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# Article

## Prevalence of paratuberculosis in the dairy goat and dairy sheep industries in Ontario, Canada

Cathy A. Bauman, Andria Jones-Bitton, Paula Menzies, Nils Toft, Jocelyn Jansen, David Kelton

**Abstract** — A cross-sectional study was undertaken (October 2010 to August 2011) to estimate the prevalence of paratuberculosis in the small ruminant dairy industries in Ontario, Canada. Blood and feces were sampled from 580 goats and 397 sheep (lactating and 2 y of age or older) that were randomly selected from 29 randomly selected dairy goat herds and 21 convenience-selected dairy sheep flocks. Fecal samples were analyzed using bacterial culture (BD BACTEC MGIT 960) and polymerase chain reaction (Tetracore); serum samples were tested with the Prionics Parachek enzyme-linked immunosorbent assay (ELISA). Using 3-test latent class Bayesian models, true farm-level prevalence was estimated to be 83.0% [95% probability interval (PI): 62.6% to 98.1%] for dairy goats and 66.8% (95% PI: 41.6% to 91.4%) for dairy sheep. The within-farm true prevalence for dairy goats was 35.2% (95% PI: 23.0% to 49.8%) and for dairy sheep was 48.3% (95% PI: 27.6% to 74.3%). These data indicate that a paratuberculosis control program for small ruminants is needed in Ontario.

**Résumé** — **Prévalence de la paratuberculose dans l'industrie des chèvres et des brebis laitières en Ontario, au Canada.** Une étude de prévalence a été entreprise (d'octobre 2010 à août 2011) afin d'estimer la prévalence de la paratuberculose dans les industries laitières des petits ruminants en Ontario, au Canada. Du sang et des fèces ont été prélevés auprès de 580 chèvres et de 397 brebis (en lactation et âgées de 2 ans et plus) qui ont été choisies au hasard parmi 29 troupeaux de chèvres laitières et 21 troupeaux de brebis laitières choisis au hasard. Des échantillons de fèces ont été analysés à l'aide d'une culture bactérienne (BD BACTEC MGIT 960) et d'une amplification en chaîne par la polymérase (Tetracore); des échantillons de sérum ont été analysés à l'aide d'un ELISA Prionics Parachek. À l'aide d'un modèle Bayésien de variable à classe latente permettant de comparer 3 tests imparfaits, la véritable prévalence à la ferme a été estimée à 83,0 % (IP de 95 % : 62,6 %–98,1 %) pour les chèvres laitières et à 66,8 % (IP de 95 % : 41,6 %–91,4 %) pour les brebis laitières. La véritable prévalence à la ferme pour les chèvres laitières était de 35,2 % (IP de 95 % : 23,0 %–49,8 %) et de 48,3 % (IP de 95 % : 27,6 %–74,3 %) pour les brebis laitières. Ces données signalent le besoin d'un programme de contrôle de la paratuberculose en Ontario.

(Traduit par Isabelle Vallières)

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### Introduction

**P**aratuberculosis (Johne's disease) is a chronic, enteric wasting disease of ruminants, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Previous research and control strategies in Ontario have focused on dairy cattle because of their agricultural importance and the proposed association between MAP and Crohn's disease in humans (1). MAP has also been detected in goat and sheep milk/food products in Europe

and Mexico (2–4). Sporadic cases of paratuberculosis have been diagnosed in sheep and goats in Canada and, in the province of Quebec, a prevalence of 3% was detected in cull sheep (5) and 10.5% in a goat mortality study (6). Demand for goat and sheep milk-based products is increasing in North America and these dairy industries have grown rapidly in Ontario. With this growth comes increased scrutiny from a food safety and welfare standpoint and an increased need for research on

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production-limiting diseases. Before resources can be allocated appropriately, however, accurate and relevant prevalence data must be determined.

