Published in final edited form as:

J Urol. 2008 February ; 179(2): 759–763. doi:10.1016/j.juro.2007.09.024.

Bacterial Cystitis is Accompanied by Increased Peripheral Thermal Sensitivity in Mice

Dale E Bjorling^{*,#}, Zun-Yi Wang^{*}, Kyle Boldon^{*}, and Wade Bushman[#]

^{*}Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin

[#]Department of Surgery, Division of Urology, School of Medicine, University of Wisconsin

Abstract

Purpose—Visceral inflammation and pain associated with chemical cystitis produces increased sensitivity to noxious stimuli within with the sacral dermatomes. This study was performed to determine whether a similar sensitization occurs in response to bacterial cystitis.

Materials and Methods—Bacterial cystitis was induced by intravesical instillation of *E. coli* 1677 in female C57BL/6N (C57) and C3H/OuJ (OuJ) mice. C3H/HeJ (HeJ) mice were used as a control because HeJ mice lack functional toll-like receptor 4 (*Tlr4*), an essential component of cellular recognition of bacterial lipopolysaccharide (LPS). Hindpaw sensitivity to a thermal stimulus was quantitatively determined 1, 2, 7 and 14 days after infection.

Results—Intravesical instillation of *E. coli* produced infection in all strains of mice. Infection persisted in all OuJ and HeJ mice but spontaneously cleared in some C57 mice. Increased sensitivity to thermal stimuli was observed in C57 and OuJ, starting one to two days after *E. coli* instillation and still present 14 days after instillation. Increased sensitivity to thermal stimuli did not occur in C3H/HeJ mice.

Conclusions—*E. coli*-induced cystitis produced increased sensitivity to peripheral thermal stimuli in mice with competent *Tlr4*.

Keywords

mice; bladder; cystitis; *E. coli*; thermal sensitivity

Introduction

Bacterial cystitis typically induces increased frequency and urgency of micturition, dysuria and suprapubic pain.¹ Antibiotic treatment usually results in rapid resolution of symptoms in most patients. However, some patients treated for presumed cystitis experience persistent painful bladder symptoms despite antibiotic treatment and documented sterile urine. These individuals comprise a subgroup of patients afflicted with painful bladder disorders of uncertain etiology characterized by chronic urinary frequency and suprapubic pain that is transiently alleviated by voiding.^{2,3}

Corresponding Author: Dale E. Bjorling, Department of Surgical Sciences, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706, E-mail: bjorlind@svm.vetmed.wisc.edu, Office: 608-263-4808, Fax: 608-263-7930.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Patients with painful bladder disorders often experience other painful somatic disorders such as irritable bowel syndrome and fibromyalgia with a frequency that exceeds the general population.⁴⁻⁶ This suggests increased sensitivity to visceral and somatic stimuli in patients with painful bladder disorders. Visceral pain is often accompanied by increased sensitivity to peripheral and somatic stimuli,⁷ but basic mechanisms that modulate perception of visceral pain, including that arising from the bladder, remain poorly understood.⁸

We and others have reported previously that cyclophosphamide-induced cystitis in mice is accompanied by increased sensitivity to peripheral mechanical stimuli.^{9,10} However, the effects of bacterial cystitis on sensitivity to application of somatic stimuli have not been reported previously. Preliminary experiments in our laboratory indicated that *E. coli* cystitis in mice reduced the threshold to peripheral application of thermal stimuli. This study addresses the specific hypothesis that bacterial cystitis in mice reduces the threshold of response to application of thermal stimuli to the hind paw and that sensitization is dependent on the inflammatory response to infection. Since persistence of infection and intensity of bladder inflammation induced by *E. coli* infection varies depending upon the presence or absence of competent toll-like 4 receptors (*Tlr4*),¹¹ we used 2 unrelated strains of mice with functional *Tlr4* receptors (C57BL/6N or C57 and C3H/OuJ or OuJ) and a third strain of mice related to the OuJ that lack functional *Tlr4* receptors (C3H/HeJ or HeJ).

