Escherichia coli Isolated from Domestic Animals
Pathogenic for Gnotobiotic Piglets

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Three strains of Escherichia coli isolated from infectious processes in a calf, a dog, and a cat were examined for their capacity to produce disease or death, or both, in newborn gnotobiotic piglets. The O groups represented by these particular strains of E. coli were O4: (canine origin), O6: (feline origin), and O39: (bovine origin). All three isolates upon oral administration proved to be pathogenic. Infection with the E. coli O4: (canine) or O39: (bovine) consistently produced signs of enteric colibacillosis and death in all 1- and 3-day-old piglets within 24 to 48 hr. The O6: (feline) isolate, on the other hand, produced a marked polyserositis and generally required 6 to 7 days to kill a piglet. Only the respective type of E. coli used in the particular trial was recovered from the diseased piglets. These findings suggest the possible role of domestic animals and household pets in the spread of potentially pathogenic E. coli to other species.

The past decade has seen marked changes in the production and management of livestock. The trend toward larger herds, confinement-type housing, and year-round farrowing for swine are excellent examples of such change. These newer procedures, by introducing many time- and labor-saving features, unquestionably provide considerable convenience to the producer, but they can, in turn, complicate disease control. Enteric infections of the neonatal pig still constitute a major problem to the swine producer, and the control and treatment of such infections, when they occur in a confinement-type production facility, may be both difficult and unsuccessful (12).

From an engineering viewpoint, a wide variety of designs for rearing large numbers of livestock in a limited area are possible, but infectious diseases and related ecological-biological problems undoubtedly will prove to be the limiting factors governing high-density confinement-type production of livestock.

The role of Escherichia coli in infections of the newborn of various species has been long recognized (15), but the sources of these pathogenic strains and the means by which they can be introduced to a herd or population has remained, in many cases, conjectural.

Pathogenic E. coli have been recovered from asymptomatic adult carriers of various species of animals and man as well as from dust, air, old litter, etc. (1, 2). Although several reports exist on the isolation of E. coli serotypes associated with human infantile gastroenteritis from household pets (7, 11) and cattle (3), the role of such animals and man as a source or reservoir of E. coli pathogenic for swine has remained largely unexplored.

The following report deals with pathogenicity studies on three isolates of E. coli in neonatal gnotobiotic swine. These strains of E. coli belonged to O groups not commonly associated with diseases of swine, and were isolated from animals other than swine: (i) E. coli O39: (bovine origin), (ii) E. coli O4: (canine origin), and (iii) E. coli O6: (feline origin).

MATERIALS AND METHODS

Gnotobiotic piglets. Only 1- and 3-day-old newborn germ-free piglets were used. All were obtained by hysterectomy and maintained in pen-tub-type isolators. Methods of procurement, rearing, and microbiological monitoring were previously described (7).

In the initial trials with the three isolates of E. coli, only 1-day-old piglets were used. With the production of clinical disease or death, or both in these piglets, the experiments were repeated two additional times with the O39: and O4: cultures and three additional times with the O6: culture by using litters of different genetic background. In these subsequent trials, some of the piglets were challenged orally at 1 day of age as before and others at 3 days. A total of 49 pigs including 36 experimental and 13 controls were used.

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Pathogenic isolates of E. coli. E. coli O39: (bovine origin) was obtained from a 3-day-old calf which died of pneumatenteritis. It was hemolytic on bovine blood-agar and was the dominant O group isolated from a number of newborn calves afflicted with a similar condition on a large dairy farm in South Central Illinois.

E. coli O4: (canine origin) was also hemolytic on bovine blood-agar and was isolated both from newborn puppies with an enteritis and from their German shepherd dam. The bitch had a history of a vaginitis before and after whelping. The puppies 2 days after birth developed severe diarrhea. Specimens obtained from the puppies and post partum vaginal discharge from the bitch yielded hemolytic O4: E. coli. In the first trial, O4: isolates both from the puppies and the bitch were used. After learning that both isolates were equally pathogenic, only the O4: isolate from the bitch was used in the succeeding trials.

E. coli O6: (feline origin) was isolated from a young cat with profuse diarrhea, in a colony of cats the majority of whose members were also afflicted. This particular O group (O6:) was the predominant one in this cat population and was hemolytic as the other above isolates (10).

Preparation of inoculum. All of the isolates were initially isolated on blood-agar plates, recloned, maintained on heart infusion agar slants, and held at room temperature until their pathogenicity for newborn piglets could be determined. The general procedures were similar to those described in a previous report (9).

