Chemical Company, St. Louis, Missouri) at a concentration of 5 mg/mL ad libitum for seven days in the drinking water. Mice in groups A, C and E received tap water. On day 0, streptomycin administration was stopped and water without antibiotics was provided to all groups until the end of the experiment. Also on day 0 and prior to inoculation with S. hyodysenteriae, two mice in groups A and B were euthanized by cervical dislocation and necropsied for pathological and bacteriological examinations. On day −1, the feed was withdrawn and on day 0 mice were inoculated twice orogastrically at eight hour intervals with 0.5 mL volume of culture broth containing $2 \times 10^8$ S. hyodysenteriae serotype 2 (groups C and D) or serotype 4 (groups E and F) (Table 1). Groups A and B were inoculated similarly with sterile TSB. Regular feeding was resumed four hours after the second inoculation. On postinoculation days (PID) 7 and 14, three mice per group were necropsied for pathological and bacteriological examinations.

**CLINICAL AND BACTERIOLOGICAL EXAMINATIONS**

Mice were monitored daily for fecal consistency. Body weights of individual mice were recorded at weekly intervals. Pooled fecal samples from each group were collected weekly and the total numbers of anaerobe bacteria was determined using a plate counting method as follows: immediately after collection, each dilution of tenfold serial dilutions of feces in sterile phosphate buffered saline was plated onto trypticase soy agar (BBL, Beckton Dickinson Microbiology Systems, Cockeysville, Maryland) containing 5% bovine blood (TSAB) and incubated anaerobically at 37°C using the Gas Pak Anaerobic System (BBL). Total anaerobic bacterial colonies per gram of feces (wet weight) were counted after 24 hours. At the time of necropsy, the cecal mucosa and its contents from each mouse were cultured using a selective agar medium for S. hyodysenteriae (17). Cultures were incubated in the Gas Pak Anaerobic System (BBL) at 42°C and examined after two, four and six days of incubation. Serpulina hyodysenteriae was identified on the basis of characteristic β-hemolysis and by the presence of spirochetes under dark field examination of smears taken at the periphery of hemolytic zones. The identity of representative S. hyodysenteriae isolates obtained from the ceca of both streptomycin-treated and -untreated mice was confirmed by comparing the restriction endonuclease pattern of genomic DNA from each mouse isolate to the corresponding parent strain by agarose gel electrophoresis after diges-

**TABLE I. Weekly fecal anaerobic bacterial counts of streptomycin-treated and -untreated, TSB-and S. hyodysenteriae-inoculated C3H/HeN mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serotype</th>
<th>Treatment</th>
<th>-7</th>
<th>0</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td>3.9 x 10^6</td>
<td>4.5 x 10^6</td>
<td>5.0 x 10^6</td>
<td>5.3 x 10^6</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td></td>
<td>6.4 x 10^6</td>
<td>3.1 x 10^6</td>
<td>2.8 x 10^6</td>
<td>3.3 x 10^6</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td>7.9 x 10^6</td>
<td>8.5 x 10^6</td>
<td>3.5 x 10^6</td>
<td>2.8 x 10^6</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>5.9 x 10^6</td>
<td>6.6 x 10^6</td>
<td>6.6 x 10^6</td>
<td>8.3 x 10^6</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td></td>
<td>6.7 x 10^6</td>
<td>6.8 x 10^6</td>
<td>1.5 x 10^6</td>
<td>1.3 x 10^6</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td>8.2 x 10^6</td>
<td>3.6 x 10^6</td>
<td>2.1 x 10^6</td>
<td>4.8 x 10^6</td>
</tr>
</tbody>
</table>

*Weekly total anaerobic bacterial colony counts per gram of feces (wet weight)*
tion with the restriction endonucleases Hind III and Hae III (4,18).

