Epidemiological study of *Yersinia enterocolitica* in swine herds in Québec

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Abstract — The objectives of this study were the identification of the different contamination sources of *Yersinia enterocolitica,* as well as the determination of the prevalence and the distribution of the different genotypes in swine herds. The owners of 20 farms, located in the Richelieu-Yamaska region, agreed to participate in the study. Each farm was visited a minimum of 5 times between May and October 1997, and, at each visit, 20 environmental and 10 fecal samples were collected. *Yersinia enterocolitica* isolates were identified, serotyped, and submitted to a genetic characterization by pulsed-field gel electrophoresis. The correlation coefficient (0.61) between prevalence in environment and in feces was significant ($P = 0.004$). Among the 153 positive samples, 93.5% belonged to serotype 0:3. The comparison of PFGE profiles revealed that all environmental *Y. enterocolitica* isolates had a profile identical to that of isolates recovered in feces from the corresponding farms. Also, when the genetic profiles of isolates recovered from feces collected at the first visit were compared with the profiles of isolates obtained from the subsequent visits, the same profile was observed on every farm. We concluded that environment does not represent the main source of contamination of swine by *Y. enterocolitica* and that, in most instances, the same strain persists in a barn from one production lot to another.

Résumé — Étude épidémiologique de l'infection à *Yersinia enterocolitica* dans les élevages porcins au Québec. Les objectifs de cette étude étaient d'identifier les différentes sources de contamination par *Yersinia enterocolitica* et de déterminer la prévalence et la distribution des différents génotypes dans les élevages de porcs étudiés. Les propriétaires de 20 fermes, situées dans la région Richelieu-Yamaska, ont accepté de participer à cette étude. Chaque ferme a été visitée un minimum de 5 fois entre mai et octobre 1997 et lors de chaque visite, 20 échantillons environnementaux et 10 échantillons de matières fécales ont été récoltés. Les isolats de *Y. enterocolitica* ont été identifiés, sérotypés, et une caractérisation génétique par l'électrophorèse en champs pulsés a été effectuée. Un coefficient de corrélation (0,61) significatif ($P = 0,004$) entre la prévalence dans l'environnement et dans le fèces a été observé. Parmi les 153 échantillons positifs, 93,5 % appartenaient au sérotype 0:3. La comparaison des profils génétiques a révélé que les profils des souches environnmentales étaient identiques à ceux des isolats provenant des fèces d'une même ferme. Aussi, les profils génétiques observés d'une visite à l'autre étaient similaires sur chaque ferme tout en étant différents dans les différentes fermes. Il a été conclu que l'environnement ne représente pas la principale source de contamination par cette bactérie et que la plupart du temps la même souche persiste d'un lot de production à l'autre.

(Traduit par les auteurs)

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Introduction

The number of infections due to *Yersinia enterocolitica* in humans has increased considerably over the past years (1). These infections are often linked to pork consumption. Contamination of meat often occurs at the time of evisceration in the abattoirs (1,2). This bacterium has been isolated from a variety of environmental sources, but pigs are recognized to be the main reservoir of the different *Y. enterocolitica* serotypes (0:3, 0:9, 0:5, and 0:8), which also are the most prevalent serotypes in human infections (1,2). In Quebec, serotype 0:3 has been shown to be the predominant serotype in swine carcasses, as well as in clinically healthy pigs (3–9).

The potential sources of *Y. enterocolitica* in swine are numerous. Studies from Europe, Japan, and the United States have reported the presence of this microorganism in rats (10–12) and other rodents (13–15). Other studies have shown its presence in flies (16) and water (1,7,9,13, 17–20).

A study carried out in Québec in 1989 revealed that 18% of fecal specimens from clinically healthy pigs were positive for the presence of *Y. enterocolitica* (21). More recent data are not available. A better knowledge of the prevalence in swine and farm environment and the identification of possible sources of contamination are prerequisites for the development of better control measures against this microorganism.

The objectives of this study were to identify the different sources of *Y. enterocolitica,* as well as to determine

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the prevalence and the distribution of the different genotypes in swine herds.

Materials and methods

Owners of 20 swine finishing farms of approximately 800 to 1200 animals agreed to participate in the study. Farms were located in the Richelieu-Yamaska region of the province of Quebec, within a 75-km radius from the Faculté de médecine vétérinaire of the Université de Montréal, located in St-Hyacinthe. All farms were visited a minimum of 5 times between May and October 1997.