Materials and Methods

Animals

All experimental protocols were reviewed and approved by the Animal Care and Use Committee of the University of Wisconsin. Ten to 12 week old C57, HeJ, and OuJ female mice (18-20 g body weight; Jackson Laboratories, Bar Harbor, ME) were used in these experiments.

Intravesical instillations

Mice were anesthetized by intraperitoneal injection of tribromoethanol (Avertin; 250 mg/kg), and PE10 tubing was gently inserted via the urethra into the bladder. The bladder was emptied by application of digital pressure to the lower abdomen. *E. coli* 1677 (10⁸ colony forming units or CFU in 0.1 ml) or sterile saline (0.9%, 0.1 ml) was instilled into the bladder. The catheter remained in place for 30 minutes prior to removal.

Bacteria

The *E. coli* used was isolated from a patient with a severe urinary tract infection and has been shown to be O6, type 1 and P fimbriated, hemolytic, and motile.¹² *E. coli* 1677 was grown overnight in Luria Broth (LB). The concentration of bacteria was determined by spectrophotometry and confirmed by intermittent culture.

Thermal nociception testing

Response to thermal stimuli applied to the hind paw was evaluated by the Hargreaves method prior to and 1, 2, 7, and 14 days after intravesical instillation of *E. coli* or saline.¹³ Mice were placed inside a plastic box, and a focused beam of radiant heat was placed under the animal's paw. Movement of the paw activated a photocell that turned off the light and timer. Latency (in seconds) for the animal to withdraw its paw was recorded, and the apparatus was set to automatically shut off after 15 seconds to prevent burning the paw. On the day of testing, the test was repeated 4 times, with intervals of at least 5 minutes between each test, and the response was recorded as the average of latencies for the 4 replicates.

Urine culture

Urine was collected into sterile Petri dishes prior to and 1, 2, 7, 10, and 14 days after instillation of saline or *E. coli*. Urine was serially diluted 1:100,000 and cultured on LB/agar plates. Plates were incubated overnight at 37° C, and colonies counted.

Euthanasia and tissue collection

Animals were sacrificed by pentobarbital overdose (100 mg/kg ip) after thermal nociceptive testing 1, 2, 7, or 14 days after intravesical instillation of saline or *E. coli*. Bladders and left kidneys were collected sterilely, weighed, and divided in half. One half of each organ was processed for histology, and the other half was used for bacterial culture.

Histology

Tissues were fixed in 4% formalin, sectioned (5 µm thick), and processed for light microscopy. Some sections were stained with hematoxylin and eosin.

Culture of tissues for bacteria

A sample of the bladder dome was excised sterilely, weighed, and placed in a sterile bag containing 500 Sterile Dulbecco's PBS. Tissues were homogenized mechanically, serially diluted to 1:100,000, and diluted solution was plated on LB/agar plates. Plates were incubated overnight at 37° C, and colonies counted. The kidney samples were weighed, homogenized, and cultured in the same manner.

Evaluation of bladder inflammation

Severity of bladder inflammation was estimated by comparing bladder weights (normalized to body weight) among mice after intravesical infusion of saline or *E. coli* and by evaluation of tissue sections stained with H&E. Three sections of each bladder separated by at least 20 µm were evaluated for evidence of edema and infiltration with leukocytes (predominantly neutrophils). Both edema (as evidenced by increased separation of the mucosa from the muscularis) and leukocytic infiltration were often regional in distribution. Each section was therefore divided into quadrants by creating a grid consisting of 2 perpendicular lines that intersected in the center of the lumen of the section. An edema index was determined for each tissue section by scoring the severity of edema in each quadrant as: 0 = no edema evident; 1 = edema present, limited to submucosal region, and width of submucosal region did not exceed combined width of urothelium and detrusor; 2 = edema present in bladder wall but not detrusor, and width of submucosal region greater than combined width of urothelium and detrusor but less than 2 times this distance; 3 = edema present in bladder wall, possibly including occasional areas of detrusor, width of submucosal region 2-4 times width of urothelium and detrusor; and 4 = edema present throughout bladder wall, including detrusor, and width of submucosal region greater than 4 times width of urothelium and detrusor. The edema score for each bladder was the average of scores for four quadrants from three tissue sections separated by at least 20 µm.

