Transmission of foodborne zoonotic pathogens to riparian areas by grazing sheep

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Abstract

The objective of this study was to determine if sheep grazing near riparian areas on pasture in Ontario are an important risk factor for the contamination of water with specific foodborne pathogens. Ten Ontario sheep farms were visited weekly for 12 wk during the summer of 2005. Samples of feces, soil, and water were collected and analyzed for the presence of *Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter jejuni* and *C. coli*, and *Yersinia enterocolitica*, by bacteriological identification and polymerase chain reaction (PCR). The data was analyzed as repeated measures over time using mixed models. No samples were positive for *Salmonella*, and no samples were confirmed positive for *E. coli* O157:H7 after PCR. Levels of *Campylobacter* were highest in the soil, but did not differ between soil where sheep grazed or camped and roadside soil that had never been grazed ($P = 0.85$). Levels of *Yersinia* were highest in water samples and were higher in soil where sheep had access ($P = 0.01$). The prevalence of positive *Campylobacter* and *Yersinia* samples were not associated with locations where sheep spent more time (*Campylobacter* $P = 0.46$, *Yersinia* $P = 0.99$). There was no effect of stocking density on the prevalence of *Campylobacter* ($P = 0.30$), but as the stocking density increased the levels of *Yersinia* increased ($P = 0.04$). It was concluded that although sheep transmit *Yersinia* to their environment, pastured sheep flocks are not major risk factors for the transmission of zoonotic pathogens into water.

Résumé

*Cette étude avait comme objectif de déterminer si des moutons broutant près de zones riveraines dans des pâturages en Ontario sont d'importants facteurs de risque pour la contamination de l'eau avec des agents de toxi-infections alimentaires spécifiques. Dix fermes ovines ontariennes ont été visitées à chaque semaine pour 12 semaines au cours de l'été 2005. Des échantillons de fèces, de sol et d'eau ont été prélevés et analysés pour la présence d'*Escherichia coli *O157:H7*, Salmonella *spp.*, Campylobacter jejuni *et* C. coli*, et* Yersinia enterocolitica*, par culture et identification bactériologique ainsi que par réaction d'amplification en chaîne par la polymérase (PCR). Les résultats ont été analysés comme des mesures répétées dans le temps à l'aide de modèles mixtes. Aucun échantillon ne s'est avéré positif pour* Salmonella*, et aucun échantillon n'a été confirmé comme positif pour* E. coli *O157:H7 après PCR. Les quantités de* Campylobacter *étaient plus élevées dans le sol, mais il n'y avait pas de différence entre les sols où les moutons avaient brouté ou séjourné et des sols routiers où aucun mouton n'a pu paître (*P = *0,85). Les quantités de* Yersinia *étaient maximales dans les échantillons d'eau et étaient plus élevées dans les sols où les moutons avaient eu accès (*P = *0,01). Les prévalences d'échantillons positifs pour* Campylobacter *et* Yersinia *n'étaient pas associées à l'endroit où les moutons ont passé le plus de temps (*Campylobacter P = *0,46;* Yersinia P = *0,99). La densité animale n'avait pas d'effet sur la prévalence de* Campylobacter *(*P = *0,30), mais à mesure que la densité animale augmentait il en était de même pour la quantité de* Yersinia *(*P = *0,04). En conclusion, bien que les moutons transmettent* Yersinia *à leur environnement, les troupeaux de mouton au pâturage ne sont pas un facteur de risque majeur pour la transmission d'agents zoonotiques dans l'eau.*

(Traduit par Docteur Serge Messier)

Introduction

Foodborne gastrointestinal illness induced by bacterial infection affects millions of people in North America each year (1). *Salmonella* spp., *Esherichia coli* O157:H, *Campylobacter* spp., and *Yersinia enterocolitica* are important causes of gastrointestinal illness and are often found in production animal settings. There are numerous routes by which these organisms can access the food chain, including direct contamination of water for consumption as well as contamination of water used at various points in the food

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chain. Each of these organisms can be carried and shed by sheep, and can be transmitted to humans through water. However, there is a paucity of information regarding the risks of sheep production to nearby water supplies.

The World Health Organization estimates that 2 million deaths per year result from drinking water that is contaminated with bacterial pathogens (2). Pathogens from livestock feces are the source of some of the water contamination. After direct feces deposition by grazing animals or manure application on farm fields, pathogens can move from manure to waterways or riparian areas (3). In Canada, a study has shown that 32% of farm wells in Ontario exceed the maximum acceptable concentrations of coliforms (4).

