Presentation of postweaning *Escherichia coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns

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Abstract

Post-weaning *Escherichia coli* diarrhea (PWECD) in Ontario was investigated using a case-control study involving 50 Ontario nurseries. The clinical signs and the impact on productive parameters were determined by means of a producer survey. The hemolytic *E. coli* serogroups involved in PWECD (O149:K91:K88) were examined in this study. Based on a polymerase chain reaction test, the hemolytic *E. coli* from 82% of the case herds were positive for 3 enterotoxins (STa, STb, and LT), those from 12% of the case herds were positive for STb and LT only, and those from one herd (6%) were positive for 3 enterotoxins, as well as for verotoxin and F18 pili. The *E. coli* involved in disease were resistant to multiple antibiotics. Case farms commonly used a wide variety of antibiotics either in the feed or water, or as injectable drugs. The most common antibiotic used to treat PWECD on the study farms was apramycin, but evidence of resistance to this antibiotic was noted. The PWECD problem was commonly seen within a week of weaning but onset of diarrhea was reported as late as the grower-finisher stage. Growth rate was poorer in case herds and mortality was higher than in control herds, demonstrating that PWECD is an economically important disease in Ontario.

Résumé

Une étude de cas contrôle impliquant 50 pouponnières fut effectuée en Ontario afin d'étudier la diarrhée en période post-sevrage associée à Escherichia coli. *Les signes cliniques ainsi que l'impact sur les paramètres de production furent déterminés au moyen d'une enquête auprès des producteurs. Les sérogroupes d'*E. coli *hémolytiques impliqués dans la diarrhée en période post-sevrage (O149:K91:K88) furent examinés dans cette étude. Suite aux résultats d'une épreuve d'amplification en chaîne par la polymérase, les isolats d'*E. coli *hémolytiques provenant de 82 % des troupeaux-cas étaient positifs pour les trois entérotoxines (STa, STb et LT), ceux provenant de 12 % des troupeaux-cas étaient positifs pour STb et LT, et ceux provenant de un troupeau (6 %) étaient positifs pour les trois entérotoxines, de même que pour la vérotoxine et le fimbriae F18. Les isolats d'*E. coli *impliqués dans les cas cliniques étaient résistants à plusieurs antibiotiques. Les troupeaux-cas utilisaient couramment une grande diversité d'antimicrobiens soit dans l'eau ou les aliments, ou par voie parentérale. L'antibiotique utilisé le plus fréquemment dans les troupeaux-cas pour traiter les cas de diarrhée en période post-sevrage était l'apramycine, mais des signes de résistance envers cet antibiotique furent notés. Les problèmes de diarrhée en période post-sevrage étaient observés principalement à l'intérieur d'un délai d'une semaine après le sevrage mais l'apparition de diarrhée fut rapportée aussi tard qu'à la période de finition. Le taux de croissance était inférieur dans les troupeaux-cas et la mortalité plus élevée que dans les troupeaux témoins, démontrant ainsi qu'en Ontario la diarrhée en période post-sevrage associée à* E. coli *est une condition importante d'un point de vue économique.*

(Traduit par Docteur Serge Messier)

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) strains that express K88 (F4) fimbriae are a major cause of diarrhea and death in neonatal and newly weaned pigs. Enterotoxigenic *E. coli* adhere to the small intestinal microvilli and produce enterotoxins that act locally on enterocytes. This action results in hypersecretion of water and electrolytes, and reduced absorption (7,18,19).

Enterotoxigenic *E. coli* implicated in post-weaning diarrhea in pigs most frequently produce either the K88 or F18 fimbrial adhesin (19). The most frequently observed enterotoxin combinations are LT and STb, or LT, STa, and STb (2). Isolates that are nonenterotoxigenic and induce attaching and effacing (AE) lesions are detected in about 6% of pigs with diarrhea in the post-weaning period (7).

