

Enteropathogenicity of *Escherichia coli* Isolated from Hamsters (*Mesocricetus auratus*) with Hamster Enteritis

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Escherichia coli isolated from ilea of hamsters with hamster enteritis were tested for enteropathogenicity in intestinal loops prepared in both adult and weanling hamsters. *E. coli* isolated from hamsters with hamster enteritis caused dilatation of loops in weanling hamsters but not in adult hamsters.

The inoculation of *Escherichia coli* into ligated loops of rabbit, pig, mouse, or sheep intestine has produced favorable results in the detection of enteropathogenic *E. coli* strains (6, 8, 11, 12). Smith and Halls (12) reported that this technique was most valid when the *E. coli* was tested in the same species from which the isolation was made. The etiological agent of hamster enteritis (HE) is unknown (3); however, *E. coli* has been implicated in the pathogenesis of HE (1, 4, 7). The present study was designed to establish the enteropathogenicity of *E. coli* from hamsters with HE.

Three different strains of *E. coli* (1056, 1126, and 4165) were isolated from ileal lesions of hamsters with naturally occurring HE. Each hamster was from a separate outbreak of the disease. *E. coli* (2995) was isolated from the ileum of a hamster without HE.

Bacteria were incubated at 37°C in Trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, Md.) for 12 h. Before inoculation, cultures were centrifuged at 1,700 × *g* for 15 min, and the supernatant was decanted. The remaining bacteria were suspended in 5 ml of sterile Trypticase soy broth, and this bacterial suspension was used for inoculation. The concentration of the suspensions ranged from 4 × 10⁸ to 1 × 10¹¹ colony-forming units, as determined by plate counts of serial dilutions.

Intestinal loops were formed in adult and weanling hamsters by tying off a section of intestine with ligatures. Anesthesia was by intraperitoneal injection of sodium pentobarbital (Diabotal, Diamond Laboratories, Des Moines, Iowa). Through an abdominal incision, a 7-cm section of intestine was tied off with two 0000 silk ligatures. The anterior ligature was placed approximately 10 cm from the pylorus, and the

second was placed 7 cm posterior to it. Ligatures were placed between mesenteric vessels so that blood flow to the ligated loop was not impaired. Loops of intestine were either not inoculated, inoculated with sterile Trypticase soy broth, or inoculated with an *E. coli* suspended in Trypticase soy broth. For inoculations, 0.2 ml was injected through a 27-gauge needle. Intestines were placed in the abdomen, and the incision was closed. After surgery, hamsters were given water ad libitum but were not given food. Hamsters were killed by chloroform inhalation 8 h after surgery.

At necropsy, intestinal loops were excised intact and graded positive or negative for distension. An index was calculated for each loop by dividing fluid accumulation (milligrams) by length (centimeters). Indexes from the groups were compared by analysis of variance.

Results of intestinal loop inoculations in adult and weanling hamsters are summarized in Table 1. Positive responses were not consistently observed from any inoculum when adult hamsters were used. Statistical analysis of the indexes of weanling hamsters revealed *E. coli* groups 1056, 1126, and 4165 to be significantly different (*P* = 0.05) from uninoculated, sterile Trypticase soy broth, and *E. coli* 2995 groups. *E. coli* isolated from hamsters with HE (*E. coli* 1056, 1126, and 4165), therefore, were enteropathogenic by the ligated intestinal loop technique only when weanling hamsters were used. *E. coli* 2995, which was isolated from a normal hamster, was not enteropathogenic.

Although isolated from a hamster with HE, *E. coli* 4165 did not produce dilatation of all inoculated intestinal loops in weanling hamsters (Table 1). A possible explanation for its comparatively lower enteropathogenicity is that it was passaged on artificial media many times before lyophilization. Arm et al. (2) found that the ability of organisms to cause dilatation when

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TABLE 1. *Detection of enteropathogenic E. coli isolated from hamsters with HE by inoculation of intestinal loops of adult and weanling hamsters*

Group	Adult hamsters		Weanling hamsters	
	No. of positive loops ^a /total no. of loops	Mean of indexes \pm SD ^b	No. of positive loops/total no. of loops	Mean of indexes \pm SD
Uninoculated	1/18	15 \pm 8	1/8	16 \pm 10
Sterile TSB ^c	— ^d	—	1/10	14 \pm 12
<i>E. coli</i> 1056	3/9	30 \pm 28	9/9	117 \pm 82
<i>E. coli</i> 1126	3/10	32 \pm 23	10/10	106 \pm 67
<i>E. coli</i> 4165	3/10	23 \pm 11	7/11	88 \pm 86
<i>E. coli</i> 2995	1/10	18 \pm 11	2/9	39 \pm 38

^a A positive loop had an index above 30.

