The seroprevalence of *Toxoplasma gondii* in Ontario sheep flocks

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Abstract

In a random sample of 103 sheep farms in Ontario, 99% of the farms had some sheep serologically positive for *Toxoplasma gondii*, based on an enzyme-linked immunosorbent assay (ELISA). The percent of sheep affected within farms ranged from 3.8% to 97.8%, with an average flock prevalence of 57.6%. When farm management variables were considered in a multivariate analysis, significantly lower rates of serologically positive sheep were associated with neutering of female cats and clipping of ewes' perineums before lambing; significantly higher prevalence rates were found on farms where sheep were purchased from other flocks, pigs were raised on the same farm, sheep shared pasture with other animals, flowing water was available at pasture, and pastured replacements had access to housing. As well, in univariate analyses, higher prevalence was positively associated with an increasing number of cat litters born over the previous two years and offering creep feed or forage to lambs, and inversely with the amount of labor expended on sheep rearing.

Résumé

La prévalence sérologique de *Toxoplasma gondii* dans les troupeaux de moutons en Ontario

Des échantillons pris au hasard dans 103 fermes d'élevage de moutons ont démontré dans 99 % des fermes des analyses sérologiques positives à *Toxoplasma gondii*, par essai immuno-enzymatique (Elisa). Le pourcentage de moutons infectés par ferme variait de 3,8 à 97,8 % avec une prévalence moyenne de 57,6 %. Lorsque les facteurs de régie ont été considérés par analyse de variance selon plusieurs critères de classification, les paramètres suivants soit la stérilisation des chats femelles et soit la tonte de la région du périnée chez les brebis avant l'agneulage ont été associés à des taux de prévalence plus bas de façon significative pour les sérologies positives. Par contre, les taux de prévalence étaient plus élevés de façon significative dans les fermes où les moutons avaient été achetés à partir d'autres troupeaux; où des porcs étaient élevés sur la même ferme; où les moutons partageaient les pâturages avec d'autres animaux; où une source d'eau naturelle était présente au pâturage et où il y avait rotation de pâturages avec accès à la bergerie. Lors d'analyses de variance à un seul critère de classification, un taux de prévalence plus élevé était relié positivement à l'augmentation du nombre de portées des chats nés durant les deux dernières années, à une alimentation à la dérobée ou à base de foin offerte aux agneaux et inversement, au travail fourni pour l'élevage de moutons.

(Traduit par Dr Thérèse Lanthier)

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Introduction

Based on serological evidence, toxoplasmosis appears to be a common and geographically widespread parasitic infection of many warm-blooded animals, including people (1).

About 30 to 60% of North American adults are estimated to have been exposed to *Toxoplasma gondii*, with some geographical variation in prevalence (1-3). Tizard *et al* (4) reported that about half of all Ontario adults were serologically positive to *Toxoplasma*. Congenital infections, of which there are more than 3000 in the U.S. every year (5), and infections in immunocompromised people have the most serious clinical course. Between five and ten percent of patients with acquired immunodeficiency syndrome develop *Toxoplasma* encephalitis (3). While members of the cat family have been identified as the reservoir of *T. gondii* (1), many people are thought to become infected by consumption of *Toxoplasma*-contaminated meat. Sheep are one of the animals which have been identified as possible sources of infection for people (6).

While various serological surveys have been done on sheep, the extent to which the results represent the true field situation is not clear from the reports, since the surveyed subjects were not randomly selected from the populations of interest (1,7). Tizard *et al* (8) reported that 65% of the Ontario sheep sera they collected from various diagnostic laboratories were serologically positive for *T. gondii*. However, because of the way the sera were collected, these workers were unable to examine flock level or geographic variations in serological reactors, nor to evaluate factors which might influence whether or not sheep were positive.

Our study was designed to determine the serological prevalence of *T. gondii* in Ontario sheep flocks, and to examine the relationship between serological reactivity and management.

Materials and methods

Selection of animals

This study was one component of a much larger undertaking, a multidisciplinary project (SHEPHERDS) undertaken to identify and address problems of disease and management in Ontario sheep flocks. One-hundred-and-seven flocks with 40 or more breeding ewes were selected, using a random number-generating

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Table 1. Factors hypothesized to be associated with the within = flock prevalence of *T. gondii* antibodies in Ontario sheep flocks, 1988

Variables	Description
Geographic area	North, south, west, east and central Ontario
Exposure to other animals	Other livestock currently raised; pasture-sharing with other livestock; pets on premises; if cats, then male or female; if female, then neutered?; if not neutered, how many litters born over past two years?
Labor patterns	Number of years of experience in sheep farming; estimated % of labor used to manage sheep; crops grown as well as sheep raised
Demographics	Size of sheep flock; age of sheep
Feeding	Use of creep feeds; concentrates and grains fed (source and type)
Housing	Housing and pasture management at various times of year; use of manure packs; density at pasture and in housing
Other	Internal and external treatments for parasites other than coccidia; giving a coccidiostat to lambs in creep feed, or to market lambs; clipping of perineum (crutching) before lambing; contact of sheep with each other during perinatal period

computer program, from a list provided by the Ontario Red Meat Plan. The selection was stratified geographically, with a sample proportionate to the number of listed sheep flocks in each of five regions: north, east, central, west, and south. Because northwestern Ontario (west of Sault Ste. Marie) has very few sheep flocks, that area was excluded from the study for logistical and economic reasons. Serum samples were taken in all flocks from adult ewes, randomly selected by tag number prior to the visit, in the summer of 1988. Sufficient numbers were sampled to determine that, if the flock was infected to at least the five percent level, we would detect at least one case, 95% of the time (9).