In the fall and winter of 1997, reports of post-weaning diarrhea and mortality caused by K88-positive *E. coli* emerged as a concern

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Table I. Proportion of farms with positive and negative isolation of K88+ *E. coli* and proportion of different toxin genes identified by the polymerase chain reaction between case and control farms as part of a study involving 50 Ontario farms in 1999

	Cases (n = 28)[a]		Controls (n = 22)		
K88	%	*n*	%	*n*	*P*-value
Positive	60.7	17	13.6	3	
Negative	39.3	11	86.3	19	< 0.001

	Positive cases (n = 17)[a]		Positive controls (n = 3)		
Pathotype	%	*n*	%	*n*	
STb LT F4	11.8	2	100	3	0.01
STa STb LT F4	82.3	14		0	0.01
STa STb LT F4 +VT F18	5.9	1		0	NS

[a] Grower-finisher farm and farm where producer did not complete the survey are included in these data

in Ontario. Information accompanying diagnostic laboratory submissions indicated that the infection sometimes progressed so rapidly that pigs 2 to 8 wk of age were found dead before clinical signs were observed. Compared with isolations from the previous year, there was a 3-fold increase in the isolation of ETEC, specifically of O149:K88 serogroup (14–16). Pure cultures of the ETEC organisms were grown from small intestine swabs of pigs with post-weaning diarrhea. There have been speculations that newly emerging problems with PWECD are sometimes caused by *E. coli* strains that are more virulent in individual animals and more persistent within a herd than previous *E. coli* strains (22).

The objectives of this study were to determine the common K88-positive *E. coli* serogroups and pathotypes involved in PWECD problems in nursery units in Ontario, to evaluate the impact of the disease on certain productivity parameters, and to characterize the presentation of the disease on these pig farms.

Materials and methods

Selection of the farms

A total of 50 farms were visited in the summer of 1999 as part of a case-control study. Case and control herds were selected from the records of the Animal Health Laboratory, Guelph, Ontario, and with the assistance of several swine practitioners. Farms were selected based on the presence or absence of K88 *E. coli* and a history of diarrhea and/or sudden death in the previous year (March–December, 1998). Twenty-five farms in each category were sought in March of 1999.

A case farm met the following criteria: post-weaning pigs that had clinical signs of *E. coli* diarrhea and mortality as well as positive cultures of K88-positive *E. coli*. A farm was classified as a control if the herd did not have a history of PWECD and the disease was not diagnosed at the time of the visit. Control farms were selected from the list of herds submitting samples to the Animal Health Laboratories during the same time period as the case farms.

Survey information

Information on average daily gain (ADG), mortality, presentation and management of diarrhea problems, and antimicrobial drug usage was collected on each farm visit. Growth rate was obtained either from farm records or by subtracting the weaning weight from the weight of the pigs when they were moved out of the nursery, divided by the number of days in the nursery. Preweaning ADG was calculated by subtracting an average birth weight of 1.5 kg from the average weight at weaning, divided by the average weaning days. Data on clinical problems that have been associated with a diarrhea problem were collected in the case and control farms. A standard protocol was followed on each farm.

Bacteriology and antibiotic sensitivity

Rectal swabs of 10 weaned pigs were collected from each of the farms visited to ensure a consistency in culturing for the presence of K88-positive *E. coli* at the time of the visit. The samples were taken from pigs within 1 or 2 wk of weaning that showed clinical signs of diarrhea; in herds where no diarrhea was present, pigs were sampled in a random manner from the same age group. Depending on the size of the farm, 1 or 2 samples were taken from each pen. These samples were sent to Gallant Custom Laboratories in Cambridge, Ontario, where *E. coli* were isolated and slide agglutination tests for K88 antigen and for O and K serogroups implicated in PWECD were performed on hemolytic *E. coli* isolates by using standard techniques (6). Only O149:K91:K88 isolates were tested for antimicrobial sensitivity.

Diffusion sensitivity testing (Kirby-Bauer) was conducted for the following antibiotics: ampicillin, carbenicillin, cefadroxil, gentamicin, amikacin, tobramycin, enrofloxacin, neomycin, ciprofloxacin, polymyxin B, spectinomycin, sulfamethoxazole, tetracycline, ceftiofur, and apramycin. The criteria used to indicate resistance versus susceptibility were based on the proposed standards of the National Committee for Clinical Laboratory Standards.

