INFECTION AND IMMUNITY, Mar. 1978, p. 883-888
0019-9567/78/0019-0883$02.00/0

Vol. 19, No. 3
Printed in U.S.A.

Differences in Serological Responses and Excretion Patterns of Volunteers Challenged with Enterotoxigenic *Escherichia coli* with and without the Colonization Factor Antigen

DOLORES G. EVANS,* TERRY K. SATTERWHITE, DOYLE J. EVANS, JR., AND HERBERT L. DuPONT

Program in Infectious Diseases and Clinical Microbiology, The University of Texas Medical School at Houston, Houston, Texas 77030

Received for publication 5 October 1977

Double-blind studies were performed to compare the virulence of enterotoxigenic *Escherichia coli* with and without the fimbriate colonization factor antigen (CFA), using young healthy adults (mean age, 23 years) as volunteers. In the first study one group of volunteers ingested 1×10^6 *E. coli* H-10407, the CFA-positive strain, and another group ingested 1×10^6 *E. coli* H-10407-P, the CFA-negative spontaneous derivative of strain H-10407. The second study was similar except that the test strains were administered at a dose of 1×10^8 viable cells. Three parameters of infection were monitored: (i) diarrhea and associated symptoms; (ii) excretion pattern of test strains; and (iii) humoral antibody response to CFA, somatic antigen, and heat-labile enterotoxin. Significant signs of illness occurred only in six of seven volunteers who ingested *E. coli* H-10407 at a dose of 1×10^8. At both doses, *E. coli* H-10407-P appeared in the stool on day 1 postchallenge and disappeared by day 4. In contrast, strain H-10407 was persistently excreted from the first to the last day of the study. Also, only those volunteers in the H-10407 challenge groups (12 of 13 analyzed) responded with a fourfold antibody titer rise to CFA, somatic antigen, and/or heat-labile enterotoxin. No reversion of H-10407-P to H-10407 was detected.

It is a generally accepted concept that bacterial colonization of small intestine is a prerequisite to diarrheal diseases caused by enterotoxigenic *Escherichia coli* (ETEC). Colonization of epithelial surfaces, in the absence of actual tissue invasion, and subsequent multiplication to a large ETEC population is consistent with both clinical and experimental observations of ETEC diarrhea in humans (4, 12, 22) and in laboratory animals (8, 13, 22). Evidence for the role of specific surface-associated, fimbriate antigens of ETEC in intestinal colonization was first obtained through the study of animal-specific ETEC. ETEC isolated from swine generally possess the antigen K88 (15), whereas those isolated from calves generally possess K99 (17, 20). K88 and K99, known collectively as adhesion factors, are morphologically indistinguishable from each other and from the so-called common pili of *E. coli* but nevertheless are antigenically and functionally distinct (14, 20). Both K88 and K99 hemagglutinate erythrocytes of different animal species (1, 16). Similarly, ETEC isolated from humans possess a fimbriate colonization factor antigen, termed CFA, distinctly different from K88, K99, and common (type I) pili (6, 7, 19). CFA-positive ETEC of several different serotypes have been isolated from cases of diarrhea in both infants and adults (5, 7, 21).

The role of CFA in mediating intestinal adhesion and colonization has been demonstrated, using an intact infant rabbit diarrhea model (7, 8). A more ideal comparison of the biological effects of live inocula of CFA-positive and CFA-negative ETEC would of course employ the natural host of the pathogen, humans, by challenging and subsequently observing healthy young volunteers. This comparison has now been made with the aid of recently described laboratory techniques (6, 10), which facilitated the rapid identification of the heat-labile enterotoxin (LT)-positive, CFA-positive *E. coli* test strain (H-10407) and its LT-positive, CFA-negative derivative (H-10407-P). A separate report describes in detail the clinical aspects of these volunteer studies; this report describes results relating to CFA as a primary virulence factor in humans and also to the immunological aspects of experimental ETEC infection.

MATERIALS AND METHODS

Volunteers. Volunteers were 15 females and 12 males attending one of several universities in Houston, Tex. These ranged from 18 to 27 years of age (mean,

23 years) and were free of underlying illness, chronic or recurrent gastrointestinal symptoms, or regular medication. Informed consent was obtained by a protocol approved by Committees for the Protection of Human Subjects at The University of Texas Health Science Center at Houston, Baylor College of Medicine, and the Methodist Hospital of Houston, Tex. Pre- and postchallenge medical examinations, medical surveillance, and diarrhea therapy, where required, are described elsewhere (T. K. Satterwhite, D. G. Evans, H. L. DuPont, and D. L. Evans, Jr., manuscript in preparation).