Sample collection

At each visit, 30 samples were collected with the following distribution: 20 environmental samples and 10 fecal samples. For fecal samples, approximately 2 g of feces were collected by swabbing 10 pens, chosen randomly, at 5 different locations. For environmental samples, 10 cm × 1 cm surfaces were swabbed (when more than one location was swabbed, all swabs were pooled in the same tube in the following locations): passage (3 different locations), fans when forced ventilation was used or edges when natural ventilation was present (4 different locations), water pipes (4 different locations), pen separations (4 different locations), exterior entry, interior floor entry, pig loading areas, stairs, shovels, brushes, boots, and wagons.

Other environmental samples were collected as follows: 1 mL of tap water and 1 mL from watering place (stagnant water from trough) or a swab of watering place suckling devices, 1 g of feed sample in feeding place and storage, and 10 flies collected on fly catchers and pooled in a tube (the fly catcher was changed at each visit). Finally, rodent cages were placed outside the farms, near the foundations of the piggery. Necropsy of captured rodents was performed at the laboratory. A one-gram (approximate) sample of intestines was ground for culture. The cages were disinfected after each use. For other samples, a similar procedure was used.

Moist swabs were used to collect material on dry surfaces. All samples were placed in 9 mL of phosphate-buffered saline (Unipath, Nepean, Ontario) for cold enrichment for a period of 18 to 21 d (1).

Isolation and identification of *Yersinia enterocolitica*

Isolation was carried out on *Yersinia*-selective agar containing cefsulodin, irgasan, and novobiocin (CIN agar; Laboratoires Quélab, Montreal, Quebec) (1). Preliminary biochemical identification of *Y. enterocolitica* was carried out according to Canadian Food Inspection Agency procedures (22), and confirmation was obtained by using a commercial biochemical identification system (API 20E system, Biomérieux Canada, Ville St-Laurent, Quebec). All *Y. enterocolitica* isolates were then serotyped by using a commercial coagglutination method (Accurate Chemical & Scientific, Westbury, New York, USA).

Genetic characterization

Pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA were used for genetic comparison of

Table 1. Prevalence of *Yersinia enterocolitica* in fecal and environmental samples from selected swine herds in Quebec

	Fecal samples		Environmental samples	
Herds	% positive	No. samples	% positive	No. samples
1	0.0	50	0.0	96
2	4.0	50	0.0	90
3	8.3	60	1.8	113
4	1.7	59	0.0	114
5	3.5	57	0.9	109
6	14.0	50	2.1	97
7	6.0	50	0.0	95
8	44.2	43	2.2	91
9	8.7	46	0.0	89
10	12.0	50	0.0	90
11	30.0	50	5.1	98
12	30.0	50	2.1	94
13	28.0	50	2.1	94
14	10.0	50	0.0	101
15	46.9	49	1.0	98
16	10.0	50	0.0	99
17	20.4	49	0.0	94
18	0.0	50	0.0	95
19	0.0	48	0.0	91
20	0.0	49	0.0	96
Mean/Total	13.5	1010	0.9	1944

Table 2. Distribution of *Yersinia enterocolitica* serotypes of all positive isolates collected on 20 swine farms in Quebec

Serotype	Number	Percentage
0:3	143	93.5
0:9	2	1.3
0:5	2	1.3
0:8	0	0.0
Nontypable	6	3.9

Y. enterocolitica isolates. Genomic DNA was isolated by a modified version of the method of Kaufmann and Pitt (23). Briefly, 25–30 colonies of a pure culture on blood agar were suspended in a saline ethylenediaminetetraacetic acid (EDTA) (SE) solution of 75 mM NaCl and 25 mM EDTA (pH 7.5), to an optical density of 1.5 to 1.8 at 540 nm. Then, 250 µL of this suspension were mixed with 250 µL of 1.5% low gelling temperature agarose (Sigma Chemical, Oakville, Ontario) dissolved in SE. The mixture was kept at 56°C until it was dispensed into molds. After 10 min at 4°C, the solidified plugs were transferred in 3.6 mL of 1% (w/v) N-lauryl sarcosine-0.5 M EDTA (pH 9.5) (lysis buffer). To this mixture was added 0.4 mL of a 10 mg/mL solution of proteinase-K (Sigma Chemical) dissolved in 50 mM Tris-1 mM $CaCl_2$ (pH 8.0). The cell lysis was carried out for 20 h at 56°C in a water bath. The following day, the agarose plugs were washed with Tris-EDTA (TE) solution (10 mM Tris and 10 mM EDTA (pH 7.5)) and were stored in this buffer at 4°C. Digestion of agarose-embedded DNA was carried out with 20 U *Not*1 (Canadian Life Technologies, Burlington, Ontario) (24) at 37°C for 18 to 24 h. Pulse-field gel electrophoresis (PFGE) was performed with a contour clamped homogeneous field apparatus (Gene Navigator system, Pharmacia Biotech, Baie d'Urfé, Québec) in a 1.2% high gelling agarose-Tris-borate-EDTA 0.5X buffer

