

A case-control study investigating risk factors associated with postweaning *Escherichia coli* diarrhea in southern Ontario

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Summary

Objectives: To investigate management, housing, and nutrition factors and potential viral infections that might be associated with postweaning *Escherichia coli* diarrhea in pigs in southern Ontario.

Methods: A case-control study involving 50 herds was conducted. Information on hygiene, management, facilities, management of diarrhea, porcine reproductive and respiratory syndrome virus (PRRSV) status, and nursery feed management was collected through a questionnaire. Rectal swabs and sera collected from ten weaned pigs in each herd were tested for F4⁺ (K88⁺) *E coli* and antibodies to two viral

pathogens. Hemolytic *E coli*-like colonies were tested for agglutination in specific anti-F4 antiserum.

Results: Twenty-six herds were designated as cases and 22 as controls. *Escherichia coli* positive for F4 were isolated from pigs in 15 case herds and 3 control herds. Postweaning diarrhea did not occur in the control herds during the study. In case herds, there were more feeder spaces per pen, the first rations offered in the nursery were more likely to be pelleted feed, and nursery pigs and gestating sows were more likely to be vaccinated against PRRSV than in control farms.

Implications: Weaning age, weaning weight, and commingling of pigs, which are commonly considered risk factors for postweaning diarrhea, did not appear to be contributing factors in this study. Vaccination for PRRSV was associated with an increased risk of postweaning diarrhea, which occurred in a variety of herd types and sizes.

Keywords: swine, *Escherichia coli*, postweaning diarrhea, nursery management, starter feed

Received: August 31, 2001

Accepted: February 25, 2002

Swine practitioners noted an increase in the number of cases of postweaning diarrhea and mortality associated with F4⁺ (K88⁺) *Escherichia coli* occurring in Ontario beginning in the fall of 1997.¹ The total number of swine enteric submissions to the Animal Health Laboratory (AHL; Guelph, Ontario) between October and May remained constant at approximately 23% of total swine submissions. However, in that period, the total number of F4⁺ *E coli* isolates from piglets increased from 20% of total enteric submissions in 1996-1997 to 42% in 1997-1998.¹ Information accompanying diagnostic laboratory submissions indicated that the infec-

tion sometimes progressed so rapidly that pigs of 2 to 8 weeks of age were found dead before clinical signs were observed. In epidemics of postweaning diarrhea, up to 70% of all pigs may show clinical signs,² and the group mortality rate may reach 25% in the absence of adequate medication.³

There is no doubt that toxigenic *E coli* is involved in the pathogenic process of postweaning digestive disorders,⁴ but may also be found in healthy piglets raised on farms with no history of postweaning diarrhea.⁵ The presence of an infectious agent is not sufficient to induce the clinical

manifestations of enzootic health disorders.⁶ Full expression of diseases such as postweaning colibacillosis not only requires sufficient infection pressure, but also is strongly dependent on environmental and management conditions. It is evident that additional factors are required for the onset of clinical signs of postweaning diarrhea.⁵⁻⁸ It has been suggested that postweaning F4⁺ *E coli* diarrhea frequently appears in commingled, multi-source early-weaned pigs.¹ Dietary change, which necessarily occurs with segregated-early-weaning management, may play an important role in pathogenesis of the disease; in addition, there may be a build-up of enterotoxigenic *E coli* (ETEC) organisms in the environment.⁶⁻⁸ Infection with rotavirus or transmissible gastroenteritis (TGE) virus may predispose pigs to postweaning diarrhea.⁹ In herds where clinical porcine respiratory and reproductive syndrome (PRRS) is prevalent, *E coli* is often involved in secondary infections.¹⁰ We investigated management, housing, nutrition, and viral infections (PRRS and TGE) as potential factors associated with the resurgence of postweaning *E coli* diarrhea (PWED) in Ontario.

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This article is available online at <http://www.aasv.org/shap.html>.

Amezcua R, Friendship R, Dewey C, et al. A case-control study investigating risk factors associated with postweaning *Escherichia coli* diarrhea in southern Ontario. *J Swine Health Prod.* 2002;10(6):245-249.

