Susceptibility of Gnotobiotic Swine to Escherichia coli Isolated from Nonenteric Human Infections

R. C. MEYER, H. E. RHOADES, AND J. SIMON

Department of Veterinary Pathology and Hygiene, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received for publication 24 February 1972

Newborn, germfree piglets were susceptible to Escherichia coli associated with human, nonenteric infections and should provide a useful model in the study of generalized E. coli infections.

The role, in infantile diarrhea, of certain enteropathogenic Escherichia coli (EEC) has been long recognized (1) and is well documented (14). An awareness has also developed in the past 10 years as to the role of certain E. coli serotypes encountered in nonenteric infections (3, 10, 15). Currently, infections caused by gram-negative bacilli represent one of the more serious challenges to the clinician and bacteriologist, and such infections constitute a growing concern both to the surgical and medical services in our hospitals.

In the case of the EEC, virulence appears dependent upon the elaboration of an enterotoxin (4, 11, 13). Little is known, however, about the virulence factors associated with the more invasive strains that are commonly found in nonenteric and often fatal generalized infections.

To date, conventional laboratory animals have been of limited value as an experimental model in the study of nonenteric E. coli infections. Although several unsuccessful attempts have been carried out to reproduce disease in piglets with EEC associated with infantile diarrhea (5, 9), the following report indicates for the first time that newborn, germfree piglets are susceptible to E. coli from human nonenteric infections and in light of previous work (7, 8) should be investigated more fully as a means to study the pathogenesis of generalized E. coli infections.

Only 2-day-old newborn, germfree (GF) piglets were used. Methods of procurement, rearing, and microbiological monitoring were as previously described (6). In the case of the pathogenic strains, three separate trials were conducted with two piglets per trial from litters with different genetic backgrounds. A total of 42 pigs (34 experimental and 8 controls) representing 8 different litters were used.

The seven E. coli isolates employed were selected from a large number provided by a local hospital. Although all had been thought to be associated with an infectious process, we had no knowledge as to their true virulence, if any, or if other bacterial agents also had been cultured from the original specimen. Those E. coli cultures used were selected primarily on the nature of the clinical specimen. Four of the cultures were associated with urinary tract infections, and the other three isolates were from (i) cerebrospinal fluid, (ii) sputum from a case of pneumonia, and (iii) an axillary abscess (Table 1).

The agents in question had been isolated on blood agar, transferred to Brain Heart Infusion (BHI) agar slants (Difco), and maintained on BHI slants until biochemically characterized and their pathogenicity for GF piglets could be determined.

The general procedures relative to preparation of inoculum, oral administration of the agents, preliminary serotyping as to O group, necropsy, etc., were as previously reported (7, 8). To obtain a more detailed serotyping relative to K antigens, the cultures were sent to G. J. Hermann, Assistant Chief, Enterobacteriology Unit, Center for Disease Control, Atlanta, Ga. The K antigens, however, were not identified. Results of the serotyping are summarized in Table 1.

Of the seven cultures examined for disease-producing potential, three were pathogenic, capable of initiating a generalized infection leading to the death of GF piglets usually.
within 2 to 5 days postinoculation. Bacteremia was a constant feature of these infections, with signs of a peritonitis usually evident at time of necropsy. The remaining four cultures, although colonizing the gastrointestinal tract of the challenged piglets, had no discernible effect and conceivably may not have had a direct role in the infectious process observed in the patient from which the agent was obtained.

All seven strains upon reisolation from infected piglets were hemolytic on bovine blood agar. The three pathogens, however, possessed readily demonstrable capsules, whereas the nonpathogens after passage in piglets still appeared to lack well-defined capsules. Attempts to produce a keratoconjunctivitis with the three pathogens in both young rabbits and guinea pigs (Séreny test) were negative, as were the four nonpathogens (2, 12).

There are many factors that contribute to, or predispose an individual to, an infection by *E. coli*. Some of these are host-related and may be associated with some pre-existing disease such as diabetes mellitus. Others are iatrogenic and stem from such things as surgical manipulations, the use of immunosuppressive drugs, etc. The remaining are those that may be considered inherent to the microorganism in that members of some serotypes simply are more invasive than others and possess virulence factors unique to the bacterium in question (3).

