
Z. Poljak\textsuperscript{a,*}, C.E. Dewey\textsuperscript{a}, S.W. Martin\textsuperscript{a}, T. Rosendal\textsuperscript{a}, J. Christensen\textsuperscript{b}, B. Ciebin\textsuperscript{c}, R.M. Friendship\textsuperscript{a}

\textsuperscript{a} Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1
\textsuperscript{b} Canadian Food Inspection Agency (CFIA), 690 University Avenue, Charlottetown, Prince Edward Island, Canada C1E 1E3
\textsuperscript{c} National Centre for Yersinia, Ontario Ministry of Health and Long-Term Care, Toronto, Ontario, Canada M5W 1R5

1. Introduction

\textit{Yersinia enterocolitica} is a Gram-negative coccobacillus classified, together with 10 other species, within the genus \textit{Yersinia} of the family \textit{Enterobacteriaceae} (Bottone, 1997). On the basis of biochemical properties, \textit{Y. enterocolitica} is frequently classified into six biotypes. Biotype 1A is considered non-pathogenic for people, whereas others are considered to be human pathogens (1B, 2, 3, 4, and 5) (Nesbakken, 2005). On the basis of serological properties associated with O-antigens, \textit{Y. enterocolitica} is further classified into \sim60 serotypes, 11 of which are reported to be associated with clinical illness in people (Bottone, 1999). Sources of \textit{Y. enterocolitica} include the intestinal tracts of mammalian, avian, and cold-blooded species (Bottone, 1997), and the environment, including water and soil (Bottone, 1997; Nesbakken, 2005). Environmental isolates are more commonly classified as “non-pathogenic”, whereas isolates of animal origin are more frequently classified as “pathogenic” (Bottone, 1997). In particular, porcine sources are often associated with...
pathogenic serotypes (O:3, O:9, and O:5,27) (Bottone, 1997; Nesbakken, 2005) and sometimes with the highly virulent serotype O:8 (Bottone, 1997).

Infection with *Y. enterocolitica* in humans can cause clinical disease, primarily affecting the gastrointestinal tract. Clinical signs of infection include watery (and occasionally bloody) diarrhea, signs suggestive of appendicitis, necrotizing enterocolitis, suppurative mesenteric adenitis, and septicemia (Bottone, 1997). Sequelae of acute infections (usually erythema nodosum) are also reported (Bottone, 1997).

Human infection with *Y. enterocolitica* might create a greater economic and public-health burden than is suggested by the incidence of reported cases (Nesbakken, 2005). Changes in farming practices (e.g., vertical integration, increase in herd size, high density of swine herds in swine-producing regions, and new mixing patterns), as well as in the food-processing industry, might have contributed to the infection frequency. The ability of *Y. enterocolitica* to multiply at temperatures near 0 °C has likely had an impact on this frequency (Nesbakken, 2005).

The annual incidence of reported cases of *Y. enterocolitica* was 3 per 100,000 people in the province of Ontario, Canada, in the period between 1997 and 2001 (Lee and Middleton, 2003). This was the fourth highest incidence among the eight reportable and laboratory-confirmed enteric pathogens, accounting for 3.9% of total cases (Lee and Middleton, 2003). Approximately 90% of clinical yersiniosis cases are considered to be of foodborne origin (Mead et al., 1999), and pork is an important source (Bottone, 1997; Jones, 2003; Nesbakken, 2005). In Ontario, 72.7% of affected humans were epidemiologically linked with pork (Lee and Middleton, 2003). In Denmark, the incidence of pork-related human yersiniosis in 1996 was estimated at 9 cases per 100,000 people—the same as pork-related human salmonellosis (Nielsen and Wegener, 1997).

The swine industry might have an interest in establishing a *Y. enterocolitica* monitoring-and-control program because specific *Y. enterocolitica* serotypes from both pork (Fukushima et al., 1997) and pig imports (Blumberg et al., 1991) have been linked with specific geographical areas. Infection with *Y. enterocolitica* does not cause clinical disease in pigs. Thus, it does not present a production problem—which disqualifies this pathogen as a subject of passive monitoring through clinical signs followed by diagnostic testing. Consequently, recent prevalence estimates and bioserotype distribution of *Y. enterocolitica* in Ontario swine herds are unknown. The most recent estimates for Ontario are based on sampling 283 pigs from a slaughterhouse in a mid-nineties study that involved swine populations from multiple provinces (Letellier et al., 1999).

Our objectives were to (i) estimate *Y. enterocolitica* shedding prevalence in finisher pigs, (ii) estimate the bioserotype distribution, (iii) assess agreement between herd-level tests based on pig-level and pooled samples, (iv) determine whether bioserotypes cluster by farm, and (v) determine whether herds positive for *Y. enterocolitica* and its bioserotypes cluster geographically. To reduce the effect of selection bias, for the first objective, we decided to report prevalence in each selection stratum as well as overall prevalence. For the second and fifth objectives, we decided to run analysis for the entire dataset as well as for the random group only. If not specified otherwise, results of analysis pertain to the full dataset.