The challenge dose for each pig consisted of 1 cc of an 18-hr Brain Heart Infusion broth culture administered orally by means of a 5-cc syringe fitted with a blunt 18-gauge needle. After the first trial demonstrated the virulence of a particular bacterium, the agent was lyophilized to preserve its properties and provide a uniform seed stock for succeeding confirmation trials.

Serotyping of isolates. Typing procedures were as previously reported (4); however, the K and H antigens were not identifiable with the reagents available either from the National Communicable Disease Center (Microbiological Reagents, 1967 ed.) or the typing-sera prepared in our laboratory.

Necropsy procedures. All necropsies were performed in an aseptic manner. Specimens collected for bacteriological examination consisted of brain, heart, blood, lung, spleen, liver, kidney, mesenteric lymph nodes, peritoneal fluid, and stomach and intestinal contents. The specimens were cultured on blood and MacConkey agar at an incubation temperature of 35°C. Gram-stained smears from such specimens were also examined by direct microscopy.

Virus isolation attempts. Virus isolation attempts were made on piglets employed in the first trial with each of the three different cultures but repeated only on piglets infected with the O6: (cat origin) culture on the fourth trial. The procedures were essentially the same as those of Hancock et al. (6). Fecal specimens and 10% tissue suspensions of lung, liver, and kidney were clarified by centrifugation, and antibiotics were added and inoculated into monolayer swine kidney cell cultures. Both 4-oz prescription bottles and tube cultures with cover slips were used. After intervals of 3, 5, and 8 days, the cells were fixed in absolute methyl alcohol stained with May-Grünwald-Giemsa stain and examined for possible virus-induced alterations.

Histopathological procedures. Tissues for histopathological examination consisting of brain, lung, liver, spleen, kidney, heart, mesenteric lymph nodes, and segments of the gastrointestinal tract were fixed in neutral 10% Formalin. Sections were cut at 5 μm and stained with hematoxylin and eosin.

RESULTS

Clinical signs. The clinical disease that resulted from infection by the O39: (bovine origin) and O4: (canine origin) was similar to that caused by agents isolated from swine with typical signs of enteric colibacillosis (9). These particular agents (bovine and canine origin) killed all pigs exposed to them, usually within 24 to 48 hr. The infected piglets generally appeared bright during the first 24 hr after exposure and then died very quickly. The signs observed were typical of acute colibacillosis: (i) loss of appetite, (ii) depression, (iii) greenish-yellow watery stools, (iv) dehydration, and (v) coma and death.

With the O6: (feline origin) culture, the piglets developed signs on the 5th day postinfection. The major signs were depression and anorexia. Diarrhea was not apparent. Of 16 piglets, 12 died between the 6th and 7th day and 3 recovered. Approximately one-half of the pigs that died had signs of respiratory distress before death.

Gross pathological findings. Piglets succumbing to infection with either the O39: (bovine) or O4: (canine) strain failed to develop significant gross lesions. The stomachs usually contained coagulated milk. The contents of the small intestine consisted of small gas pockets and a greenish-yellow watery fluid. The contents of the large intestine were also fluid in nature. There appeared to be little or no inflammation of the intestinal tract or the mesenteric lymph nodes. However, areas of the stomach of O39: (bovine)-infected piglets had small areas of hyperemia. The gross lesions of piglets infected with the O6: (feline) culture were extensive and markedly different. Unlike that observed in O39: and O4: infected piglets, a polyserositis was present which involved the peritoneal, pericardial, and pleural surfaces. In numerous instances, the serofibrinous exudate was so profuse it literally encased the thoracic and abdominal viscera. The intestinal contents of most pigs appeared normal, and fecal material was semisolid, not watery as in the O4: and O39: infected piglets.

Microbiological findings. Blood and tissue specimens collected at necropsy for bacteriological examination yielded only the respective E. coli employed. A bacteremia was a consistent feature of the pathogenesis of the disease. No
other bacterial agents were demonstrable in the experimental piglets either by culture or by direct microscopic examination of stained smears prepared from various tissues. Invariably *E. coli* of the respective type used in each trial was readily recovered from all sections of the gastrointestinal tract.

The inoculation of prepared fecal and tissue suspensions into primary swine kidney cell cultures failed to reveal the presence of a cytopathic virus.

**Histopathological results.** The histological changes of the O39: (bovine) and O4: (canine) infections were neither marked nor well defined and may be summarized as follows. Areas of congestion and submucosal edema were observed in the stomach. In the small intestine, evidence of a mild enteritis was observed with some necrotic debris in the lumen. In the spleen and liver, congestion with mild degenerative changes was noted.

