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"New" updates on "old" pathogens: *Escherichia coli* and rotavirus

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Introduction

There seems to be little new or exciting regarding neonatal pig diarrhea of late. The major pathogens involved are still the same – *Escherichia coli*, rotavirus, *Clostridium perfringens*, and *Clostridium difficile*. However, if we take a closer look at these microorganisms, we find that they are indeed changing in both dramatic and subtle ways. In this article, we examine two pathogens – *E. coli* and rotavirus – in more depth with the purpose of understanding the pathogenesis and facilitating treatment and control methods.

Escherichia coli

E. coli is a common infection of pigs that may cause significant morbidity, mortality and economic loss. The disease syndromes associated with *E. coli* infections include watery diarrhea caused by enterotoxigenic *E. coli* (ETEC), edema disease caused by Shiga toxin-producing *E. coli* (STEC) and diarrhea caused by attaching/effacing *E. coli* (AEEC) (1). The *E. coli* virulence factors present that induce disease are enterotoxins and fimbrial adhesins. Porcine ETEC may express K88 or F18 (2134 P) fimbria and heat-labile (LT) and/or heat-stable (STa, STb) enterotoxins (2). Recently, the role of additional virulence factors such as EAST1 toxin, adhesin involved in diffuse adherence (AIDA-I) and porcine attaching and effacing-associated factor (paa) has been explored as they may also be expressed by ETEC strains associated with diarrhea (2). ETEC, STEC and AEEC can act as sole or primary pathogens. However, multiple mixed infections with other pathogens such as rotavirus, TGE virus, cryptosporidium and coccidia are common. There is evidence that these additional agents can predispose the host to colonization by pathogenic *E. coli*.

The MVDL receives thousands of specimens for porcine diarrhea diagnostics yearly. A retrospective study was initiated to characterize porcine intestinal isolates of *E. coli* using culture phenotype, fluorescent antibody (FA) for pilus antigen, and polymerase chain reaction (PCR) for virulence and toxin genes. *E. coli* characteristics were then correlated with pig age and clinical signs in an attempt to better understand the role *E. coli* play in varying disease syndromes.

3,960 *E. coli* were isolated at the University of Minnesota Veterinary Diagnostic Laboratory (MVDL) between January 1, 2002 and June 30, 2005. Twenty-seven percent (n = 1,107) were non-hemolytic and 73% (n = 2,853) were beta-hemolytic. 653 beta-hemolytic *E. coli* isolates were pilus-typed by FA; 79.5% (n = 519) were K88 pilus antigen positive while 20.4% (n = 133) were F18 (2134P) positive. Of 440 non-hemolytic isolates pilus-typed by FA, only 5.5% (n = 24) were found to possess pilus antigens by FA; 16 isolates possessing K99 antigen, 8 with 987P antigen and 1 with K88 antigen. 1,380 beta-hemolytic and 322 non-hemolytic isolates were tested by PCR for surface antigen and toxin genes. In the beta-hemolytic group, 77% of the isolates possessed one or more of the virulence and toxin genes included in the current test (K88, K99, F18, F41, 987P, LT, StaA, StaB, and Shiga-like IIe). Of those virulence and/or toxin positive beta-hemolytic *E. coli* isolates, 586 contained K88, 480 contained 2134P while only 2 and 1 isolates contained K99 and 987P, respectively. Within the non-hemolytic group, only 16% of the isolates possessed any virulence and toxin genes included in the current test (K88, K99, F18, F41, 987P, LT, StaA, StaB, and Shiga-like IIe). Of those, 10% (n=32) contained K88 gene, 3% (n = 10) contained K99 gene, 1.9% (n = 6) contained 2134P gene and 1.1% (n = 4) contained 987P gene.

The FA results for each isolate were compared to the PCR results to roughly determine the level of agreement between the tests. When compared to PCR, the FA was only able to detect 87% of the K88 positive isolates and 27% of the F18 positive isolates. It is important to note that the 2134P FA used in this study detects only F18ac, so the inability of the FA to identify 2134P positive isolates can be explained by its failure to detect F18ab subtypes of *E. coli*.

The most prevalent toxin genes detected by PCR for K88 positive isolates were heat-stable toxin B (93.8%), heat-labile enterotoxin (87.3%), heat-stable toxin A (16.2%) and Shiga-like toxin IIe (1.1%). The most prevalent toxin genes detected by PCR for F18 positive isolates were heat-stable toxin B (65.8%), Shiga-like toxin IIe (59.0%), heat-stable toxin A (58.6%), and heat-labile enterotoxin (10.9%).