Challenge strains. *E. coli* strain H-10407 (O78:H11) was isolated in 1971 from the stool of an adult with severe non-*Vibrio* cholera in Dacca, Bangladesh (9). H-10407 has been the subject of extensive investigations including challenge of a variety of experimental animals without demonstrating tissue invasiveness or systemic infection (8, 9). Strain H-10407, which is CFA positive, and its stable CFA-negative spontaneous derivative H-10407-P were used as challenge organisms. Both strains are equally positive for LT (8) and differ antigenically only in the presence or absence of CFA; both strains are equally reactive with anti-078 sera. Both strains produce so-called common pili but only in liquid media (5, 8), so that monospecific anti-CFA rabbit sera prepared against purified CFA (7) and also by adsorption of anti-H-10407 serum with H-10407-P cells were available for differentiation purposes (see below). Also strain H-10407 but not H-10407-P was readily detectable by mannose-resistant hemagglutination (MR-HA) of human erythrocytes (6). Neither strain possessed unusual antibiotic resistance properties so that intestinal clearance at the end of the study was easily achieved with ampicillin or tetracycline.

Challenge of volunteers with test strains. Two challenge studies were performed. In the first study seven volunteers were administered 1×10^6 viable *E. coli* H-10407 cells, and an equal number were given 1×10^6 H-10407-P cells from containers coded such that neither the volunteers nor the medical team had knowledge of the code. The same protocol was followed in the second study except that 1×10^8 viable cells of H-10407 and H-10407-P, respectively, were administered.

Challenge inocula were prepared by harvesting cells from Casamino Acids-yeast extract (CYE) agar media (6) that had been inoculated 4 h previously for confluent growth from an overnight aerated brain heart infusion broth culture. Cells were harvested in brain heart infusion broth, adjusted to the appropriate optical density at a 640-nm wavelength, and finally diluted to either 1×10^6 or 1×10^8 bacteria per 50 ml of phosphate-buffered saline (0. 1 M, pH 7.2) for ingestion. These suspensions were prepared within 1 h of challenge. Volunteers were given 2.0 g of sodium bicarbonate (baking soda) in 4 ounces (ca. 120 ml) of water 3 min before ingestion of bacteria. Inoculum size was confirmed by standard plate-count techniques.

Volunteers were administered the challenge organisms in the late afternoon so that the next day was registered as day 1 and so forth. Prestudy blood samples were drawn before challenge, and prechallenge stool samples were also obtained to test for the presence of ETEC, CFA-positive, or O78-positive bacteria.

Clinical observations and specimen collection. Volunteers were monitored for symptoms such as diarrhea, nausea, vomiting, abdominal cramps, and/or fever. Stool was collected, and volume and weight were recorded. Detailed clinical observations are presented elsewhere (Satterwhite et al., manuscript in preparation). Daily stool specimens were transported to the laboratory for bacteriological processing. Postchallenge blood samples were obtained on the last day of the study and approximately 1 month therafter. Sera were divided into small fractions and stored at −65°C.

Detection and identification of challenge strains. *E. coli* were isolated by streaking each stool specimen on Tergitol agar and on CYE agar plates. After 18 h of incubation of the plates at 37°C, well-isolated lactose-positive colonies were selected from the Tergitol agar and inoculated into 2% peptone–0.5% NaCl–2% agar deeps in airtight screw-cap tubes so that each stool specimen was represented by 10 randomly selected *E. coli*-like isolates. CYE agar isolates were used to test the efficiency of the MR-HA assay in detecting strain H-10407 and also to search for possible CFA-positive revertants in stools of volunteers challenged with the CFA-negative strain H-10407-P.

All Tergitol agar isolates were assayed for LT enterotoxin within 72 h of isolation by the direct passive immune hemolysis assay recently described in detail (10). The enterotoxigenicity of both challenge strains was confirmed by the cultured Y-1 adrenal cell assay for LT (3) before each study. Also, randomly chosen CFA-positive and CFA-negative enterotoxigenic (passive immune hemolysis-positive) isolates from the volunteers were confirmed by the Y-1 adrenal cell assay during each study.

All isolates were also tested for CFA by subculturing on CYE agar and testing zones of confluent growth for MR-HA by the slide hemagglutination method recently described (6). Presence of CFA was confirmed by slide agglutination with monospecific serum. Fifty percent of all isolates were randomly selected and tested for O78 antigen.