PATHOLOGICAL EXAMINATION

After examination for the presence of macroscopic changes, transverse and longitudinal sections were made of the cecum, proximal colon, transverse colon, and descending colon, and immersed in 10% phosphate buffered formalin for light microscopy. For morphometric analyses each tissue specimen was sectioned transversely and longitudinally, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin-eosin (HE). Warthin-Starry staining was employed for the demonstration of spirochetes in-situ (19).

MORPHOMETRY

Computer-assisted morphometric measurements were performed using a video-imaging morphometric system (PC VISION plus Frame Grabber Version of JAVA™, Jandel Scientific, California). Tissue sections labelled with number codes were evaluated blindly for cytokine and inflammatory parameters. Equations used for the derivatization of cytokinin and inflammatory parameters were as described (20). Cytokine parameters were recorded by examining four to six well-oriented crypt columns at 40× magnification in two sections for each intestinal segment from three mice at each sampling and expressed as means ± standard error of the mean (SEM) of longitudinal crypt length (LCL), cross-sectional crypt diameter (CSCD), number of epithelial cells per crypt column (EC:CC), goblet cell to epithelial cell percentage ratio (GC:EC), and mitotic figure to epithelial cell percentage ratio (MC:EC) of mice from the same treatment group necropsied on the same PID. The LCL and CSCD were reported respectively as means of linear measurements of length and diameter in cm of cecal crypts taken from an image displayed onto a video monitor. The mean density of inflammatory cells (DIC) per 100 cm² ± SEM was determined by examining two separate fields at 40× in two sections for each intestinal segment from three mice necropsied on the same PID. The field of interest on the video monitor was defined as the muscularis mucosa as the lower boundary, the superficial epithelium as the upper boundary and a constant width of 75 mm.

STATISTICAL ANALYSIS

To determine if inoculation of mice with S. hyodysenteriae had an effect on the development of enteric lesions, the mean ± SEM of the LCL, CSCD, EC:CC, GC:EC percentage ratio, MC:EC percentage ratio, and DIC of the cecum, proximal colon, transverse colon, and descending colon of mice inoculated either with TSB or S. hyodysenteriae serotypes 2 or 4 were compared between groups necropsied on the same PID using the Student’s t-test (H0: A=C and H0: A=E; H0: B=D and H0: B=F) (21). Additionally, to determine if administration of streptomycin had an effect on the development of enteric lesions, the mean ± SEM of the LCL, CSCD, EC:CC, GC:EC percentage ratio, MC:EC percentage ratio, and DIC of the cecum, proximal colon, transverse colon, and descending colon of mice inoculated either with TSB or S. hyodysenteriae serotypes 2 or 4 were compared between streptomycin-treated and -untreated groups necropsied on the same PID using the Student’s t-test (H0: A=B, H0: C=D, and H0: E=F). Differences between groups were considered significant if the p value was less than 0.01 (p < 0.01) or 0.05 (p < 0.05).

RESULTS

CLINICAL AND BACTERIOLOGICAL EXAMINATIONS

Soft fecal pellets were present in streptomycin-treated (groups D and F) and -untreated (groups C and E) S. hyodysenteriae-inoculated groups on PID 7. No significant differences in body weights were present between TSB- and S. hyodysenteriae-inoculated groups, irrespective of streptomycin pretreatment (p < 0.05) (data not shown). Results of weekly fecal anaerobic bacterial counts are presented in Table 1. Oral administration of streptomycin resulted in a 100 to 1000-fold reduction in the numbers of fecal anaerobic bacteria, from day 0, the day of inoculation with S. hyodysenteriae, until the end of the study on PID 14. Following challenge-inoculation, S. hyodysenteriae was consistently isolated from the ceca of inoculated mice, except on PID 7, in one out of three mice in group E; the overall recovery rate was 95.8%. The recovery rates of serotype 2 and serotype 4 from the ceca of inoculated mice were 100 and 91.7%, respectively. Spirochetes were not isolated from any mice in the TSB-inoculated groups A and B. Restriction endonuclease analyses of representative isolates of S. hyodysenteriae from streptomycin-treated and -untreated infected mice yielded restriction patterns identical to the parent strains (data not shown).

