Pig and herd level prevalence of *Toxoplasma gondii* in Ontario finisher pigs in 2001, 2003, and 2004

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Abstract

The objective of this study was to estimate the apparent and true prevalence of exposure to *Toxoplasma gondii* in Ontario finisher pigs. During the study period (2001 to 2004), sera from 6048 pigs were tested with a commercial enzyme-linked immunosorbent assay (ELISA); 103 farms were included 1 to 3 times in the study. True prevalence was estimated using a Bayesian approach. Apparent prevalence at the pig level was 1.59% [95% confidence interval (CI): 0.45, 2.99] in 2001, 0.06% (95% CI: 0.00, 0.46) in 2003, and 0.26% (95% CI: 0.00, 0.82) in 2004. Apparent prevalence at the herd-level was 13.7% (95% CI: 7.5, 22.3) in 2001; 1.25% (95% CI: 0.03, 6.77) in 2003, and 3.75% (95% CI: 0.78, 10.6) in 2004. Similarly, posterior Bayesian estimates of true prevalence at the pig level were 1.7% [95% probability interval (PI): 1.2, 2.2] in 2001, 0.2% (95% PI: 0.04, 0.4) in 2003, and 0.3% (95% PI: 0.1, 0.7) in 2004. At the herd level, posterior estimates of prevalence were 11.6% (95% PI: 7.4, 16.8) in 2001, 0% (95% PI: 0.0, 2.5) in 2003, and 1.2% (95% PI: 0.0, 5.0) in 2004 when a herd cut-point ≥ 1 was used. Exposure to *T. gondii* in finishing pig farms in Ontario appears to be infrequent.

Résumé

L'objectif de la présente étude était d'estimer les prévalences apparente et réelle de l'exposition à Toxoplasma gondii *chez des porcs en finition en Ontario. Au total, 103 fermes ont été incluses 1 à 3 fois durant la période d'étude allant de 2001 à 2004 et des échantillons de sérum provenant de 6,048 porcs ont été analysés à l'aide d'une trousse ELISA commerciale. La prévalence réelle a été estimée par une approche bayesienne. La prévalence apparente au niveau de l'animal était de 1,59 % (IC 95 % : 0,45, 2,99) en 2001, de 0,06 % (IC 95 % : 0,00, 0,46) en 2003, et de 0,26 % (IC 95 % : 0,00, 0,82) en 2004. La prévalence apparente au niveau du troupeau était de 13,7 % (IC 95 % : 7,5, 22,3) en 2001; 1,25 % (IC 95 % : 0,03, 6,77) en 2003 et de 3,75 % (IC 95 % : 0,78, 10,6) en 2004. Également, les estimés bayesiens à posteriori de la prévalence réelle au niveau de l'animal était 1,7 % (PI 95 % : 1,2, 2,2) en 2001, 0,2 % (PI 95 % : 0,04, 0,4) en 2003, et de 0,3 % PI 95 % : 0,1, 0,7) en 2004. Au niveau du troupeau, les estimés bayesiens à posteriori de la prévalence réelle étaient 11,6 % (PI 95 % : 7,4, 16,8) en 2001, 0 % (PI 95 % : 0,0, 2,5) en 2003, et 1,2 % (PI 95 % : 0,0, 5,0) en 2004 lorsqu'un seuil ≥ 1 au niveau du troupeau était utilisé. En Ontario l'exposition à* T. gondiii *sur les fermes de porcs en finition semblent peu fréquentes.*

(Traduit par Docteur Serge Messier)

Introduction

Toxoplasma gondii, a parasite with worldwide distribution, is able to infect and invade multiple cell types of all warm-blooded animals (1). The life cycle of this parasite is divided into 2 parts: sexual and asexual. The sexual cycle occurs in the intestines of *Felidae*, which are the definitive hosts. This cycle results in the production of environmentally resistant oocysts, each containing 4 sporozoites. The asexual cycle occurs in tissues of many mammalian and avian species, which are the intermediate hosts. Tissue cysts have an affinity for muscular and neural tissues and may have lifelong persistence (1). These cysts stimulate the immune system, so that infected hosts become serologically positive and immune to new infections (1). Hence, seropositivity correlates with potential infectivity of the meat in food-producing animals (2). *Toxoplasma gondii* may infect definitive and intermediate hosts through different routes. For example, orally through the ingestion of meat that contains tissue cysts and tachyzoites (foodborne; horizontal transmission); through food and water that is contaminated with oocysts (foodborne; horizontal transmission); or through a transplacental route with tachyzoites (congenital; vertical transmission).