Leukocytic infiltration was highly variable and ranged from no or a few cells to focal areas containing too many cells to count individually. Leukocytic infiltration was therefore assessed by again dividing the circumference of the of the tissue section into four quadrants and assigning a score of 0=no leukocytes and 1=leukocytic infiltration for each quadrant. The leukocyte infiltration score was the average of the total score for each of the 3 sections examined.

Statistical analysis

Data were collected chronologically, and group sizes were not less than 6 mice/strain/time interval (e.g., 6 mice of each strain and treatment were sacrificed at each time point). Therefore, data were collected for 30 mice/strain prior to and at 1 day after intravesical instillation of saline or *E. coli*, and 6 mice/strain 14 days after intravesical instillation of saline or *E. coli*. Observations made over time or among groups or strains of mice were compared by analysis of variance (ANOVA). Comparisons of multiple groups were performed using ANOVA *pre hoc* and the Bonferroni method to determine *post hoc* significance. Significance was set at 95% confidence limits. (SAS Institute, Cary, NC, USA)

Results

Thermal nociception testing

Tlr4-competent C57 and OuJ mice were significantly more sensitive to application of thermal stimuli within 1-2 days of intravesical instillation of *E. coli* (Figure 1). There were no significant differences in response to thermal stimuli among strains of mice prior to intravesical instillation of saline or *E. coli* (baseline), and intravesical infusion of saline had no effect on the threshold to thermal nociceptive testing (data not shown). Increased sensitivity of C57 and OuJ mice lasted for the duration of the experiment. However, HeJ mice that are genetically similar to OuJ but lack functional *Tlr4* were unique in that there was no change in threshold of response to application of thermal stimuli after intravesical instillation of *E. coli*.

Urine culture

Positive urine cultures were obtained from all strains of mice after intravesical instillation of *E. coli* but not after intravesical instillation of saline (Figure 2). Urine from OuJ mice contained significantly more bacteria on days 1 and 10 than that from HeJ or C57 mice, but persistent positive urine cultures were obtained from all three strains. However, negative urine cultures were obtained from several C57 mice 10 and 14 days post-instillation.

Culture of tissues for bacteria

Bladder tissues from mice that received intravesical saline were sterile, but positive bacterial cultures were obtained from bladder tissues from all strains of mice 1, 2 and 7 days after intravesical instillation of *E. coli*. Fourteen days after infection, bacteria were cultured from bladder tissue from HeJ and OuJ mice but not in C57 mice (Figure 3). Clearance of bacteria from bladder tissue in C57 mice confirms the somewhat unique capacity of C57 mice to spontaneously resolve bacterial infection within 14 days. Positive cultures were obtained from the kidneys of all mice 1, 2, and 7 days after infusion of *E. coli*. Bacteria were not cultured from kidneys of C57 mice 14 days after instillation of *E. coli* (Figure 4). Although bacteria were cultured from kidneys of HeJ and OuJ mice 14 days after infection, the numbers of bacteria were not significantly increased.

Bladder weight

There were no differences in bladder weights (normalized to body weights) among strains of mice prior to treatment (control), and intravesical saline had no effect on bladder weight. Bladder weight in C57 and OuJ mice was significantly increased 1, 2, and 7 days after intravesical instillation of *E. coli* (Table 1). Intravesical instillation of *E. coli* had no effect on bladder weight of HeJ mice, and bladder weight was not significantly increased in any strain of mice 14 days after inoculation.

Histological evaluation of bladder inflammation

Histological evidence of inflammation appeared generally more severe in C57 and OuJ mice than in HeJ mice (Figure 5). Edema scores and leukocytic infiltration scores for bladders infused with saline remained unchanged from baseline. Edema scores for C57 mice were increased 1, 2 and 7 days after instillation of *E. coli*. Edema scores for OuJ mice were increased 1 day after instillation of *E. coli* but returned to baseline 2 days post-instillation (Table 1). Leukocytic infiltration scores were significantly increased in both C57 and OuJ mice at all time points after intravesical instillation of *E. coli* (Table 1). Neither edema scores nor leukocytic infiltration scores were significantly increased in HeJ mice after intravesical instillation of *E. coli* at any time point studied.