Serology. Serum antibody against LT enterotoxin of *E. coli* (anti-LT) was determined by microtiter titration, using LT-sensitized sheep erythrocytes to quantitate passive immune hemolysis activity as recently described (11).

Serum antibody against CFA (anti-CFA) was determined by microtiter titration of *E. coli* H-10407 cells, the titer being defined as the reciprocal of the highest dilution of serum producing 100% agglutination of the bacterial cells, as recently described (6, 7). *E. coli* H-10407-P cells were used as a control, and a serum control consisting of rabbit monospecific anti-CFA serum was also used (7).

Serum antibody against the *E. coli* O78 somatic antigen (anti-O78) was determined by passive hemagglutination using O78-sensitized sheep erythrocytes as described by Neter et al. (18). *E. coli* H-10407-P was the source of O78 antigen. The titer was defined as the reciprocal of the highest dilution of serum producing 100% agglutination of the O78 erythrocytes. Each plate contained a control anti-O78 serum obtained from the Center for Disease Control, Atlanta, Ga. The control

consistently produced an agglutination titer of 1:512.

All of the above microtiter titration assays were performed in duplicate, and a fourfold increase in titer (two wells) was considered a significant increase in antibody titer.

RESULTS

Essential clinical observations. A detailed clinical analysis is reported elsewhere (Satterwhite et al., manuscript in preparation). The results may be summarized as follows. At a dose of 1×10^6, there was no discernible difference between the seven volunteers challenged with the strain H-10407 and the seven challenged with strain H-10407-P; only one student in each group had one watery stool each during the study. At a dose of 1×10^8 H-10407 (CFA-positive *E. coli*), six of seven volunteers had watery diarrhea, particularly on days 2 through 4. In the group of six volunteers challenged with the CFA-negative strain H-10407-P, none had watery diarrhea. None of the volunteers experienced fever during the studies.

Excretion patterns of challenge strains at a dose of 1×10^6. From the group of seven volunteers challenged with 1×10^6 H-10407, 390 isolates were analyzed (day 1 to 7), and 192 (49.2%) were positive for both LT and CFA. No LT-positive isolates lacked CFA and no CFA-positive isolates lacked LT. From the group of seven volunteers challenged with 1×10^6 CFA-negative H-10407-P, 400 isolates were analyzed (day 1 to 7), and 27 (6.7%) were Lt positive, although all isolates tested were CFA negative. As can be seen from the excretion patterns presented in Fig. 1, the excretion of H-10407 and H-10407-P were markedly different. Strain H-10407 was present in stools of all volunteers analyzed on day 6, and 60% (three or five available for analysis) were excreting the CFA-positive strain on day 7. On the other hand, strain H-10407-P was present in five of five stools analyzed on day 2, only one of six on day 3, and thereafter none was detected.

Excretion patterns of challenge strains at a dose of 1×10^8. Analysis of *E. coli* isolates for LT production and CFA was performed in a double-blind fashion as described above. From the group of seven volunteers challenged with 1×10^8 H-10407, 460 isolates were analyzed (day 1 to 7), and 333 (72.4%) were positive for both LT and CFA. No LT-positive isolates lacked CFA and no CFA-positive isolates lacked LT. In addition, stool from each volunteer was analyzed on day 8, and 36 of 70 isolates were strain H-10407. From the group of 6 volunteers challenged with 1×10^8 CFA-negative H-10407-P, 400 isolates were analyzed (day 1 to 7) and 92 (23%) were LT positive, although all were CFA negative. As the data in Fig. 1 shows, the excretion of H-10407 and H-10407-P was again very different. H-10407 was present in all stools analyzed on day 7, whereas H-10407-P was present in only one of six stools on day 4 and in none thereafter. H-10407 was excreted by the volunteers for days after diarrhea and other symptoms had subsided, since the mean duration of diarrhea for this group was 43.8 h (range, 21 to 81 h), and total stool volume was only slightly above normal on day 5 and was normal on day 6.