Estimates of seroprevalence vary between countries and geographical regions, but overall seroprevalence in the global human population is high (1). In a recent serological survey of the human population of the United States 22.5% tested positive (3). Acute infection with *T. gondii* (toxoplasmosis) in healthy people most frequently is asymptomatic or manifests with nonspecific symptoms, although outbreaks of clinical disease have been recorded (4).

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Similarly, chronic toxoplasmosis in healthy people is most frequently a dormant, asymptomatic, and persistent infection.

In contrast, during acute toxoplasmosis in pregnant women, tachyzoites may transplacentally infect the unborn fetus and cause conditions that range from asymptomatic infection to death or serious disability of children (5). Moreover, toxoplasmosis in immunocompromised people may manifest as a serious clinical disease with lesions located in the central nervous system (6) or other organs (5). Toxoplasmosis has been reported as the 3rd leading cause of mortality due to foodborne illness in the American population (7).

Toxoplasmosis in pigs is not a production problem (8); however, pork is considered as one possible source of foodborne toxoplasmosis in people (9). Changes in the pig-farming systems over time have decreased the contact of swine with the outside environment, thus decreasing the *T. gondii* prevalence to a low level (1). This low prevalence is also reflected in the way researchers currently look at swine toxoplasmosis. For example, van Knapen et al (10) recommended the use of within-herd seroprevalence of *T. gondii* infection as an indicator of pig contact with the outside environment, and Blaha (11) considered the production of *T. gondii*-free pork as one of the objectives of quality assurance programs.

The most recent report of *T. gondii* seroprevalence in pigs in Ontario dates from 1991 and 1992 in market-age or finisher pigs (12) and 1990 in sows (13). The apparent seroprevalence of 6.6% in finisher pigs and 16.2% in sows was determined in these studies. Since then, the swine industry of Ontario has undergone an increase in the number of pigs marketed and a decrease in the number of producers, accompanied by further intensification of swine production. Hence, the primary objective of this study was to estimate pig- and herd-level apparent and true prevalence of *T. gondii* in finisher pigs in Ontario for the years 2001, 2003, and 2004.

Materials and methods

A network of sentinel swine herds — the Ontario Sentinel Swine Project

Farms participating in this study were part of a multi-year project started in 2001 called the "Ontario Swine Sentinel Project" conducted by the Department of Population Medicine, University of Guelph. The primary objective of the project was to monitor diseases and farm management practices of public health importance in swine farms in Ontario. For this project, a swine operation was defined in terms of ownership, a swine farm in terms of location, and a swine herd in terms of age group at the farm (sows in a sow herd and finisher pigs in a finisher herd). Each year between 80 and 103 swine operations of different management systems across southern Ontario were visited for blood sampling of sows and finisher pigs close to market weight. This sentinel project was established in part from conveniently selected operations close to Guelph, Ontario; purposively selected operations based on geographical and herd type distribution of farms in Ontario; and randomly selected operations based on the swine producers willingness to participate after termination of another study (Deckert, personal communication). Swine operations that declined to participate in one or more years, either temporarily or permanently, were replaced with new farms, selected by convenience sampling. Consequently, swine operations included in this sentinel network were not a true random sample of Ontario farms. However, samples were taken from all 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 multi-site swine operations with directed flow.

Samples were collected between May 2001 and April 2002 for 2001 data, between April and November in 2003, and between January and June in 2004. At each visit, blood samples were taken from 30 finisher pigs from a cohort of the swine operation that housed the animals closest to market weight. Finisher pigs were sampled conveniently, without predefined criteria and formal sampling frame. Blood was centrifuged and sera were separated and stored in a serum bank at –20°C until tested.