PATHOLOGICAL EXAMINATION

No significant gross and histological changes were present in the intestines of mice necropsied on day 0. On PID 7 and 14, soft fecal pellets were present in the lumen of the large intestine of mice inoculated with S. hyodysenteriae. In addition, on PID 14, a marked reduction in the size of the cecum together with replacement of the cecal contents by mucus was present in mice from the S. hyodysenteriae-inoculated groups, except for one culture-negative mouse in group E. Mild focal infiltration of the lamina propria by polymorphonuclear leukocytes was present in the cecal mucosa of S. hyodysenteriae-inoculated mice on PID 7. On PID 14, moderate numbers of mixed mononuclear inflammatory cells were present in the lamina propria and occasional polymorphonuclear leukocytes were seen between epithelial cells and near areas of focal superficial mucosal epithelial erosions in the ceca of S. hyodysenteriae-inoculated mice. Warthin-Starry staining disclosed large numbers of spirochetes individually or in clumps in the lumens of cecal crypt columns and occasionally within goblet cells in the ceca of mice inoculated with S. hyodysenteriae. No significant differences in the severity of gross and histological lesions was detected in the ceca of mice from the streptomycin-treated and -untreated S. hyodysenteriae-inoculated groups. No significant gross and histological changes were present in other segments of intestines in mice inoculated with S. hyodysenteriae and in all segments of intestines in the TSB-inoculated mice in groups A and B.

MORPHOMETRY

Statistically significant differences in intestinal mucosal cytokinin and
TABLE II. Mean values of cytokinetic and inflammatory parameters in ceca of control and *S. hyodysenteriae*-inoculated C3H/HeN mice with or without streptomycin pretreatment

<table>
<thead>
<tr>
<th>Parameters§</th>
<th>A</th>
<th>B</th>
<th>C†</th>
<th>D†</th>
<th>E†</th>
<th>F†</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCL (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID 7</td>
<td>2.77±0.307*</td>
<td>3.47±0.307*</td>
<td>5.33±0.307*</td>
<td>3.93±0.307*</td>
<td>4.14±0.307*</td>
<td>4.59±0.307*</td>
</tr>
<tr>
<td>PID 14</td>
<td>2.70±0.530*</td>
<td>3.70±0.530*</td>
<td>4.31±0.530*</td>
<td>4.86±0.530*</td>
<td>4.94±0.530*</td>
<td>4.05±0.530*</td>
</tr>
<tr>
<td>CSCD (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID 7</td>
<td>1.21±0.129*</td>
<td>1.11±0.129*</td>
<td>1.56±0.129*</td>
<td>1.57±0.129*</td>
<td>1.40±0.129*</td>
<td>1.21±0.129*</td>
</tr>
<tr>
<td>PID 14</td>
<td>0.94±0.142*</td>
<td>1.04±0.142*</td>
<td>1.28±0.142*</td>
<td>1.58±0.142*</td>
<td>1.64±0.142*</td>
<td>1.14±0.142*</td>
</tr>
<tr>
<td>EC/CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID 7</td>
<td>216.37±13.17*</td>
<td>214.58±13.17*</td>
<td>484.77±13.17*</td>
<td>334.52±13.17*</td>
<td>203.10±13.17*</td>
<td>233.87±13.17*</td>
</tr>
<tr>
<td>PID 14</td>
<td>375.37±40.03*</td>
<td>277.89±40.03*</td>
<td>352.25±40.03*</td>
<td>304.89±40.03*</td>
<td>300.33±40.03*</td>
<td>270.02±40.03*</td>
</tr>
<tr>
<td>GC/EC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID 7</td>
<td>16.92±3.27*</td>
<td>19.12±3.27*</td>
<td>12.55±3.27*</td>
<td>14.64±3.27*</td>
<td>19.46±3.27*</td>
<td>14.14±3.27*</td>
</tr>
<tr>
<td>PID 14</td>
<td>07.70±1.82*</td>
<td>13.68±1.82*</td>
<td>3.30±1.82*</td>
<td>7.13±1.82*</td>
<td>10.63±1.82*</td>
<td>13.44±1.82*</td>
</tr>
<tr>
<td>MC/EC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID 7</td>
<td>0.60±0.13*</td>
<td>0.40±0.13*</td>
<td>0.28±0.13*</td>
<td>0.90±0.13*</td>
<td>1.33±0.13*</td>
<td>1.59±0.13*</td>
</tr>
<tr>
<td>PID 14</td>
<td>0.36±0.08*</td>
<td>0.25±0.08*</td>
<td>2.68±0.08*</td>
<td>3.5±0.08*</td>
<td>2.76±0.08*</td>
<td>2.82±0.08*</td>
</tr>
<tr>
<td>DIC/100cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID 7</td>
<td>8.16±1.10*</td>
<td>5.07±1.10*</td>
<td>8.15±1.10*</td>
<td>10.92±1.10*</td>
<td>10.90±1.10*</td>
<td>12.66±1.10*</td>
</tr>
<tr>
<td>PID 14</td>
<td>9.19±1.21*</td>
<td>8.91±1.21*</td>
<td>9.43±1.21*</td>
<td>12.64±1.21*</td>
<td>15.45±1.21*</td>
<td>14.38±1.21*</td>
</tr>
</tbody>
</table>

