Colibacillosis in pigs and its diagnosis

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Summary

Colibacillosis is a major cause of illness and death in young pigs. The condition is caused by enterotoxigenic and other strains of *Escherichia coli*. Characteristics of the causative agents and the animals at risk are discussed. Methods for diagnosis of the condition are given.

Keywords: *Escherichia coli*, colibacillosis, swine, diarrhea, scours

Colibacillosis is a major cause of illness and death in neonatal and recently weaned pigs. The disease is usually caused by enterotoxigenic strains of the bacterium *Escherichia coli*, although nonenterotoxigenic strains of that organism may also occasionally cause the disease. Diarrhea is typically fluid and profuse, and frequently results in severe dehydration and circulatory shock. Enterotoxins produced by the enterotoxigenic *E. coli* (ETEC) strains pathogenic to pigs include heat-labile enterotoxin (LT), and/or heat-stable enterotoxins STa (STI) or STb (STII). These organisms also produce fimbrial adhesins that mediate the adherence of the bacterium to the mucosal surface. The fimbriae produced include K88 (F4), K99 (F5), 987P (F6), F41, and F18 (F107 and 2134P). Although less common, some strains produce a Shiga toxin (Stx2e) and may cause edema disease in addition to colibacillosis. Also uncommon are strains that produce no toxins, but efface the microvilli of the epithelial cells to which they attach (Helie P, et al. *Proc Int Congr Vet Soc*. 1990).[1] Such strains contain *eae* genes, which have been associated with attachment/effacement. Porcine attaching-effacing *E. coli* strains are very similar to those that cause diarrhea in human infants, and are known as enteropathogenic *E. coli* (EPEC). Because many strains of *E. coli* isolated from animals are nonpathogenic, it is important to identify the virulence factors produced by ETEC or EPEC strains, or the genes that encode those factors, to establish the etiology of diarrhea.

Inherent susceptibility or resistance of pigs to ETEC appears to be a function of age and/or genetic background. Resistance of pigs to *E. coli*-expressing K99 or 987P arises with age. Age-associated resistance develops gradually and becomes more-or-less complete by 2 weeks of piglet life.[2–4] Interestingly, age-acquired resistance of calves to K99+ *E. coli* occurs very rapidly and is complete by 48 hours of age.[5] Pigs are resistant to infection by F18+ *E. coli* at birth, but become susceptible after several weeks of life.[6] Inheritable resistance to colibacillosis caused by K88+ and F18+ ETEC is well documented, but has not been reported with regard to *E. coli* which produces other adhesive fimbriae.[7–9] Inherent resistance to, attachment of, and susceptibility to K88+ and F18+ *E. coli* are autosomal recessive traits. Resistance is achieved by failure to produce the receptor to which the fimbriae adhere on epithelial brush border membranes.[9] Enterotoxigenic *E. coli*-expressing K88 and F18 account for essentially all postweaning colibacillosis in pigs.[2,10,11] K88+ *E. coli* is believed to be responsible for a majority of neonatal colibacillosis cases as well.[12] Recent studies suggest that about 50% of pigs in common breeds inherit resistance to K88+ organisms.[13] Thus it appears that pigs inherently susceptible to K88+ ETEC account for a disproportionately high proportion (at least 75%) of all colibacillosis. Therefore, selective breeding for resistance to K88+ and perhaps F18+ *E. coli* could have a significant economic impact on the swine industry.

The technology for identifying inherently resistant animals is currently quite primitive and cumbersome, yet testing for and selecting such animals may be economically justified. Currently, methods of susceptibility/resistance phenotype analysis require either laboratory analysis of intestinal biopsy specimens or specimens from progeny at slaughter. Two laboratory tests for phenotype analysis are available. The most definitive of these tests employs a Western blot protocol.[14] This test requires larger specimens than can be collected by biopsy, expensive reagents, and considerable technical expertise.