Discussion

Intravesical instillation of *E. coli* induced culture-positive bladder infection in all 3 strains of mice. However, we observed significant differences in the degree of associated inflammation and changes in sensitivity to peripheral thermal stimuli among the strains of mice studied. C57 and OuJ mice both exhibited considerable inflammatory responses to bacterial infection, as measured by bladder weight, edema and leukocyte infiltration. They also both developed increased sensitivity to peripheral thermal stimuli 1-2 days after infection. Interestingly, the more rapid appearance of increased thermal sensitivity in C57 mice appeared to parallel a more robust and/or rapid development of inflammation as indicated by bladder weight and histological appearance (edema and leukocyte infiltration). Increased sensitivity to peripheral thermal stimuli persisted in both C57 and OuJ mice up to 14 days after instillation of *E. coli*.

Differential capacity to spontaneously resolve bacterial cystitis induced by intravesical instillation of *E. coli* 1677 among strains of mice has been reported previously, and it has been observed that C57 mice frequently spontaneously resolve experimental bacterial cystitis within 14 days.^{11,14} We observed decreasing numbers of bacteria in bladder tissue and urine of C57 mice, as well as reduction in bladder weight and edema scores to control values by 14 days. Despite these events, C57 mice continued to exhibit increased leukocyte infiltration scores at all time points examined. The persistence of increased sensitivity to peripheral thermal stimuli 14 days after instillation of bacteria in these mice suggests either that chronic inflammation manifested by leukocyte infiltration is sufficient to maintain the increased sensitivity to thermal stimuli or that the change in sensitivity to thermal stimuli can persist after resolution of acute bacterial cystitis.

The intensity of inflammation induced by *E. coli* cystitis has been reported to be less in HeJ than in C57 or OuJ mice.^{11,14-16} In our study, intravesical instillation of *E. coli* produced numbers of bacteria in the urine and bladder tissue of HeJ mice that were approximately intermediate between those observed in C57 and OuJ mice. In contrast to the other two strains, however, HeJ mice developed minimal histological evidence of cystitis as measured by bladder weight, histological appearance, edema, and leukocyte infiltration. HeJ and OuJ are coisogenic strains of mice, but over time, a dominant negative proline-to-histidine substitution in the gene for *Tlr4* emerged in HeJ.¹⁷ *Tlr4* is thought to be the transmembrane component of the cell membrane-associated complex that recognizes lipopolysaccharide (LPS) formed by gram-negative bacteria, and the absence of *Tlr4* results in a reduced inflammatory response to LPS.¹⁷ The absence of the inflammatory response in HeJ mice following instillation with *E. coli* presumably reflects this attenuated response to LPS. Comparison of the threshold response to peripheral thermal stimuli following bacterial instillation revealed a striking absence of induced hypersensitivity in HeJ mice. This finding suggests that the inflammatory response is necessary to reduce the threshold for response to peripheral thermal stimuli. It has been shown previously that turpentine-induced cystitis in rats was accompanied by increased sensitivity to peripheral thermal stimuli.¹⁸ Nonetheless, it is important to note that we cannot rule out the possibility

that the *Tlr4* receptor is specifically mediates changes in sensitivity independent of its role in the inflammatory response to LPS.

Hypersensitivity to various peripheral noxious stimuli has been demonstrated in patients with chronic pain disorders, including increased sensitivity to mechanical and/or thermal stimuli in patients with irritable bowel syndrome, vulvodynia and painful bladder disorders.^{5,7,19} It is generally recognized that women diagnosed as having painful bladder disorders frequently report a history of recurrent urinary tract infection; however, a consistent relationship between urinary infection and the onset of painful bladder disorders remains unproven.²⁰ The observations reported here suggest that significant changes in afferent sensitivity induced by bacterial cystitis associated with inflammation may contribute to perceived hypersensitivity to peripheral stimuli.

Conclusions

E. coli infection of the bladder accompanied by bladder inflammation induced increased sensitivity to peripheral thermal stimuli. This response was observed in both C57 and OuJ mice. *E. coli* infection of the bladder in HeJ mice was not accompanied by histological evidence of inflammation, and HeJ mice failed to develop increased sensitivity to peripheral thermal stimuli. These experiments provide the first conclusive evidence that bacterial cystitis induces increased sensitivity to somatic stimuli.