Mead et al (5) estimated that 0.5% of all foodborne infections and 3% of deaths from foodborne infections in the United States were due to *E. coli* O157:H7. The main reservoir species for *E. coli* O157:H7 are ruminants, such as cattle and sheep (6). Pollution of groundwater with livestock manure can be an important cause of *E. coli* O157:H7 outbreaks (7); however, the risk from sheep production is unclear. Estimates of overall prevalence of *E. coli* O157:H7 in sheep range from 0 to 60% (8,9), with differences stemming from animal age, production type, and season.

Salmonella is thought to be responsible for the highest cost per case of foodborne gastroenteritis in Canada and the US (1). A study in the United States estimated that 9.7% of foodborne infections and 31% of deaths were due to nontyphoidal *Salmonella* (5). There has been no study on the prevalence of *Salmonella* in sheep in Canada. A survey in Great Britain by Davies et al (10) found a fecal prevalence of *Salmonella* in sheep of only 0.1%. However, Hutchinson et al (11) found that 8% to 11% of sheep manure samples contained *Salmonella*. Contamination of the environment by manure outflow may lead to significant levels of this pathogen in surface water. Johnson et al (12) found that 6.2% of river water samples in Alberta, Canada were positive for *Salmonella*.

Campylobacter, particularly *C. jejuni* and *C. coli,* is the most common foodborne pathogen in the developed world. These organisms cause gastroenteritis, but this is usually not fatal (13). The main reservoirs of these bacteria are poultry and wild birds (14,15), but they are commonly found in ruminants and other production animal species. A study of sheep on pasture in the UK found that the prevalence of shedding of *Campylobacter* varied from 0 to 100%, with the highest prevalence following lambing and very low shedding during other times (16). There has been no study on the prevalence of *Campylobacter* in sheep in Canada. Commonly transmitted by water, *Campylobacter* has been found in 50% of river water samples in New Zealand (17). Contamination of streams, rivers, ponds, and groundwater is strongly associated with upstream agricultural locations, or sewage contamination (15).

There is a much higher prevalence of *Y. enterocolitica* in pigs than in ruminants, and serotypes associated with human disease are more likely to be isolated from pigs (18). Estimates of the prevalence of *Yersinia* infection in humans in Canada are not available. There has been no study on the prevalence of *Yersinia* in sheep from Canada. In the UK, McNally et al (18) found 10.7% of samples from slaughter sheep feces were positive for *Yersinia enterocolitica*. *Yersinia* survives well in spring, river, or groundwater (19), and is often isolated from water (20).

According to recent figures (21), there are 230 000 sheep in Ontario, which comprises 27% of the national flock. There is increasing concern in Ontario and elsewhere regarding the health risks of livestock grazing near waterways and riparian areas. This is partly due to large outbreaks of human disease associated with cattle manure (22). However, it is known that sheep and cattle exhibit different behavior patterns on pasture (23). Combined with the lack of foodborne pathogen data associated with sheep, it has been difficult to estimate the risk of transmission of zoonotic pathogens from sheep.

The objective of this study was to determine whether sheep pastured near riparian areas are an important risk factor for the contamination of water with foodborne zoonotic pathogens.

Materials and methods

Study design and sample collection

Written surveys were mailed to all producers in the Ontario Sheep Marketing Agency District 5 to determine the existence of riparian areas on sheep farms (24). Ten farms were selected to participate in the study, based on proximity to laboratory facilities, presence of a suitable waterway, and willingness to participate. From the period of May 30th until August 19th, 2005, each of the 10 farms was visited weekly. Order of visits was formally randomized for day of the week and time of day (morning or afternoon). Over the course of this study period, each farm received 5 morning visits (07:30–11:30) and 5 afternoon visits (15:30–19:30), during which time behavioral observations were taken for an associated study.

Soil, water, and fecal samples were collected each day after the end of the morning behavioral observations. Water samples (50 mL) were collected within 30 cm of the water's edge, in open water, using a sterile conical tube. Because of their strong flocking behavior, sheep typically access water sources from particular locations. Hence, water samples were collected from 3 different locations, 1 taken at the location where sheep access the stream (access), 1 taken 10 m upstream from the access point (upstream), and 1 taken 10 m downstream from the access point (downstream). If the sheep were contaminating the water source, pathogen levels would be higher at the access and downstream locations than at the upstream locations.