Although it can be less specific, the other test—a brush border/bacteria aggregation test—requires only small specimens, no expensive reagents, and minimal training to perform. However, some laboratory equipment, including a centrifuge and a microscope with a phase-contrast condenser, is required. K88+ bacteria are incubated with whole enterocytes or osmotically prepared enterocyte brush-border vesicles obtained from the pig in question. Bacteria/brush border suspensions are viewed by phase contrast microscopy for the adherence of bacteria to brush borders (Figure 1). Adherence of numerous bacteria to brush borders is highly correlated with piglet susceptibility. Specimens from pigs < 6 weeks of age may give false-positive results.[9] Sows of the resistant phenotype should not be mated with boars of the susceptible phenotype or boars that remain uncharacterized because such sows may be unable to protect susceptible offspring from K88+ ETEC (susceptibility is dominant over resistance). Sows of the resistant phenotype do not produce anti-K88 antibody subsequent to oral exposure with K88+ ETEC or K88 antigen.[15] However, they probably produce circulating (IgG) anti-K88 antibody following parenteral vaccination.

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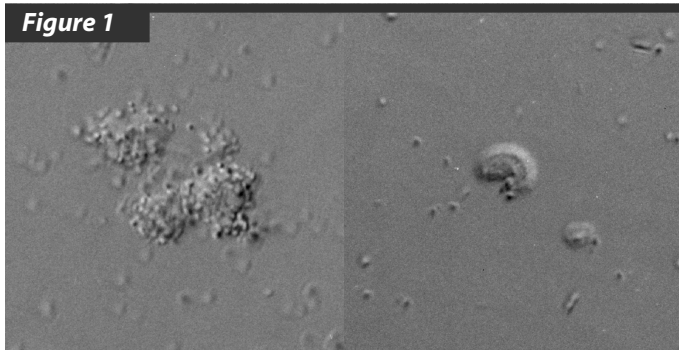
Figure 1

Left: Bacteria adhere in large numbers to brush border vesicles prepared from enterocytes from pigs of the susceptible phenotype, but not pigs of the resistant phenotype (**right**).
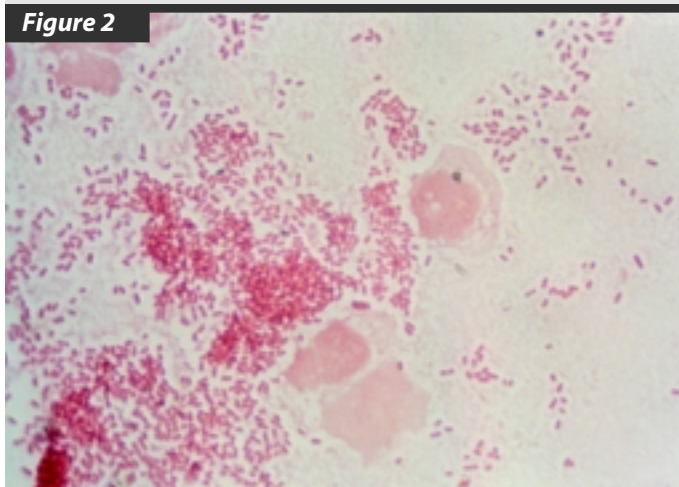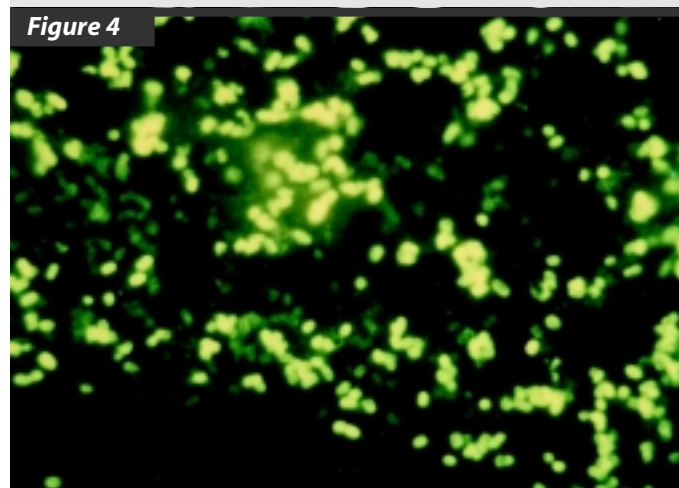
Figure 2