Subsequently, blood agar plates with a single representative colony type from each of the positive farms were submitted to the Faculté de médecine vétérinaire, Université de Montréal. Isolates were tested for the presence of genes for K88 (F4) and F18 fimbriae and for toxins associated with PWECD (STa, STb, LT, and VT) by a polymerase chain reaction (PCR) technique as described previously (3) using primers for the detection of the appropriate genes (8,12,17,20,21).

Table II. Average daily gains and total nursery mortality rate between case and control farms before and after a postweaning *E. coli* problem

	Cases (n = 26)			Controls (n = 22)[a]			
Productivity parameters	n	mean	SD	n	mean	SD	P-value
Pre-weaning average daily gain (g/d)	21	229	36	19	238	35	NS
Average daily gain of nursery (g/d)	22	414	76	20	452	54	0.07
Mortality before *E. coli* problem (%)	26	2	1.1	20	1.8	0.7	NS
Mortality after *E. coli* problem (%)	26	7.7	7.3	20	1.8	0.7	< 0.001

SD — standard deviation; NS — nonsignificant

[a] Same data used to represent control farms in before and after categories

Statistical analysis

The simple association between case and control herd status and putative disease factors was determined using the chi-squared test for qualitative variables and Student's t-test for quantitative variables. Numerical variables that were not normally distributed according to the Wilk-Shapiro test were tested with the Mann-Whitney test. Variables different at $P \leq 0.05$ were considered significant. Variables with a P-value between 0.06 and 0.1 were considered numerically reportable as potential trends. Statistical analyses were completed by using a statistical software program (Statistix v. 1.0 for Windows; Analytical Software, Tallahassee, Florida, USA).

Results

Three control herds developed diarrhea problems and were diagnosed with K88-positive *E. coli* prior to the farm visit. These 3 farms were considered cases. One survey from a case farm was not completed at the time of the visit. The farmer was asked to complete and return the survey but failed to do so. Another case farm was a grower-finisher operation. Both these farms were dropped from the survey analysis, although rectal swabs were taken from each farm; these were cultured, and the hemolytic *E. coli* isolates were serogrouped and tested for sensitivity (Table I).

Nursery inventory ranged from 20 to 4400 (1099.2 ± 1086) pigs on case farms and from 60 to 3000 (1194 ± 926.2) pigs on control farms. The type of management system used by case and control farms included farrow-to-finish, farrow-to-partly finish, farrow-to-feeder, and off-site nurseries.

There were 28 case farms and 22 control farms in the analysis for the serogroup and antibiotic resistance study. Seventeen case farms and 3 control farms had pigs whose hemolytic *E. coli* were identified as O149:K91:K88. The 3 control farms that had pigs with K88-positive *E. coli* were left in the control group because no diarrhea was observed at the time of the study and there was no history of an *E. coli*-related diarrhea problem prior to the study. The PCR results are summarized in Table I. Three case farms with K88 *E. coli* also had isolates of serogroup O139:K82, and *E. coli* of serogroup O138:K81 were isolated on a case farm where K88 *E. coli* were not isolated at the time of the visit. Two case farms whose pigs lacked K88-positive *E. coli* had pigs from which VTF18-positive *E. coli* of serogroups O138:K81 and O139:K82 were isolated.