Soil samples were collected from 5 locations, and a minimum of 1 cm^3 of soil, free of vegetation, was collected using a sterile scupula. One sample was taken at the edge of the water at the sheep access point (Access), 1 in the open field where the sheep were observed to graze (field), and 1 from the road edge where sheep had never had access (roadside). The behavior of pastured sheep includes grazing bouts interspersed with camping bouts where sheep congregate to lie down, ruminate, and rest. Therefore, 2 samples were collected from each camping area. On farms where sheep had barn access, camping tended to occur in the barn with a 2nd camping site located in the pasture.

Two fecal samples of approximately 5–10 g each were collected from the ground at the sheep camping site. Where possible, feces were collected immediately after excretion. If the sheep were not on the camping site at the end of the observation period, the freshest feces were collected.

All fecal and soil samples were placed in Whirl-pak plastic bags (Nasco; Fort Atkinson, Wisconsin, USA), and transported to the laboratory within 1 h of collection.

Methods were approved by the University of Guelph Animal Care and Use Committee in concordance with the Canadian Council for Animal Care (CCAC) guidelines (AUP#05R093, "Risks of water borne pathogens from pastured sheep").

Environmental parameters

During each farm visit, the air temperature, relative humidity and black-globe temperature were recorded hourly. Air temperature data were collected using a thermometer (VWR International, Mississauga, Ontario), black globe temperature using an identical thermometer located inside a black globe (10 cm-diameter copper sphere painted matt black) (25), and relative humidity data were collected using an electronic thermohygrometer (Mannix Testing and Measurement, Lynbrook, New York, USA). The equipment was always situated in direct sunlight, in or beside the field containing the sheep. The black globe humidity index (BGHI) was calculated using the equation:

$$(0.8 \times T) + [RH \times (T - 14.3)/100] + 46.3$$

where: T = the temperature on the black globe, and

RH = the relative humidity (26).

Microbial analysis

Samples were enriched as previously described (27) with appropriate sample size modifications. Briefly, 2 g (+/- 0.5 g) of each of the soil and fecal samples were weighed and individually placed into 20 mL of Brilliant Green Bile Broth 2% (Fisher Scientific, Hampton, New Hampshire, USA). Five mL of each of the water samples was pipetted into 45 mL of Brilliant Green Bile Broth 2%. All samples were enriched for 18 h at 37°C.

Escherichia coli O157:H7

Following enrichment, samples were processed for *E. coli* O157:H7 as described previously (27) with appropriate sample size modifications. Briefly, 1 mL aliquots were removed and added to a suspension of *E. coli* O157 antibody coated immunomagnetic beads (Dynal, Oslo, Norway). The resulting suspensions were processed by immunomagnetic separation (IMS) according to the manufacturer's instructions. Fifty μL of the resuspended bead-bacteria complexes were plated onto sorbitol MacConkey (SMAC; Difco, Becton Dickson, Sparks, Maryland, USA) supplemented with cefixime/tellurite (CT-SMAC; SMAC Media Cefixime-Tellurite Supplement, Oxoid, Nepean, Ontario) and incubated overnight at 37°C. Nonsorbital fermenting colonies on CT-SMAC agars were tested for the presence of β-glucuronidase and the ability to ferment lactose using *E. coli* broth containing 4-methylumbelliferyl-β-D-glucuronide (MUG) and MacConkey agar, respectively. Lactose-fermenting and MUG negative isolates were tested for the presence of the *E. coli* O157 antigen gene locus, *rfb*E_{O157}, using PCR as previously described (28). Isolates positive for *rfb*E_{O157} were also tested for the presence of genes encoding for the H_7 flagellum (*fli*C-H_7), by PCR analysis as previously described (29).

Salmonella spp.

Following enrichment, *Salmonella* spp. were cultured, detected, and confirmed as described previously (30). A 1-mL aliquot was placed in tetrathionate broth (Difco; Becton Dickson) for 48 h at 37°C using a ratio of 1 mL of sample to 10 mL of media. After 48 h of incubation, 100 μL of tetrathionate media was transferred to 10 mL Rappaports broth (Difco; Becton Dickson) and incubated at 37°C for 24 h. Subsequently, the broth was streaked to XLT4 agar (Difco; Becton Dickson) and incubated as described previously. Suspect colonies were tested on TSI and LIA agar slants (Difco; Becton Dickson) for reactions typical of *Salmonella* spp.