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 AHL, with the assistance of several swine practitioners. Criteria for selection of herds as cases or controls included history of F4⁺ *E coli* infection in the herd (present or absent) and a history of diarrhea or diarrhea and sudden death in the previous year (March-December 1998). Twenty-five herds in each category were sought in March of 1999. Herds were classified as case herds if clinical signs of *E coli* diarrhea and mortality occurred in postweaning pigs, and F4⁺ *E coli* was isolated. Herds were classified as control herds if there was no history of PWECD and the disease was not diagnosed at the time of the visit. These herds were randomly selected from those submitting samples to

the AHL during the same period as the case herds, but where a diagnosis of F4⁺ *E coli* was not made.

Survey information

Information on farm hygiene, nursery management, nursery facilities, management of postweaning diarrhea, PRRS virus (PRRSV) status, and feed management in the nursery was collected through a survey. Definitions of variables included in the survey are listed in Table 1. Factors in addition to those described in Table 1 included producer-reported average daily gain in the nursery, average weaning weight, average age at weaning, continuous flow management, and all in-all out by room.

Sample collection

Rectal swabs were collected from ten weaned pigs in each herd at the time of the herd visit to ensure consistency in culturing

for the presence of F4⁺ *E coli*. Sample size was calculated on the basis of a herd prevalence of 36% using a 99% confidence interval for a population of 1000 animals. Samples were collected 1 to 2 weeks after weaning from pigs that showed clinical signs of diarrhea. In herds where no diarrhea occurred, pigs were sampled in a random manner from the same age group. Depending on the size of the herd, one or two samples were taken from each pen. Samples were submitted to Gallant Custom Laboratories Inc, Cambridge, Ontario, for isolation of *E coli*. Slide agglutination tests for F4 antigen and for O and K serogroups implicated in PWECD were performed on hemolytic *E coli* isolates.

Serum samples were obtained from the same ten weaned pigs in each herd, using the orbital sinus method for blood collection.¹¹ Blood samples were refrigerated overnight, then centrifuged the following

Table 1: Description of variables examined in a survey of case and control herds¹ in a study investigating risk factors associated with postweaning *Esherichia coli* diarrhea in Ontario²

Variables	Description
Total inventory	Average number of pigs in the nursery
Number of pigs per pen	Average number pigs per pen
Number of feeders per pen	Number of feeder spaces per pen
Total space per pig (m ²)	Area of pen divided by the average number of pigs per pen
Off-site vs farrowing and nursery in the same building	Farms which were off-site nurseries, compared to farrow-to-finish, farrow-to-feeder pigs, and farrow-to-partly finish
Type of floor used in the pen	100% solid, 100% slotted, or combination of both
Pelleted first nursery feed	Texture of the first feed: mashed, crumble or pelleted
Levels of protein, fat, and fibre (%)	Percentage obtained from the feed label
Sources per pen in the nursery	Did the pen house pigs from one litter or more than one litter, more than one farrowing room, or more than one sow farm?
Sources per room in the nursery	Did the room house pigs from one farrowing room, more than one farrowing room, or more than one sow farm?
Cleaning and disinfection procedures	Did the producer scrape residual feed and manure off the pen before washing; use high pressure hose, hot water, or both for washing; use a detergent solution before disinfection; disinfect the nursery, feeders and drinkers; allow the room to dry 12 or 24 hours; disinfect water lines between batches of pigs?
PRRS ³ vaccination status	Were nursery pigs, suckling pigs, gestating sows, lactating sows, open sows, or replacement gilts vaccinated against PRRS virus?
Full access feed vs limited feed	Did the pigs in the nursery access feed all day or they were fed several times a day?
Blending of feed between phases	Was the current feed blended with the next phase?

¹ Case herd: postweaning pigs showed clinical signs of postweaning *E coli* diarrhea and mortality, and fecal samples collected during a herd visit were positive for F4⁺ *E coli*; control herd: no history or evidence of postweaning *E coli* diarrhea and no F4⁺ *E coli* cultured from pigs during the postweaning period.

² Case and control herds submitted samples from pigs within 1–2 weeks after weaning to the Animal Health Laboratory, University of Guelph, Ontario, during March to December, 1998.