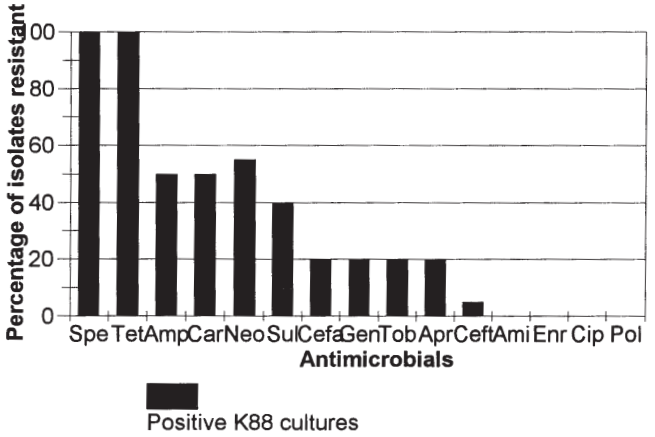

Figure 1. Resistance patterns of 75 K88-positive isolates from 20 K88-positive farms. Results were expressed as percentage. The black bar represents the percentage of antimicrobial resistance to an specific antibiotic. The antimicrobials tested were: Spe (spectinomycin), Tet (tetracycline), Amp (ampicillin), Car (carbenicillin), Neo (neomycin), Sul (sulfamethoxazole), Cefa (cefadroxil), Gen (gentamicin), Tob (tobramycin), Apr (apramycin), Ceft (ceftiofur), Ami (amikacin), Enr (enrofloxacin), Cip (ciprofloxacin), Pol (polymyxin B).

Case herds were more likely than control farms to have *E. coli* of pathotype STa STb LT (P = 0.01). The K88-positive *E. coli* from pigs on 3 control farms were of pathotype STb LT, and among the K88-negative control farms, 2 were *eae*-positive (*E. coli* attaching and effacing).

A total of 75 isolates from 20 K88-positive case farms were assayed for antibiotic sensitivity. Multiple antimicrobial resistance, as typified by resistance to at least 2 distinct antimicrobial classes, was observed in 100% of the isolates that were assayed (Figure 1).

For the survey analysis, 26 farms were designated as cases and 22 farms were designated as controls. K88 *E. coli* were isolated from 15 of the 26 case farms during this study.

In 23 case farms (88.5%), the diarrhea and mortality began within a week of weaning, whereas only one control farm reported severe diarrhea problems within this period (P < 0.001). Three case farms (11.5%) had the worst problem of diarrhea at 2 to 3 wk after weaning, and 4 case farms (15.38%) also reported diarrhea problems in the preweaning period.

There were no significant differences in growth rate of pigs in the preweaning period between case and control farms. After weaning, growth rate in the nursery tended to be better on control farms than on case farms (452 g/d vs 414 g/d, respectively) (P = 0.07). There was

Table III. Management of diarrhea between case and control farms as part of a study involving 48 Ontario farms in 1999 (1)

		Cases (n = 26)		Controls (n = 22)		
Antibiotic usage	Antibiotics	%	n	%	n	P-value
Farms that used antibiotic in water[a]		73.1	19	13.6	3	< 0.001
Antibiotics used[b]	Apramycin	73.6	14		0	< 0.001
	Neomycin, oxytetracycline	5.2	1	33.3	1	NS
	Tiamulin		0	33.3	1	NS
	Tylosin	5.2	1		0	NS
	Neomycin	15.7	3	33.3	1	NS
Second antibiotic used in water[a]		26.9	7	4.5	1	0.05
Antibiotics used[b]	Neomycin, oxytetracycline	28.5	2		0	NS
	Penicillin, streptomycin		0	33.3	1	NS
	Neomycin	71.4	5		0	0.05
Farms that used injectable antibiotics		65.3	17	18.1	4	0.001
Antibiotic used[b]	Ceftiofur	11.7	2		0	NS
	Lincomycin		0	25	1	NS
	Gentamicin	11.7	2	50	2	NS
	Tiamulin	11.7	2		0	NS
	Trimethoprim, sulfadoxine	41.1	7		0	0.01
	Tylosin	17.6	3	25	1	NS
	Enrofloxacin	5.8	1		0	NS

[a] Percentage based on total of case and control farms

[b] Percentage based on farms that used antibiotics

no difference in mortality rate between the 2 groups before the *E. coli* outbreak occurred. After the disease outbreak, the mortality in case farms (7.7%) was higher than on control farms (1.8%) (P < 0.001) (this P value was obtained from the Mann-Whitney test). The mean mortality in case herds ranged from 0.5% to 10–30% (Table II).

The prevalence of clinical signs associated with an outbreak of PWECD in a case farm were: sudden death (84.6%), watery diarrhea (73%), a thin or unthrifty appearance (50%), vomition (19.2%), and dehydration (77%). Other observations reported by the farmers were: pigs with purple coloration of the body before death, and the impression that certain pens were more frequently affected than other pens.