Gram's stained impression smear from the small intestine of a piglet with colibacillosis. Numerous gram-negative bacteria are present.
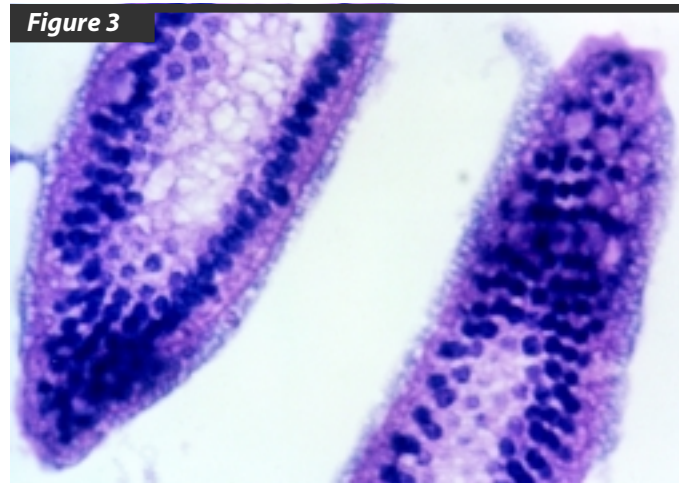
Figure 3

Hematoxylin-eosin (H&E)-stained histologic section of small intestine. Bacteria form a confluent layer on villous surface.

Figure 4

Intestinal impression smear stained by indirect immunofluorescence with anti-K88 serum and fluorescein-isothiocyanate-conjugated anti-immuno-globulin serum. Numerous fluorescing bacteria are present.

Diagnostic methods

Colibacillosis is a disease of the small intestine, and the condition of that organ must be assessed in diagnosing the disease. Strains of some serotypes of ETEC colonize the entire small intestine, while strains of other serotypes colonize only the distal portion of the small intestine. For that reason, specimens examined by the clinician or submitted to a laboratory for examination should include distal ileum. High concentrations of *E. coli* in pure or nearly pure culture in the ileum are indicative of colibacillosis.

Simple clinical methods

The concentration of *E. coli* in the intestines can easily be estimated by preparing and examining Gram-stained impression smears of the mucosal surface of the small intestine (Figure 2). More than 100 bacteria per 1000× microscopic field is indicative of colibacillosis.[17] Alternatively, one may estimate the *E. coli* concentration by culture of the mucosa of the ileum on blood and/or a differential medium agar such as Tergitol-7 or MacConkey. Abundant growth of *E. coli* of a single colony type is indicative of colibacillosis.

Laboratory methods

In addition to the above mentioned methods for estimating the concentration of *E. coli* in the intestine, determining whether such organisms adhere to epithelial cell brush borders is useful in diagnosing colibacillosis. Only *E. coli* that are capable of adherence are considered significant in the disease. Adherent bacteria, if present, can be observed by examining Hematoxylin-eosin (H & E)-stained or Wright-Giemsa-stained ileal tissue sections (Figure 3), or unstained wet mount preparations of mucosal scrapings.[16] The wet mounts should be examined using phase-contrast microscopy. Perhaps one of the better approaches to determining the existence of ETEC infection is by examining immunofluorescence- or immunohistochemical-stained ileal impression smears or histologic sections for bacteria expressing adhesive fimbriae (Figure 4).[16] Examination of anti-fimbriae antibody-

stained intestinal sections reveals the fimbriae, if any are expressed by the *E. coli*, provides an estimate of the concentration of the organisms, and shows whether they adhere to the epithelium. Rabbit antisera or monoclonal antibodies specific for the various adhesive fimbriae are used in immunofluorescent or immunohistochemical tests. However, a limitation of this approach is that only *E. coli* expressing the fimbriae to which the antibodies are directed will be detected. Cases involving attaching-effacing *E. coli* or *E. coli*-expressing uncharacterized adhesive fimbriae will be missed. The ideal specimen for diagnosing colibacillosis is a pig euthanized during acute disease and subjected to necropsy immediately after euthanasia. Because the mucosal epithelium of the small intestine is subject to rapid autolysis and postmortem colonization by colonic bacteria, use of animals that have succumbed to diarrhea is discouraged in attempting diagnosis.