Campylobacter spp.

Following enrichment, the presence of *Campylobacter* spp. was evaluated by plating the sample onto Blood-free Karmali plates (Oxoid). Plates were incubated at 42°C for 48 h in a microaerophilic atmosphere generated by Campypak with catalyst (Fisher Scientific, Fort Atkinson, Wisconsin, USA). Plates were subsequently examined for the presence of typical *Campylobacter* colonies. All suspect colonies were selected and subjected to identification and processed for PCR confirmation. Isolates were confirmed using methods described by Persson and Olsen (31) for identification of *Campylobacter* spp. and differentiation of *C. coli* and *C. jejuni*. Primers were directed towards the following loci: a universal 16S rDNA gene sequence serving as an internal positive control for *Campylobacter* with a 1062 kb product, the hippuricase gene (*hip*O) with a 344 kb product characteristic of *C. jejuni*, and a sequence partly covering an aspartokinase gene characteristic of *C. coli* with a 500 kb product.

Yersinia spp.

For detection of *Yersinia* spp. following enrichment, each sample was streaked onto CIN agar (Oxoid) and incubated at 25°C for 48 h. Suspect colonies were further tested for lactose fermentation on MacConkey agar plates. Nonlactose-fermenting colonies were evaluated for Rhamnose (10 g rhamnose/L) fermentation using Rhamnose MacConkey (Fisher Scientific) agar at 25°C for 18 h. Nonrhamnose-fermenting colonies were selected and subjected to PCR. Isolates were confirmed using methods described by Wannet (32) for identification of *Yersinia enterocolitica*. The *ail* gene was identified with a PCR product size of 454 kb which identifies a large proportion of the *Y. enterocolitica* which are considered to be pathogenic.

Statistical analyses

In order to test if differences exist between prevalence of pathogens in soil, feces and water, a Fisher's exact test was used, SAS proc freq (33). Where there was significance, a mixed model (SAS proc glimmix) was used to estimate the probability of finding a positive sample in different sample types. The model used test results (positive and negative coded as 1 and 0) as the response, with farm, date, and sample type (feces, soil, or water) as class statements. A model was also used to determine if there were differences between prevalence of positive samples in different soil sample and water sample locations.

A model was used (SAS proc glimmix) to test whether temperature or stocking density affected the prevalence of organisms recovered.

Table I. Description of the 10 farms used in this study. Note: Farm D rotated the sheep between 4 different pastures. On some of these pastures strip grazing was practiced, with the result that the stocking density varied between visits

Farm	Number of sheep	Stocking density (sheep per acre)	Riparian area	Location of riparian area	Shade near water	Barn access
A	14	4.6	Pond	Edge of pasture	Yes	Yes
B	21	1.1	Pond	Edge of pasture	No	Yes
C	12	2.6	Pond	Inside pasture	No	No
D	250	166.7/93.9	Pond	Edge of pasture	Yes	No
		75.7	Pond	Edge of pasture	Yes	No
		55.6	Stream	Edge of pasture	Yes	No
		92.6	Stream	Edge of pasture	Yes	No
E	19	1.6	Stream	Edge of pasture	No	No
F	40	0.75	Stream	Edge of pasture	No	Yes
G	70	1.4	Stream	Inside pasture	Yes	Yes
H	150	6.3	Stream	Inside pasture	No	Yes
I	200	11.1	Pond	Inside pasture	No	Yes
J	100	2.2	Stream	Inside pasture	Yes	Yes

The final model included type of sample, BGHI (or dry temperature) and stocking density. Farm was included as a random effect, with farm, date and sample type as class statements.

Contrasts were used to test for differences between farms that had ponds and those farms that had streams, and for differences between farms where the waterway was inside or on the edge of the pasture.

Results

Flock size for the 10 farms used in this study varied from 12 to 250 sheep. The stocking density varied from approximately 0.75 to 167 sheep per acre (Table I). Four of the farms had ponds, 5 had streams and 1 farm rotated sheep between different pastures of which 2 had pond access and 2 had stream access.