The bacteriologist can do little relative to the first two areas; however, he can make a contribution in the last area by devising tests that will facilitate the rapid identification of the potential pathogen. The standard biochemical reactions alone are not sufficient to assess the pathogenicity of *E. coli* and other gram-negative bacilli. The question as to the pathological significance of an isolate of *E. coli* has not in all cases been satisfactorily answered, and there are as yet no all-inclusive criteria to be met in proving that an isolate is indeed a true pathogen. However, once the virulence factors are recognized, diagnostic tests for the presence of these factors undoubtedly will follow.

We feel this animal host system and technique more closely resemble a natural situation when compared to intraperitoneal injections of mice with bacterial suspensions in 5% hog gastric mucin or to the starvation of guinea pigs for 4 days followed by the administration of relatively large volumes of material via a stomach tube.

Additional work will be required, but preliminary findings with *E. coli* of human origin indicate that the GF piglet may provide a very useful laboratory model for studying the pathogenesis of generalized *E. coli* infections and the virulence factors associated with invasive strains of this bacterial species.

We thank H. P. Friedman, Carle Foundation Hospital, Urbana, Ill., for his cooperation in securing the *E. coli* isolates used in this study, and G. J. Hermann, Center for Disease Control, Atlanta, Ga., for serotyping. The assistance of Charles Campbell and Jane Hinch is also acknowledged.

This study was supported by funds from the Swine Disease Research Program, Illinois Department of Agriculture.

**LITERATURE CITED**

1. Bray, J., and T. E. D. Beavan. 1948. Slide agglutination of *Bacterium coli* var. *neapolitanum* in summer diarrhea. J. Pathol. Bacteriol. 60:395–401.

2. Cross, W. R., and M. Nakamura. 1970. Analysis of the virulence of *Shigella flexneri* by experimental infection of the rabbit eye. J. Infect. Dis. 122:394–400.

3. Erickson, A. L., M. W. Fisher, L. A. Gagliardi, I. A. Pearson, and B. A. Waisbren. 1961. Characteristics of strains of *Escherichia coli* associated with severe infection in adults. J. Infect. Dis. 108:189–194.

4. Gyles, C. L., and D. A. Barnum. 1969. A heat-labile enterotoxin from strains of *Escherichia coli* enteropathogenic for pigs. J. Infect. Dis. 120:419–426.

5. Meyer, R. C., E. H. Bohl, R. D. Henthorne, V. L. Tharp, and D. E. Baldwin. 1963. The procurement and rearing of gnotobiotic swine. Lab. Anim. Care 13(Part 2):655–663.

6. Meyer, R. C., E. H. Bohl, and E. M. Kohler. 1964. Procurement and maintenance of germ-free swine for microbiological investigations. Appl. Microbiol. 12:295–300.

7. Meyer, R. C., H. E. Rhoades, S. P. Saxena and J. Simon. 1971. *Escherichia coli* isolated from domestic animals pathogenic for gnotobiotic piglets. Infect. Immun. 3:735–738.

8. Meyer, R. C., S. P. Saxena, and H. E. Rhoades. 1971. Polysaccharides induced by *Escherichia coli* in gnotobiotic swine. Infect. Immun. 3:41–44.

9. Minias, O. P., L. Mitchell, and D. A. Barnum. 1970. Response of gnotobiotic pigs to *Escherichia coli*. Can. J. Comp. Med. 34:260–276.

10. Rantz, L. A. 1962. Serological grouping of *Escherichia coli*: a study in urinary tract infection. Arch. Intern.
11. Sack, R. B., S. L. Gorbach, J. G. Banwell, B. Jacobs, B. D. Chatterjee and R. C. Mitra. 1971. Enterotoxogenic Escherichia coli isolated from patients with severe cholera-like disease. J. Infect. Dis. 123:378-385.
12. Sőreny, B. 1957. Experimental keratoconjunctivitis shigellosa. Acta Microbiol. Acad. Sci. Hung. 4:367-376.
13. Smith, H. W., and S. Halls. 1967. Studies on Escherichia coli enterotoxin. J. Pathol. Bacteriol. 93:531-543.
14. Tennant, B. 1971. Neonatal enteric infections caused by Escherichia coli. Ann. N.Y. Acad. Sci. 176:9-405.
15. Turck, M., and R. C. Petersdorf. 1962. The epidemiology of nonenteric Escherichia coli infections: prevalence of serologic groups. J. Clin. Invest. 41:1760-1765.