2. Materials and methods

2.1. Herd and pig selection

This study was a part of larger active monitoring system of pig herds in Ontario established in 2001 under the name “The Ontario Swine Sentinel Project.” The primary objective of the monitoring system was to monitor infections and practices of public-health importance in the swine industry. The goal was to establish a network of ~100 farms that would be visited yearly. This number was deemed to be manageable for visits, considering the restrictions imposed by the biosecurity protocols of the farms (1 farm per day) and the limited personnel resources available to the investigators (1 team). A total of 113 different swine operations located in Ontario were included in this monitoring system over a period of 5 years (2001–2005). A subset of these 113 operations was visited once a year. The system was established in 2001 by including swine operations through random, purposive, and convenience sampling. Randomly selected operations were originally selected in 1999 by means of computer-assisted stratified random sampling without replacement from a sampling frame of producers who marketed their finishing pigs through Ontario Pork. The latter is the swine marketing board in the province of Ontario. In 2001, 4185 producers marketed their pigs through Ontario Pork (Ontario Pork Producers Marketing Board, 2002), while 3968 producers reporting having finishing pigs at Agricultural Census 2001 (Anonymous, 2005a) (This discrepancy is likely due to the cumulative versus cross-sectional nature of measurement.). The inclusion criterion for swine operations was to select those that marketed > 500 finisher pigs/year. The four strata considered for this sampling were based on sow-herd size: (i) 50–200 sows, (ii) 201–500 sows, (iii) 501–1000 sows, and (iv) >1000 sows. These sow-herd sizes were believed to represent different farming styles. In total, 106 operations were included in this 1999 study, and their owners were asked to participate in the on-going monitoring system for infections and practices of public-health importance. A total of 53 randomly selected producers agreed to participate in this project and were visited in the first season of sampling. Operations selected through convenience sampling (n = 18) were included because they were located close to University of Guelph, or because they were owned by producers with good rapport with researchers involved in this project. Purposely selected operations (n = 32) were selected according to two criteria. First, 21 were located in regions of Ontario that were deemed either under-represented or important from the perspective of possible incursion of pathogens due to local spread from other regions of North America (Niagara region and Eastern Ontario). Second, 11 operations were in multisite system with directed flow. There were two reasons for including the latter group of herds. First, farrow-to-wean
and farrow-to-grow operations that do not market finishing pigs were not included in the sampling frame due to inclusion criteria; and second, producers owned finishing-only operations – but not sow herds – were also not included because they could not have a number reported for sows. Operations that permanently dropped out of the study (n = 10) were replaced by 7 conveniently and 3 purposively selected operations. Reasons for refusing to continue participating included family reasons, complete depopulation, and halting pig production. To assess the representativeness of operations included in this study in terms of herd demographics, the number of finisher pigs at the time of sampling was compared with the mean number of finisher pigs in Ontario on 2001 agricultural census (mean = 456; N operations = 3968; N pigs = 1,807,530). Finisher size in the overall study population and in each sampling strategy group were compared with the mean finisher size in the target population by a one-sample t-test. In addition, finisher size of convenience and purposive samples were compared to the finisher size of the random sample using a 2-sample t-test. To assess the potential impact of the selection bias on the results further, we compared prevalence of Y. enterocolitica of different sampling groups to that in the randomly selected group. Groups were compared at the herd-level using a logistic regression model and at pig-level using random-intercept logistic regression with the Wald test. Additionally, herd prevalences in subgroups of the purposive sample (location and multi-site membership) were compared for each year separately to herd prevalence in the random group by the Fisher’s exact test. To assess the potential extent of geographical bias in the study population, the sampling proportions of finisher herds in each Statistics Canada census division (CD) were compared by inspection to the sampling proportion of finisher herds at the level of Ontario, also using Statistics Canada data.

For the Y. enterocolitica study, herds were included if sentinel swine operations owned market-age pigs in any of the three study periods. In total, 100 farms producing finisher pigs were included over a period of 3 years, with 92 herds sampled in 2001, 78 herds in 2002, and 80 herds in 2004. The three study periods were January 9, 2001–April 5, 2002 (referred to as “2001”); February 18–August 28, 2002; and January 13–June 15, 2004. In 2001 and 2002, 85% and 95% of herds, respectively, were sampled between May and August, whereas in 2004, herds were sampled evenly throughout the study period with the exception of January and June when the study started and ended. Locations of barns were obtained by a hand-held global positioning system (GPS) receiver (Etrex Legend; Garmin, Olathe, Kansas). A map of Ontario for 2001 at the level of CD (Anonymous, 2005b) and the total number of pig operations at the CD level (Anonymous, 2005a) were downloaded from the Statistics Canada Web page through the University of Guelph Library Web page under the Data Liberation Initiative Licence Agreement.

In all three study periods, finisher pigs in rooms that contained animals closest to market weight in a barn were included. In 2001 and 2002, finisher pigs from one room were conveniently selected regardless of pen membership. Fifteen individual-pig fecal samples per herd in 2001, and 15 individual-pig samples per herd in 2002 were collected using sterile gloves. In 2004, 5 pens were selected from one finisher room or, alternatively, from more rooms if the initially selected room had <5 pens. These rooms were also purposively identified by the herdsperson as containing finishers closest to market weight. We tried to include pens by systematic sampling, although we could not strictly adhere to this procedure due to large variation in room design and our inability to predict all scenarios. In each pen, fecal material was pooled from five different places for a total of approximately 200 g (pooled samples). If a barn had less than 5 pens, 5 pooled samples were collected from all available pens. In addition, 10 fecal samples per herd were collected from individual animals by conveniently sampling two pigs per pen for a total of ~200 g (pig samples). Fresh fecal samples were collected from animals as they were defecating. If <5 pens were present in a barn, adjustments were made so that the number of pigs would be equally distributed across the available number of pens per farm. Pen and pooled-sample membership was recorded.