Acknowledgements

Supported by NIH awards R01DK57258 and R01DK066349

References

1. Malterud K, Baerheim A. Peeing barbed wire. Symptom experiences in women with lower urinary tract infection. *Scand J Prim Health Care* 1999;17:49. [PubMed: 10229994]
2. Kelada E, Jones A. Interstitial cystitis. *Arch Gynecol Obstet* 2007;275:223. [PubMed: 17021774]
3. Mayer R. Interstitial cystitis pathogenesis and treatment. *Curr Opin Infect Dis* 2007;20:77. [PubMed: 17197886]
4. Alagiri M, Chottiner S, Ratner V, Slade D, Hanno PM. Interstitial cystitis: unexplained associations with other chronic disease and pain syndromes. *Urology* 1997;49(Suppl 5A):52. [PubMed: 9146002]
5. Caldarella MP, Giamberardino MA, Sacco F, Affaitati G, Milano A, Lerza R, et al. Sensitivity disturbances in patients with irritable bowel syndrome and fibromyalgia. *Am J Gastroenterol* 2006;101:2782. [PubMed: 17227524]
6. Clauw DJ, Schmidt M, Radulovic D, Singer A, Katz P, Bresette J. The relationship between fibromyalgia and interstitial cystitis. *J Psychiatr Res* 1997;31:125. [PubMed: 9201654]
7. Bohm-Starke N, Hilliges M, Brodda-Jansen G, Rylander E, Torebjork E. Psychophysical evidence of nociceptor sensitization in vulvar vestibulitis syndrome. *Pain* 2001;94:177. [PubMed: 11690731]
8. Sandner-Kiesling A, Bantel C. New models for visceral pain. *Curr Opin Anaesthesiol* 2003;16:535. [PubMed: 17021508]
9. Guerios S, Wang Z-Y, Bjorling DE. Nerve Growth Factor mediates peripheral mechanical hypersensitivity that accompanies experimental cystitis in mice. *Neurosci Lett* 2006;392:193. [PubMed: 16203088]
10. Bon K, Lichtensteiger CA, Wilson SG, Mogil JS. Characterization of cyclophosphamide cystitis, a model of visceral and referred pain, in the mouse: species and strain differences. *J Urol* 2003;170:1008. [PubMed: 12913760]
11. Hopkins WJ, Gendron-Fitzpatrick A, Balish E, Uehling DT. Time course and host responses to *Escherichia coli* urinary tract infection in genetically distinct mouse strains. *Infect Immun* 1998;66:2798. [PubMed: 9596750]

12. Uehling DT, Hopkins WJ, Jensen J, Balish E. Vaginal immunization against induced cystitis in monkeys. *J Urol* 1987;137:327. [PubMed: 3543412]
13. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988;32:77. [PubMed: 3340425]
14. Hopkins WJ, Gendron-Fitzpatrick A, McCarthy DO, Haine JE, Uehling DT. Lipopolysaccharide-responder and nonresponder C3H mouse strains are equally susceptible to an induced *Escherichia coli* urinary tract infection. *Infect Immun* 1996;64:1369. [PubMed: 8606102]
15. Mysorekar IU, Mulvey MA, Hultgren SJ, Gordon JI. Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic *Escherichia coli*. *J Biol Chem* 2002;277:7412. [PubMed: 11744708]
16. Schilling JD, Mulvey MA, Vincent CD, Lorenz RG, Hultgren SJ. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. *J Immunol* 2001;166:1148. [PubMed: 11145696]
17. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 1998;282:2085. [PubMed: 9851930]
18. Jaggar SI, Scott HCF, Rice ASC. Inflammation of the rat urinary bladder is associated with a referred hyperalgesia which is nerve growth factor dependent. *Br J Anesth* 1999;83:442.
19. Ness TJ, Powell-Boone T, Cannon R, Lloyd LK, Fillingim RB. Psychophysical evidence of hypersensitivity in subjects with interstitial cystitis. *J Urol* 2005;173:1983. [PubMed: 15879797]
20. Warren JW, Diggs C, Brown V, Meyer WA, Markowitz S, Greenberg P. Dysuria at onset of interstitial cystitis/painful bladder syndrome in women. *Urology* 2006;68:477. [PubMed: 16979747]