Paratuberculosis often goes unrecognized in small ruminant species due to a lack of producer awareness, poor sensitivity of diagnostic tests at the individual animal level (7), and clinical similarity to other wasting diseases (8). Furthermore, small ruminants exhibit diarrhea in only 20% of cases (9), in contrast to cattle in which intractable and profuse diarrhea is a main clinical sign (8). As there is no pathognomonic sign for paratuberculosis in small ruminants, the infection may be well-established in a herd before the first case is diagnosed (8).

The transmission of MAP is mainly fecal-oral; infectious animals shed the bacteria in their feces and contaminate the environment, feed, water, and skin surfaces (10). Infection is often contracted early in life (8), but it may take 2 to 14 y before the clinical disease appears, depending on the dose of MAP ingested (10), species (10), and within-farm prevalence (11). During this period of latency, antemortem diagnosis is challenging. As an animal progresses from infected to infectious to diseased (12), antibody titers can fluctuate (13) and fecal shedding can be intermittent (12). Even when animals are shedding, it is challenging to detect the bacterium in feces by means of culture or polymerase chain reaction (PCR) (8,14).

In summary, there is no perfect antemortem test and most such tests demonstrate low sensitivity (15) and test performance (i.e., sensitivity and specificity), can vary by farm contingent based on factors such as the age distribution in the herd (12) and stages of infection present (16). Apparent prevalence calculations based on a single test result will therefore underestimate the true level of infection (10) and estimating true prevalence with traditional frequentist statistical methods does not account for variability in test performance (17).

Latent class analysis (LCA) is an alternative method of prevalence estimation that is based on using a combination of 2 or more tests conducted and interpreted in parallel (18). This method may demonstrate increased accuracy if the tests used cover more than 1 stage of the infection (19) and when there is low agreement between tests. For example, a fecal test would detect the pathogen and therefore the shedding or "infectious" state, while a blood test would detect an immune response to infection or the "affected/diseased" state (12). Latent class analysis is often combined with Bayesian statistical analysis when determining paratuberculosis prevalence because it represents test sensitivity and specificity as distributions rather than as constant fixed parameters, thus accounting for test variation. To further reduce uncertainty in the prevalence estimate (20), Bayesian modeling incorporates previous knowledge about test performance, known as "priors," and combines this with the study data, also modeled as a distribution, to obtain an estimate of true prevalence (21).

To date, no randomized herd-/flock-level studies of paratuberculosis in goats or sheep have been published in Ontario or any other Canadian province. Furthermore, there are few well-designed dairy goat and dairy sheep studies elsewhere (22), even though the disease is well-recognized globally (8). Therefore, the objective of this study was to estimate the true farm-level

and within-farm prevalence of paratuberculosis in milking dairy goats and dairy sheep in Ontario.

## Materials and methods

### Herd and animal sampling

A cross-sectional study was conducted from October 2010 to August 2011. Farm sample size calculations were based on allowable error of 5%, 95% confidence, intra-class correlation coefficient of 0.1, expected prevalences of 15% (goats) and 10% (sheep), and the equation to estimate prevalence (Eq 2.4) provided in Dohoo et al (23), which did not account for test performance. This resulted in a need for 29 dairy goat herds and 20 dairy sheep flocks. Animal sample size was subsequently determined by a combination of budget and feasibility. Twenty lactating females, 2 y of age or older, were selected from each farm, for a goal of 580 goats and 400 sheep.

Goat herd inclusion criteria were: being located in Ontario, licensed to produce goat milk, and currently milking more than 20 goats over the age of 2 y. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) maintains contact information on licensed producers, of which there were 263 at the time of sampling. Seventy-three dairy goat producers were selected from this list for recruitment by mail, using randomized, stratified sampling based on size of the breeding herd: small (< 100 animals), medium (100 to 400 animals), and large (> 400 animals) and anticipating a 40% response rate. From 38 responding producers, 36 indicated willingness to participate and 29 herds that fulfilled the inclusion criteria were randomly selected.