At the time of the visit, observers collected information related to the size of the operation, integration level, management practices, biosecurity measures, location of the sampled farm, and vaccination and medication protocols. Part of the questionnaire was completed by the observer, and the rest by interviewing the owner/manager in a face-to-face dialogue. Questionnaires varied in design by year. In 2001, farm-level information related to the management system was collected through the interview with farm personnel during the farm visit. In 2003 and 2004 farm-, room-, and pen-level information was collected through an interview with farm personnel, and through scoring by an observer. In addition, in 2004, pig-level information was collected including weight, taken by measuring tape, and by recording the general health status of the pigs that were sampled. This information was entered into an Access 2000 database (Microsoft Corporation, Redmond, Washington, USA).

Study population and serological tests

Serum samples were extracted from the serum bank of the "Ontario Sentinel Swine Project" and submitted for testing: all 30 finisher pig sera from 95 herds sampled in 2001; 20 randomly selected finisher pig sera from 80 randomly selected finisher herds (2003); and 20 randomly selected finisher pig sera from all 80 herds sampled in 2004. Only 80 herds were included in 2004 and 2003 due to logistic reasons. The within-herd sample size, calculated in available software (14), was sufficient to detect truly exposed herds ($\geq$ 25% expected prevalence) with 95% probability and truly non-exposed herds ($<$ 25%) with probability $>$ 80%, assuming sampling from an infinite population, and using estimates of sensitivity and specificity as outlined in the following text. Sera from all 3 y were tested with an enzyme-linked immunosorbent assay (ELISA) (SafePath Laboratories, Carlsbad, California, USA) according to the manufacturer's instructions, and an optical density (OD) $\geq$ 0.2 was considered as the positive cut-off. Testing was done at the Animal Health Laboratory of the University of Guelph and the Agriculture Alberta Laboratory in Edmonton (2001 sera); the results were entered into an Access database.

Analysis of apparent prevalence

Data from the *T. gondii* test results, demographic information on the farms, and potential risk factors were imported from the Access database into SAS 9.1 (SAS Institute, Cary, North Carolina, USA) for further manipulation and descriptive statistics. Apparent

prevalence at the pig level and the 95% confidence interval (CI) were calculated using an exact method assuming a binomial distribution, and using an empty logistic regression model that accounted for within-herd dependence using 2 approaches for each year. First, logistic regression with farm as a random intercept was fitted using a maximum likelihood approach based on adaptive quadrature (Proc Nlmixed; SAS 9.1). The intercept and 95% CI were transformed to the probability scale to yield a conditional estimate of the apparent prevalence. Next, estimates of coefficients for intercept and variance were used to yield a population-averaged apparent prevalence and 95% confidence limits using a method reported elsewhere (15) with 1000 simulated datasets. Finally, the within-herd dependence was modeled through a generalized estimating equation approach assuming exchangeable correlation structure (Proc Genmod; SAS 9.1), and the estimates of intercept and standard error were used to construct an additional estimate of the population-averaged apparent prevalence and the 95% CI.

Herds were assumed to be apparently positive if at least 1 pig tested positive on ELISA. The apparent prevalence at the herd level and the 95% CI were calculated using an exact method and assuming a binomial distribution.

Epidemiological information

In 2001, the farm was recorded as positive for cats if the farmer said cats had access to the barn or the feed. In 2003, a farm was classified as positive if cats were occasionally (score 3) or regularly present in the barn (score 4). In 2004, a farm was classified as positive if investigators observed at least 1 (score 3) or many (score 4) cats in the barn.

For the study population in 2004, pig weight was described at the individual level using mean, median, minimum and maximum weight, and standard deviation (*s*). Individual pig weights were collapsed at the farm level using means, and farm-level estimates of average pig weight were also calculated using the same type of statistics as for individual pig weight. These estimates could be considered as being representative of weights in the 2001 and 2003 study populations because the same criteria were used to include pigs in the study.

Bayesian analysis of true prevalence

Bayesian analysis was used to estimate the true prevalence at the pig- and herd-levels. In Bayesian analysis the likelihood of the observed data is combined with prior information for each of the unknown parameters into a joint posterior distribution of parameters through Bayes' theorem (16).