Laboratory methods

The test used was an indirect sequential enzyme-linked immunosorbent assay (ELISA) for sheep IgG antibodies to *T. gondii*. The method was a modification of an ELISA previously described for human IgG antibodies to *T. gondii* (10). Briefly, purified soluble antigen derived from *T. gondii* (RH strain, supplied courtesy of Dr. Ian Tizard) tachyzoites immobilized on polystyrene microwells was incubated with sheep serum diluted 1/50 in phosphate buffered saline containing 0.05% Tween 20 (PBST) and 0.5% normal human serum previously shown to be unreactive with *T. gondii*. Following a 37°C incubation and wash step with PBST, optimally diluted enzyme labelled anti-ovine IgG horseradish peroxidase conjugate (Sigma Chemical Co., St. Louis, Missouri, USA) was added to the microwells. Any previously bound sheep IgG was recognized by the conjugate. The final stage following another PBST wash was the addition of 200 μL of 200 mM o-phenylenediamine 2HCl in 0.05 M citrate buffer of pH 5.5/0.03% H_2O_2. Incubation was at 25°C for 15 minutes. Following the addition of 50 μL of 4N H_2SO_4, the results were spectrophotometrically evaluated at 492 nm.

The derivation of our cut-off value was based on the comparison of known negative and positive sheep sera as determined by a commercially available indirect hemagglutination inhibition (IHA) test for *Toxplasma* IgG antibodies (Toxo — IHA TEST™; Carter Wallace Inc., Dist., Cranbury, New Jersey, USA). Additional support for this cut-off value was derived from a recent study on a flock of "pathogen free" sheep. The mean ELISA value obtained for 94 presumably immunologically naive sheep was 0.11, with SD = 0.065. Over 98% of samples classified as immune by other tests yielded ELISA values >0.30 which is above 2 SDs from the mean value of "negative" samples.

The criteria for the determination of the immunological status of the ovine sera were as follows: $\times < 0.1$ = negative, no previous exposure, IHA <16; $0.1 < \times < 0.15$ = equivocal/borderline, IHA 16–32; $0.15 < \times < 0.2$ = low titer, immune, IHA 32–64; and >0.2 = immune; IHA 128+. For analysis, all values below 0.15 (negative and borderline) were classed as negative, and those above that value as positive.

Management information

Management and demographic information was collected with a comprehensive questionnaire administered in a standardized fashion at each farm by one of three trained technicians. Only variables which could be reasonably hypothesized to have an effect on *T. gondii* status of the sheep, or which might serve as surrogate measures for general aspects of flock management, were selected for analysis (Table 1).

Statistical methods

All analyses were done using the commercial statistical package SAS (SAS Institute Inc., SAS/STAT Guide for Personal Computers, Version 6 Edition, 1988). Univariate analyses were used for initial screening — Student's *t*-test for bi-level variables, analysis of variance for multi-level variables, and regression for continuous variables. Those factors which were statistically significant at $p < 0.10$ on univariate analysis were presented for possible inclusion in multiple regression analysis. The combination of variables which explained the greatest amount of variation in prevalence rates, with the least amount of bias, was selected using Mallow's Cp statistic (11). Our outcome was the proportion of ewes serologically positive per farm.

Results

Sera were collected and tested from 3872 sheep, representing 103 flocks, with an average of 37 sampled per flock (range 12–51). Flock sizes varied from a

Table 2. Factors associated with the proportion of sheep positive to *T. gondii* within Ontario sheep flocks in univariate analysis ($p < 0.10$)

Factor	Associated with higher (+) or lower (−) prevalence	p-value
Female cats neutered vs intact	−	0.001
Cat litter born on the farm in the last two years	+	0.007
No. cat litters born last two years	+	0.017
Pigs raised as well as sheep	+	0.001
Pasture shared with other animals	+	0.018
Pasture shared with cattle	+	0.021
Indoor housing in summer, vs none	+	0.015
Crops grown as well as sheep raised	+	0.020
Estimated % of labor used to raise sheep	−	0.083
Water source at pasture: flowing vs standing	+	0.014
Ewes' perineums clipped before lambing	−	0.057
Sheep purchased from other flocks	+	0.018
Creep feed offered to lambs	+	0.026
Forage offered to lambs	+	0.049
Lambing ewes have contact with other ewes	−	0.011

Table 3. Factors associated with the proportion of sheep seropositive to *T. gondii* in Ontario sheep flocks (multiple regression)