§ LCL: longitudinal crypt length, CSCD: cross-sectional crypt diameter, EC/CC: number of epithelial cells per crypt column, GC/EC: goblet cell to epithelial cell percentage ratio, MC/EC: mitotic figure to epithelial cell percentage ratio, DIC: density of inflammatory cells, PID: postinoculation day
† Mice inoculated with *S. hyodysenteriae* serotype 2
‡ Mice inoculated with *S. hyodysenteriae* serotype 4

Inflammatory parameters between TSB- and *S. hyodysenteriae*-inoculated mice were found only in sections taken from the ceca (Table II). Of the cytokinetic parameters examined, the mean LCL was significantly increased (p < 0.01) in the ceca of the serotype 2- and serotype 4-inoculated and streptomycin-untreated mice (groups C and E), and also in the serotype 4-inoculated, streptomycin-treated mice (group F) on PID 7 (p < 0.05). Significantly increased mean LCL (p < 0.05) persisted until PID 14 in group E only. Significantly increased mean CSCD (p < 0.05) was present in the ceca of serotype 2-inoculated and streptomycin-treated mice (group D) but not in the corresponding serotype 4-inoculated mice (group F) on PID 7 and 14. A significant increase in the mean CSCD (p < 0.01) was also present in the ceca of the serotype 4-inoculated and streptomycin-untreated mice (group E) on PID 14. The mean EC/CC was increased significantly (p < 0.01) on PID 7 in the ceca of mice inoculated with serotype 2 (groups C and D), irrespective of streptomycin treatment prior to inoculation. Although the mean GC/EC percentage ratio was unaltered on PID 7 in the ceca of the *S. hyodysenteriae*-inoculated mice (groups C, D, E and F), and on PID 14 in the serotype 4-inoculated mice, it was significantly decreased on PID 14 in the serotype 2-inoculated mice (groups C and D; p<0.05), irrespective of streptomycin treatment. A significant increase in the mean MC/EC percentage ratio (p < 0.01) was present in the ceca of the serotype 4-inoculated mice (groups E and F) on PID 7. However, on PID 14, the mean MC/EC percentage ratio in the ceca of mice inoculated with *S. hyodysenteriae* (groups C, D, E and F) were significantly increased (p < 0.01), irrespective of the serotype or streptomycin pretreatment. No statistically significant differences were found when cytokinetic and inflammatory parameters were compared between streptomycin-treated and -untreated, TSB- and *S. hyodysenteriae*-inoculated groups.