The Government of Ontario has no current list of producers shipping sheep milk for human consumption. Using an out-of-date government list of 41 producers, of which only 16 were still producing milk, all producers were contacted and licensed processors were asked for contact information of farms supplying them with sheep milk. Flocks were recruited (26 in total) until 20 farms were enrolled. There was no stratification based on flock size as no information was available on the distribution of flock sizes in Ontario. Farm inclusion criteria were: milking at least 20 ewes in Ontario, and shipping milk to an off-farm processor. As 1 farm did not have 20 milking ewes over 2 y of age, an additional farm was recruited to achieve the desired sampling target of 400 animals.

Each farm was visited once, at milking time. At each farm, 20 milking does or ewes over the age of 2 y were randomly selected as follows: lactating herd/flock size was determined pre-visit and a random number list was generated. Before collecting fecal and blood samples, the owner checked the ear tag or tattoo to verify that the animal was > 2 y of age. If not, then the next animal in the milking line > 2 y was selected and sampled. The recorded tag numbers had to be checked after milking in 2 sheep flocks. Signalment data were recorded where available. Feces were obtained per rectum and blood was drawn into a 10-mL serum tube by jugular venipuncture. No animals had been vaccinated against paratuberculosis and researchers were unaware of their paratuberculosis status at the time of the study.

**Table 1.** Prevalence, sensitivity, and specificity priors for fecal culture, fecal polymerase chain reaction, and the serum ELISA test used in a 3-test Bayesian model estimating farm-level and within-farm paratuberculosis prevalence in 29 dairy goat herds and 21 sheep flocks from October, 2010 to August, 2011 in Ontario, Canada

Parameter	Goats			Sheep		
	Prior data		Beta distribution (a,b)	Prior data		Beta distribution (a,b)
	Mode	% sure		Mode	% sure	
Se <sub>FCUL</sub>	0.4	90% < 0.6	(4.98, 6.96)	0.15 <sup>a</sup>	90% < 0.4 <sup>a</sup>	(2.15, 7.52) <sup>a</sup>
Sp <sub>FCUL</sub>	0.9975	95% > 0.995	(2291.17, 6.74)	0.9975	95% > 0.995	(2291.17, 6.74)
Se <sub>FPCR</sub>	0.3	90% < 0.6	(2.41, 4.29)	0.3	90% < 0.6	(2.41, 4.29)
Sp <sub>FPCR</sub>	0.98	95% > 0.95	(107.20, 3.17)	0.98	95% > 0.95	(107.20, 3.17)
Se <sub>ELISA</sub>	0.3	90% < 0.5	(4.33, 8.77)	0.28 <sup>a</sup>	90% < 0.4 <sup>a</sup>	(9.26, 22.23) <sup>a</sup>
Sp <sub>ELISA</sub>	0.95	95% > 0.9	(99.7, 6.19)	0.95	95% > 0.9	(99.7, 6.19)
Farm-level prevalence	NI	NI	(1,1)	NI	NI	(1,1)
Within-farm prevalence	NI	NI	(1,1)	NI	NI	(1,1)

<sup>a</sup> Parameters differ from goats.

Se — sensitivity; Sp — specificity; FCUL — fecal culture; FPCR — direct real-time fecal polymerase chain reaction; ELISA — enzyme-linked immunosorbent assay; NI — non-informative.

### Sample handling and testing

All samples were stored at 4°C to 8°C until submitted to the appropriate laboratories within 18 h of collection. Feces were processed fresh when possible, although samples from 7 goat and 10 sheep farms had to be frozen at -80°C due to high work volume at the laboratory. Blood was centrifuged for 10 min at 1000 × *g* within 12 h of collection and the serum was frozen at -80°C. Three tests were carried out on the samples collected: fecal culture (FCUL), direct real-time fecal polymerase chain reaction (FPCR) on feces, and Parachek enzyme-linked immunosorbent assay (ELISA) (Prionics Schlieren-Zurich, Switzerland) on serum (ELISA).