Samples were stored at ~4 °C during transport and overnight storage and processed the following day. In 2001, samples were processed and stored at ~70 °C until the summer of 2002 when they were tested with Protocol 1 (see below). In 2002, fresh fecal samples were processed and tested with Protocol 1. In 2004, samples were submitted to Laboratory Services Division, Guelph, Ontario, and tested with Protocol 2 (see below).

2.2. Diagnostic testing in 2001 and 2002 (Protocol 1)

Fecal samples were processed by a cold-enrichment technique. Nine millilitre of phosphate-buffered saline (PBS) prepared to a pH of 7.4 with 15 g of bile salts No. 3 (Difco) per litre and 10 g of D-sorbitol (Difco) per litre was inoculated with 1 g of the fresh or frozen fecal sample and was incubated for 21 days at 4 °C. Cefsulodin irgasan novobiocin (CIN; BD Biosciences, Mississauga, ON, Canada) agar (Difco) plates were spread plated by means of a sterile glass rod with 50 μL of the PBS–fecal solution. Plates were incubated at 30 °C and checked for growth at 24 and 48 h. Characteristic bull’s-eye colonies were streaked for isolation on CIN agar plates without antibiotic supplements and were then further tested by biochemical methods using the BBL™ Enterotube™ II rapid identification system. Biochemically positive isolates were sent to the National Centre for Yersinia, Ontario Ministry of Health and Long-Term Care (Toronto, ON), for further biotyping and serotyping using standard methods (Bottone, 1997).

2.3. Diagnostic testing in 2004 (Protocol 2)

Pig and pooled fecal samples were processed according to the MID-116—Detection of Y. enterocolitica protocol of Laboratory Service Division of the University of Guelph (Guelph, ON, Canada). In the selective-enrichment step, a 25-g aliquot of each pooled or individual sample was added to 100 mL of 0.1% peptone diluent and homogenized for 1 min (Seward Stomacher 400; Seward, Norfolk, UK). In total, 5 mL of the homogenate was added to 100 mL of
In the differential plating step, a loop of ITCB broth was plated in parallel to MacConkey agar (MAC) and to CIN agar, and incubated at 24°C for 48 h. A sample was declared "negative" if no typical colonies were observed after 48 h. Typical colonies (at least 2 from each plate) were biochemically tested using the automated MicroScan WalkAway 40 system (Dade–MicroScan International, West Sacramento, CA, USA), according to the manufacturer’s instructions. Biochemically positive isolates were submitted to the National Centre for Yersinia, Ontario Ministry of Health and Long-Term Care for further analyses.

2.4. Assumptions about diagnostic test accuracy and calculation of herd sensitivity

Diagnostic test sensitivity (Se) and specificity (Sp) for the target population that was investigated by either diagnostic protocol were not known exactly. Nowak et al. (2006), using samples from commercially slaughtered pigs and 6 different combinations of enrichment and selective steps, reported the Se of these Y. enterocolitica culturing techniques to be 0% to 35.7%, based on multiplex PCR as a reference method. Test Sp in their study was between 98.7% and 100%. The method that corresponded to our testing Protocol 1 was the most sensitive (35.7%), with high Sp (100%). A method corresponding to our Protocol 2 was not considered in their study. However, for two reasons, it is unlikely that the Se of Protocol 2 was lower than that of Protocol 1. First, Protocol 2 used two selective media in parallel during differential plating and assuming they are not perfectly correlated, this alone should increases Se of the test. Second, Protocol 2 used 25 g of fecal sample. Funk et al. (2000) showed that the relative Se of a bacteriological culture for Salmonella in pig feces was 78% for a 25-g fecal sample, and 22% for a 1-g fecal sample. In addition, keeping everything else constant and Sp at 100%, it would be illogical to observe a higher apparent prevalence using a test with lower Se. Thus test Se and Sp of Protocol 2 were also assumed to be 35.7% and 100%, respectively.

Theoretical herd sensitivity (HSe) based on individual samples for a given sampling scenario was calculated in Herdacc (Jordan, 1995) using the above assumptions about test accuracy, average herd size of 1000 pigs, and minimum pig-level prevalence of 10% and of 50%. For HSe in 2004, total number of samples (pig and pooled) (n = 15) and total number of pig samples (n = 10) were used as a sample size in two separate analyses. In addition, theoretical herd sensitivity based on pooled samples (HPSe) in 2004 was calculated with the previous assumptions about test accuracy (e.g., pooled Se = test Se), and a sample size of 5 pools per herd, with each pooled sample containing 5 individual floor samples, using an approach described elsewhere (Christensen and Gardner, 2000).

2.5. Data management and statistical analyses

Data were stored in an Access database (Microsoft Corporation, Redmond, WA, USA) and imported to SAS 9.1 (SAS Institute Inc., Cary, NC, USA) for further manipulation. Herds were classified according to their infection status with respect to all Yersinia species and the Y. enterocolitica bioserotypes that were detected in that year.

