Escherichia coli sequence type 73 bloodstream infections in a centralized Canadian region and their association with companion animals: an ecological study

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Abstract

Purpose: Extraintestinal pathogenic *E. coli* (ExPEC) are important pathogens causing community-acquired infections in humans, including bloodstream infections (BSIs), and may also colonize and infect animals. Our aim was to investigate associations between incidence rates (IRs) of BSIs caused by ExPEC and number of dogs and cats in communities in Calgary.

Methods: We used a well-characterized collection of blood isolates (n = 685) from Calgary, Alberta, Canada (2016). We used a combination of a seven-single-nucleotide-polymorphism quantitative PCR to type ExPEC into sequence types (STs). Calgary census data were used to estimate IRs per city community, as well as to investigate associations between number of companion animals per community, as obtained from licensing data, and IR of BSIs caused by each dominant ST.

Results: From the 685 isolates available, ExPEC ST131 was most prevalent (21.3% of included isolates), followed by ST73 (13.7%), ST69 (8.2%), ST95 (6.7%), and ST1193 (5.3%), respectively. Incidence of BSIs caused by ExPECs among Calgary residents was 48.8 cases per 100,000 resident-years, whereas communities had on average of 1.7 companion animals per 10 residents. No association between the number of dogs and IR of BSIs caused by ExPECs was detected for any ST. Conversely, the incidence rate of BSIs caused by ST73 was 3.6 times higher (95%CI 1.3–9.99) for every increase of 1 cat per 10 habitants in communities.

Conclusions: Number of cats per habitant was positively associated with the incidence of BSIs caused by ExPEC ST73.

Keywords: Bloodstream infections; Cats; Dogs; *Escherichia coli*; Population-based surveillance

Introduction

Escherichia coli is an important pathogen causing intestinal and extraintestinal infections in animals and humans worldwide. The *E. coli* pathotypes associated with extraintestinal infections are also known as ExPEC (Extraintestinal Pathogenic *E. coli*). ExPEC are responsible for nearly 70–95% of community-onset urinary tract infections (UTIs) in humans [1], and a few limited ExPEC lineages are frequently tied to most infections globally. Most prevalent ExPEC sequence types (STs) include ST131, ST69, ST10, ST95, ST73 and ST1193 [2].

Primary reservoir of ExPEC is the intestinal tract of people. Yet, ExPEC lineages that infect humans are also able to colonize and infect animals, which in turn can serve as potential reservoirs of ExPEC to humans. Within-household transmission of ExPEC between humans and companion animals has been documented [3], and pandemic ExPEC lineages have been detected in dogs [4]. The role of companion animals in transmission of ExPEC to humans has been reviewed [5], and recent evidence has suggested that companion animals are spillover hosts rather than reservoirs of some human-associated ExPEC sequence types (STs) such as ST131 and ST1193 [6]. Yet, ExPEC are a large non-homogenous group of bacteria, and determinants of their presence in a population will be dependent on each individual lineage. Indeed, risk factors for presence of ExPEC in humans depend on the ST and also on specific clades and subclades [7, 8]. Similarly, the role of companion animals as reservoirs of ExPEC could potentially be ST or even clade specific. Of note, ST73 is the most prevalent clonal group associated with UTIs in cats and is also associated with bloodstream infections (BSI) in humans [9].

Most studies reporting on the link between ExPEC in humans and companion animals are focused on the molecular characterization and comparison of diverse ExPEC strains across species. Alternatively, companion animals are often surveyed for presence of human-associated ExPEC. Although such studies are invaluable to demonstrate potential interspecies transmission, the magnitude and directionality of events, in general, cannot be ascertained in such studies. Calgary has a centralized laboratory system that performs all clinical microbiology work for the community, including hospital sites. Such a system is an excellent model for population-based studies, where selection bias is minimized and risk factors for pathogen transmission within a well-defined human community can be investigated. Concurrently, the City of Calgary has long implemented Responsible Pet Ownership principles and accompanying bylaws. Under this framework, pet owners are required to license their companion animals every year, which provides a reasonably robust estimation of number of companion animals in the city. These data are integrated into the City of Calgary census data and are available for epidemiological studies. Altogether, Calgary is ideally suited to carry out ecological investigations of the potential role of companion animals as reservoirs of ExPEC to humans.

