Brief Communication Communication brève

Prevalence of *Campylobacter* spp. isolated from grower-finisher pigs in Ontario

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Abstract — This study aimed to establish the prevalence of *Campylobacter* spp. in 80 Ontario grower-finisher pig herds. Ninety-nine percent of the isolates yielded *Campylobacter, C. coli* being the most common species detected. Control of this microorganism must rely on careful food processing and storage of pork, rather than on an on-farm approach.

Résumé — Prévalence de *Campylobacter* spp. isolés chez des porcs en croissance-finition de l'Ontario. Cette étude visait à établir la prévalence de *Campylobacter* spp. chez 80 troupeaux de porcs en croissance-finition de l'Ontario. On a retrouvé du *Campylobacter* dans 99 % des isolats, *C. coli* constituait l'espèce la plus souvent détectée. La lutte contre ce microorganisme doit se fonder à la fois sur la transformation alimentaire et un entreposage minutieux de la viande de porc plutôt que sur des actions entreprises à la ferme.

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Food safety has become an important issue in Ontario. From 1997 to 2001, there were over 44 450 reported cases of illness attributable to enteric pathogens (1). Of all foodborne pathogens, *Campylobacter* spp. accounted for the highest annual average incidence rate at 42.3 cases per 100 000 people during a 4-year period (1).

Pork is considered a possible source of *Campylobacter* infection of humans as a result of carcass contamination at slaughter. Improving food safety by reducing the level of *Campylobacter* at the farm level, or at least gaining better understanding of the epidemiology of the disease organism is important in order to maintain consumer confidence. The primary goal of this study was to improve epidemiological understanding of *Campylobacter* by determining the prevalence of *Campylobacter* spp. on certain grower-finisher pig herds in Ontario. Secondarily, because *C. jejuni* and, less commonly, *C. coli* are the usual causes of campylobacteriosis in humans, all *Campylobacter* isolates had to be biotyped in order to quantify the diversity of *Campylobacter* spp. isolated from these pigs and to determine which *Campylobacter* spp. are most commonly isolated from pigs.

A sample of 80 farms were selected, in part, from a) conveniently selected operations close to Guelph, Ontario; b) purposively selected operations, based on geographical distribution of farms in Ontario and based on herd type; and c) randomly selected operations, based on the willingness of swine producers to participate after the termination of a previous study. Swine operations were not a true random sampling; however, they did represent farms from all the swine producing regions of southern Ontario, and in terms of management style, varied from single-site farrow-to-finish operations to specialized farms of large multisite swine operations with direct pig flow. The 80 farms were visited in 2004 between January and June, and a fixed number of samples were collected from healthy animals on each farm.

For each all-in/all-out grower-finisher barn, 5 pens were randomly selected and a total of 15 specimens were collected. In herds using a continuous-flow system of management of the grower-finisher barn, the 5 pens were those identified with the largest pigs. In each pen, 1 freshly voided fecal sample from each of 2 different hogs and a 3rd sample that combined fecal samples from 5 different places in the pen (a pooled environmental sample), were collected. Samples were stored in sterile containers (Starplex Scientific; Etobicoke, Ontario), transported to the Laboratory Services Division (LSD), University of Guelph, in coolers containing ice packs, and processed within 24 h of collection.

Upon arrival at the laboratory, the following procedures were used to isolate and categorize *Campylobacter:* enrichment in a selective broth, selective plating, and biochemical characterization, based on the USDA/FSIS methodology (2). Ten grams of each fecal sample were mixed with 90 mL of 0.1% buffered peptone water (BPW) (Oxoid Company; Nepean, Ontario) and homogenized in a sterile stomacher bag. One milliliter of the rinse was added to a 9-mL tube containing Hunt enrichment broth (HEB), and 2 subsequent dilutions were prepared in HEB tubes. All the tubes, including controls, were placed in microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) and

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incubated at 37°C ± 1.0°C while being shaken at 100 rpm for 4 h. After this incubation period, 36 μL of a sterile cefoperazone solution was added to bring the final concentration to 30 mg/L. The microaerobic atmosphere was then reestablished and the solution was incubated at 42°C ± 1.0°C while being shaken at 100 rpm for 24 h.

Modified Campylobacter charcoal differential agar plates (MCCDA) containing cefoperazone and amphotericin B were inoculated with serial enrichments and incubated at 42°C in microaerophilic conditions for 48 h. Well-isolated typical colonies were selected from each plate and examined by dark-field microscopy. *Campylobacter* colonies on MCCDA are either smooth, shiny, convex-shaped with a defined edge, or flat, translucent, spread out with an irregular edge. They are usually colorless, greyish, or light cream, and usually 1 to 2 mm in diameter, but they may also grow to be several millimeters in diameter (2).

The following medium controls were inoculated with each batch of tests to ensure a proper medium formulation, sterility, and atmospheric conditions: *Campylobacter jejuni* ATCC 49432, *Escherichia coli* ATCC 25922, and without bacteria, as positive, negative, and blank controls, respectively.