Factor	Coefficient[a]	p-value[a]
Female cats neutered, vs intact	−0.134	0.045
Pigs raised on same farm	+0.288	0.045
Ewes' perineums clipped before lambing	−0.175	0.008
Pasture shared with other animals	+0.099	0.069
Sheep are purchased from other flocks	+0.205	0.003
Indoor housing in summer for replacements vs no housing	+0.120	0.059
Source of water at pasture: flowing vs pipe or standing	+0.119	0.047

[a]Coefficients and p-values are those for the full model selected on the basis of the lowest Mallow's Cp statistic ($R^2 = 0.47$; $Cp = 5.7$)

stated initial inventory of 21 to 500 adult ewes, with a mean of 81 and a median of 61. Because of the way in which sample sizes were calculated, the percentage of adult ewes sampled in each flock varied from about 8% to 100%, with an average of 58% and a median of 56%. In general, we sampled a higher percentage of smaller flocks than of larger flocks; we sampled more than 40% of the adult ewes in 75% of the flocks. Thus, although the sampling strategy was designed to detect disease, we are reasonably certain that we sampled sufficient numbers of ewes to estimate within-farm prevalence.

Our initial strategy had been to compare farms positive for *T. gondii* with those that were negative. However, 99% of farms were classed as positive; this did not provide an adequate negative comparison group. In order to obtain the maximum amount of information from the data, we used the proportion of positive sheep as our outcome. The percentage of positive sheep ranged from 3.8% to 97.8%, with a mean of 57.6%. Mean flock prevalences did not differ significantly among the five regions we looked at.

In univariate analysis (Table 2), increased prevalence of serological reactors within farms was associated with intact female cats on the farm. Furthermore, the more litters that had been born on the farm in the previous two years, the higher the prevalence was likely to be. The presence of pigs on the farm was also associated with higher seroprevalence, as was the practice of having sheep share pasture with "other animals". In almost all cases, the "other animals" were cattle. Other managerial factors associated with higher seroprevalence were giving creep feed and forages to lambs, purchasing replacements from other flocks, having flowing water available at pasture (versus piped or standing water), and giving pastured replacements access to housing during the summer. Clipping of ewes' perineums (crutching), and allowing lambing ewes to have contact with other sheep were associated with lower prevalence of serological reactors.

In multivariate analysis (Table 3), lower seroprevalence rates were associated with neutering of female cats and crutching of ewes, while higher rates were associated with raising pigs on the same farm, having the sheep share pasture with other animals (which in most cases meant cattle), purchasing sheep from other flocks, having flowing water available at pasture, and having housing available for pastured replacements. All together, the factors in the model were able to account for 47% of the variation in seroprevalence rates.

Discussion

The results of this study confirm those reported by Tizard *et al* (8), suggesting that many Ontario sheep flocks have had some exposure to *T. gondii*. Our results suggest, furthermore, that there is considerable variation in the proportion of positive sheep between flocks.

The findings reported here support the hypothesis that young kittens can serve as a source of infection for sheep, and that, as has been suggested, the neutering of farm cats may be an effective control measure (12). The positive associations of increased seroprevalence with creep feed and forage given to lambs (in univariate analysis), and the use of indoor housing for replacement animals in the summer, may all be explained with reference to increased potential for exposure of the sheep to infected kitten feces. At least one human outbreak has been attributed to drinking creek water contaminated by wild cats (13); the association between higher seroprevalence and running water at pasture in our study might be explained

similarly. Piped water would not likely be contaminated by defecating kittens; it would be of ethological as well as biological interest to know if kittens are more likely to frequent the banks of streams (flowing water) than the edges of ponds (standing water).

The associations reported here between cattle, pigs, and increased serological prevalence to *Toxoplasma* remained strong even after accounting for the presence of newborn cats on the farm. This suggests that pigs and cattle (and perhaps sheep) may play roles in the epidemiology of this parasite on the farm. The seroprevalence of *Toxoplasma* in Ontario swine was reported by Tizard *et al* to be 45% (8); other North American authors have reported rates varying from less than 1% to almost 70% (1,14,15). While Tizard *et al* (8) reported that 17% of the Ontario cattle sera that they tested were positive, serological testing for *Toxoplasma* in cattle has been shown to be unreliable, and the true prevalence is probably lower than that reported (1,16). One biologically plausible explanation for the associations seen in our study is that cats are getting reinfected from eating aborted, infected fetal material and/or infected dead piglets, lambs, and calves.

The association between lower seroprevalence and the practice of clipping the ewes' perineums (crutching) before lambing could be explained by hypothesizing that infected fetal and placental material may cling to the unclipped wool and serve as a source of infection for the cats, or for the sheep directly if they ingest this material.

The associations of growing crops with higher seroprevalence, and percent of farm labor effort expended on the sheep with lower seroprevalence, can perhaps be interpreted as indicators of how much (or little) time, energy, and money are spent on the management of sheep. Finally, the association between lower prevalence and allowing lambing ewes to have contact with other sheep, which disappeared when other variables were controlled for, also has no readily apparent biological explanation; this variable appeared to be associated with a wide range of other managerial factors, and this may represent either a spurious chance association, or be an indicator for something we did not measure. CVJ

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