³ Porcine reproductive and respiratory syndrome.

day. Serum was separated and stored at -70°C. Sera were tested at the AHL for antibodies to TGE and PRRSV. Results were used to determine whether there was an association between these diseases and PWECD status. A virus neutralization test was used as a screening test on all sera to detect coronavirus antibodies, and then a blocking ELISA (Svanovir; Svanova Biotech Uppsala, Sweden) was used to differentiate TGE from respiratory coronavirus antibodies. The IDEXX HerdChek ELISA (IDEXX Laboratories Canada Corporation, Toronto, Ontario) was used to detect PRRSV antibodies. Herds were grouped by PRRS serological status as follows: serologically negative: sample:positive (S:P) ratio <0.4 in all samples; low S:P ratios: S:P ratio 0.4 to 2.5 in at least one sample; high S:P ratios: S:P ratio >2.5 in at least one sample. Low S:P ratios (as defined) are usually a result of maternal antibodies, past infection, or vaccination. High S:P ratios (as defined) imply recent field infection, usually referred to as active PRRSV infection.

Statistical analysis

The simple associations between case and control herd status and management and disease factors were determined using a chi-square test for qualitative variables, and a Fisher's exact test or a Student *t* test for quantitative variables. Fisher's exact test was used in cases when expected values in the two-by-two table were <5 in at least one of the cells. Odds ratios (OR) of qualitative variables and their confidence intervals were calculated. A value of *P*<.05 was considered significant; *P* values between

Table 2: Quantitative variables tested¹ for association with postweaning *Escherichia coli* diarrhea (PWECD) in 48 Ontario nurseries² in 1999

Variables measured	Cases			Controls			P
	n	mean	SD	n	mean	SD	
Number of feeders per pen	25	7.7	3.5	19	5.2	2.3	.01
Total space per pig (m ²)	26	0.29	0.09	21	0.31	0.07	.54
Average weaning weight (kg)	22	6.30	2.4	19	6.05	0.8	.64
Average weaning age (days)	26	19.8	3.8	22	19.1	2.5	.44
Protein in first feed (%)	23	21.2	1.5	22	21.7	1.7	.31
Fat in first feed (%)	23	7.2	1.4	22	7.6	2.2	.37
Fibre in first feed (%)	23	2.8	1.2	22	2.3	1.3	.18

¹ Two-sample *t* test used to determine statistical significance.

² Cases: herds with a history of PWECD; Controls: herds with no history of PWECD.

.06 and .1 were considered numerically reportable as potential trends. Risk factors related to the case and control classification (*P*<.20) were re-examined in a multivariate model using logistic regression. Models were built using backwards elimination: those with the highest *P* values were removed one at a time until all factors left in the model were statistically significant at *P*<.05. Statistical analysis was completed in Statistix, (Statistix7, Version 1.0, 1996; Analytic Software, Tallahassee, Florida) and SAS System Program (Version 8.2; SAS Institute Inc, Cary, North Carolina).

Results

In three herds originally selected as controls, pigs developed postweaning diarrhea and were diagnosed with F4+ *E coli* just

before the farm was visited. These three herds were designated as cases. One case herd was dropped from the study because the survey was not completed at the time of the visit, and a second case herd was dropped because it was a grower-finisher operation. Finally, 26 herds were designated as case herds and 22 as control herds. *Escherichia coli* positive for F4 was cultured from pigs in 15 of the 26 case herds, and in three of the 22 control herds, but PWECD did not occur in the control herds. Case herds were 8.6 times more likely to yield positive culture results for F4+ *E coli* than control herds (*P*=.002).

Nursery inventory ranged from 20 to 4200 pigs (1099.2 ± 1086) in case herds and from 60 to 3000 pigs (1294 ± 926.2) in

Table 3: Qualitative variables tested¹ for association with occurrence of postweaning *Escherichia coli* diarrhea (PWECD) in 48 Ontario nurseries² in 1999

Variables measured	Cases	Controls	Odds ratio	P	95% CI of OR ⁵
Off-site nursery	9/26	12/22	0.44	.16	0.13-1.41
Sources per pen from one litter	5/26	1/22	5	.19	0.53-46.52
Sources per pen from more than one sow farm	4/26	7/22	0.38	.17	0.09-1.56
Source per room from more than one sow farm	5/26	8/22	0.41	.18	0.11-1.53
Detergent	7/26	12/22	0.31	.05	0.09-1.02
Pelleted first nursery feed	22/25 ⁴	13/22	5.07	.02	1.16-22.20
Creep feed before weaning	20/26	13/22	2.3	.18	0.66-8.03
Blended feed between phases	15/26	8/22	2.38	.14	0.74-7.65
Nursery pigs vaccinated against PRRSV ³	10/25 ⁴	3/22	4.22	.04	0.98-18.12
Gestating sows vaccinated against PRRSV	13/23 ⁴	5/20 ⁴	3.9	.03	1.05-14.38

¹ Variables with *P* values <.20 (chi-square test) were tested in a multivariate model using logistic regression.