True prevalence at the pig-level was calculated for pig-level data using the Rogan–Gladen estimator (Greiner and Gardner, 2000) using apparent prevalence and assumptions about the test accuracy. To further account for uncertainty in diagnostic test accuracy, we used a Bayesian modeling approach described elsewhere (Branscum et al., 2004). In this approach, prior distribution of test Se was assumed to be uniform distributed between the lower and upper limits of 18.6% and 56.9%, respectively, in agreement with 95% confidence intervals on the test Se calculated from data reported elsewhere (Nowak et al., 2006). Furthermore, prior distribution of test Sp was assumed to be uniformly distributed between the lower and upper limits of 99% and 100%, respectively. The lower limit was set to 99% recognizing that all positive samples were bioserotyped, which was expected to further increase Sp of a test. Prior information about prevalence was assumed to be uniformly distributed between 5% and 65%. Burn-in period was 5000 samples, posterior distribution was updated using 20,000 iterations, herd membership was ignored for simplicity, and median, 2.5, and 97.5 percentiles of prevalence from the posterior distribution were used to express prevalence and associated 95% probability intervals. The latter analysis was performed using WinBugs 1.4 (Spiegelhalter et al., 2004). Distribution of within-herd apparent pig-prevalence was inspected using histograms and frequency tables. Median and quartiles of within-herd prevalence were calculated using only positive farms. Prevalence was examined in Stata 8 (StataCorp, College Station, TX, USA).

At the pen-level, pooled and pig samples were first aggregated from 799 pig and 397 pooled samples to 374 pen samples. Then, the proportions of positive pooled samples and pens were calculated. Prevalence at the pen- and herd-level was calculated under the assumption of the binomial distribution.

Data were examined descriptively using frequency tables. Herds were considered Yersinia-positive if at least one pooled sample tested positive for Y. enterocolitica (pooled herd test) or at least one pig sample tested positive (pig-level herd test). The two herd-level tests were then compared using the McNemar's exact test to evaluate whether discordant pairs were biased, and by the kappa statistic to assess extent of agreement (Dohoo et al., 2003). Association between Y. enterocolitica herd prevalence and herd type (sows present or not) was evaluated stratifying on year of sampling using the Mantel-Haenszel approach and a χ² test (Dohoo et al., 2003; Stata 8).

A final analysis considered only bioserotypes 2, O:5,27 and 4, O:3. We evaluated whether, if a herd had positive isolations in >1 year, the isolations were more likely to be either of the same (or different) bioserotypes than would be expected by chance. First, we included only herds that were tested in all 3 years (n = 65) and cross-classified and inspected the number of times a herd tested positive with two bioserotypes over a period of 3 years. Second, in 72 herds that had such information available, we used exact
logistic regression to evaluate previous herd-positivity for a specific bioserotype as a risk factor for *Yersinia* bioserotype status in 2004 (bioserotype 4, O:3 and 2, O:5,27 in separate models). The following discrete “levels of exposure” were assumed, taking into consideration all previous testing: (0) herd was negative for any *Yersinia* bioserotype, (1) herd was positive only for the bioserotype specified as the outcome bioserotype, (2) herd was positive only for bioserotypes other than the outcome bioserotype, and (3) herd was positive for any combination of bioserotypes including the one specified as the outcome bioserotype in 2004. Level 0 was used as baseline group for all other levels. Additional comparisons were made for level 1 against 2, for levels 1 and 3 against 0, and levels 1 and 3 against 0 and 2.

Spatial analysis was performed for each study period separately. Distances between all possible combinations of farms, and between each farm and its first neighboring farm, were calculated using the spatstat library (*Baddeley and Turner, 2005*) of the R statistical computing environment (R Development Core Team, 2005; version 2.2.1), and examined descriptively. Two methods were used to assess spatial clustering of *Y. enterocolitica* and of *Yersinia* bioserotypes that were detected in at least 3 herds. First, spatial clustering of positive farms was assessed by the Cuzick and Edward’s global test of clustering (*Cuzick and Edwards, 1990*); (ClusterSeer version 2; TerraSeer, Crystal Lake, IL, USA). The method was based on evaluating possible clustering in up to the 10th neighborhood level (*k*). Both normal approximation and a Monte Carlo test based on 9999 simulations were used to evaluate spatial clustering at each *k*, and the Simes adjustment was used to produce a summary *P*-value. Second, a purely spatial scan Bernoulli model (*Kulldorff and Nagarwalla, 1995*) with a scanning window of up to 50% of the population at risk was used to scan for the areas with increased risk. Analyses were performed in SatScan 5.1 (*Kulldorf and Information Management Services Inc., 2004*) and were based on 9999 Monte Carlo iterations.

### 3. Results

In total, 3747 fecal samples were collected from 250 cohorts of finisher pigs, sampled from 100 farms that were included at any time during the monitoring. In 2001, 92 herds and 1381 pigs were tested, whereas 78 herds and 1170 pigs were tested in 2002. In 2004, 80 herds and 1196 samples were tested, out of which 799 were individual-pig samples and 397 were pooled samples. In total, 65 farms were tested in all 3 years, 20 farms were tested in 2 years only, and 15 farms were tested in 1 year only. This was because individual farms dropped out of the study, either permanently or temporarily, and new farms were added over time.

In 2001, 16.3% (*n* = 15), 28.3% (*n* = 26), and 55.4% (*n* = 51) of study herds were selected by convenience, purposive, and random sampling, respectively. In 2002, 16.7% (*n* = 13), 26.9% (*n* = 21), and 56.4% (*n* = 44) study herds were selected by convenience, purposive, and random sampling, respectively. In 2004, 23.7% (*n* = 19), 22.5% (*n* = 18), and 53.7% (*n* = 43) study herds were selected by convenience, purposive, and random sampling, respectively.