Inferences about the parameters are based on draws from their joint posterior distribution, which are generated by Gibbs sampling, a Monte Carlo Markov chain (MCMC) procedure (17).

The model and method for estimating true *T. gondii* prevalence was based on Tu et al (18); subsequently implemented for paratuberculosis and explained in detail by van Schaik et al (19).

For convenience, we used the same terminology as van Schaik et al (19). Briefly, the number of test positive pigs per herd (*k*) was assumed to follow a binomial distribution with apparent prevalence (AP_k) and number of animals (n_k) tested per herd as parameters. Pig-level AP_k was modeled as a function of true prevalence (TP_k), test sensitivity (Se), and test specificity (Sp) through Bayes' theorem. The logit of TP_k was modeled as a regression model with a binary variable representing the presence of cats on the farm (as defined previously and with β_1 as a regression coefficient corresponding to presence of cats), 2 indicator variables representing year 2003 (β_2) and year 2004 (β_3) included as fixed effects, and a herd membership included as a random effect (r_k) (random herd effect). An intercept (with coefficient β_0) was included in the model.

Herd sensitivity (HSe_k) was calculated for all herds using the approach originally presented by Martin et al (20) and explained in detail in van Schaik et al (19). The HSe_k was calculated using 2 approaches: i) when a herd cut-off was ≥ 1, and ii) when a herd cut-off was ≥ 2 positive animals. Then, a herd was classified as a "true positive" if HSe_k was $> 50\%$ (19), using both approaches. Following this, a posterior estimate of true herd prevalence in each year was calculated as the proportion of herds in which HSe_k was $> 50\%$ using the two approaches separately. This can be interpreted as the proportion of herds in which within-herd prevalence was sufficient to produce a probability of correctly classifying a diseased herd $> 50\%$ (HSe_k) using: i) cut-off of ≥ 1, and ii) cut-off of ≥ 2 positive animals.

The prior distributions of pig-level Se and Sp were modeled as informative prior distributions through the parameters of the distribution (a and b), which were calculated in BetaBuster (University of California, Davies, California, USA). The prior beta distribution for Se was based on its most likely value of 88.6% and the 95% probability that it was $> 78.7\%$ (a = 44.53; b = 6.60). The prior beta distribution for Sp was based on its most likely value of 98% and the 95% probability that it was $> 95.1\%$ (a = 159.15; b = 4.23). These estimates were calculated from data provided by Gamble et al (21). Additionally, in the model evaluation phase we specified uniform distribution for Se and Sp with minimum value of 78.7% and 95.1%, respectively, and with maximum value of 99.9%.

The priors for fixed effects (βs) were assumed to be normally distributed with a mean of 0 and a precision of 1. The mean of the random herd effect was assumed to be 0. The standard deviation of the random herd effect is the square root of the inverse of the precision parameter (tau). Tau (τ) was assumed to follow a gamma distribution ($\tau = 1$; $\lambda = 25$). These parameters were used as they produced distribution of standard deviations of random effects that were considered as plausible at the farm level in the commercial swine production. When simulated, these parameters provided 2.5th, 50th, and 97.5th percentile of standard deviation (*s*) at 2.6, 6, and 32, respectively.

Posterior median, and 2.5th and 97.5th percentiles were calculated for true prevalence at the pig- and herd-levels, sensitivity, and specificity. In addition, posterior estimates of coefficients for fixed effects and random herd effect were calculated (median, and 2.5th and 97.5th percentile). For interpretation purposes, the coefficients for fixed effects in the logistic regression model (LRM) were considered to be associated with TP_k if the 95% PI did not include 0.

The Bayesian model was fitted in WinBUGS, Version 1.4 (22) based on 2×10^6 iterations with a burn-in period of an 2×10^5 initial iterations. Model convergence was assessed visually by examining parameters from multiple chains starting from different initial points (23), and by examining the change in parameter estimates after

running chains for more iterations, discarding more initial iterations, and running the model under a non-informative prior distribution for Se and Sp (19). In addition, models were rerun using flatter priors for coefficients of the fixed effects {N(0,01)}, using absence of cats as an indicator variable, as well as with priors for fixed parameters and random effect reported by van Schaik et al (19). The model was considered stable if the posterior estimates of prevalence did not change by an amount considered to be important.