Management and feed changes to control diarrhea problems were more commonly reported in case farms than in control farms (P < 0.001). The most common management changes were: increased age at weaning, better control of temperature and ventilation, creation of sick pens, decreased density in pens, and reduced mixing of pigs. The most common feed changes reported were: purchasing feed from a different supplier, changing the feed medication, offering a limited amount of feed 4 or 5 times per day, blending of feed between phases, adding high levels of zinc oxide, decreasing the level of protein, and increasing the level of fibre.

Case farms commonly switched feed antibiotics when diarrhea problems were present (46.2%). The most common antibiotics used on farms that had switched in-feed antibiotics were: carbadox (30.8%), chlortetracycline (23%), and a combination of chlortetracycline, sulfamethazine, and procaine penicillin (15.4%). However, significant differences were not found between the use of these antibiotics and the ones used on control farms. Case farms were more likely to use antibiotics in water (73.1%) than control farms (13.6%) (P < 0.001), with apramycin being the first water medication of choice in case farms. Case farms were more likely to change to a second choice of antibiotic in water when no results were observed with the first choice of antibiotic. Neomycin was the antibiotic of choice in these cases (P = 0.05) (Table III).

Case farms were more likely to use injectable antibiotics on individual pigs than were control farms (63.4 and 18.2%, respectively) (P = 0.001). The injectable antibiotic most commonly used to treat PWECD was trimethoprim with sulfadoxine (41.2%) (P = 0.01) (Table III).

The use of *E. coli* vaccines in nursery pigs, probiotics, and acidifiers in water or feed were other forms of prevention employed by a few of the case farms. Sanitizers in water were used on 19.2% of the case farms and just in one (4.5%) of the control farms. More case farms (61.5%) used high levels of zinc oxide (≥ 2.5 kg/T) than control farms (36.4%) (P = 0.08). Case farms (42.3%) tended to be more likely to use electrolytes for nursery pigs than control farms (18.2%) (P = 0.07) (Table IV).

Discussion

This study demonstrated that PWECD is an economically important disease in pigs. Farmers reported that, on average, mortality increased from 2% to 7% following an outbreak of PWECD, and some farms reported having had the problem for more than one year. If it is assumed that a newly weaned pig is worth approximately $40, then this level of mortality in a 500-sow herd would equal a loss of about $20 000 annually. However, some farms experienced mortalities as high as 20–30%, over a 1- to 2-month time span. Average daily gain (ADG) tended to be lower on farms with the problem compared with the farms that did not have the disease (452 g/d and 414 g/d, respectively). Similar decreases in growth rates and

Table IV. Management of diarrhea between case and control farms as part of a study involving 48 pig Ontario farms in 1999 (2)

	Cases (n = 26)		Controls (n = 22)		
Type of management	%	n	%	n	P-value
Vaccine against *E. coli* diarrhea in nursery pigs[a]	7.6	2	—	0	NS
Acidifiers in feed[a]	11.5	3	4.5	1	NS
Acidifiers in water[a]	7.6	2	—	0	NS
Probiotics[a]	3.8	1	—	0	NS
Sanitizers in water[a]	19.2	5	4.5	1	NS
Use of electrolytes when needed[a]	42.3	11	18.2	4	0.07
Zinc oxide 2500 ppm	61.53	16	36.4	8	0.08
Management changes at the moment of the problem[a]	57.6	15	9	2	< 0.001
Feed changes at the moment of the problem[a]	73	19	13.6	3	< 0.001

[a] Percentage based on total of case and control farms

mortality were reported by Josephson et al (14). In addition, case farms used more antibiotics and other treatments such as vaccines, acidifiers, probiotics, high levels of zinc oxide, and injection of individual pigs, which reflect an increased production cost and labor cost.

In this study, it was noted that PWECD occurred most commonly in the first week after weaning, but in agreement with Fairbrother (7), the disease was also observed to affect pigs after 2 to 3 wk following weaning, or in at least one case diarrhea occurred following the transfer of pigs to the grower-finisher unit.