Fecal culture was conducted using the BD BACTEC MGIT 960 Mycobacterial detection system and BACTEC MGIT Para TB medium (Becton Dickinson, Franklin Lakes, New Jersey, USA). Culture-positive samples underwent both acid-fast staining and PCR confirmation that targeted the *hspX* gene (Culture Confirmation Protocol, MAP Extraction System; Tetracore, Rockville, Maryland, USA). Fecal culture (FCUL), direct real-time fecal polymerase chain reaction (FPCR) was conducted directly on the feces before decontamination with the MAP extraction system by Tetracore using a cycle threshold (Ct) of ≤ 42.0. The serum Parachek ELISA was interpreted using the optical density cutoff of > 0.3 (mean negative control + 0.2), which is the specific cutoff for small ruminants stipulated by the manufacturer.

### Bayesian statistical analysis

To determine true herd-level prevalence, a herd/flock was considered infected if it contained 1 or more “infected” animals. An animal was considered infected if it became infectious (shed the bacteria detected by PCR or culture), affected (developed an immune response detectable by the ELISA), or both. The case definition was the “mutual condition” where this occurs (12).

A 3-test Bayesian model was constructed separately for each species, as previous work demonstrated that species-specific differences in test accuracy and disease pathogenesis are likely (24). Models based on binomial distributions were used in each case and no adjustment was made for whether fecal samples were fresh or frozen. An adaptation of the Branscum et al (21) 2-stage cluster model was used, which allows for the possibility

that any herd/flock had the potential to be disease free and using the cutoff of 1 test-positive animal to designate herds/flocks as “infected” (complete model coding, without data, is available from the corresponding author). In both models, ‘p.true.pos’ represents the probability that a farm was infected, ‘whp’ is the true prevalence within an infected farm (assumed to be the same level for all 3 tests), and ‘*n*’ represents the number of animals tested.

$$p.\text{true.pos} \sim \text{dbeta}(\alpha_{\text{inf}}, \beta_{\text{inf}})$$

$$\text{whp} \sim \text{Beta}[\text{fvar} * \text{fmean}; \text{fvar} * (1 - \text{fmean})] \text{ with probability } p.\text{true.pos}$$

$$\text{whp} = 0 \text{ with probability } 1 - p.\text{true.pos}$$

Assuming a random effects model for within farm prevalence, fmean and fvar are distributions describing the mean and variability of the prevalence within infected farms:

$$\text{fmean} \sim \text{Beta}(a_m; b_m)$$

$$\text{fvar} \sim \text{Gamma}(s; r)$$

The basic assumptions and relationships are also maintained for test positives (*r*) and the sensitivity (Se) and specificity (Sp) prior information distributions for each of the serological (ELISA), culture (FCUL), and RT-PCR (FPCR) tests:

$$r_{\text{FCUL}} | \text{whp}; \text{Se}_{\text{FCUL}}; \text{Sp}_{\text{FCUL}}; \sim \text{Bin}(\text{whp} * \text{Se}_{\text{FCUL}} + (1 - \text{whp})(1 - \text{Sp}_{\text{FCUL}}); n_{\text{FCUL}})$$

$$r_{\text{FPCR}} | \text{whp}; \text{Se}_{\text{FPCR}}; \text{Sp}_{\text{FPCR}}; \sim \text{Bin}(\text{whp} * \text{Se}_{\text{FPCR}} + (1 - \text{whp})(1 - \text{Sp}_{\text{FPCR}}); n_{\text{FPCR}})$$

$$r_{\text{ELISA}} | \text{whp}; \text{Se}_{\text{ELISA}}; \text{Sp}_{\text{ELISA}}; \sim \text{Bin}(\text{whp} * \text{Se}_{\text{ELISA}} + (1 - \text{whp})(1 - \text{Sp}_{\text{ELISA}}); n_{\text{ELISA}})$$