Attempts were also made to correlate clinical signs (when reported) with *E. coli* pilus type and toxin genes. The clinical signs reported for the F18 *E. coli* cases were: diarrhea (214 cases), central nervous system (CNS) disease (73), wasting (14), sudden death (46) and edema (12). 61

of 73 CNS cases were associated with Shiga-like toxin IIe gene positive *E. coli*. Other potential causes for neurological disease in these pigs included non-enteric bacterial meningitis or encephalitis and porcine reproductive and respiratory syndrome virus (PRRSV). The clinical signs reported for K88 cases included diarrhea (400), sudden death (68), wasting (14), CNS (45), and edema (4). 1 of 45 K88 positive CNS cases were also Shiga-like toxin IIe positive.

The majority (233 of 486) of F18 *E. coli* were cultured from pigs between 3 and 8 weeks of age with 104 isolates cultured from 4 and 5 week old pigs. Only 14 F18 *E. coli* were recovered from pigs less than 3 weeks of age. Of K88 *E. coli* isolates, 263 of 600 were from pigs 3 to 8 weeks of age with 111 isolates from pigs less than 3 weeks old. Isolation of either F18 or K88 *E. coli* was rare in pigs over 8 weeks of age. Nevertheless, pathogenic *E. coli* were isolated and attributed to clinical disease in 84 cases of growing pigs over 8 weeks of age.

Escherichia coli infections in swine are common and can be difficult to control. Characterization of the isolates by PCR can be helpful in determining whether or not they are causing the reported clinical signs of disease in the herds and may be useful for the veterinarian when designing herd specific *E. coli* control and prevention programs. PCR characterization has been demonstrated as more sensitive than FA, thus FA is rarely used at MVDL for *E. coli* pilus typing. Future strategies for characterizing *E. coli* isolated from pigs with diarrhea should include PCR tests for EAST1 toxin, the adhesion AIDA-I and the virulence factor paa because studies at SDSU revealed that AIDA-I and paa adhesin genes were commonly present in *E. coli* strains isolated from young pigs with diarrhea (2).

Rotavirus

Historically, rotavirus infection has been described as a cause of diarrhea in 3 to 8 week old pigs. In the last decade, rotavirus infections have been increasingly identified (by presence of characteristic lesions, localization of antigen to lesions and/or presence of antigen in feces) as causes of diarrhea in neonatal pigs 1 to 7 days old.

Rotaviruses are non-enveloped, segmented, double-stranded RNA viruses classified in the family Reoviridae. There are 7 serogroups of rotaviruses as determined by their intermediate layer of the viral protein capsid - VP6 (3). Pigs can be infected most with rotavirus groups A, B, and C and rarely group E (3). Diagnostic tests for the detection of rotavirus at the MVDL include electron microscopy (EM), immunohistochemistry (IHC), and a rapid immunoassay (ImmunoCard STAT! Rotavirus – Meridian, Bioscience, Inc.). The IHC and STAT tests detect only serogroup A while EM detects all rotavirus groups through visualization of rotavirus particles in feces. So, use of only serogroup A specific rotavirus detection tests may give false negative results and EM may provide more sensitivity. However, EM technology is expensive to maintain, requires skilled technicians, has low throughput, and requires significant sample preparation time prior to testing. If rotavirus is strongly suspected as a cause of diarrhea or if diarrhea continues to be a problem despite treatment and control of the other pathogens previously identified, submission of additional samples from acutely affected, untreated pigs is recommended and tests such as EM be used for detection.

Through the use of molecular diagnostic tests performed at Ohio State, rotaviruses can be serogrouped as A, B, or C and subsequently serotyped within groups. Both serogroups A and C have been identified simultaneously in rooms of pigs and concurrently in pigs on the same site but of different ages (e.g., serogroup A in pigs 1 week of age and serogroup C in pigs 4 weeks of age). In an Italian study, the majority of serogroup C rotavirus infected pigs were found to be co-infected with other enteric viruses (4). Infection with different strains of rotavirus complicates diarrhea control and prevention. For example, if the "feedback" or rotavirus inoculum/seed given to pigs is of a different serogroup than the infecting strain, there will be less than optimal immune protection as cross-protection is minimal at best (3).

Although rotaviruses can be considered ubiquitous in swine farms and most pigs are endemically infected, they can indeed be significant enteric pathogens. Several tests are available for the diagnosis of rotavirus diarrhea in pigs. In addition, characterization of rotaviruses may prove beneficial for troubleshooting problems with control measures. Finally, it is important to remember that rotaviruses are RNA viruses and as such, have inherent genetic diversity and a propensity to change (5). Never underestimate a virus.

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