The O6: (feline)-infected piglets had a marked peritonitis, pericarditis, and myocarditis, pleuritis associated with bronchopneumonia, marked splenic congestion, hepatitis, lymphadenitis, renal congestion, and edema of the large intestine as well as congestion of the brain and spinal cord.

**DISCUSSION**

During the past several years, our laboratory has been engaged in characterizing pathogenic *E. coli* strains and assessing their role in diseases of baby pigs (4, 9, 10; R. C. Meyer et al., *Bacteriol. Proc.*, p. 69, 1969). During this period, *E. coli* strains representing 16 specific O groups isolated from swine and various other animals have been examined for their capacity to produce disease in germ-free piglets. In each case, the isolate was thought to be associated with the disease process in the animal from which it had been obtained and was selected on that basis. Of these, a total of three have been identified as highly pathogenic for 1- and 3-day-old piglets. These agents resulted in enteric colibacillosis and generally killed piglets in 24 to 48 hr. Of these three, one was of swine origin (9) and the second, the O39: (bovine), and the third, O4: (canine), as reported in this study. The O6: (feline) is also considered a pathogen, but, as noted (10), it did not consistently kill all piglets and the resultant disease did not fit any of the classical clinical forms ascribed to *E. coli* infections in swine (15). Two additional agents of swine origin, and O70: and O139:, were of moderate virulence and usually killed less than half of the piglets challenged (Table 1). The remaining 10 isolates of swine origin had no discernible adverse effect upon newborn germ-free piglets. No further work has been done with these isolates since they did not appear to possess a significant disease-producing potential.

<table>
<thead>
<tr>
<th>O group</th>
<th>Species of origin</th>
<th>Virulence*</th>
<th>Clinical disease induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>O4:</td>
<td>Dog</td>
<td>++++</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>O6:</td>
<td>Cats</td>
<td>+++</td>
<td>Polyserositis</td>
</tr>
<tr>
<td>O8:</td>
<td>Swine</td>
<td>+++++</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>O39:</td>
<td>Calf</td>
<td>+++++</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>O70:</td>
<td>Swine</td>
<td>++</td>
<td>Septicemia</td>
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<tr>
<td>O139:</td>
<td>Swine</td>
<td>++</td>
<td>Septicemia</td>
</tr>
</tbody>
</table>

* Symbols: ++++, 100% mortality rate of 1- to 3-day-old piglets; ++, 75% mortality rate; +, 50% mortality rate.

There can be little doubt that a variety of subsidiary factors can play a role in *E. coli* infections. The role of age in susceptibility and resistance is recognized, and it is well known that enteropathogenic strains generally are without effect or induce only mild, transitory reactions in adults. In the case of our studies with germ-free piglets, it was seldom possible to produce disease by the oral administration of known pathogenic *E. coli* to piglets over 1 week of age. Thus, even with conditional pathogens, there usually are mechanisms whereby the newborn animal may resist bacterial invasion and adjust itself to an equitable host-parasite relationship.

Household pets such as dogs and cats have been reported capable of serving as carriers of *E. coli* that are pathogenic for man (7, 11), and *E. coli* serotypes responsible for scour in calves have also been encountered in human infantile gastroenteritis (3, 13).

In general, the strains isolated from disease states in various domestic animals and fowl tend to be of specific serotypes, that is certain serotypes tend to be associated with a given species of animal (15). However, little is known about the host species specificity and interspecies susceptibility to such agents. Moreover, as indicated by this study, a given isolate of *E. coli* may be pathogenic for more than one species, and the adults of one animal species, although not clinically ill, may still harbor strains of *E. coli*, which to the young of another species could conceivably constitute a serious threat.

Since most farms have pet dogs and cats, it would be advisable to exclude such animals from the farrowing area. Generally, however, it has been observed that on many, if not most, farms a pet dog or cat will have complete access of the premises.

Although it is believed that other swine still
constitute the major source of *E. coli* that are pathogenic for newborn pigs, other members of the animal kingdom including the swine producer himself should not be excluded as a source.

Apparently, the virulence factors and mechanisms associated with different strains of *E. coli* and their disease potential are variable. Since many of the enteropathogenic *E. coli* associated with diarrhea are believed to produce a diffusible enterotoxin (5, 14), other strains apparently possess a mechanism which enable them to invade the tissues circumventing normal host defenses. Indeed, the diseases and types of infections associated with *E. coli* are numerous and may prove as varied as the members of the "so-called" species of *E. coli* itself.

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**LITERATURE CITED**