A total of 542 samples were collected over the course of the study, 279 of which were soil samples, 143 were water samples, and 102 were fecal samples. Soil samples could be subdivided by location of collection into 51 from field soil, 49 from roadside soil, 57 from sheep access soil, and 122 from camping soil. Of the camping soil samples, 64 were from inside barns, 20 from field camping sites, and the remaining 38 from camping areas on farms where the sheep did not have barn access. The 143 water samples could be further subdivided into 52 from the site where the sheep had water access, 24 from upstream of this site, and 23 downstream. The remaining 44 water samples were from pond water to one side or the other of the sheep access point. All fecal samples were collected from camping areas, with 64 samples from barn camping areas, 8 from field camping areas, and 30 from "near barn" camping areas on farms where the sheep did not have barn access.

Microbial analysis

No *Salmonella* spp. were recovered from the samples collected in this study. Preliminary confirmation classified 54 out of 524 (10.3%) of the samples as positive for *E. coli* O157:H7. However, none of the samples were confirmed as true *E. coli* O157:H7, since they were lacking one or more genotypic traits.

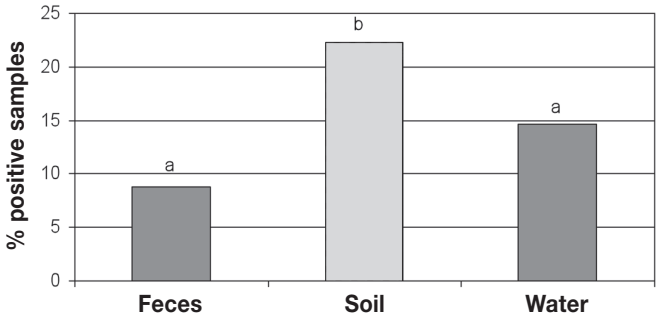

Figure 1. Proportion of positive samples of *Campylobacter* from different sample types. Samples from soil were significantly more likely to be positive for *Campylobacter* than samples from feces or water ($P < 0.05$).

There were 92 out of 524 (17.6%) samples positive for *Campylobacter* spp. The sample type most commonly positive for *Campylobacter* was soil (22.3%), compared with water (14.7%), and feces (8.7%). There was a significant difference in the prevalence of *Campylobacter* between types of sample ($P = 0.0063$), with higher levels in the soil than feces ($P = 0.0034$) and a tendency towards being higher in soil than water ($P = 0.0677$), but not significantly different between feces and water ($P = 0.1553$) (Figure 1). The overall difference between soil locations was not quite significant ($P = 0.068$). There was a significantly higher level of *Campylobacter* recovered from access soil than from field soil ($P = 0.0140$), but none of the other sample location comparisons were significantly different (camping soil versus field soil $P = 0.0835$; camping soil versus roadside soil $P = 0.8395$; camping soil versus access soil $P = 0.1958$; field soil versus roadside soil $P = 0.2088$; access soil versus roadside soil $P = 0.1877$). There was no difference between levels of *Campylobacter* in samples from different water locations ($P = 0.2588$).

There were 97 out of 524 (18.5%) samples positive for *Yersinia* spp. There was a significant difference in levels of *Yersinia* between

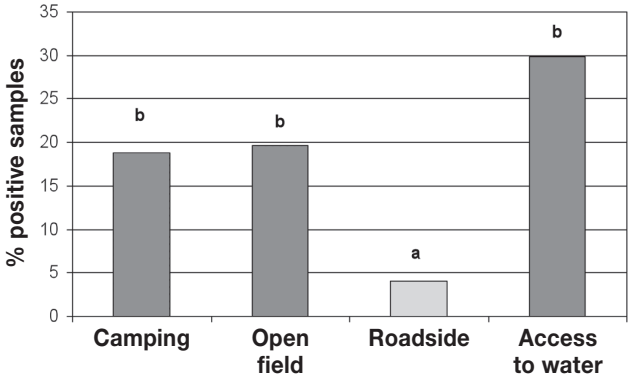

Figure 2. Proportion of soil samples from different locations that tested positive for *Yersinia* spp. Samples from the roadside soil (negative control) were less likely to be positive for *Yersinia* than samples from camping soil (*P* = 0.0161), open field soil (*P* = 0.0165), or soil from the sheep access to water point (*P* = 0.0007)

different types of samples ($P = 0.0122$), with higher levels found in water samples than in feces ($P = 0.0164$), but not significantly higher in soil than feces ($P = 0.0689$) or in soil than water ($P = 0.3384$). When the soil samples were examined by collection location (camping, field, access, and roadside), Fisher's exact test indicated a significant difference in prevalence of *Yersinia* between soil types ($P = 0.0047$). Samples from the roadside soil were lower in *Yersinia* compared with camping ($P = 0.0161$), field ($P = 0.0165$), and access ($P = 0.0007$) soils. None of the other differences were significant (camping versus field soils $P = 0.8968$; camping versus access soils $P = 0.1078$; field versus access soils $P = 0.1772$) (Figure 2).