² Cases: herds with a history of PWECD; Controls: herds with no history of PWECD.

³ Porcine reproductive and respiratory syndrome virus.

⁴ Information not available for all herds.

⁵ 95% confidence interval of the odds ratio.

control herds. There was no difference in size between case and control herds ($P>.05$). Management systems used in both case and control herds included farrow-to-finish, farrow-to-partly finish (where only a portion of grower-finisher pigs were raised to market on the farrowing site), farrow-to-feeder, and off-site nurseries.

There were no differences between case and control herds in the following parameters: population density, average age at weaning, weight at weaning, and levels of protein, fat, and fiber in the first feed offered to the pigs in the nursery (Table 2). No differences ($P>.05$) were found between cases and controls for the following variables: multiple sources of pigs per pen or room; type of flooring; disinfection procedures; use of creep feed; or use of all in-all out compared to continuous flow management by room or building.

Case herds commonly reported changes in feeding and management in response to diarrhea. The common feed changes reported were purchasing feed from a different supplier; changing the in-feed medication; offering a limited amount of feed several times per day; blending of feed between phases; decreasing the level of protein; and increasing the level of fiber. The common management changes reported were increasing weaning age; improving control of temperature and ventilation; creation of sick pens; decreasing density in pens; and reducing the mixing of pigs.

Case herds had more feeder spaces per pen than control farms (Table 2). Case herds were more likely to use pelleted and crumbled feed in the first ration offered in the nursery, compared to control herds (Table 3). Control herds more commonly used detergent prior to disinfection than case herds (Table 3). The type of feed in the first nursery ration (mash or pelleted feed) and the use of detergent before disinfecting were tested for interaction within PRRS-positive and PRRS-negative herd status. No interaction was found between PRRS status and either the use of detergent ($P>.05$) or the type of feed in the first nursery ration ($P>.05$).

Among the 26 case herds, seven were serologically negative for PRRSV; 17 herds had low S:P ratios (between 0.4 and 2.5), possibly resulting from vaccination or passive immunity; and two herds had high S:P ratios (>2.5), indicative of recent PRRSV infection. Among the 22 control herds, six were serologically negative for PRRSV, 16

Table 4: Final multi-variable analysis¹ of risk factors for postweaning *Escherichia coli* diarrhea in a study in 48 Ontario nurseries in 1999

	Odds ratio	P	95% CI of OR ⁴
Gestating sows vaccinated against PRRSV ²	5.7	.05	0.98 - 32.8
Pelleted feed ³	20.9	.03	1.30 - 334.3
Number of feeder spaces per pen	1.5	.02	1.07 - 2.26

¹ Final multivariable logistic regression model describing the association between case herds and management factors.

² Porcine reproductive and respiratory syndrome virus.

³ Pelleted versus mash (first nursery feed).

⁴ 95% confidence interval of the odds ratio.

had low S:P ratios, and none had high S:P ratios indicative of active PRRSV infection. There was no association between PRRS serological status and occurrence of PWECD ($P=.4$). However, case herds were more likely to vaccinate their nursery pigs against PRRSV than control herds (Table 3). The only PRRS vaccine used in both case and control herds was Ingelvac PRRS MLV (Boehringer Ingelheim, Burlington, Ontario). Case herds were more likely than control herds to vaccinate gestating sows against PRRSV (Table 3). In PRRS-positive herds, after controlling for sow PRRS vaccination, nursery vaccination was not important as a risk factor for *E. coli*. After controlling for nursery vaccination, sow vaccination was also not important as a risk factor for *E. coli*. These factors were confounded, ie, associated with one another ($P=.008$).

