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Jerome C. Nietfeld

T Yeary

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Abstract

A retrospective survey of the Kansas State Veterinary Diagnostic Laboratory records was made for Escherichia coli isolated from pigs with diarrhea. There were 111 E. coli isolates that carried genes for attachment pili that are necessary for E. coli to cause diarrhea. Of the 111 isolates, 103 had one pilus gene and eight had two pilus genes. The most common pilus type was the K88 pilus accounting for 73% of the isolates. All but one of the K88 isolates also carried at least one toxin gene indicating that they were virulent for pigs. The next most common pilus type was F18 accounting for 21% of the isolates. However, more than half of the F18 isolates did not have detectable toxin genes. F41, K99, and 987P pilus types made up 7%, 4%, and 2% of the isolates, respectively (percentages total greater than 100% because some isolates had 2 pilus genes). Escherichia coli expressing pilus types K88 and F41 are currently the major causes of colibacillosis in pigs.; Swine Day, Manhattan, KS, November 14, 2002

Keywords

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PILUS GENES IN *Escherichia coli* ISOLATED FROM PIGS WITH DIARRHEA

J. C. Nietfeld[1] and T. Yeary[1]

Summary

A retrospective survey of the Kansas State Veterinary Diagnostic Laboratory records was made for *Escherichia coli* isolated from pigs with diarrhea. There were 111 *E. coli* isolates that carried genes for attachment pili that are necessary for *E. coli* to cause diarrhea. Of the 111 isolates, 103 had one pilus gene and eight had two pilus genes. The most common pilus type was the K88 pilus accounting for 73% of the isolates. All but one of the K88 isolates also carried at least one toxin gene indicating that they were virulent for pigs. The next most common pilus type was F18 accounting for 21% of the isolates. However, more than half of the F18 isolates did not have detectable toxin genes. F41, K99, and 987P pilus types made up 7%, 4%, and 2% of the isolates, respectively (percentages total greater than 100% because some isolates had 2 pilus genes). *Escherichia coli* expressing pilus types K88 and F41 are currently the major causes of colibacillosis in pigs.

Introduction

Enterotoxigenic *Escherichia coli* is an important cause of diarrhea in nursing and weaned pigs. In order for *E. coli* to cause diarrhea it must have two virulence factors: 1) an attachment factor or pilus that allows the bacteria to adhere to epithelial cells lining the small intestine, and 2) the ability to produce one or more toxins that cause active secretion of fluid by the intestinal epithelial cells into the intestine. The pili that are known to occur on enterotoxigenic *E. coli* that affect pigs are K88 (F4), K99 (F5), 987P (F6), F41, and F18. Strains of *E. coli* that express K99 and 987P pili cause diarrhea only during the first week of life. Strains that express F18 pilus cause diarrhea only in weaned pigs, and K88 positive strains cause diarrhea in both nursing and weaned pigs. The majority of strains that express F41 also express other pili, usually K99. F18 pilus also occurs on strains of *E. coli* that cause edema disease in weaned pigs. Toxins associated with enterotoxigenic *E. coli* are heat labile toxin (LT), heat stable toxin a (STa), and heat stable toxin b (STb). Edema disease-causing strains of *E. coli* produce what is known as Shiga-like toxin variant 2e (SLT-2e). The purpose of this study was to determine the *E. coli* pilus types associated with diarrhea in pigs that had samples submitted to the Kansas State University Veterinary Diagnostic Laboratory.

Procedures

Escherichia coli isolates from nursing and weaned pigs with diarrhea are routinely tested by a multiplex polymerase chain reaction (PCR) technique for genes that encode the following pili and toxins: K88, K99, 987P, F18, F41, LT, STa, STb, and SLT-2e. The *E. coli* PCR records for the past 3.5 years were examined and all isolates that contained one or more pilus genes were identified. The types of toxin gene(s) carried by the isolates were also recorded.

[1]Department of Diagnostic Medicine/Pathobiology.

Results and Discussion

There were 111 isolates of *E. coli* carrying genes for pilus production: 103 isolates with one pilus gene and eight isolates with two pilus genes. Eighty-one isolates (73%) possessed genes for K88 pilus and all but one of those isolates carried genes for one or more toxins. Of the K88 positive isolates, 73 carried genes for heat labile toxin (LT) and heat stable toxin b (STb) and one isolate carried the gene for F18 pilus. One isolate had no toxin genes.