Table 3. Distribution of *Yersinia enterocolitica* in the environment of 20 swine farms in Québec

Type of sample	No. samples	No. positive	Percentage
Passage	309	2	0.6
Fans	103	0	0.0
Piping	103	3	2.9
Pen separations	103	3	2.9
Interior floor entry	119	1	0.8
Exterior entry	97	0	0.0
Shovels	90	2	2.2
Brushes	33	0	0.0
Boots	64	1	1.6
Wagons	16	0	0.0
Tap water	101	1	1.0
Watering place (stagnant)	155	0	0.0
Suckling devices	45	2	4.4
Food from reserve	96	0	0.0
Food from feeding place	203	1	0.5
Flies	371	0	0.0
Rodents	15	0	0.0
Pig loading areas	9	0	0.0
Stairs	8	1	12.5
Others	4	0	0.0
Total	1944	17	0.6

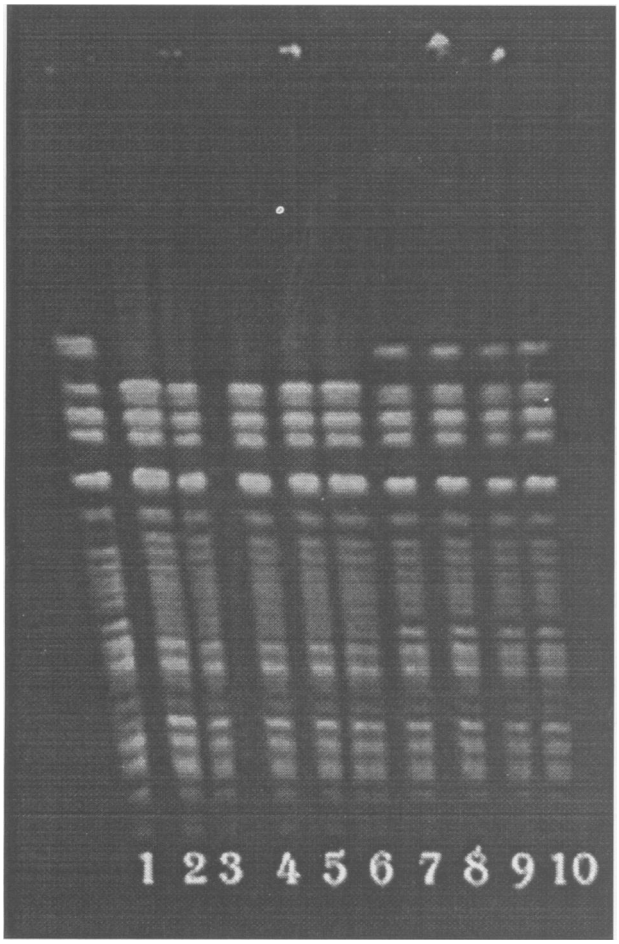

Figure 1. Pulsed-field gel electrophoresis profiles comparison of genomic DNA of environmental and feces *Yersinia enterocolitica* isolates. Lane 1: λ ladder; lanes 2 to 6: isolates from feces and environment of farm 8; lanes 7 to 10: isolates from feces and environment of farm 3.

gels, in accordance with the manufacturer's instructions (Sigma Chemical). The gel was run for 16 h at a constant voltage of 200 V, pulse time 5 to 25 s with linear ramping, an electrical field angle of 120°, and a temperature of 10°C. Gels were stained with ethidium bromide, destained in distilled water, and photographed by using an instant sheet film apparatus (Type 667 Polaroid, Polaroid Canada, Etobicoke, Ontario) under UV illumination. A lambda ladder PFGE marker (Bio Rad Laboratories, Mississauga, Ontario) was used for the calculation of molecular weights.