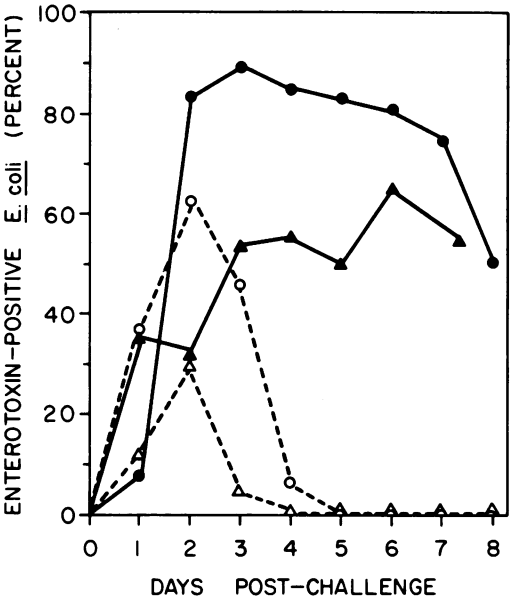

FIG. 1. *Percentage of stool E. coli isolates found positive for LT enterotoxin. Volunteer groups represented are:* (●) *H-10407, 1×10^8 dose;* (○) *H-10407-P, 1×10^8 dose;* (▲) *H-10407, 1×10^6 dose;* (△) *H-10407-P, 1×10^6 dose.*

It should be noted here that vomitus from one volunteer challenged with H-10407, collected on day 3 of the study, was analyzed for the challenge strain. H-10407 was present at 5×10^8 per g of material.

Humoral antibody responses to *E. coli* H-10407 and H-10407-P. Anti-CFA, anti-LT, and anti-O78 antibody determinations were performed on sera collected from the volunteers before challenge and 10 and 30 days after challenge. Table 1 lists data obtained for sera of volunteers challenged with 1×10^6 H-10407 and H-10407-P, respectively. Only those volunteers exposed to the CFA-positive challenge strain demonstrated significant, i.e., fourfold, rises in anti-CFA, anti-LT, and anti-O78 antibody titers. Individually (not shown in Table 1), two volunteers (H-10407, 1×10^6 dose) responded only to

the O78 somatic antigen, one volunteer responded to both CFA and O78, and two volunteers responded with at least a fourfold rise in titer against CFA, O78, and LT antigens. One volunteer in this group had only twofold rises in anti-CFA and anti-LT titer (not significant), and one volunteer's prechallenge serum was unavailable for analysis.

Table 2 lists data for sera of volunteers challenged with 1×10^8 H-10407 and H-10407-P, respectively. As with the lower dose, only those volunteers exposed to the CFA-positive challenge strain demonstrated significant, i.e., fourfold, rises in anti-CFA, anti-LT, and anti-O78 antibody titers. Individually (not shown in Table 2), two volunteers responded to the O78 antigen and LT but not to CFA. One volunteer, who had the highest prechallenge anti-O78 titer (1:256), did not respond to O78 antigen but did respond to both LT and CFA. One volunteer who had the highest prechallenge anti-LT titer (1:64) failed to respond to LT but did respond to both O78 and CFA. Finally, two volunteers showed seroconversion with respect to CFA, O78, and LT antigens.

DISCUSSION

There is ample evidence that colonization of small intestine is a primary facet of the pathogenesis of enterotoxic diarrhea caused by ETEC (4, 12, 22). Since the normal small intestine of humans is essentially free of coliform contamination (2), it follows that, for colonization by ETEC to occur in the absence of intestinal abnormalities, the organisms must be capable of gaining residence in spite of normal host defenses. This further implies that ETEC possess one or more specific virulence factors that facilitate colonization. The surface-associated fimbriate K88 and K99 antigens of animal-specific ETEC function as such colonization factors (15, 20). In 1975, Evans and co-workers reported on the existence of CFA on *E. coli* H-10407 (8). More recently, we demonstrated both the presence of CFA on ETEC isolates from Mexico and the United States and that CFA is not restricted to *E. coli* of serotype O78:H11 (7, 21). Also, CFA activity in an infant rabbit model has been confirmed by immunofluorescent techniques with the interesting result that CFA-positive, but not

TABLE 1. *Serological determinations performed with sera from volunteers challenged with 1×10^6 dose of E. coli H-10407 and H-10407-P, respectively*

Type of antibody	Serum sample (day)	Challenge strain and dose: H-10407 at 1×10^6			H-10407-P at 1×10^6		
		No. of seroconversions	Geometric mean titers	Range of titers	No. of seroconversions	Geometric mean titers	Range of titers
Anti-CFA	0		2.5	(2–4)		2.2	(2–4)
	10	1 of 6	4.0	(2–8)	0 of 7	2.2	(2–4)
	30	3 of 6	6.5	(4–8)	0 of 7	2.2	(2–4)
Anti-LT	0		11.3	(2–32)		19.5	(4–32)
	10	2 of 6	23.8	(16–32)	0 of 7	21.5	(8–32)
	30	2 of 6	29.0	(16–32)	0 of 7	21.5	(8–32)
Anti-O78	0		25.4	(8–128)		43.1	(16–128)
	10	5 of 6	231.9	(64–1024)	0 of 7	47.6	(16–128)
	30	3 of 6	70.6	(32–128)	0 of 7	43.1	(8–256)

