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Coxiella burnetii seropositivity and associated risk factors in goats in Ontario, Canada

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ABSTRACT

Coxiella burnetii is a zoonotic bacterium, and infection in goats with this bacterium can result in abortion, stillbirth or birth of non-viable kids. A cross-sectional study was conducted to identify the seroprevalence and risk factors for *C. burnetii* exposure in Ontario goats. Sera were collected between August 2010 and February 2012, and tested for *C. burnetii* specific antibodies using an enzyme-linked immunosorbent assay (IDEXX).

Overall, 63.2% (48/76, 95% CI = 51.9–73.4) of farms had one or more seropositive goats. A higher farm-level seroprevalence of 78.6% (33/42) was found on dairy goat farms, compared to 44.1% (15/34) on meat goat farms ($p < 0.01$). At the overall individual-animal level, 32.5% (714/2195, 95% CI = 30.6–34.5) of goats were seropositive. Similarly, a higher individual-level seroprevalence was identified for dairy goats (43.7%, 633/1447) compared to meat goats (10.8%, 81/748) ($p < 0.001$).

A mixed multivariable logistic model that controlled for farm-level clustering identified risk factors associated with seropositivity ($p < 0.05$). Increases in the female herd size (logarithmic scale) were associated with increased odds of seropositivity, while increases in male herd size had a negative association with seropositivity. If other sheep or goat farms were located in a 5-km radius, goats had 5.6 times (95% CI = 1.01–30.8) times the odds of seropositivity compared to those that were not. Relative to goats from farms where all kidding pen hygiene was practiced (adding bedding, removing birth materials and disinfection after kidding), goats from farms which only added bedding and removed birth materials had a higher odds of seropositivity (OR = 19.3, 95% CI = 1.1–330.4), as did goats from farms which practiced none of these measures (OR = 161.0, 95% CI = 2.4–10822.2). An interaction term revealed kidding outdoors when there were no swine on farm had a protective effect on seropositivity compared to kidding indoors, or kidding outdoors with swine on the farm. These results can inform strategies to mitigate exposure to *C. burnetii* in Ontario.

1. Introduction

Coxiella burnetii (*C. burnetii*) is a zoonotic intracellular bacterium that can infect a wide variety of mammals, birds and arthropods (Astobiza et al., 2011; Enright et al., 1971; Maurin and Raoult, 1999; Thompson et al., 2012). In goats, *C. burnetii* infection can result in clinical disease, called coxiellosis (Lang, 1990). Human *C. burnetii* infection, or Q fever, has most frequently been attributed to direct or indirect contact with infected ruminants, primarily sheep, goats or cattle (Lyytikäinen et al., 1998).

A large human Q fever epidemic occurred in the Netherlands between 2007 and 2009, and was attributed to transmission of *C. burnetii* from infective dairy goats, and to a lesser extent, dairy sheep (Roest et al., 2011a). This epidemic demonstrated the potential impacts of coxiellosis on goat production and subsequently, public health (Roest et al., 2011a). Important gaps in knowledge were also identified, including the impact of *C. burnetii* infection in humans and animal reservoirs, risk factors for infection in these populations, and the effectiveness of various prevention and control strategies (De Valk, 2012). Q fever in humans and coxiellosis in goats have been recognized as endemic in Ontario since 1980s (Palmer et al., 1983; Simor et al., 1984; Simor, 1987). In 1984, research indicated that 20.0% (4/20) of goat farms had at least one seropositive animal using two different ELISAs (Lang, 1988). Since this time, case reports have been published after goat-related

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epidemics in Ontario (Sanford et al., 1994), yet the *C. burnetii* seroprevalence in Ontario goats has not been updated.

In 2013, coxiellosis in animals became an immediately notifiable disease in Ontario. The passive surveillance system currently used to monitor coxiellosis in Ontario goats relies on the reporting of positive diagnostic tests, primarily abortion diagnoses conducted at reference laboratories, to the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) (McEwen et al., 2011). However, this passive system likely underestimates the true prevalence of *C. burnetii* infection due to a high proportion of asymptomatic infections anticipated where no abortions/stillbirths occur (Sidi-Boumedine et al., 2010). If abortions or stillbirth do occur, the likelihood of identifying a case of coxiellosis is still influenced by the probability of a detailed veterinary investigation occurring, in which the causative agent is identified in the laboratory (Georgiev et al., 2013), and by the sensitivity of the diagnostic test used. Diagnostic sample submission, particularly following a single abortion in a herd may be uncommon (Georgiev et al., 2013).

During abortion and normal deliveries, large quantities of *C. burnetii* bacteria can be shed by does in the birth fluids, placenta and fetal membranes (Berri et al., 2001). In aborted goat placentas submitted in Ontario for an abortion diagnostics project, the average number of *C. burnetii* DNA copies per μl of aborted placenta was 3.20×10^{10} (Hazlett et al., 2013). Therefore, during kidding season, the risk of disease transmission is high due to heavy bacterial loads contaminating the kidding environment (Maurin and Raoult, 1999; Astobiza et al., 2010). However, intermittent shedding may continue in the feces, vaginal discharge, urine and milk for several weeks or months following kidding (Rodolakis et al., 2007; Porter et al., 2011). *C. burnetii* can also remain infective for months in aerosols or contaminated dust that continue to be released as the shed material desiccates (Woldehiwet, 2004).