A biotyping scheme was used to differentiate between the 3 classical thermophilic *Campylobacter* spp: *C. jejuni, C. coli,* and *C. lari.* Hippurate hydrolysis, production of hydrogen sulphide, DNA hydrolysis, and indoxyl acetate hydrolysis were performed on suspected *Campylobacter* colonies. The following parameters were taken into consideration: colonies showing ability to hydrolyze both hippurate and indoxyl acetate were classified as *C. jejuni;* colonies able to hydrolyze indoxyl acetate, but not hippurate were classified as *C. coli;* and colonies showing the capacity to hydrolyze neither hippurate nor indoxyl acetate were classified as *C. lari.* This procedure was based on the method used by Health Canada's National Laboratory for Enteric Pathogens, described in 1984 by Lior (3), with revisions by Lior and Patel (4). Colonies identified as *C. coli* were inoculated into 1.5-mL microtubes (Sarstedt; Montreal, Quebec) with 0.6 mL of Mueller-Hinton broth (Difco Laboratories; Detroit, Michigan, USA), containing 50% glycerol (Fisher Scientific, Nepean, Ontario), and stored at -70°C for future susceptibility testing.

Data were entered into a spreadsheet program (Excel 2000; Microsoft, Redmond, Washington, USA) and transferred into a statistical analysis program (SAS/STAT, version 8.2; Statistical analysis system — SAS Institute, Cary, North Carolina, USA) for statistical analyses (previous verification for entry data accuracy). Prevalences and standard errors were calculated in SAS software and confidence intervals were calculated by using R software (R; Copyright 2005, The R Foundation for Statistical Computing Version 2.2.1, Vienna, Austria). In addition, a pig was considered positive to *Campylobacter* if at least 1 strain of *Campylobacter* was found, using the isolation technique described above. Likewise, a farm was considered positive, if at least 1 of the 15 samples tested was positive.

Of the 1200 samples taken from 80 farms, 800 were categorized as fecal samples and 400 were categorized as environmental samples. *Campylobacter* was recovered from all but 1 fecal and 5 environmental samples; 4 of the negative environmental samples were from the same farm, so a total of 1194 samples yielded *Campylobacter* and all but 9 isolates (independent colonies) were identified as *C. coli.* The prevalence of *C. coli, C. lari,* and *C. jejuni* were 99.2% (1185 of 1194), 0.6% (7 of 1194), and 0.2% (2 of 1194), respectively, resulting in 95% confidence intervals of 98.6%–99.7% for *C. coli,* 0.24%–1.20% for *C. lari,* and 0.02%–0.60% for *C. jejuni.*

Campylobacter was isolated from almost all the samples and it is quite possible that on the rare occasion when *Campylobacter* was not isolated, the reason may have been related to the transportation/storage and culture technique, rather than the sample actually being negative. *Campylobacter* was present on every farm and was very widespread within the pig population, suggesting that *C. coli* is a normal gut inhabitant of pigs. These results are consistent with previous Canadian studies that reported high prevalence rates of *Campylobacter* spp. in swine fecal material (5,6).

The authors of previous studies, carried out at slaughter, have suggested that the high prevalence of *C. coli* in pigs is caused by the practice of mixing pigs from different farms before slaughter (5), whereas this study at the farm level demonstrates that *C. coli* is an ubiquitous inhabitant of pigs. A high isolation rate of *C. coli* in pig feces has also been reported worldwide, ranging from 66% to 100% isolation rates; however, *C. jejuni* was isolated rarely, if at all (6). Some researchers, by the use of enrichment broth and restrictive media, suggest that a relative high prevalence of *C. jejuni* in pigs may be found (7). It is possible that *C. coli* is more easily detected than other *Campylobacter* species in pigs or that pigs carry a larger amount of *C. coli* than *C. jejuni* in their feces, making *C. coli* more likely to be isolated in a particular sample. Both direct plating and selective enrichment protocols might need to be employed for optimal surveillance of *C. jejuni* in fecal material (8). Further research is needed to clarify whether *C. jejuni* is also present in the swine intestinal tract at a prevalence higher than that observed in this study. The use of molecular techniques may increase the diagnostic efficiency, allowing for a better estimate of the prevalence of different *Campylobacter* spp. in pigs.

Reports from retail studies suggest that most chickens are contaminated with *Campylobacter,* whereas this microorganism is not detected on pork carcasses after overnight chilling, possibly reflecting differences between poultry and swine slaughter practices (9). It has been shown that the ability to survive on skin is similar for *C. coli* and *C. jejuni* and, therefore, that differences in prevalence at the retail level between pork and chicken cannot be explained by the low prevalence of *C. jejuni* in pigs (10). High prevalence levels of *C. coli* identified in this study were achieved by the use of an improved isolation technique. Isolation of fastidious organisms, such as *Campylobacter,* has improved significantly since Lior first isolated this organism. The use of the indoxyl acetate hydrolysis test for the differentiation between species of *Campylobacter* allows for superior differentiation and avoids misclassification (11).

This work demonstrates that *C. coli* is the dominant *Campylobacter* species in Ontario pigs, and because it is isolated from over 99% of pigs in Ontario, *C. coli* can be regarded as a

normal inhabitant of a pig's gastrointestinal system. Attempts to eradicate *Campylobacter* in pig farms would appear to be ill-advised, since levels of *Campylobacter* spp. at the farm level appear to be extremely high. Therefore, control of this organism must rely on careful food processing and storage of pork rather than on an on-farm approach.

Because the sampling process was a mixing of convenience and random sampling at the province and farm levels, respectively, the results can be regarded as representative for the 80 farms, but not for the province. However, it is interesting that within this sample of farms, there were farms with excellent health status and good biosecurity rules. Therefore, it is likely that these findings are also valid for the Ontario swine industry. The fact that some surveys have found a lower prevalence may reflect poorer laboratory techniques and ability to culture the bacteria rather than a true difference in prevalence.

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