Two of the 26 case herds and one of the 22 control herds were serologically positive for TGE (coronavirus differential ELISA test). No association was found between PWECD and positive TGE titres ($P=.6$).

In the final-multivariable model, case herds were more likely than control herds to use PRRS vaccine in sows, case herds were more likely than control herds to feed pelleted feed in the nursery rather than mash, and case herds allowed more feeder spaces per pen than control herds (Table 4).

Discussion

Culture of rectal swabs from scouring piglets is an effective method of detecting F4⁺ *E. coli* when clinical disease is present. However, some of the case herds were visited at a time when diarrhea was not a clinical problem. Isolation of F4⁺ *E. coli* from nursery pigs in only 15 of the 26 case

herds (57%) at the time of the herd visit reflects the sporadic occurrence of the disease.

The wide range in the estimates of the confidence intervals of the odds ratios for qualitative variables in this study might be due to the small sample size of herds included. There were no differences between case and control herds for a number of factors commonly thought to be associated with nursery pig diseases.⁶⁻⁸ In particular, early weaning age and commingling of pigs from several herds into one nursery room, practices commonly associated with segregated-early-weaning production systems, were not associated with a higher likelihood of PWECD. In this study, multi-site and single-site herds were represented in case and control herds in approximately equal proportions, and weaning weights and ages were similar between case and control herds. Weaning at an early age has been considered a risk factor for PWECD because the physiological immaturity of the gut allows undigested substrate to pass along the digestive tract and supply nutrients to pathogenic bacteria.¹² In addition, immunity is not fully developed in the very young pig, which relies on passive protection from colostrum and milk.

Differences between case and control herds included feeding pellets or mash as the first nursery feed, and number of spaces at the feeders. It has been suggested that heat-treated cereals, for example, in pelleted feed, might provide a better environment for *E. coli* proliferation.¹³ It is believed that pelleted feed provides more nutrients for *E. coli* in the following two ways: first, pigs eat more of the pelleted feed; and second, pelleted feed moves through the gut more rapidly so that fewer of the nutrients are

absorbed by the pig, leaving more for the organisms. The results of this study support this concern that pelleted feed is a risk factor for PWECD. Greater feeder space availability might allow or encourage pigs to overeat after weaning, which may contribute to diarrhea.¹⁴ Feed restriction has been advocated as a method of reducing PWECD.^{3,9,14} However, other workers have suggested that reduced feeder space is a risk factor for PWECD.⁶

Our study supports the view that unlimited access to feed in the early weaning stage is a risk factor for PWECD. Hygiene was expected to be a major risk factor for PWECD, with poor cleaning procedures resulting in increased bacterial challenge. Most farms cleaned pens between groups of pigs whether or not *E coli* was a problem. Power washing with a surfactant reduces bacteria and enables emulsification of the organic matter that protects the pathogens.¹⁵ More control farms used a detergent prior to disinfection, and therefore control herd facilities might have been cleaner compared to case herd facilities, but on the basis of visual inspection, the nurseries in both groups appeared similar in cleanliness. In all likelihood, pigs carry F4⁺ *E coli* into the nursery in intestinal flora,¹⁶ and therefore PWECD may occur no matter how thorough the cleaning procedure is.

Use of PRRS vaccine has been associated with the birth of weak viremic pigs,¹⁷ and clinical PRRS may exacerbate *E coli* problems.^{10,18} There is some biological basis to support the suggestion that vaccination of pregnant sows or weanling pigs might reduce gut immunity and contribute to *E coli* diarrhea, and this association should be further investigated.

Many factors associated with PWECD in experimental studies were not significant in this observational herd level study. This is consistent with the view that multiple risk factors must be present, as well as F4⁺

E coli, in order for clinical diarrhea to occur. In herds in which PWECD did not occur despite younger weaning age or other possible risk factors, undocumented procedures may have been counteracting the negative influences. It is also possible that in at least some of the control herds, risk factors for PWECD were present, but F4⁺ *E coli* was not.

Implications

- Weaning age, weaning weight, and commingling of pigs from multiple sources were not risk factors for PWECD in this case-control study.
- Vaccination for PRRS appeared to be associated with an increased risk of PWECD.
- Pelleted feed and larger number of feeder spaces were associated with an increased risk of PWECD.
- PWECD occurred on a variety of farm types and sizes.

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