$$\text{Se}_{\text{ELISA}} \sim \text{Beta}(a_{\text{Se-ELISA}}; b_{\text{Se-ELISA}})$$

$$\text{Sp}_{\text{ELISA}} \sim \text{Beta}(a_{\text{Sp-ELISA}}; b_{\text{Sp-ELISA}})$$

$$\text{Se}_{\text{FCUL}} \sim \text{Beta}(a_{\text{Se-FCUL}}; b_{\text{Se-FCUL}})$$

$$\text{Sp}_{\text{FCUL}} \sim \text{Beta}(a_{\text{Sp-FCUL}}; b_{\text{Sp-FCUL}})$$

$$\text{Se}_{\text{FPCR}} \sim \text{Beta}(a_{\text{Se-FPCR}}; b_{\text{Se-FPCR}})$$

$$\text{Sp}_{\text{FPCR}} \sim \text{Beta}(a_{\text{Sp-FPCR}}; b_{\text{Sp-FPCR}})$$

**Table 2.** Characteristics of 29 goat herds and 21 sheep flocks sampled for *Mycobacterium avium* subsp. *paratuberculosis* from October 2010 and August 2011 in Ontario, Canada

Farm species	Herd/ Flock size <sup>a</sup>	# Farms in each category in Ontario	Total farms sampled	Total animals sampled	Mean herd/flock size <sup>a</sup> (95% CI)	Mean age animals sampled in months (95% CI)	Mean days in milk (95% CI)
Goat						<i>n</i> = 256	<i>n</i> = 436
	Small (< 100)	30	3	60	71.3 (70.0 to 72.7)	40.8 (36.8 to 44.7)	227.2 (184.2 to 270.3)
	Medium (100 to 400)	217	23	460	231.3 (224.4 to 238.3)	39.9 (38.4 to 41.4)	197.1 (180.4 to 213.7)
	Large (> 400)	16	3	60	896.7 (799.9 to 993.4)	43.8 (38.5 to 49.0)	207.9 (173.8 to 242.0)
All herds		263	29	580	283.6 (262.8 to 304.3)	40.4 (39.0 to 41.7)	201.7 (187.5 to 216.0)
Sheep						<i>n</i> = 89	<i>n</i> = 200
	Small (< 100)	NK	16	297	66.4 (63.4 to 69.5)	51.8 (46.3 to 57.4)	83.3 (75.1 to 91.5)
	Medium (100 to 400)	NK	4	80	250.0 (231.6 to 268.4)	40.0 (36.6 to 43.3)	125.6 (93.6 to 157.5)
	Large (> 400)	NK	1	20	1000.0 (—)	42.4 (37.6 to 47.3)	174.1 (169.2 to 179.1)
All flocks			21	397	167.4 (139.9 to 195.0)	45.6 (42.7 to 48.6)	124.5 (115.2 to 133.7)

<sup>a</sup> Number of breeding females.  
CI — confidence interval; NK — not known.

A noninformative prior was used for herd/flock-level prevalence, given the lack of literature on this in North America. Informative priors were used for the test characteristics (Se and Sp) and were estimated from the literature and modified by paratuberculosis researcher Dr. M. T. Collins, University of Wisconsin (Table 1). Beta distributions for these priors were then generated using the BetaBuster software (<http://betabuster.software.informer.com/1.0>). A prior for the variation of within-farm prevalences was generated by the method described in Hanson et al (25). Author P. Menzies, who has more than 20 years of experience with the Ontario sheep and goat industries, was asked to give her prior belief of the median and 95% upper limit of within-farm prevalences, which were used as the priors for a beta distribution of within-farm prevalences. (In the final model, within-farm prevalences were modeled with a noninformative prior, although this information was used only to determine the priors for between herd variability.)