Abbreviations

C57	C57BL/6N strain of mice
OuJ	C3H/OuJ strain of mice
HeJ	C3H/HeJ strain of mice
<i>Tlr4</i>	toll-like receptor 4
PBS	phosphate-buffered saline
LB	Luria broth
CFU	colony forming units
LPS	lipopolysaccharide

Figure 1. Thermal Nociceptive Testing

The threshold to response to peripheral thermal stimuli as determined by latency (i.e., seconds until paw moved) was significantly reduced from baseline values in C57 mice from day 1 through day 14 and in OuJ mice from day 2 through day 14. Sensitivity to thermal stimuli was unchanged in HeJ mice for the duration of the experiment. (* $p<0.05$, ** $p<0.01$, and *** $p<0.001$, for comparison made between values observed on specific days compared to baseline values for each individual strain)

Figure 2. Bacterial Culture of Urine

Positive bacterial cultures were obtained from all strains of mice at all time intervals; however, three C57 mice had negative urine cultures 10 and 14 days after instillation of *E. coli*. Urine cultures from OuJ mice produced significantly more colony forming units (CFU) than C57 and HeJ mice on day 1 (** $p < 0.01$ for both) and day 14 (* $p < 0.05$ for both).

Figure 3. Bacterial Culture of Bladder Tissue Collected on Day 14

Graphs illustrate distribution and group means of number of CFU/mg bladder tissue on day 14. There was no significant difference between CFU/mg bladder tissue in infected bladders from C57 mice compared to controls (0 CFU/mg bladder tissue), but CFU/mg bladder tissue was increased in HeJ (* $p < 0.05$) and OuJ (** $p < 0.001$) mice compared to controls.

Figure 4. Bacterial Culture of Kidney Tissue Collected on Day 14

Graphs illustrate distribution and group means of number of CFU/mg kidney tissue on day 14.

There were no significant differences between CFU/mg kidney tissue from animals that received intravesical infusion of *E. coli* compared to controls (0 CFU/mg kidney tissue).

Figure 5. Histological Appearance of Bladders

Representative histological slides of bladders from C57, OuJ, and HeJ mice 24 hours after intravesical instillation of saline or *E. coli*. Note significant submucosal edema and leukocytic infiltration in response to *E. coli* that appears more regional in HeJ bladder. (4x magnification; scale bar = 100 μm)

Table 1
Bladder Weights, Edema Scores, and Leukocyte Infiltration Scores

	Control	1 day	2 days	7 days	14 days
Bladder weight (mg/g)	C57	1.13 ± 0.05	1.96 ± 0.17**	1.87 ± 0.13**	1.63 ± 0.09*
	Hel	0.96 ± 0.07	1.22 ± 0.05	1.24 ± 0.03	1.15 ± 0.06
	OuJ	1.06 ± 0.02	1.35 ± 0.04*	1.38 ± 0.11*	1.49 ± 0.07*
Edema score	C57	0	2.25 ± 0.6*	2.1 ± 0.6*	1.29 ± 0.7*
	Hel	0.17 ± 0.09	0.83 ± 0.4	1.04 ± 0.31	0.7 ± 0.28
	OuJ	0	2.52 ± 0.4*	0.88 ± 0.27	0.93 ± 0.21
Infiltration score	C57	0.17 ± 0.17	3.96 ± 0.04**	3.62 ± 0.28*	3.19 ± 0.33*
	Hel	0.11 ± 0.11	1.5 ± 0.42	1.54 ± 0.31	2.3 ± 0.51
	OuJ	0.17 ± 0.17	3.24 ± 0.35*	3.33 ± 0.34*	3.5 ± 0.35*

E. Coli-induced cystitis is characterized by increased bladder weight (mg/g body weight and edema formation and leukocytic infiltration of the submucosa. All values are mean ± SEM. * p<0.05 and ** p<0.01 for comparisons between values observed on specific days after infection and control values for each individual strain.