The next most common pilus type was F18 with 23 (21%) isolates. However, 13 of the 23 isolates did not have genes for any of the toxins. Of the 10 isolates with toxin genes, nine had genes for both heat stable toxin a and b, and one carried only the heat stable toxin b gene. Five isolates with both heat stable toxin genes also had genes for Shiga-like toxin 2e (SLT-2e), which is associated with edema disease. Two toxin positive F18 isolates also possessed genes for F41 pilus and one isolate carried the gene for K88.

Eight (7%) isolates possessed the gene for F41 pilus. Five of the isolates also had genes for K99 pilus and for heat stable toxin a, and two isolates had genes for F18 pilus plus both heat stable toxins. One F41 isolate had no other pilus or toxin genes.

There were five (4%) isolates that had genes for K99 and all five also had genes for F41 and heat stable toxin a. Two (2%) isolates were positive for the 987P pilus gene. One 987P positive isolate had a gene for heat stable toxin a, and one had genes for both heat stable toxins.

The results of this survey had similarities and differences with those of a similar study from South Dakota State University (SDSU) in 1986 that examined 223 strains of *E. coli* from pigs with colibacillosis (Wilson RA, Francis DH. Fimbriae and enterotoxins associated with *Escherichia coli* isolated from pigs with colibacillosis. American Journal of Veterinary Research 1986;47:213-217). In both studies, K88 was the most common pilus type identified in *E. coli* isolates from pigs, but the percentage of K88 positive isolates in our study (73%) was considerably higher than in the South Dakota study (48%). The primary reason for the increase in the percentage of K88 isolates appears to be a decrease in 987P and K99 positive isolates. In the South Dakota study, 987P accounted for 30% and K99 for 13% of the enterotoxigenic *E. coli* from pigs, whereas their prevalences in our study were 2% and 7%, respectively. These changes are not just restricted to the Kansas State Diagnostic Lab. The K99 and 987P pilus types have almost disappeared from submissions to the SDSU Laboratory, which also tests *E. coli* isolates for the Veterinary Diagnostic Laboratories at Iowa State University and the University of Minnesota (D.F. Francis, personal communication).

The second most common pilus type, F18, is associated with diarrhea and edema disease in weaned pigs and had not been identified when the South Dakota study was performed. The prevalence of F18 positive *E. coli* strains isolated at the Kansas State Laboratory is lower than at some other Midwestern laboratories. The interesting thing about the F18 positive isolates is that over half did not have genes for any of the toxins. If these isolates do not produce an as-of-yet unknown toxin, they should not cause disease.

In the instances where there was an age given for the pigs from which the *E. coli* isolates originated, 87% of the pilus positive isolates were from weaned pigs (data not shown). Based on the results of this survey, the *E. coli* pilus types that swine producers need to be most concerned about are K88 and F18 in re-

cently weaned pigs. Vaccinating sows for the various pilus types is effective in preventing diarrhea in nursing pigs and may be the reason that 987P and K99 pilus types have become uncommon. Both K88 and F18 positive *E. coli* can cause diarrhea in weaned pigs and it is much more difficult to induce protective immunity in the immediate postweaning period. Immunity from the sow's milk and colostrums can inhibit a protective response to vaccination in young pigs, and the level of antibodies in the pigs' sera that were derived from the sow is usually beginning to decline at weaning. Also, when pigs are weaned there is a sudden complete lack of milk antibodies in the intestinal tract, which makes them more susceptible. The trick is to induce immunity during the nursing period in spite of interference by passive colostral and milk antibodies from the sow.

Susceptibility to colonization and diarrhea caused by K88 and F18 pilus positive *E. coli* is inherited. It is possible that there has been inadvertent selection for susceptibility to K88 *E. coli* while selecting for other traits and that this is a major factor in the increase in the importance of the K88 pilus. Currently, different groups are working on control of diarrhea caused by F18 and K88 pilus producing *E. coli* by production of pigs genetically resistant to infection.