From this distribution, the mean was estimated as  $f_{mean} = a_m / (a_m + b_m)$ . Dr. Menzies was also asked to estimate the median and 95% upper limit for the distribution of the 90th percentile of the within-farm prevalences. We then estimated the median ( $f_{var_m}$ ) and 95% upper limit ( $f_{var_u}$ ) associated with the 90th percentile of the Beta [ $f_{var} * f_{mean}$ ;  $f_{var} * (1 - f_{mean})$ ] distribution. The 2 estimates,  $f_{var_m}$  and  $f_{var_u}$ , were then used to fit a gamma distribution using the statistical program R (26) (R-code available from the 4th co-author on request).

The 2 separate models were fitted in WinBUGS (27) using Markov Chain Monte Carlo (MCMC) sampling and the Gibbs

sampler. Posterior distributions were derived and presented using the medians of the posterior distributions and the 5% and 95% percentiles as the 95% probability interval limits after 50 000 iterations, with the first 10 000 iterations discarded as the burn-in period.

Convergence was assessed using visual inspection of parameter traces, histories, Monte Carlo (MC) errors, and autocorrelations. Diagnostic convergence was assessed using convergence diagnosis and output analysis (CODA) software (28). CODA outputs from WinBUGS were evaluated in R using the Raftery-Lewis statistic (29) and examining the I statistic for variations exceeding 5 (30).

To assess the influence of the initial values, the Gelman-Rubin (31) statistic was carried out using 3 different sets of starting points. To assess the effect of the prior selections on the model, a sensitivity analysis was conducted. The model was re-run with different priors (non-informative (1,1); and wider intervals (4.2, 29.3) and (42.6, 5.6) for each of test sensitivity and specificity, and the posterior estimates were evaluated for any subsequent changes. Lastly, dependence between the fecal tests in the model was evaluated through monitoring the probability intervals of covariance parameters for the presence of zero and comparing the Deviance Information Criteria for variations of > 2 (32).

Data were stored in Microsoft Office Excel (Microsoft, Redmond, Washington, USA) and all statistics were calculated using Stata Version 11.2 (StataCorp LP, College Station, Texas, USA).

**Table 3.** Distribution of fecal culture *versus* serum ELISA *versus* fecal PCR positive and negative paratuberculosis dairy goat herds in Ontario

Fecal culture	Serum ELISA				Total herds
	Positive		Negative		
	Fecal PCR	Fecal PCR	Fecal PCR	Fecal PCR	
Positive	14	1	5	3	23
Negative	0	1	5	0	6
Total herds	14	2	10	3	29

ELISA — enzyme-linked immunosorbent assay; PCR — polymerase chain reaction.

## Results

### Descriptive statistics

The response rate to recruitment letters in the goat industry was 52.1% (38/73). No data were available on nonresponders as the initial list of licensed producers used for recruitment was confidential. The response rate was not quantifiable for the sheep industry as there is no accurate database of producers in the province.

Fecal and blood samples were collected from 580 goats, with 20 goats sampled from each of 29 herds. Descriptive statistics of the 29 herds are listed in Table 2. The exact birthdate of sampled animals was available for 256 goats (44.1%) and exact kidding dates were available for 436 goats (75.2%). The study sample included Saanen, Alpine, Toggenberg, Nubian, and La Mancha breeds and crossbreeds. Proportions of animals sampled per breed could not be determined as breed could not be definitively identified in some animals due to the large number of crossbred animals.

Fecal and blood samples were available from 397 sheep (3 sheep were excluded from the study due to age < 24 mo) from 21 flocks (Table 2). Age data were available for 89 (30.0%) sampled sheep and lambing dates for 200 sheep (67.3%). Sampled animals were East Friesian, British Milk sheep, or their crosses. Proportions of animals sampled per breed could not be determined confidently due to the large number of mixed-breed animals. Farm status based on test results is listed in Table 3 for goats and in Table 4 for sheep.