Results

Study population

In total, 103 different swine farms were included in this study; with 64.1% of them being tested in all 3 y, 19.4% tested in 2 y, and 16.5% tested in only 1 y. Descriptive statistics of the study population are provided in Table I. In 2001, 56.8% of the farms were classified as having cats. In 2003 and 2004, 36.2% and 10% of farms, respectively, were classified as having cats. In 2004, the mean weight of included pigs was 95 kg (median = 95 kg; min = 38, max = 133; *s* = 13). The mean of farm-level averages was 95 kg (median = 96; min = 47; max = 113; *s* = 10).

Apparent prevalence

Pig- and herd-level apparent *T. gondii* prevalence by year, and the respective 95% CI are reported in Table II. Estimates assuming independence, conditional estimates (conditional on random intercept being zero), and population-averaged estimates using 2 approaches are presented for comparison. The approach for population averaged estimates used earlier (15) yielded the widest confidence intervals and was identified as a recommended approach. Estimates in 2004 and in 2003 were based on only 4 and 1 apparently positive pig, respectively, and should therefore be interpreted with care.

In 2001, 1 herd had an apparent prevalence close to 50%, and 6% of herds had an apparent prevalence of $\geq$ 10%. In contrast, only 1 herd in 2004 had an apparent prevalence of 10% (2 out of 20 tested), while other apparently positive herds in 2003 and 2004 each had only 1 positive pig. One herd had 14 positive pigs in 2001 (Figure 1), and was positive for cats. The same herd was tested in 2003 when it was cat-positive and in 2004 when it was cat-negative with 0 positive pigs detected in both subsequent testings.

Posterior estimates of coefficients for fixed effects and model robustness

Point estimates (medians from posterior distribution) of coefficients for the fixed effects of years 2003 and 2004 were both negative indicating lower true pig-level prevalence in these years than in 2001. In addition, the 95% probability intervals (PI) of both of these coefficients did not include 0, indicating that this was not a chance finding (Table III). In contrast, the point estimate of coefficient for the variable that indicated presence of cats in the barn was positive, but the 2.5th percentile was negative (Table III). Despite this, the variable indicating presence of cats was kept in the model as evidence of their presence was considered an important component of prior information for *T. gondii* prevalence in pigs. Moreover, when flatter prior distribution on coefficients for fixed effects was specified, the negative coefficients (intercept, year 2003 and 2004) became more negative, and the positive coefficient (cats), became more positive including the 2.5th percentile for coefficient from posterior distribution. Similarly, when absence of cats was specified as a factor, the point estimate was negative and the 95% PI did not include 0. The coefficient for presence of cats, therefore, was not sufficiently stable. Nevertheless, the point estimates of true prevalence at the pig level and at the herd level did not change materially.

Table I. Study population used to determine the prevalence of *Toxoplasma gondii* in Ontario by year of sampling

	Year of sampling		
	2001	2003	2004
Animals tested	2848	1600	1600
Animals apparently positive	**40**	**1**	**4**
Farms tested	95	80	80
Farms apparently positive	13	1	3
Animals tested per farm	30	20	20
Type of farm — sampling site (%)			
Farrow-to-finish (%)	60	61.3	71.3
Finishers only (%)	33.7	32.5	26.3
Nursery and finishers (%)	6.3	6.3	—
Farrowing and GF	—	—	2.5
Integration level (%)			
Company (%)	18.9	16.3	16.3
Family loops (%)	11.6	12.5	13.8
Independent (%)	69.5	71.3	70
Number of sites (%)			
1 site (%)	52.6	52.5	50
2 sites (%)	25.3	21.25	25
3 and more sites (%)	22.1	26.25	25
Number of finisher pigs on-site (Mean)	1232	1190	1050
Number of finisher pigs on-site (s)	1150	1109	821
Proportion of farms with cats (%)	56.8	36.3	10.0

s = standard deviation; GF — growing-finishing phase.