In 2001, 60.9% of sampled locations were farrow-to-finish farms, 33.7% were finishing-only herds, and 5.4% were wean-to-finish farms. In 2002, 66.7% of farms were farrow-to-finish, whereas 28.2% were finishing-only and 5.1% were wean-to-finish farms. In 2004, 70% of sampling sites were farrow-to-finish facilities, 26.3% were finishing-only, and 3.8% were farrowing and finishing operations, but without the nursery stage. Over the entire study period, 26% of herds with sows on-site and 18% of herds without sows on-site were *Y. enterocolitica*-positive. The difference in prevalence between these two herd types was not significant (*P* = 0.15), and adjusting for sampling period did not change measures of association substantially. Mean numbers of finisher pigs on site were 1198, 1302, and 1051 in 2001, 2002, and 2004, respectively. This was higher (*P*-values < 0.01) than the target population mean number of finisher pigs of 456 pigs. For conveniently selected herds, mean numbers of finishing pigs were 1730, 1500, and 1147, in 2001, 2002, and 2004, respectively. For purposively selected herds, mean numbers of finishing pigs were 964, 1114, and 1107, in 2001, 2002, and 2004, respectively. For randomly selected herds, mean numbers of finishing pigs were 1161, 1334, and 984, in 2001, 2002, and 2004, respectively. These herd sizes were also higher (*P*-values < 0.01) than the target population mean. In addition, the difference in herd size between the conveniently and randomly selected herds was marginally significant (*P* = 0.06).

Herd sizes in all 3 years were sampled throughout southern Ontario. The median pairwise distance between each farm and its nearest neighbor was 8.1 km in 2001, 9.0 km in 2002, and 7.6 km in 2004. The minimum distance between farms was 1.2 km in 2001, 1.4 km in 2002, and 2.2 km in 2004. The maximum distance between a farm and its nearest neighboring farm was 76 km in all 3 years. The maximum distance between two farms was 754 km in 2001 and 2002, and 689 km in 2004. At the level of the Statistics Canada Censuses, operations having finisher pigs were reported in 38 CD, and 3887 operations, based on the 2001 Agricultural Census.

Over all 3 years, farms were included from 22 CDs representing an area that included 85% of swine operations in southern Ontario and 83% of swine operations of Ontario. Overall sampling proportions in 2001, 2002, and 2004 were 2.4%, 2.0%, and 2.1%, respectively. Sampling proportions at the level of CD varied between 1.0%, and 7.7%, with 5 sampling proportions > 4.0%. Only 1 of those 5 sampling proportions was in a CD with >100 operations. This was the CD with farms close to Guelph. Only one CD with >100 operations was not included, and this was a CD north of the one with farms close to Guelph.

Table 1 shows estimates of true prevalence at the pig-level, as well as prevalence at the pen and herd-level in 3 years, stratified by type of sampling. In 2001, apparent prevalence of *Y. enterocolitica* at the herd (*P* = 0.07) and pig-level (*P* = 0.05) was lower in purposively selected herds than in randomly selected herds. In 2004, apparent prevalence of *Y. enterocolitica* at the herd (*P* = 0.05) and pig-level (*P* = 0.001) was lower in purposively selected herds than in randomly selected herds.
herds than in randomly selected herds. In 2001, only the purposively included herds selected for their locations had lower herd prevalence than randomly selected herds (P = 0.05). In contrast, in 2004 only the purposively included herds selected for their membership in the multi-site system had lower herd prevalence than randomly selected herds (P = 0.07; considered marginally significant because of importance of discovering selection bias). In addition, purposively included herds due to membership in the multi-site system had numerically lower herd prevalence than randomly selected herds in all 3 years.

Table 2 shows the summary of within-herd prevalences in herds that had at least one Y. enterocolitica-positive sample. Calculation of expected herd-level test characteristics was based on assumptions of diagnostic test accuracy and within-herd prevalence. Due to assumed perfect Sp of diagnostic tests, calculated herd specificity was 100%. In contrast, under assumptions used for herd test, calculated HSe for expected within-herd prevalence of 10% was 42.5% in all three study periods, and for expected within-herd prevalence of 50%, was 94.8%. In 2004, for herd test based on pig samples (n = 10), predicted HSe was 31% and 86% for within-herd prevalences of 10% and 50%, respectively. Predicted HPSe for an expected prevalence of 10% and 50% was 55% and 88%, respectively. Frequency distribution of within-herd prevalences in all 3 years is shown in Fig. 1. Fig. 2 shows the line plot of herd-level Y. enterocolitica prevalence over a time period categorized by the month of sampling.

The most common bioserotype was 4, O:3, followed by 2, O:5,27 (Table 3). In addition, only two bioserotypes were identified in 2001 and 2002. Herds were detected with up to four bioserotypes at one point in time (Table 4). Agreement between herd-level classification by using pig and pooled samples was moderate (Table 5). Overall, herd test based on individual-pig samples appeared to be more sensitive than herd testing based on pooled samples for detecting Y. enterocolitica, and in particular, its bioserotype 4; O:3 (Table 5) despite similar theoretical HSe for the herd testing based on two different sample types.

Among 65 farms tested in all 3 years, 57% were negative in all 3 years, only one bioserotype was identified in 40% of farms (in 34%, bioserotype 4, O:3, and in 6%, bioserotype 2, O:5,27), and both bioserotypes were identified in 3% of farms (Table 6). Herds in which bioserotype 4, O:3 alone was historically detected were 3.8-times more likely to have the same type detected than were herds that were historically negative for any Y. enterocolitica (Table 7).
Other comparisons were based on only a few herds (Table 7); all \( P \) were \( \geq 0.10 \) even without any corrections for multiple comparisons. Although we feel that there is a suggestion of consistency in association between “current” and historical positivity for a bioserotype (regardless of the specific underlying hypothesis tested); with the low power in this dataset, we are unable to demonstrate this clearly.