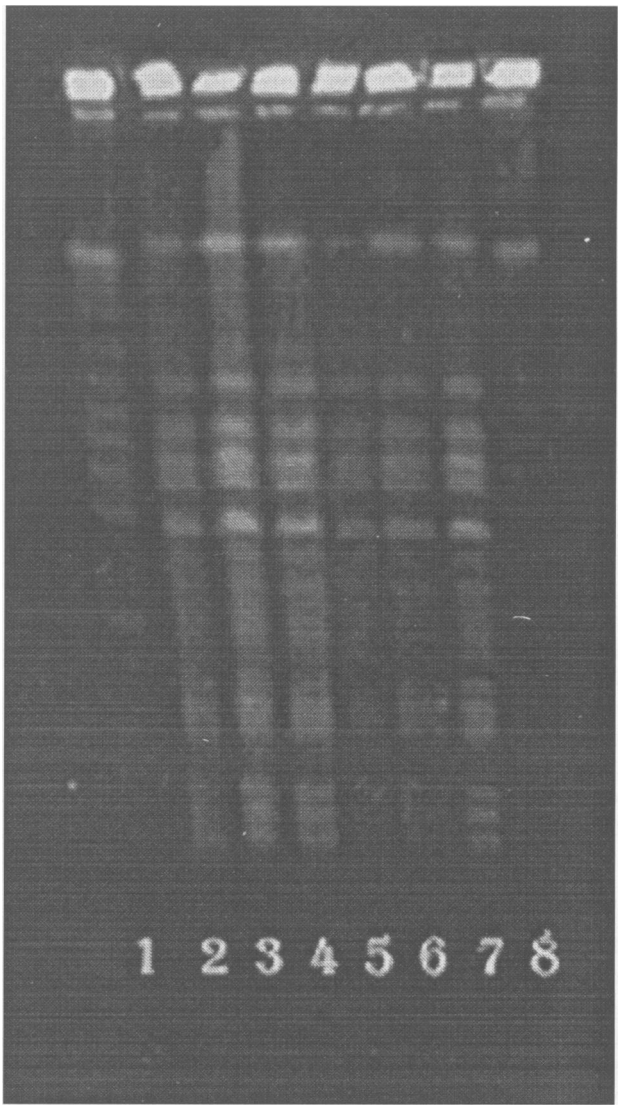

Figure 2. Pulsed-field gel electrophoresis profiles comparison of genomic DNA of *Yersinia enterocolitica* isolates recovered at first and following visits. Lanes 1 and 8: λ ladder; lanes 2 to 7: isolates from feces of farm 12 at different times.

Statistical analyses

A correlation coefficient was computed between the prevalence of *Y. enterocolitica* in the environment and the prevalence of this bacterium in feces. Also, medians were computed on *Y. enterocolitica* prevalence in feces for farms where positive and negative environmental samples, respectively, were found.

Results

Sixteen out of 20 farms visited were positive for *Y. enterocolitica* in fecal samples and 8 of these farms were

found positive for *Y. enterocolitica* in environmental samples (Table 1). The correlation coefficient between prevalence in the environment and in feces was significant at 0.61 ($P = 0.004$). The median of *Y. enterocolitica* prevalence in feces collected on farms where environmental samples were found to be positive was 29%. In farms where environmental samples were negative, the median of prevalence was 5%. Among the 153 positive samples, 93.5% belonged to serotype 0:3 (Table 2).

None of the 15 rodent and 271 flies samples were found to be positive for *Y. enterocolitica.* Watering places with stagnant water were also negative. Positive samples were found in feeding places, tap water, and watering place suckling devices (Table 3).

The comparison of PFGE profiles revealed that all environmental *Y. enterocolitica* isolates, except one, had a profile identical to that of an isolate recovered in feces from the corresponding farms (Figure 1). Also, with this same exception, the same and unique genotypic profile was found in samples collected at every visit within a given herd. In addition, when the profiles of isolates recovered from feces at the first visit were compared with the profiles of isolates at the subsequent visits, the same genetic profile was observed on every farm (Figure 2). However, the genetic profiles observed were, in most cases, different from one farm to another (Figure 2).