Posterior estimates of true prevalence at different levels

Posterior distributions of true pig- and herd-level prevalence by year are reported in Table III. Statistics reported include mean, median, and 2.5th and 97.5th percentiles. The interval between the posterior 2.5th and 97.5th is referred to as the 95% probability interval (PI), also known as the credible interval. It is interpreted as the 95% posterior probability that the true value is between these 2 values. The posterior distributions of animal level test sensitivity and specificity are reported in Table III. The maximum change in posterior estimates of true prevalence was 0.2% at the pig level and 3.5% at the herd level, even if coefficients for fixed effect changed after specifying different priors. Thus, for the main purpose of this study, these models were sufficiently robust and estimates from the model that yielded the highest estimates are reported here.

Discussion

Apparent seroprevalence of *T. gondii* in this study was lower than in the study conducted 10 years ago in Ontario finisher pigs (12).

Table II. Apparent prevalence at the pig- and herd-level in Ontario finishing pig population in 2001, 2003, and 2004

	Pig apparent prevalence (95% CI) Year of study		
	2001	2003	2004
Assuming independence and binomial distribution	1.40 (1.01, 1.91)	0.06 (0.00, 0.35)	0.25 (0.07, 0.64)
Conditional estimates[a]	0.07 (0.01, 0.44)	0.06 (0.01, 0.46)	0.03 (0.00, 1.71)
Population-averaged estimates based on random effect model[a]	1.59 (0.45, 2.99)	0.06 (0.00, 0.18)	0.26 (0.00, 0.82)
Population-averaged estimates based on GEE model[b]	1.40 (0.63, 3.08)	0.06 (0.01, 0.44)	0.25 (0.08, 0.81)
	Herd apparent prevalence (95% CI)		
Assuming independence and binomial distribution	13.68 (7.49, 22.26)	1.25 (0.03, 6.77)	3.75 (0.78, 10.57)

GEE — generalized estimating equations.

[a] Based on logistic regression with herd as a random effect.

[b] Based on logistic regression with dependence modeled through the GEE approach.

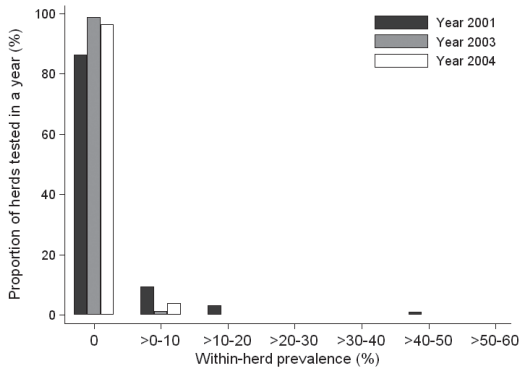

Figure 1. Frequency distribution of within-herd apparent prevalence of *Toxoplasma gondii* in 103 Ontario finisher herds sampled in 2001, 2003, and 2004.

In that study, pig sera were tested with a latex agglutination test (LAT) and a titer of 32 was considered positive. The sensitivity and specificity of LAT for sows were estimated to be 45.9% and 96.9% when a titer of 64 was considered positive (2). The sampling strategy used herein was different than that used by Gajadhar et al (12), in which pigs were blood sampled in an abattoir. This sampling strategy might have had a two-fold impact on the comparison. In theory, pigs sampled in the previous study might have been older than pigs sampled in our study. This is illustrated by descriptive statistics of individual pig- and farm-level weights in 2004; pigs in some farms were sampled in earlier phases of finishing-pig production. It is reasonable to assume that a similar study population was sampled in earlier years as well. Since *T. gondii* prevalence increases with age (24,25), this might partly explain the higher apparent seroprevalence in their study (12). In contrast, pigs sampled in our study may have had a higher likelihood of being seropositive because of the presence of maternally derived antibodies (MDA). These MDA can persist up to 3 mo of age (26), and in some surveys age of selected pigs was limited to 4 mo (27). Although both the age and MDA theories are possible, they likely did not contribute significantly to the estimates of apparent prevalence, and a direct comparison between studies is not possible. Most likely, the lower apparent prevalence can be attributed to the further intensification of the Ontario swine industry, a process that similarly has lowered the *T. gondii* seroprevalence in other swine-producing regions (1).