Cohen's kappa for the 3 test combinations are as follows: fecal culture and fecal PCR:  $\kappa = 0.209$  (dairy goats),  $\kappa = 0.135$  (dairy sheep); fecal PCR and serum ELISA:  $\kappa = 0.206$  (dairy goats),  $\kappa = 0.064$  (dairy sheep); and fecal culture and serum ELISA:  $\kappa = 0.287$  (dairy goats),  $\kappa = 0.057$  (dairy sheep).

### Prevalence

The true farm-level prevalence estimate using the 3-test Bayesian model was 83.0% (95% PI: 62.6% to 98.1%) for goat herds and 66.8% (95% PI: 41.6% to 91.4%) for sheep flocks. The median within-farm true prevalence was 35.2% (95% PI: 23.0% to 49.8%) and 48.3% (95% PI: 27.6% to 74.3%) for infected goat herds and infected sheep flocks, respectively. Probability intervals were wide for all estimates.

**Table 4.** Distribution of fecal culture *versus* serum ELISA *versus* fecal PCR positive and negative paratuberculosis dairy sheep flocks in Ontario

Fecal culture	Serum ELISA				Total flocks
	Positive		Negative		
	Fecal PCR	Fecal PCR	Fecal PCR	Fecal PCR	
Positive	6	1	4	2	13
Negative	0	1	4	3	8
Total flocks	6	2	8	5	21

ELISA — enzyme-linked immunosorbent assay; PCR — polymerase chain reaction.

### Bayesian model

The final models converged well. Posterior estimates varied by < 0.02% with larger burn-in periods of 15 000 to 20 000 or more iterations (100 000 to 150 000). Autocorrelations became low before a lag of 20 and Monte Carlo (MC) errors were very small (< 1% of the probability intervals). The graph of the Gelman-Rubin diagnostic supported convergence as well, as  $\hat{R}$  quickly approached 1 and the inter- and intra-sample variations were stable. The Raftery-Lewis diagnostic demonstrated that the burn-in period and iteration numbers were adequate and the parameter I was < 5, which indicated no thinning was necessary. Two models were evaluated per species: 1 assuming dependence between fecal PCR and culture and 1 assuming all tests were independent. As the probability intervals of the covariance parameters for sensitivity and specificity in the dependent model contained 0, the independent model was chosen for the primary analysis. Posterior estimates were unchanged with either model.

### Sensitivity analysis

Alternative priors for all test sensitivities had negligible impact on prevalence estimates; increasing the prior for test specificity of serum ELISA and fecal PCR increased both farm-level and within-farm prevalences, with little impact when reduced. Fecal culture specificity had the greatest influence. Reduction in fecal culture specificity caused the farm-level prevalence to drop as low as 70.6% (within-farm prevalence: 35.1%) in goats and 57.0% (within-farm prevalence: 37.4%) in sheep with a noninformative prior.

## Discussion

The objective of this study was to estimate the true farm-level and within farm-level prevalences of paratuberculosis in both the small ruminant dairy industries, while compensating for variation in prevalence of infection at the herd-level, low test sensitivity, and the long latency period characteristic of this chronic disease.

The observed farm-level prevalence of paratuberculosis in dairy goat herds (83.0%) and dairy sheep flocks (66.8%) was high. As few, large-scale, randomized studies have been carried out in dairy small ruminants (24), there is a relative lack of prevalence estimates for comparison. A recent study from Cyprus,

which sampled a population similar to ours (animals > 24 mo) and also used Bayesian methods, found a goat herd-level prevalence of 48.6% (95% PI: 30.4% to 68.5%) and a sheep flock-level prevalence of 60.8% (95% PI: 42.3% to 78.8%) (22). A study of dairy cattle herds in Ontario in 2005 yielded an apparent herd-level prevalence of 58% based on serum ELISA-positive animals (33).

Our estimated within-farm true prevalence estimates were also high (35.0% in goats and 48.3% in sheep). As most producers visited during this study were unaware of their paratuberculosis disease status, these results support the suspicion that paratuberculosis is often well established on farms by the time the disease is recognized (9). These estimates are consistent with those reported in Norway, where infected goat farms had apparent prevalences close to 50% (34).