**DISCUSSION**

Results from this study indicated that significant pathological changes associated with *S. hyodysenteriae* infection in C3H/HeN were restricted to the cecum. These findings are in agreement with those reported previously and confirm the usefulness of quantitative measurements of cytokinetic and inflammatory parameters for assessment of large bowel injury and repair during enteric infection of laboratory mice with *S. hyodysenteriae*. Although oral administration of streptomycin for seven days prior to *S. hyodysenteriae* inoculation resulted in a 100 to 1000-fold reduction in the numbers of fecal anaerobe bacteria, it did not affect the colonization, distribution, severity, or progression of cecal lesions associated with cecal infection by *S. hyodysenteriae* in C3H/HeN mice.

The infection rate of C3H/HeN mice inoculated either with *S. hyodysenteriae* serotypes 2 or 4 was nearly 100%, making this strain of mice a reliable model for studies on the pathogenesis of *S. hyodysenteriae* infection (7,8). Isolation of *S. hyodysenteriae* in association with cecal lesions indicated that colonization was required for the initiation of pathological changes. Streptomycin treatment prior to inoculation with *S. hyodysenteriae* had no effect on the colonization of the ceca of mice inoculated with *S. hyodysenteriae*.

Earlier reports on experimental infection of CF-1 mice with *S. hyodysenteriae* (strain DJ70P3) described diarrhea and soft feces with mucus and occasional...
hemorrhagic diarrhea similar to classical swine dysentery of pigs (22). In contrast, studies conducted by us and others using C3H/HeN mice orally gnotobiologically inoculated with various strains of S. hyodysenteriae have indicated soft fecal pellets without evidence of diarrhea (7,8). Serpulina hyodysenteriae strain variation may account for the discrepancies. Unlike the disease in pigs where lesions are present in the cecum and spiral colon, in C3H/HeN mice, lesions and therefore pathophysiological alterations in mucosal transport mechanisms were limited to the cecum (1,7,8). Consequently, compensatory fluid reabsorption in distal segments of large intestine may account for a lack of clinical diarrhea in this model. This is consistent with the lack of a significant effect of S. hyodysenteriae infection on the body weight gain of mice reported here and in previous studies (8).

Histological changes in the ceca of S. hyodysenteriae-inoculated mice were similar to previous descriptions (6,8). In contrast with pigs, in which infection results in diffuse superficial erosion and necrosis of the cecal and colonic mucosa with severe suppurative exudation, mice developed crypt epithelial cell hyperplasia which resulted in significant elongation of the longitudinal length of the cecal crypt columns on PID 7 and 14 (1,6,8). Only slight differences in the onset and the pattern of mucosal response were found between S. hyodysenteriae serotype 2 and serotype 4. On PID 7, an increase in the mean LCL occurred in the ceca of mice inoculated with serotypes 2 and 4; however, a corresponding increase in the mean EC:CC and the mean CSCD occurred only in the ceca of mice inoculated with serotype 2. A statistically significant increase in the mean MC/EC percentage ratio without a significant change in the mean EC:CC occurred on PID 14 in the ceca of mice inoculated with serotypes 2 and 4. These changes are suggestive of accelerated rate of epithelial cell loss resulting from mucosal damage. Differences in the onset of mitotic cell activity between serotypes was attributed to relative differences in the ability of each strain to establish active cecal infection. Progression of the infection resulted in progressive depletion of goblet cells which was statistically significant on PID 14 in the serotype 2-inoculated mice (groups C and D), irrespective of streptomycin pretreatment.