Following PCR confirmation, 26 of 524 samples were confirmed positive for *Yersinia enterocolitica* specifically. The sample type most commonly positive for *Yersinia enterocolitica* was water (6.3%), compared to soil (4.7%), and feces (3.9%). There was no significant difference in *Y. enterocolitica* levels between different types of samples ($P = 0.8775$). There was also no difference in levels of *Y. enterocolitica* between different soil sample location ($P = 0.4159$), or different water sample location ($P = 0.8223$). None of the samples tested positive by PCR for the presence of the *Y. enterocolitica ail* gene associated with pathogenicity.

Farm level factors

The prevalence of positive *Campylobacter* samples recovered per farm varied between 6% and 32%. The results of the mixed model indicate that there was no effect of stocking density on the prevalence of *Campylobacter* ($P = 0.1772$). There was no difference in likelihood of recovering *Campylobacter* from farms with waterways inside the pasture (so that the sheep could access all sides) ($P = 0.8911$) or on the edge of the pasture (so that sheep could only access one side), or between farms with ponds or streams ($P = 0.2770$).

The prevalence of *Y. enterocolitica* recovered per farm varied between 0 and 12% of samples. As the stocking density increased, the probability of recovering *Yersinia* increased ($P = 0.0358$), but the probability of recovering *Yersinia enterocolitica* did not change ($P = 0.7807$). There was no difference in likelihood of recovering *Yersinia* from farms with different types of waterway ($P = 0.99$) location of waterway in the pasture ($P = 0.99$).

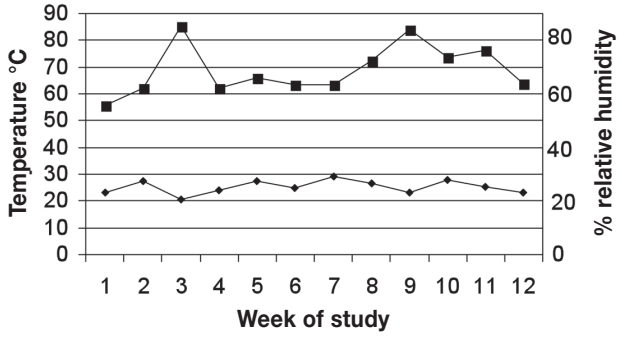

Figure 3. Variation in temperature (◆) and % relative humidity (■) over the course of the 12-week study period (May 30 to August 19, 2005). Each point is the average of the measures taken on farms visited during that week.

Environmental parameters

Over the 12-week study period, the average dry temperature ranged from 12°C to 38°C, with an average of 25°C. The relative humidity ranged from 25% to 100% and the temperature in the black globe ranged from 13°C to 47°C, corresponding to a BGHI range from 52.70 to 109.70, with an average BGHI of 81.9 (Figure 3).

There was a significant effect of temperature on the probability of recovering *Yersinia enterocolitica* ($P = 0.0025$). With each increase in one degree Celsius on the day of the observation, the odds of recovering *Yersinia enterocolitica* decreased by 0.21. There was also a significant effect of temperature on the probability of recovering *Campylobacter* ($P = 0.0022$). With each increase in one degree Celsius on the day of the observation, the odds of recovering *Campylobacter* decreased by 0.17. The BGHI also was associated with pathogens recovered in this study. With each increase in one unit of BGHI, the odds of recovering *Yersinia enterocolitica* decreased by 0.096 ($P = 0.0005$). For each increase in one unit of BGHI, the odds of recovering *Campylobacter* decreased by 0.049 ($P = 0.0139$).

Discussion

Under certain circumstances cattle can cause negative effects on natural water sources by shedding zoonotic pathogens into the environment (34). Being ruminants, sheep carry and shed many of the same zoonotic organisms as cattle including *E. coli* O157:H7 (8,35), *Salmonella* spp. (10), *Campylobacter* spp. (16), and *Yersinia enterocolitica* (36). One of the factors that make cattle a risk for transmission of pathogens into water sources is how they use riparian areas and their behavior in and around water sources (37). Even though sheep may pose similar risks, there are few data available on the impact of sheep production on natural water sources, and to the authors' knowledge there have been no previous studies on how sheep behavior may affect zoonotic pathogen load in surface water and riparian areas.