TABLE 2. *Serological determinations performed with sera from volunteers challenged with 1×10^8 dose of E. coli H-10407 and H-10407-P, respectively*

Type of antibody	Serum sample (day)	Challenge strain and dose: H-10407 at 1×10^8			H-10407-P at 1×10^8		
		No. of seroconversions	Geometric mean titers	Range of titers	No. of seroconversions	Geometric mean titers	Range of titers
Anti-CFA	0		2.8	(2–4)		2.5	(2–4)
	10	4 of 6	17.9	(4–32)	0 of 6	2.5	(2–4)
	30	4 of 6	12.7	(4–32)	0 of 6	3.2	(2–8)
Anti-LT	0		16.0	(4–64)		28.5	(16–32)
	10	4 of 7	47.6	(16–128)	0 of 6	32.0	(16–64)
	30	3 of 7	39.0	(16–64)	0 of 6	32.0	(16–64)
Anti-O78	0		47.5	(16–256)		25.4	(16–64)
	10	6 of 7	689.1	(256–2,048)	0 of 6	28.5	(16–64)
	30	2 of 7	105.0	(32–256)	0 of 6	28.5	(16–64)

CFA-negative, ETEC colonize only the upper portion of the small intestine in intact animals (7). CFA-positive isolates, but not CFA-negative isolates or derivative strains, hemagglutinate human erythrocytes, and this hemagglutination activity is mannose resistant (6). Availability of the MR-HA assay for *E. coli* H-10407 determined the feasibility of the studies reported here.

Briefly, the following results, derived from oral challenge of four groups of volunteers with 1×10^6 and 1×10^8 *E. coli* H-10407 and H-10407-P, respectively, clearly indicate that CFA is a necessary prerequisite for virulence of ETEC in humans. At a dose of 1×10^6 bacteria, neither the H-10407 nor the H-10407-P groups showed overt diarrhea, but strain H-10407 showed an extended excretion pattern, indicative of colonization, whereas H-10407-P was not detectable in stool after the day 4 postchallenge. Colonization was also indicated by fourfold antibody titer rises against CFA, O78, and LT, none of which occurred in volunteers challenged with H-10407-P. At a dose of 1×10^8 bacteria, six of seven volunteers challenged with H-10407 experienced diarrhea, and H-10407- , but not H-10407-P- , challenged volunteers showed seroconversion to the three antigens tested. At a dose of 1×10^8, strain H-10407 became the predominant coliform isolated, approaching 89% on day 3 postchallenge and remaining above 50% through and including day 8, several days after overt diarrhea had spontaneously subsided. One reason for expressing the recovery of the challenge strains of *E. coli* in terms of percentage data is the unavoidable fact that plating techniques only detect the most populous *E. coli* flora. An important observation was that vomitus from one volunteer obtained 3 days after challenge with H-10407 contained 5×10^8 H-10407 per g, indicating a large population of the challenge strain in the upper gastrointestinal system.

It is noteworthy of mention that no CFA-positive isolates were recovered from the 13 volunteers challenged with H-10407-P, although 800 *E. coli* isolates were examined in a double-blind fashion from Tergitol agar and at least 1,000 isolates were examined after primary isolation on CYE agar. This result indicates that reversion of H-10407-P to H-10407 (CFA negative to CFA positive) did not occur in vivo.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant RR-00350 from the Clinical Research Centers Branch, grants AI-12699 and AI-13385 from the National Institute of Allergy and Infectious Diseases, and by contract no. 200-75-0536 from the Center for Disease Control, Atlanta, Ga.

We acknowledge the support and cooperation of Robert B. Couch, Director of Volunteer Studies, Vernon Knight, and the staff of The Methodist Hospital at Houston, Tex. Also, the technical support and assistance of P. Byers, C. Geib, B. H. George, S. Hamilton, D. Johnson, D. Satterwhite, M. K. Scepansky, W. S. Tjoa, and H. West is greatly appreciated.