### 4. Discussion

The study population was not a random sample of the target population, and therefore a potential for selection bias existed. Among 4185 producers that marketed at least one finishing pig through Ontario Pork in 2001, 20.3% marketed < 51 pigs/year, and additional 32.1% marketed between 51 and 500 pigs/year. However, their contribution to the 4,743,018 pigs marketed in 2001 was 0.3% and 6.3% for producers in the two respective categories. From the descriptive statistics, it was apparent that herd size in the study population, as well as in all sampling groups, was larger than herd size in the population of all Ontario swine herds. Herd size in the target population (number of pigs on premises) was not available to the authors, so we were unable to explore the extent of the selection bias with respect to the actual target population.

This study population was distributed across most swine-producing regions of Ontario, which are concentrated mainly in southern and particularly in southwestern Ontario, with the area surrounding Guelph being over-represented due to conveniently selected farms. Hence, the target population could be defined as swine herds that marketed > 500 market pigs per year, distributed across Ontario, owned by producers who were willing to participate in the long-term project that involved repeated herd visits. Moreover, the prevalence of \( Y. enterocolitica \) was consistently lower in the purposely selected group relative to the randomly selected group. The reasons for that are not clear. Therefore, subgroups analyses were conducted when this was considered essential.

Results of our study confirm that pigs are an important reservoir of \( Y. enterocolitica \). Similar results, based on PCR assay and culture, were recently reported in the USA swine population, where 13.1% of finisher pigs and 53.2% of farms were positive for \( ail \)-sequence in one study (Bhaduri et al., 2005). The \( ail \) locus is one of the two chromosomal loci present in pathogenic \( Yersinia \) (Nesbakken, 2005). In Canada, the most recent data are based on two studies during the mid-1990s. Letellier et al. (1999) found that 20.9% of carcasses from 6 abattoirs, mostly in Quebec, were contaminated with \( Y. enterocolitica \) in 1995/1996. Similarly, Pilon et al. (2000) found that 13.5% of fecal samples and 80% of 20 herds located in Quebec, were positive for \( Y. enterocolitica \) in a farm-level longitudinal study in 1997. Estimates from other pig-producing countries also suggest that infection with \( Y. enterocolitica \) in finishing pigs is widespread (Nielsen and Wegener, 1997; Skjerve et al., 1998; Gurtler et al., 2005; Nesbakken, 2005). However, the true pig-level prevalence in 2004 in the present study might have been overestimated because
test Se was likely higher than the one we used for calculation.

Multiple factors might have led to the disparate estimates of *Y. enterocolitica* prevalence among different years of our study period. First, culture techniques differed among years in quantity of sample that was initially used (as well as in overall protocol). The quantity of sample is known to influence the Se of culture for *Salmonella enterica* in pig feces (Funk et al., 2000), and a similar presumption may hold for *Y. enterocolitica*. Second, frozen samples were used in 2001, whereas fresh samples were used in 2002 and 2004. Although *Y. enterocolitica* survives well and multiplies at refrigerator temperatures, the impact of long-term storage at $-70^\circ$C on *Y. enterocolitica* survival and recovery is unknown to the authors. Third, most samples in 2001

### Table 3

<table>
<thead>
<tr>
<th>All study herds</th>
<th>Randomly selected herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Yersinia frederiksenii</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Yersinia kristensenii</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Yersinia mollaretii</em></td>
<td>4</td>
</tr>
</tbody>
</table>

**2004**

1A: O:5
1A: O:6,30
1A: untypeable
2: O:3
2: O:5,27
2: O:Rough
3: O:1,2,3
3: O:5,27
4: O:3
4: O:Rough

**2001**

2: O:5,27 *a*
4: O:3 *a*

**2002**

2: O:5,27
4: O:3

*a* One-sample detected with both bioserotypes.

### Table 4

Number of swine herds detected with different numbers of *Y. enterocolitica* bioserotypes in three study periods in Ontario.

<table>
<thead>
<tr>
<th>Number of serotypes per herd</th>
<th>2001</th>
<th>2002</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>78</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* Positive for 2, O:5,27; and 4, O:3.

*b* Positive for 1A untypeable and 1A, O:6, 30 or 3, O:1,2,3 and 1A, O:5 or 4, O:3 and 4, O:Rough.

*c* Positive for and 2, O:5,27; 3, O:5,27; 2, O:3; and 2, O:Rough.

### Table 5

Cross-classification of herd infection status for *Y. enterocolitica*; bioserotype 4, O:3; and biotype 1A in 2004 on the basis of pig and pooled samples.