^b Index, Fluid accumulation (milligrams)/length of loop (centimeters). SD, Standard deviation.

^c TSB, Trypticase soy broth.

^d —, Not done.

inoculated into intestinal loops diminished rapidly when bacteria were repeatedly transferred on artificial media.

Examination of intestinal sections from loops of weanling hamsters by light microscopy was of little value. There was no correlation between distension and lesions of enteritis. Enteritis was observed in the majority of loops, irrespective of group, and was thought to be due to surgical manipulation or distension, or both. Other investigators have also found that microscopic lesions in loops were of little value (10, 13).

The somatic antigens of the enteropathogenic *E. coli* were as follows: for 1056, O103; for 1126, O158; and for 4165, O138; it appears that HE may be similar in this regard to colibacillosis of calves and swine; that is, many different *E. coli* serotypes are enteropathogenic (5).

Loops of weanling hamsters were more sensitive to enteropathogenic *E. coli* than corresponding loops in adult hamsters. Moon and Whipp (9) obtained similar results in pigs. They found that some enteropathogenic *E. coli* could cause positive loops only when pigs were less than 2 weeks old. They thought that swine intestine developed resistance to the effects of certain strains of *E. coli* during the neonatal

period. The present study suggests that a similar resistance may develop in hamster intestine. The fact that weanling hamsters were more sensitive to enteropathogenic *E. coli* was also consistent with the epidemiology of HE; that is, HE occurs most frequently in weanling hamsters.

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LITERATURE CITED

- Amend, N. K., D. G. Loeffler, B. C. Ward, and G. L. Van Hoosier. 1976. Transmission of enteritis in the Syrian hamster. *Lab. Anim. Sci.* **26**:566-572.
- Arm, H. G., T. M. Floyd, J. E. Faber, and J. R. Hayes. 1965. Use of ligated segments of rabbit small intestine in experimental shigellosis. *J. Bacteriol.* **89**:803-809.
- Frisk, C. S., and J. E. Wagner. 1977. Hamster enteritis: a review. *Lab. Anim.* **11**:79-85.
- Frisk, C. S., and J. E. Wagner. 1977. Experimental hamster enteritis: an electron microscopy study. *Am. J. Vet. Res.* **38**:1861-1868.
- Glantz, P. J. 1971. Serotypes of *Escherichia coli* associated with colibacillosis in neonatal animals. *Ann. of N.Y. Acad. Sci.* **176**:67-79.
- Gyles, C. L., and D. A. Barnum. 1967. *Escherichia coli* in ligated segments of pig intestine. *J. Pathol. Bacteriol.* **94**:189-194.
- Jacoby R. O., G. W. Osbaldistron, and A. M. Jonas. 1975. Experimental transmission of atypical ileal hyperplasia of hamsters. *Lab. Anim. Sci.* **25**:465-473.
- McNaught, W., and G. B. S. Roberts. 1968. Enteropathogenicity of strains of *Bacterium coli* isolated from cases of gastro-enteritis. *J. Pathol. Bacteriol.* **76**:155-158.
- Moon, H. W., and S. C. Whipp. 1970. Development of resistance with age by swine intestine to effects of enteropathogenic *Escherichia coli*. *J. Infect. Dis.* **122**:220-223.
- Namioka, S., and M. Murata. 1962. Studies on the pathogenesis of *Escherichia coli*. II. The effect of a substance obtained from pseudoeosinophils of rabbits on the organisms. *Cornell Vet.* **52**:289-296.
- Semjen, P., and L. Pesti. 1973. OK serogroups and loop-dilating effect of *E. coli* strains isolated from pigs. *Acta Vet. Acad. Sci. Hung.* **23**:219-225.
- Smith, H. W., and S. Halls. 1967. Observations by the ligated intestinal segments and oral inoculation method on *Escherichia coli* infections in pigs, calves, lambs and rabbits. *J. Pathol. Bacteriol.* **93**:499-529.
- Taylor, J., and M. P. Wilkins. 1961. The effect of *Salmonella* and *Shigella* on ligated loops of rabbit gut. *Indian J. Med. Res.* **49**:544-549.