A limitation of these results is the wide probability intervals surrounding our estimates; studies that do not account for imperfect test performance often have confidence intervals that are too narrow (35). Using 3 tests in this latent class model may help account for uncertainty in test performance over a 1- or 2-test model (35), especially in this situation where there was little agreement between test results.

Initially, there was concern that this study may be susceptible to selection bias and only producers free of MAP infection would participate. The high prevalence of disease in the study herds/flocks indicates that this is unlikely to have happened. A lack of an official sampling frame for sheep milk producers hampered our effort to conduct a complete random sample of this population. Based on the profile of the producers sampled, however, and our current knowledge of the industry, we feel it is fairly representative of the overall industry. Therefore, while a high proportion of producers contacted agreed to participate (21/26, 80.8%), we are still unsure of the size of the overall population and if the results obtained may still be influenced by the characteristics of those producers who chose to respond or not to respond.

Sensitivity analysis of the LCA/Bayesian model indicated that, by increasing the specificity priors for fecal PCR and serum ELISA, a higher true prevalence would be generated than the one reported here. We were unwilling to place higher priors, however, due to uncertainty about the performance of these tests in small ruminants. The fecal culture prior for specificity had the greatest influence on the estimates. We are confident in the high specificity prior used for this parameter, however, since an animal must shed large numbers of bacteria in order to be culture-positive. Furthermore, culture-positive results were confirmed with acid-fast staining and a MAP-specific PCR (36). Other than these 3, the remaining priors had little influence on the estimates.

Currently, Ontario has a voluntary dairy cattle paratuberculosis control program (37). The results of this study indicate that a similar voluntary control program needs to be developed in the small ruminant dairy industries in this province. The high farm-level prevalence in both the dairy goat and dairy sheep industries precludes eradication as an option, as there may be few, if any, producers without the disease from which to purchase replacements. It is a potential option only for breed-

ers and those with low within-farm prevalence. At this time, a vaccine against paratuberculosis is not available in Canada. For most producers, control programs that lower exposure to young stock and culling infected animals become the default strategy.

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## Book Review

### Compte rendu de livre

#### The Welfare of Animals Used in Research, Practice and Ethics

Hubrecht RC. UFAW Animal Welfare Series. Wiley Blackwell, West Sussex, UK. 2014. 284 pp. ISBN: 9781-1199-6707. \$62.99.

The mandate of the *Universities Federation for Animal Welfare* (UFAW) may be summed up in the quote by Sir Peter Medawar, in 1957; “*Improvements in the care of animals are not likely to come of their own accord, merely by wishing them; there must be research... and it is in sponsoring research of this kind, and making its results widely known, the UFAW performs one of its most valuable services.*” This text, by research scientist Robert Hubrecht is one such offering.

The aim of this text is to be an introductory resource for issues related to the use of animals in medical research. Animals are used as models to test drug efficacy, drug safety, and for the advancement of knowledge. It is a controversial subject where sides are taken, often most heatedly. At the core of the debate, I think, lies a deep concern that respect and animal welfare be paramount.

Without ruffling more feathers than needed, Robert Hubrecht provides detail regarding why and how animals are used with arguments for and against, judgement for the benefits gained *versus* harm to these animals, and global legislation in place to protect them. The concept suggested in the 1940's, “The 3 R's: Replacement, Reduction, and Refinement,” is outlined. Modifications of their original definition continue to make great strides in improving the welfare of animals used in research. Attitudes have changed and basic beliefs have been challenged, as discussed in this text.

A highly readable, well-organized volume, this book supports the mandate of the UFAW. With thoughtful, intelligent, and as much as is possible, unbiased offering of evidence-based material, the author presents as a credible guide to the future advancement of the field of animal research. The text would be most useful for all staff and students involved in the areas of animal research, animal behaviour, and animal welfare, as it succeeds in offering much insight and guidance.

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