Reduction in the numbers of fecal anaerobe bacteria following oral administration of streptomycin for seven days confirmed earlier reports on the effect of this antibiotic on the intestinal bacterial flora of mice (13,14). Absence of significant differences in gross and histological changes between streptomycin-treated and -untreated S. hyodysenteriae-inoculated mice confirmed our previous studies using subjective evaluation of enteric lesions (8). Although a role for indigenous bacteria of the large intestine in the development of enteric lesions in swine dysentery is well established (6,9,10,11,12), disruption of the delicate balance between the indigenous obligate and facultative gram-negative anaerobes of the cecum by streptomycin did not appear to hinder the colonization of the cecum by S. hyodysenteriae or enhance the host response to infection in this model. These findings suggest that the enteropathogenicity of S. hyodysenteriae is not affected by the colonization resistance conferred by the indigenous bacterial flora of the cecum.

ACKNOWLEDGMENTS

Dr. J.M. Kinyon of Iowa State University at Ames, kindly provided the bacterial isolates for use in this experiment. The authors thank M. Mathiesen, M. Ramanathan, C. Klintworth, R. Schafer, T. Bargar, R. Ellis, R. Olmscheid and L. Johns for their technical assistance. This research paper is published as paper No. 10455, Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska.

REFERENCES


It is generally thought that parasites, in the widest sense (viruses, bacteria, protozoa, etc.), are ill-served by being pathogenic. If they depend on a particular host, it is in the interest of the parasites to preserve the host. Accordingly, the pathogens prevalent today should be mutants in the process of evolutionary attenuation. Not necessarily so, says Dr. Ewald in his book, it may be of evolutionary survival advantage to the parasite to be virulent, if it serves transmission. On the other hand, if transmission is efficient, it may promote increased virulence. The message in this book is provocative and deserving of attention from pathobiologists, epidemiologists, public health researchers, and others who are concerned with control of infectious disease problems. Dr. Ewald uses examples from human medicine, but it should not be difficult to transplant his ideas to the veterinary field. Rather than review the book chapter by chapter the following is an interpretive summary of the essential ideas in the book. (See also the diagram below).

If a parasite has increased opportunities for transmission, for example because the population of potential hosts is large and the individuals are mobile, the fastest growing strains and the most virulent ones, would be favored and selected. This would tend to preserve those with virulence genes. Virulence would also in many circumstances promote transmission, because it might result in a behavior or clinical manifestation which was conducive to transmission or simply increase the number of parasites in the environment. Virulence carries a cost to the parasite, usually an extra energy demand. If the opportunities for transmission are poor, those strains which can persist without detriment to the host and its mobility, and perhaps also persist in the environment, would be favored, since their patience would tend to increase the chance of finding another host. Thus, depending upon the opportunities for transmission, virulence may under one set of circumstances be selected for and under another set of circumstances be selected against. Interestingly, many virulence genes in bacteria are located on mobile genetic elements, which would tend to facilitate relatively fast adaptation, without loosing the main gene pool of the parasite. It is well known that pathogenic bacteria can attenuate quickly during in vitro propagation, but also regain virulence upon passage in suitable host animals. With the very short generation time of many parasites the evolutionary selection needs very little time, relative to the generation time of the host animals, to modulate virulence.

As infections spread in a population, both natural and adaptive resistance emerge. Increased natural resistance is a result of survival of the fittest, and increased adaptive immunity may be a result of homologous immunization or vaccination. This would not favor virulence since the expense of virulence is no longer justified; however, if antigenic variants emerge, this may open new opportunities for transmission and virulence. Similarly, treatment or prevention using antimicrobial drugs would have a strong negative effect on transmission and therefore on virulence, but it is well known that most parasites quickly develop resistance.

If the ideas of Dr. Ewald are correct, they will have implications for prevention and control of infectious diseases in animals. Breeding genetically resistant animals, using vaccines (even if they do not prevent infection), improving sanitation, implementing all-in-all-out production methods, and even the use of chemotherapeutic treatments should promote attenuation and host independence of virulent parasites in animal populations. The empirical observation that infectious diseases seem to come and go, without a good understanding of the reasons, may now generate testable hypotheses, inspired by the ideas presented in the book.

Søren Rosendal.