The apparent prevalence in our study was numerically higher in 2001 than in the other 2 y. This was due, in part, to 1 herd that had apparent prevalence of almost 50%, and a higher proportion of herds with an apparent prevalence $\geq$ 10% than in the 2 later years. This may be because of sampling variation, or minor changes in the study population, or sampling period. Sampling period might have affected our results because kittens are most likely to shed oocysts, and cats have a seasonal reproductive cycle. It is possible that we were more likely to sample finisher pigs that had been exposed to kittens earlier in their lives in 2001 than in 2003 and 2004.

Producers were given the results of the 2001 study and informed about toxoplasmosis. This might also have had an effect on the later results by lowering the prevalence of cats, but this influence is probably low because toxoplasmosis is not a production disease, the number of positive animals was low in almost all cases, and there is no penalty imposed for either having cats in the barn or having *T. gondii*-positive pigs. The number of herds that were classified as having cats decreased over years. This change may not necessarily be due to a decrease in cats, but rather a reflection of the approaches used to determine the presence of cats. The classification of farms for the presence of cats in our study likely increased in specificity, at the expense of sensitivity over the study period. Consequently, some farms might have been misclassified, likely nondifferentially.

Point estimates of apparent prevalence at the pig level varied slightly among the methods implemented in their calculation. In earlier studies, the method based on random effect maximum-likelihood logistic regression that incorporated normally distributed random effects through the simulation approach was identified as one of the recommended approaches to obtain population-averaged prevalence estimates (15). In this study, it yielded the widest confidence intervals (except in 2003) and should be used as the most reliable estimate of apparent prevalence.

In the Bayesian model reported here, presence of cats could not be identified as a risk factor. The posterior estimates of coefficients for cat presence in a farm and 95% PIs were influenced by priors in the model evaluation phase, making the posterior median more negative and 95% PI not containing 0. For this reason this coefficient was considered to be unstable. However, regardless of priors, its posterior distribution did not materially affect posterior distribution of true prevalence. This variable (cats) was kept in the model as the

Table III. Posterior estimates of true pig-level prevalence (%) and herd-level prevalence (%) of *Toxoplasma gondii* in Ontario 2001, 2003, and 2004 and for coefficients of random effect logistic regression model

Node	Mean	2.5 p[a]	Median	97.5 p[a]	Prior
Tests					
Test sensitivity	85.28	73.73	85.79	93.94	Beta (44.5, 6.6)
Test specificity	99.88	99.75	99.89	99.96	Beta (159.1, 4.2)
Logistic regression model (LRM)					
Intercept	−6.22	−7.17	−6.21	−5.35	N (0,1)
Presence of cats	0.23	−0.85	0.24	1.26	N (0,1)
Year 2003	−2.00	−3.36	−1.98	−0.75	N (0,1)
Year 2004	−1.47	−2.78	−1.45	−0.26	N (0,1)
Herd (random)	2.15[e]	1.68[e]	2.13[e]	2.73[e]	N [0, Gamma(1,25)][d]
Prevalence					
Pig true prevalence in 2001	1.69	1.22	1.67	2.25	(LRM)
Pig true prevalence in 2003	0.19	0.04	0.17	0.45	(LRM)
Pig true prevalence in 2004	0.33	0.10	0.31	0.69	(LRM)
Herd[b] prevalence in 2001	11.28	7.37	11.58	16.84	(LRM)
Herd[b] prevalence in 2003	0.52	0.00	0.00	2.50	(LRM)
Herd[b] prevalence in 2004	1.54	0.00	1.25	5.00	(LRM)
Herd[c] prevalence in 2001	5.84	3.16	6.32	8.42	(LRM)
Herd[c] prevalence in 2003	0.05	0.00	0.00	1.25	(LRM)
Herd[c] prevalence in 2004	0.33	0.00	0.00	1.25	(LRM)

N — Normal distribution; LRM — logistic regression model.

[a] p — percentile.

[b] Based on ≥ 1 as a herd cut-off.

[c] Based on ≥ 2 as a herd cut-off.

[d] Specified in a form as required by WinBugs (as precision, not as standard deviation).