Discussion

In this study, as many as 80% of swine herds had at least one animal infected with *Yersinia enterocolitica.* Although this prevalence rate is somewhat higher than rates reported in earlier studies, it is in accordance with studies that have identified swine as an important reservoir of this microorganism (4,26,27). The presence of *Y. enterocolitica* was also detected in the environments of the herds that were visited. The number of isolates in environmental samples was directly proportional to the prevalence of this microorganism in feces. This would suggest that, in herds in which the number of carrier animals is low, the transmission of the infection by fomites is limited. The fact that the only 2 farms showing the same genotypes purchased their animals from the same source (data not shown) would also suggest an animal-to-animal transmission pattern.

Serotyping of *Y. enterocolitica* isolates recovered in this study revealed that 93.5% of them belonged to serotype 0:3. This is in accordance with previous Canadian studies mentioning that in eastern provinces, serotype 0:3 predominated in slaughtered pigs (4,5). This same serotype has also been shown to be the most prevalent in isolates of human origin (3,6).

Potential sources of infection of *Y. enterocolitica* are numerous. Among them, rodents, which are frequently seen in piggery surroundings, have been shown to carry this microorganism, particularly serotype 0:3 (10–15). In this study, none of the 15 captured rodents were positive for the presence of *Y. enterocolitica* in their intestines. This could be explained by our field observation showing that most of swine producers have put emphasis on the control of vermin within their herds. It must also be underlined that rodents were captured outside the building, and that the results might have been different if rodents inside the barn had been captured. Flies have also been implicated as a potential source of *Y. enterocolitica* for pigs (16). In this study, as for rodents, all fly specimens were negative for the presence of this microorganism.

Different studies have reported the presence of *Y. enterocolitica* in water samples and that, in several epidemics in humans, water was the common source of infection (1,13,17,18). It has been demonstrated that under experimental conditions, this bacterium can survive for several months in water in the presence of organic matter (19). In this study, only one water sample contained *Y. enterocolitica.* Interestingly, the genotypic profile of this isolate was similar to that of swine isolates on the same farm, indicating that in some swine operations, water could contribute to the transmission of the infection.

It is also noteworthy that genotypic profiles of *Y. enterocolitica* isolates were similar within a given farm and for all visits. This suggests that in a herd, a minimum of strains are present and that it or they persist for at least a few months.

Finally, previous studies have indicated that the isolation rate of *Y. enterocolitica* was higher in the cold months (20,27,29). This parameter was not evaluated in the present study.

In conclusion, the low prevalence of *Y. enterocolitica* observed in the herds' immediate environment suggests that this microorganism has a low survival rate under these conditions. Thus, the environment would not represent the main source of contamination of swine by *Y. enterocolitica.* Since we recovered only one genotype per farm, this suggests that, generally, not more than one strain is present in a given herd, and that the importance of external sources of *Y. enterocolitica* is minimal.

Acknowledgments

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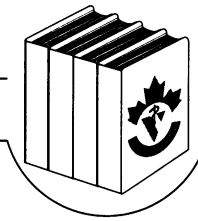
BOOK REVIEW



COMPTE RENDU DE LIVRE

Rothschild MF, Ruvinsky A, eds. ***The Genetics of the Pig.*** CAB International, Wallingford, Oxon, UK, 1998, 622 pp. ISBN 0-85199-229-3.

This book is one of a series of such texts published and seemingly coordinated by Dr. Ruvinsky who is an author of this one and one about cattle. The preface says that this book is intended "for a diverse audience including students, researchers, veterinarians, and pig breeders." I am a geneticist and would assume that only veterinarians specializing in pigs would want to purchase this book, because there is little medical content. There is one very handy chapter by Frank Nicholas containing a list of more than 130 genetic traits and disorders in the pig, with a few sentences about each one and a starting reference.

Some chapters cover material beyond the narrow "genetic" definition. For example, the chapter entitled Biology and Genetics of Reproduction summarizes data on conception rates, perinatal survival, etc. in European versus Chinese pig breeds. For veterinarians advising clients on whether to purchase Meishan-influenced pigs, this information could be quite pertinent.

Each of the 18 chapters is written by an expert in that area and is extensively referenced. The detail is very thorough and quite up to date. I would have wished for more pictures or drawings, being a visually oriented reader. Most of the chapters are written in textbook style. The book can be previewed on the Web at www.cabi.org/readingroom/frameset.asp?ISBN = 0851992293 so you have a chance to form your own opinion.

Reviewed by ***Sheila M. Schmutz, PhD, Professor,*** *Department of Animal and Poultry Science, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4.*