Identifying markers of virulence

In many cases it is desirable to characterize the *E. coli* isolate obtained from an animal to provide evidence that the isolate is virulent. Characteristics that suggest virulence include serogroup, adhesive fimbriae, and exotoxins. A serologic approach to characterization may be pursued. Serogrouping and fimbriae testing should include analysis for the serogroup and fimbrial antigens (Table 1). The serologic approach to *E. coli* isolate characterization is relatively simple to accomplish, and reagents and equipment are not expensive. This approach has weaknesses in that some (albeit few) *E. coli* strains diarrheogenic in pigs do not belong to a defined set of serogroups.[3] In addition, the expression of some fimbriae is subject to phase variation or growth conditions difficult to duplicate in the laboratory.[6,17,18] Thus, test sensitivities are limited, and false-negative results may occur relatively frequently.

Another approach to the characterization of *E. coli* strains isolated from diarrheic pigs is genetic. One may test for presence of DNA sequences consistent with those that encode virulence determinants of interest. DNA-based tests could include assays for the genes of adhesive fimbriae, enterotoxins, Stxe, and *eae*. An example of a DNA-based test for genes of *E. coli* virulence determinants is the multiplex polymerase chain reaction (PCR) test recently developed at the National Animal Disease Center in Ames, Iowa (Bosworth BT, et al. *97th Gen*

Figure 5
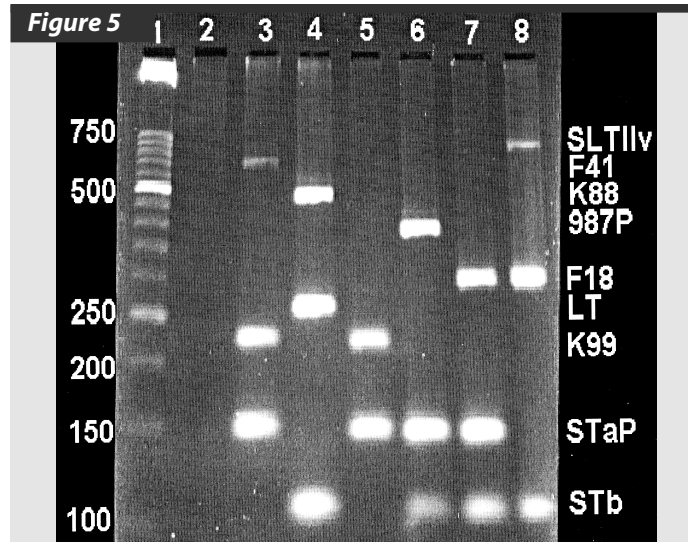
Multiplex PCR electrophoresis gel. Lane 1- base pair ladder used to estimate size of PCR products; subsequent lanes-*E. coli* strains containing genes for: Lane 2- no virulence factors (negative control); Lane 3- K99, F41, STa; Lane 4-K88, LT, STb; Lane 5- K99, STa; lane 6- 897P, STa, STb; Lane 7- F18, STa, STb; Lane 8- Stx2e (also known as SLTIIv), F18, STb.
Photograph courtesy of Dr. Tom Casey, National Animal Disease Laboratory, Ames, Iowa.

Meet Amer Soc Microbiol. 1997; 116), which is now available in some diagnostic laboratories (Figure 5). While gene-based tests are technically more demanding than serologic tests, they overcome the problems associated with poor expression of some virulence determinants under in vitro conditions. However, these tests do not determine whether a gene is actually encoding a specific virulence factor, only whether a specific segment of DNA is present in the *E. coli* strain being tested. DNA segments from genes containing mutations that render them functionally inactive, or that are similar in sequence but different in function, can lead to false positive results.

Table 1

Important serogroups, typical virulence determinants, and pigs targeted by enteropathogenic strains of *Escherichia coli*

Serogroup	Fimbriae	Enterotoxin	Hemolysin	Pigs at risk
0149	K88	LT and/or STa or STb	+	nursing and weaned
0157	K88 or F18	LT and/or STa or STb or Stx2e	+/–	nursing and weaned
08	K88 or K99	LT and/or STa or STb	+	nursing and weaned
0138	F18	LT and/or STa, STb or Stx2e	+	weaned
0139	F18	LT and/or STa, STb or Stx2e	–	weaned
0141	987P	STa	–	nursing
020	987P	STa	–	nursing
09	987P or K99. F41	STa	–	nursing
0101	K99 and F41	STa	–	nursing
045	–	–	–	weaned

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