It has been reported that problems of PWECD occur sporadically. The findings in this study are consistent with this observation as case farms with a recent history of PWECD were sometimes free of clinical signs at the time of the survey and K88-positive *E. coli* were not isolated. Producers have often experienced short periods of success in combating the disease, only to have the disease reappear a few weeks later. The clinical signs observed by the farmers in this study are similar to those described elsewhere (1,7,10).

The O149:K91:K88 ETEC *E. coli* strains were the most commonly isolated in pure cultures from pigs with PWECD problems. This agrees with a previous report (13). The O139:K82 serogroup was isolated from some farms, and this is the serogroup most commonly isolated from cases of edema disease (9). The most frequently observed enterotoxin combination was STa, STb, LT, which agrees with reports made by Celemin et al (2). The emergence of this *E. coli* with 3 enterotoxins has prompted Fairbrother (7) to suggest this may indicate the emergence of a new and more virulent pathotype. The combination of just 2 toxins (STb, LT) was observed less commonly in case farms, but was present on the 3 positive control farms, where clinical problems were not apparent. The presence of multiple adhesins and toxins was observed in at least one farm which had a serious diarrhea problem (STa, STb, LT F4 and VT+F18). Nagy and Fekete (19) reported that some ETEC strains may produce more than one adhesin factor. Other authors have suggested that the presence of verotoxin (VT), specifically VT2e, is associated with edema disease, but can play a role in PWECD (7,10,18). Attaching-effacing isolates were found in pigs from 2 control farms, but were not associated with a diarrhea problem.

In agreement with other studies (1,5,7,18) ETEC isolates from pigs with post-weaning diarrhea showed a high frequency of resistance to multiple antibiotics. Increased risk of resistance among *E. coli* has been associated with the use of various antimicrobials (1,5). The use of various antibiotic drugs via feed, water, or injection was a common finding on farms that had PWECD problems.

More antibiotics were used on case farms, so it is not surprising that the *E. coli* on case farms showed more resistance in general. Antibiotics appear to be a short term solution and as farmers move from one antibiotic to the next, as illustrated in this study, antibiotic resistance is likely to develop.

Dunlop et al (4) reported that, in Canada, the medications most commonly added to creep and starter rations were a combination of chlortetracycline-sulfamethazine-penicillin, carbadox, and tylosin plus furazolidone. In agreement with Dunlop's findings, except for furazolidone, which has been withdrawn as an approved in-feed medication for swine rations, farms in this study tended to use these antimicrobial drugs as growth promoting agents and as therapeutic drugs to treat diarrhea.

According to Fairbrother (7), apramycin remains the antimicrobial of choice for use in water. In the Fairbrother study, most isolates showed sensitivity to apramycin and amikacin. In this present study, apramycin was a commonly used treatment and the development of resistance was evident (23.5% of the *E. coli* isolates). In contrast, no resistance was observed for amikacin (a drug not used in swine production) in any of the isolates from this study. In a survey study developed in the United Kingdom (11), a high level of resistance was also found to apramycin in pigs of all ages and in humans, including hospital patients and one pig worker. Also, apramycin-resistant *E. coli* were found to persist in a dry environment in a pig pen that had been empty for 10 mo (11). Resistance to apramycin provides cross-resistance with other aminoglycosides, such as gentamicin and tobramycin (11), as well as neomycin, which makes this drug a curious second choice on farms that have switched from apramycin. There were some other antibiotics included in the study which are not commonly used in the swine industry; however, it is interesting to note the resistance patterns because of their relevance in human medicine.

This was the first reported study of PWECD in Ontario. The disease appears to be of economic significance and poses a challenge as far as control. The common approach of treating PWECD with mass antibiotic medication will likely lead to a build up of antibiotic

resistance. There is a need to conduct more carefully designed and evaluated field trials with controls to examine the true efficacy of the many treatments and control measures instituted on case farms as revealed by this study. The *E. coli* involved in PWECD tend to persist in spite of attention to the usual management, environmental and hygiene factors.

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