LITERATURE CITED

1. **Burrows, M. R., R. Sellwood, and R. A. Gibbons.** 1976. Haemagglutinating and adhesive properties associated with the K99 antigen of bovine strains of *Escherichia coli*. J. Gen. Microbiol. **96**:269–275.
2. **Dickman, M. D., A. R. Chappelka, and R. W. Schaedler.** 1976. The microbial ecology of the upper small bowel. Am. J. Gastroenterol. **66**:57–62.
3. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183**:334–336.
4. **DuPont, H. L., S. B. Formal, R. B. Hornick, M. J. Snyder, J. P. Libonati, D. G. Sheaman, E. H. LaBrec, and J. P. Kalas.** 1971. Pathogenesis of *Escherichia coli* diarrhea. N. Engl. J. Med. **285**:1–9.
5. **Evans, D. G., D. J. Evans, Jr., and H. L. DuPont.** 1977. Virulence factors of enterotoxigenic *Escherichia coli*. J. Infect. Dis. **136**:S118–S123.
6. **Evans, D. G., D. J. Evans, Jr., and W. S. Tjoa.** 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. **18**:330–337.
7. **Evans, D. G., D. J. Evans, Jr., W. S. Tjoa, and H. J. DuPont.** 1978. Detection and characterization of colonization factor of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea. Infect. Immun. **19**:727–736.
8. **Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12**:656–667.
9. **Evans, D. J., Jr., and D. G. Evans.** 1973. Three characteristics associated with enterotoxigenic *Escherichia coli* isolated from man. Infect. Immun. **8**:322–328.
10. **Evans, D. J., Jr., and D. G. Evans.** 1977. A direct serological assay for the heat-labile enterotoxin of *Escherichia coli* using passive immune hemolysis. Infect. Immun. **16**:604–609.
11. **Evans, D. J., Jr., G. Ruiz-Palacios, D. G. Evans, H. L. DuPont, L. K. Pickering, and J. Olarte.** 1977. Humoral immune response to the heat-labile enterotoxin of *Escherichia coli* in naturally acquired diarrhea and antitoxin determination by passive immune hemolysis. Infect. Immun. **16**:781–788.
12. **Gorbach, S. L., J. G. Banwell, B. D. Chatterjee, B. Jacobs, and R. B. Sack.** 1971. Acute undifferentiated diarrhea in the tropics. I. Alterations in intestinal microflora. J. Clin. Invest. **50**:881–889.
13. **Guerrant, R. L., U. Ganguly, A. G. T. Casper, E. J. Moore, N. F. Pierce, and C. C. J. Carpenter.** 1973. Effect of *Escherichia coli* on fluid transport across canine small bowel: mechanism and time-course with enterotoxin and whole bacterial cells. J. Clin. Invest. **52**:1707–1714.
14. **Isaacson, R. E.** 1977. K99 surface antigen of *Escherichia coli*: purification and partial characterization. Infect. Immun. **15**:272–279.
15. **Jones, G. W., and J. M. Rutter.** 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun. **6**:918–927.
16. **Jones, G. W., and J. M. Rutter.** 1974. The association of K88 with haemagglutinating activity in porcine strains of *Escherichia coli*. J. Gen. Microbiol. **84**:135–144.

17. **Myers, L. L., and P. A. M. Guinee.** 1976. Occurrence and characteristics of enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. Infect. Immun. **13**:1117-1119.
18. **Neter, E., L. F. Bertram, D. Z. Zak, M. R. Murdock, and C. E. Arbesman.** 1952. Studies on hemagglutination and hemolysis of *Escherichia coli* antisera. J. Exp. Med. **96**:1-15.
19. **Ørskov, I., and F. Ørskov.** 1977. Special O:K:H serotypes among enterotoxigenic *E. coli* strains from diarrhea in adults and children. Med. Microbiol. Immunol. **163**:99-110.
20. **Ørskov, I., F. Ørskov, H. W. Smith, and W. J. Sojka.** 1975. The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen, previously called "Kco," possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. **83**:31-36.
21. **Ryder, R. W., I. K. Wachsmuth, A. E. Buxton, D. G. Evans, H. L. DuPont, E. Mason, and F. F. Barrett.** 1976. Infantile diarrhea produced by heat-stable enterotoxigenic *Escherichia coli*. N. Engl. J. Med. **295**:849-853.
22. **Sakazaki, R., K. Tamura, A. Nakamura, T. Kurata, A. Gohda, and S. Takeuchi.** 1974. Enteropathogenicity and enterotoxigenicity of human enteropathogenic *Escherichia coli*. Jpn. J. Med. Sci. Biol. **27**:19-33.