Risk factors identified for *C. burnetii* exposure in animals have included animal management practices and contact with local infected animal reservoirs (Enright et al., 1971; Schimmer et al., 2011). In Canada, there is evidence that sheep, rodents, cats, cattle, and goats shed *C. burnetii* (Lang, 1988; Marrie et al., 1988; Thompson et al., 2012). Contact with infected livestock or being in the vicinity of infected livestock have been identified as significant risk factors for human exposure and disease (Van Der Hoek et al., 2011). However, data on prevalence and risk factors for Ontario livestock exposure and disease are limited. Further investigation of seroprevalence and risks for goat exposure to *C. burnetii* could inform veterinarians, goat producers and policy makers, and aid in the development and implementation of practical prevention and control strategies.

The objectives of this study were to: (i) determine the herd-level and within-herd seroprevalence of *C. burnetii* exposure in Ontario goat farms, and (ii) identify demographic and management practices that are associated with exposure to *C. burnetii* in Ontario goats.

2. Materials and methods

This study used a cross-sectional design and multi-stage random sampling. Three goat producer databases were used to construct the farm-level sampling frame: Ontario Goat, Canadian Meat Goat Association, and a list of dairy goat producers provided by OMAFRA, the Ontario provincial ministry responsible for licensing goat milk production. Farms were selected from 250 dairy goat farms and 170 meat goat farms registered with the aforementioned organizations. To estimate the herd-level prevalence using an *a priori* estimate of 24% with 95% confidence and 10% allowable error, 76 goat farms were sampled; 42 dairy goat farms and 34 meat goat farms. The number of farms sampled in each sector was proportional to the total estimated number of farms in that sector in Ontario. Goat producers were randomly selected from the sampling frame using an online random number generator (http://stattrek.com/statistics/random-number-generator.aspx), and were solicited for enrolment via letter or telephone. To allow for assessment of selection bias, all solicited producers were asked to complete information regarding their farm's industry sector and herd numbers. Farm inclusion criteria were: having at least 10 does that gave birth in the previous 12 months, and being located in Ontario within 800-km of the University of Guelph. Farms beyond this distance were excluded due to logistical concerns and small goat populations. Sampling occurred between August 2010 and February 2012.

To estimate within-farm seroprevalence using an *a priori* estimate of 10% with 95% confidence and 10% allowable error, 35 does that had kidded in the previous 12 months were randomly sampled per farm. If the farm had less than 35 does that kidded in the previous 12 months, samples were taken from all does that met this requirement.

A questionnaire was administered at each farm to the producer considered to be the farm manager, in order to gather information on farm demographics (*e.g.*, male herd size, female herd size), sources of replacement stock, kidding management practices, goat contact with other animal species (livestock, dogs, cats, rodents, wildlife *etc.*), proximity to other farms, and biosecurity practices.

Blood samples were collected into 10 ml red top serum BD vacutainer tubes® (Becton, Dickson and Company, Franklin Lakes, New Jersey, USA) via jugular venipuncture. Vacutainer tube samples were centrifuged for 10 min at $1000 \times g$ and 2 ml aliquots of the separated serum were pipetted into serum micro tubes (Fisher Scientific, Ottawa, CA). Serum micro tubes were submitted to the Animal Health Laboratory (AHL), Laboratory Services Division, University of Guelph.

Serology was performed by the AHL using the IDEXX CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX Laboratories, Broomfield, CO, USA) which detects both phase I and phase II antibodies to provide a cumulative serological outcome. In accordance with the manufacturer's instructions, the ratio of the optical density of the sample(s) to that of the positive control ≥40% was considered seropositive.

Questionnaire and serological data were entered into EpiData® v2.2 for file management (EpiData Association®, Odense Denmark). A mixed logistic regression model of seropositivity was constructed in Stata Intercooled Version 10.1 (StataCorp®, 2007), using the xtmelogit procedure. The model was constructed using manual backwards elimination and controlling for farm-level clustering by including farm as a random intercept. The initial step in model building was a univariable screening of all covariates for association with goat seropositivity. Likelihood ratio tests (LRT) were used for categorical variables with more than two categories and Wald's tests for dichotomous variables; variables were reserved for model inclusion if the level of significance was $\alpha < 0.20$. Linearity of continuous variables was assessed graphically by plotting lowess smoother curves and transforming the variable, if necessary (Dohoo et al., 2003). Explanatory variables associated with the outcome at $p < 0.20$ were then screened for pair-wise collinearity using Spearman's rank correlation; variable pairs were considered collinear if Spearman's rho was $\geq |0.8|$ (Mason and Perreault, 2013). If collinear, the covariates were compared with respect to univariable significance, missing data, and biological relationship, and the most relevant variable was retained (Dohoo et al., 2003). Retained variables were then used to construct the multivariable model with significance set at $\alpha < 0.05$. Variables with more than 25% missing values were excluded from analysis. After examination of the distribution of missing values, this cut point of 25% was chosen as it maximized both the number of variables examined for inclusion in multivariable model building and the number of observations used in the multivariable model. Eliminated variables were tested for

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