Ruminants, particularly cattle, are considered the main reservoirs for *E. coli* O157:H7. In the present study, no *E. coli* O157:H7 was recovered from the water, the soil or the sheep feces. Reliance on biochemical tests alone to identify and differentiate *E. coli* O157:H7 from other organisms and *E. coli* types would have resulted in a number of false positives. However, further confirmation with PCR eliminated all suspects. The objective of this study was to determine

which organisms sheep are contributing to their environment, rather than to measure the prevalence of zoonotic organisms in the sheep per se. Therefore, the flocks studied may have been colonized with *E. coli* O157:H7, but at levels not detected in this study.

Other factors that have been shown to contribute to shedding of *E. coli* O157:H7 by ruminants are sudden changes in diet, environment, or antimicrobial therapy (6,35). These factors were not present in any of the flocks studied. Furthermore, levels of *E. coli* O157:H7 are lower in feces from sheep and cattle fed forage rather than concentrate diet (6,11), which is typical of pastured sheep production in general and of the flocks studied here specifically. Hence, results from the present study support the hypothesis that the amount of *E. coli* O157:H7 contributed to the environment by pastured sheep is low.

No *Salmonella* was detected in this experiment; again this is likely a function of study design, sample type, and the low prevalence expected in pastured sheep. Similar to *E. coli* and *Campylobacter,* animals fed a pasture or forage-based diet shed less *Salmonella* in their feces than those fed concentrate based diets (11). Further study on the epidemiology of *Salmonella* in sheep would be useful for determining how to manage this pathogen in sheep flocks.

Campylobacter were recovered in this study from sheep feces, water, and soil samples. *Campylobacter* spp. are found frequently in all animals including wild birds and poultry (14,15), which may contribute to environmental levels of *Campylobacter.* One study found that 42% of wild geese and other waterfowl feces were contaminated with *Campylobacter jejuni* (38). In the present study, wild geese were observed near the water sources on each of the farms. The hypothesis that wildfowl are at least partly responsible for the environmental contamination is supported by the fact that there was no significant difference in levels of *Campylobacter* between soil that had never been grazed by sheep (the roadside control) and soil where sheep grazed and camped. Therefore it would seem likely the *Campylobacter* recovered from the soil and water was not exclusively from ovine sources. Again, stress such as lambing or calving, moving animals, weaning, transport or changing feed can cause an increase in the shedding of *Campylobacter* (15,39). None of these were factors in the study herein.

In the present study, more *Yersinia* spp. was recovered from soils where the sheep had access than from roadside soil; however, there were no differences in levels of *Yersinia enterocolitica* from the different soil locations. This suggests that sheep are at least partially responsible for contaminating the environment and the water, but serotypes of *Yersinia* that were shed are unlikely to cause disease in humans. There has been little or no research on the effect of feeding and management in the shedding of *Yersinia* from ruminants, and therefore it is not clear how management affects *Yersinia* prevalence, if at all.

The lack of association between amount of time that sheep spent in one location and the levels of pathogens in samples from that location was not expected. This lack of association was also found in the related behavioral component to our study (24). To the authors' knowledge, no other studies have examined these parameters with regards to sheep production. Soils where sheep camp receive higher levels of feces relative to noncamp areas (40) and, therefore, would be expected to have higher levels of pathogens. Also higher levels of pathogens would be expected in the water on farms where the sheep spent more time near the water compared with farms where the sheep were never seen near the water. These results indicate that under the management conditions observed, sheep do not have a significant impact on foodborne zoonotic pathogen levels in riparian areas and surface waters. This result is likely due to a combination of the organisms and levels that sheep typically shed, the behavior patterns specific to sheep, and the typical management of sheep in Ontario.

In conclusion, sheep on pasture in Ontario do not appear to be an important source of water contamination with *Escherichia coli* O157:H7 or *Salmonella.* Although in this study *Campylobacter* and *Yersinia* were recovered from samples of sheep feces, soil, and water; there was insufficient evidence to implicate sheep as an important cause of environmental contamination with these pathogens. The lack of association between prevalence of these pathogens and behavior of sheep supports the hypothesis that grazing sheep are not a major risk factor in the transmission of foodborne zoonotic pathogens to water sources.

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