<table>
<thead>
<tr>
<th>“Pig Test”</th>
<th>“Pooled Test”</th>
<th>McNemar’s P</th>
<th>Kappa</th>
<th>95% CI (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td><em>Yersinia enterocolitica</em></td>
<td>0.15</td>
<td>0.65</td>
<td>0.46, 0.83</td>
</tr>
<tr>
<td>Positive</td>
<td><em>Yersinia enterocolitica</em></td>
<td>50</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>Bioserotype 4; O:3</td>
<td>0.04</td>
<td>0.67</td>
<td>0.48, 0.87</td>
</tr>
<tr>
<td>Positive</td>
<td>Bioserotype 4; O:3</td>
<td>58</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>Biotype 1A</td>
<td>1.0</td>
<td>0.56</td>
<td>0.11, 1.00</td>
</tr>
<tr>
<td>Positive</td>
<td>Biotype 1A</td>
<td>75</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
and 2002 were collected during the summer, whereas in 2004, most samples were collected during a colder period of the year. A firmer conclusion about possible seasonality is precluded by the existence of confounding factors (i.e., possibly different Se of culture methods), and absence of longer overlapping seasons between years when different detection methods were used. Fourth, the proportion of farms with sows differed among years. These differences in sampling seasons, diagnostic techniques, and study-herd population among different study periods were some limitations of this study.

Within-herd distribution of *Y. enterocolitica* in 2001 and in 2002 showed an unimodal distribution, and in 2004 showed a bimodal distribution with modes at 0% and 40%.

### Table 6
Cross-classification of number of times farms were repeatedly positive for bioserotype 4, O:3 and bioserotype 2, O:5,27 over a period of 3 years in Ontario swine finishing herds.

<table>
<thead>
<tr>
<th>Number of times positive for 2, O:5,27</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of times positive for 4, O:3</td>
<td>0</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 7
Previous herd-positivity for a specific bioserotype as a risk factor for herd-positivity for *Y. enterocolitica* bioserotype in 2004 in 72 Ontario swine herds that were tested for *Y. enterocolitica* in previous years.

<table>
<thead>
<tr>
<th>Baseline groupa</th>
<th>Comparison groupa</th>
<th>OR</th>
<th>( p^b )</th>
<th>95% CIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome = herd-positivity for bioserotype 4, O:3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for 4, O:3 only (1)</td>
<td>4</td>
<td>0.04</td>
<td>0.9, 16</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for other bioserotypes only (2)</td>
<td>2</td>
<td>1.00</td>
<td>0.22</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for multiple bioserotypes (3)</td>
<td>2</td>
<td>1.00</td>
<td>0.22</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for 4, O:3 only or multiple bioserotypes including 4, O:3 (1 and 3)</td>
<td>3</td>
<td>0.10</td>
<td>0.8, 12</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for 4, O:3 only (1)</td>
<td>2</td>
<td>0.47</td>
<td>0.1, +Inf</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Negative for 4, O:3 (0 and 2)</td>
<td>3</td>
<td>0.10</td>
<td>0.8, 12</td>
</tr>
<tr>
<td>Outcome = herd-positivity for bioserotype 2, O:5,27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for 2, O:5,27 only (1)</td>
<td>12</td>
<td>1.00</td>
<td>0.188</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for other bioserotypes only (2)</td>
<td>2</td>
<td>1.00</td>
<td>0.21</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for multiple bioserotypes (3)</td>
<td>21</td>
<td>0.10</td>
<td>0.2, ~2100</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for 2, O:5,27 only or multiple bioserotypes including 2, O:5,27 (1 and 3)</td>
<td>8.0</td>
<td>0.19</td>
<td>0.1, 202</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Positive for 4, O:3 only (2)</td>
<td>NEd</td>
<td>– –</td>
<td>–</td>
</tr>
<tr>
<td>Previously negative herds for any bioserotype (0)</td>
<td>Negative for 4, O:3 (0 and 2)</td>
<td>10</td>
<td>0.16</td>
<td>0.1, 254</td>
</tr>
</tbody>
</table>

### Table 8

<table>
<thead>
<tr>
<th>Bioserotype</th>
<th>Year</th>
<th>All study herds</th>
<th>Randomly selected herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cuzick-Edwards Method</td>
<td>Spatial Scan Procedure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cases (n)</td>
<td>( p^a )</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>2001</td>
<td>15</td>
<td>0.18</td>
</tr>
<tr>
<td>2, O:5,27</td>
<td>2001</td>
<td>5</td>
<td>0.54</td>
</tr>
<tr>
<td>4, O:3</td>
<td>2001</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>2002</td>
<td>14</td>
<td>0.62</td>
</tr>
<tr>
<td>2, O:5,27</td>
<td>2002</td>
<td>3</td>
<td>0.42</td>
</tr>
<tr>
<td>4, O:3</td>
<td>2002</td>
<td>11</td>
<td>0.71</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>2004</td>
<td>30</td>
<td>0.35</td>
</tr>
<tr>
<td>2, O:5,27</td>
<td>2004</td>
<td>3</td>
<td>0.77</td>
</tr>
<tr>
<td>4, O:3</td>
<td>2004</td>
<td>22</td>
<td>0.73</td>
</tr>
<tr>
<td>1A, O:5</td>
<td>2004</td>
<td>3</td>
<td>0.77</td>
</tr>
</tbody>
</table>

a,b All individual \( P \)-values >0.13.

a Assuming normal approximation.

b Using Monte Carlo sampling.
This difference in distributions among years was probably a result of the factors discussed above.

Different study periods differed not only in prevalence estimates, but also in terms of the diversity of serotypes. In 2004, eight different serotypes were identified, but it is possible that there were only six of them. This is because untypable and rough isolates were identified in herds where isolates classified into the same biotype were detected. Hence, these might be isolates belonging to the same serotype, but detection method failed to fully describe them serologically. This higher diversity discovered in 2004 might be a consequence of a higher sensitivity of the testing scheme used in 2004. Additionally, if the seasonal trend indeed exists, it is reasonable to assume that within-herd prevalence of all or some serotypes increases during cold months when most of the herds were visited in 2004. This might also have increased the HSe.