[e] Specified as standard deviation.

presence of cats in the barn was considered as the most important measured contributor to prior information about pig-level *T. gondii* prevalence in swine barns.

Naive cats, especially those younger than 13 wk of age (28), shed oocysts 3–21 d after exposure to the infectious stage of *T. gondii* (29). It is also believed that once infected, cats have long-lasting immunity to re-infection and re-excretion. It has been reported, however, that repeated challenge with *T. gondii* at 77 mo after a primary exposure (30), infection with *Isospora felis* (31), or corticosteroid-induced immunosuppression (28) may induce reshedding. Hence, under certain conditions older cats may shed oocysts as well. In addition, feed and water contamination can be a problem since oocysts can persist in the environment for at least 200 d when the temperature is between 10°C and 25°C, and up to 54 mo when the temperature is 4°C (32).

Most prevalence studies for *T. gondii* only report apparent prevalence, which has been reported here for comparison. Estimates of true prevalence, however, are what we are really seeking. True prevalence can be calculated from apparent prevalence given known values of test sensitivity and specificity. In practice, however, sensitivity and specificity vary according to the population and therefore are more accurately depicted as random variables (33).

Bayesian analysis provides a natural framework to incorporate both uncertainty in test accuracy and important epidemiological information, into the estimates of *T. gondii* prevalence. The uncertainty about the test sensitivity and specificity in our prevalence model was included in the analysis by specifying a prior distribution based on the most likely value and the 95% confidence that each of these parameters is greater than a certain value, calculated from data provided by Gamble et al (21). These estimates were included as informative prior, but the posterior distributions of prevalence were very similar for the model based on an uniform prior distribution of test Se and Sp. The posterior distribution of test sensitivity for the reported model was very similar to its prior distribution. In contrast, the posterior distribution of test specificity was different than its prior distribution, with posterior indicating very high specificity. This should be interpreted with care because our model was not built to estimate test accuracy, but rather to incorporate the uncertainties into the prevalence estimation.

If ≥ 1 test positive is used as a herd cut-off, then herd-level true prevalence, as defined here, is the prevalence of herds with very low expected within-herd *T. gondii* prevalence. This is because HSe of at least 50% at a cut-off of at least 1 positive pig was used to declare a herd truly positive. To illustrate this, a frequentist approach could be used with a value of Se at the 2.5th percentile of the posterior distribution for test sensitivity, and a value of Sp at the 97.5th percentile of the posterior distribution, in a scenario when a herd cut-off is ≥ 1 in a sample of 20 animals. By doing this, we would be able to detect within-herd prevalence of at least 5% and at least 15% with herd sensitivities of more than 50% and more than 90%, respectively. Hence, the posterior estimate of true herd prevalence (≥ 1) determined the prevalence of herds that could be exposed to *T. gondii* at a very low

within-herd level prevalence. Similarly, sample size obviously had an impact on true herd prevalence in 2001 as herd sensitivity increases with a larger sample size.

The approach of estimating true prevalence at the pig level implemented here was conservative, and resulted in high estimates because: i) we accounted for imperfect accuracy of serological tests, ii) the posterior median of test specificity was very high indicating that the model assumed very low false-positive fraction for observed data, and iii) a logistic regression model with a variable indicating presence of cats was used as a prior information. The latter is indicative not only of the situation at the sampling occasion, but also of the cumulative risk of acquiring toxoplasmosis in swine farms that had cats present. Obviously, due to different classification of this covariate over years, it is likely that prevalence in 2001 was slightly overestimated, due to a high sensitivity of our question about cats in 2001, and slightly underestimated due to a lower sensitivity of the question in 2004.

Conclusions

Bayesian modeling in our study provided a conservative approach to estimate the prevalence of *T. gondii.* Posterior estimates of true pig-level prevalence in the population was low, even when the posterior test specificity was high and the posterior test sensitivity relatively low. Posterior median true prevalence of *T. gondii* was higher in 2001 than in 2003 and 2004. The data provided evidence of a low prevalence of *T. gondii* in pigs in our Ontario study herds. Further intensification of the swine industry and restricting cats from barns may further reduce pig- and herd-level prevalence of *T. gondii*.

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