The bioserotype distribution was characterized by a dominant role of the bioserotype 4, O:3 in all 3 years, followed by 2, O:5:27. This finding was in full or partial concordance with earlier reports from Canada (Toma and Deidrick, 1975; Schiemann and Fleming, 1981; Letellier et al., 1999; Pilon et al., 2000). In the current study, serotype O:9 and serotype O:8, which is highly pathogenic in humans, were not detected. This finding is in agreement with some previous reports from Canada (Schiemann and Fleming, 1981; Letellier et al., 1999), but not with others who reported the O:9 serotype (Pilon et al., 2000), the O:8 serotype (Toma and Deidrick, 1975), or both (Letellier et al., 1999). This suggests the existence of a temporal difference among different study periods, or spatial differences in prevalence among provinces.

The distribution of bioserotypes observed in our study is in qualitative agreement with the historical proportional distribution of *Yersinia* cases in humans in Canada up to 1990, with 8% of human isolates phenotyped as O:3, 5% as O:5:27, 5% as O:8, and 3% as O:9 (Preston et al., 1994). Assuming the latter bioserotype distribution is still present, data from our study suggest a link between human illness and infection in pigs, as other reports have also indicated (Bottone, 1997; Nesbakken, 2005). However, for definitive epidemiological evidence, “denominator” data in the form of the serotype distributions in other animal species and food sources should also be available.

One such study was recently conducted in Great Britain using bioserotyping of concurrent isolates from human disease cases and swine, sheep, and cattle populations (McNally et al., 2004). The authors of that study were unable to clearly link a specific animal source and human illness because of the considerable overlap in biotype distributions of *Y. enterocolitica* isolated from animal populations and a high prevalence of the 1A biotype. The absence of the O:9 serotype might also be of interest if a survey to substantiate freedom from *Brucella suis* were to be conducted, because *Brucella* false-positive reactions could occur as a consequence of cross-reactivity with the O:9 serotype (Jungersen et al., 2006).

Comparison of the 2004 herd results based on individual samples and pooled samples suggested agreement for *Y. enterocolitica*, but also a presence of systematic bias for serotype 4, O:3. The herd test for this serotype based on individual samples was more sensitive in detection of shedding. Therefore, investigators need to be cautious about the type of samples they collect on farms if routine monitoring is to be initiated. It is possible that 250 g of fecal material distributed over 10 individual samples from pigs, may have overcome the Se of 125 g of fecal material distributed over 5 pooled samples collected from 5 different places in a pen. However, other than sampling variation, the reason this impacted only bioserotype 4, O:3 is unclear.

Two findings of this study help to describe the epidemiology of *Y. enterocolitica* in Ontario swine herds. Firstly, herds tested in all three study periods in all 3 years tended to be affected with one bioserotype when inspected descriptively, as well as when this question was analyzed using exact logistic regression. Bioserotype 4, O:3 was influencing this finding. We hypothesize that this finding suggests the existence of an in-house *Y. enterocolitica* population that is adjusted to the environmental conditions of that farm, and that cycles within finisher pig herds. This finding is in concordance with the results of a farm-level longitudinal study in Quebec herds, whose authors used more discriminatory techniques (Pilon et al., 2000). Two limitations of our analysis were low HSe in 2001 and 2002, which served to provide exposure data, and low number of herds available for some comparisons.

Secondly, study herds infected with *Y. enterocolitica* or any of its bioserotypes did not cluster spatially. This is in agreement with our results for *Salmonella enterica* and its most common serotypes (Poljak, 2006), in part with similar argumentation. Trade between farms located in different regions is likely a more important contributor to the spread of *Y. enterocolitica* than indirect contact of neighboring herds through wildlife or humans (Berends et al., 1996). The Ontario situation, however, might not be directly applicable to areas where the density of pig herds is much higher and where biosecurity is poorer. In addition, a spatial analysis based on herd status that is determined by more discriminatory techniques might provide more power to address this question. The results of the spatial analysis pertaining to the study population should be interpreted with particular caution for at least three reasons. First, the reader should be reminded that farms included in the study were not a random sample of all Ontario herds. Second, study farms included were not a random sample of either the *Yersinia*-positive herds or the *Yersinia*-negative herds from the target population, although results from the random subset yielded the same conclusion as the results for the overall study population. Third, the scale considered in this study was provincial. Not detecting clustering at this scale does not imply that clustering does not exist at either the lower scale (i.e., limited area) or the larger scale (i.e., country).

5. Conclusions

Our results confirm that finishing pigs shed *Y. enterocolitica*, which was detected in all 3 years of the study period at different levels. The different prevalences may be attributed either to our diagnostic techniques or sampling strategies or both. The most frequently identified
Y. enterocolitica in all 3 years was phenotype 4, O:3, a bioserotype frequently associated with clinical disease in people. The same bioserotype was more likely to be detected by a pig-based herd test. Our data also demonstrated that herds tended to be repeatedly positive with the same bioserotype, suggesting either the presence of farm environmental contaminants or a cycle of repeated infections in pigs.

Acknowledgements

This study was funded by Ontario Pork and Ontario Ministry of Agriculture, Food, and Rural Affairs. We thank the producers who participated in this study. The effort by Population Medicine personnel during data collection, and by Laboratory Services Division personnel during specimen testing, is also greatly appreciated.

References