Here we investigated associations between incidence of BSIs caused by ExPEC STs and number of dogs and cats in a community. Our overarching goal was to provide valuable population-level information about the potential role of companion animals as reservoirs of specific ExPEC STs.

Methods

Ethics

Ethics approval for this study was obtained through the University of Calgary Conjoint Health Research Ethics Board (REB16-2457).

Population and case definition

The study was carried out in Calgary (Alberta, Canada), using a well-characterized collection of bacterial isolates. The Calgary Zone provides all publicly funded healthcare services to nearly 1.8 million people residing in Calgary and adjacent communities. Alberta Precision Laboratories provides all clinical microbiology services for both the hospital and community care sites within the Calgary Zone through a regional centralized laboratory system. Here we used all *E. coli* clinical isolates from blood cultures processed by the Alberta Precision Laboratories in 2016 ($n = 685$), including bacteria from adult and paediatric patient blood-culture samples from both inpatient and outpatient settings.

A case of *E. coli* BSI was defined as a patient with signs of systemic inflammation response (e.g. fever, tachycardia and leukocytosis) and growth of *E. coli* in at least one blood culture. Repeated cases within 365 after the first isolation were not considered. Patient-level data including age, gender, postal code and source of infection (community-acquired [CA], hospital acquired [HA], healthcare-associated [HCA]), were extracted from the National Ambulatory Care Reporting System and Discharge Abstract Database, and defined as described [10].

Bacteria identification and molecular typing

Bacteria identification was done using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO). We used a combination of a seven-single-nucleotide-polymorphism (SNP) quantitative PCR, sequencing and multiplex PCR to type ExPEC into STs, as described [7]. In brief, the seven-SNP qPCR was used to type *E. coli* into septatypes, which were further converted to STs or clonal complexes (CCs). Multiplex PCR was used to classify *E. coli* ST131 into clades and subclades, and to inspect CC14 isolates for ST1193. We defined dominant STs or clades as those representing at least 5% of isolates. The identification of dominant STs (i.e. ST131, ST73, ST69, ST95 and ST1193) was confirmed by multilocus sequence typing.

Statistical analysis

Population data including number of companion animals per community were obtained from the 2016 City of Calgary Civic Census [11]. Incidence rates (IRs) of BSIs caused by ExPEC per 100,000 habitants and respective 95% confidence intervals (95%CI) were estimated for each dominant ST separately using Poisson models. Thereafter, patients postal code data were mapped to Calgary community census subdivisions, which were defined according to the 2016 City of Calgary Census [11]. Prior to mapping, non-residential areas and isolates obtained outside Calgary were excluded.

Prior to the risk analysis, we explored pairwise correlations between explanatory factors known to be associated with the IR of BSIs caused by ExPEC and our primary exposures using the Pearson correlation coefficient. In brief, dominant STs are known to be more frequent among the elderly whereas ST73 is common among young females [7]. First, we estimated the proportion of elderly residents (>64 years old) as well as the proportion of young female residents (20–34 years old) within each community using census data. Thereafter, we computed correlation matrices involving the two proportions as well as the number of dogs or cats in each community.

Two approaches were used to estimate associations between the number of dogs or cats in a community and IR of each dominant ExPEC ST. First, we used overdispersed Poisson models ignoring the spatial structure of the data. The observed number of BSIs caused by each dominant ST was selected as outcome and number of companion animals (dogs, cats) per 10 habitants were used as predictors in separate models. Number of residents in the community was used as offset in all models. Residuals from each regression model were examined for evidence of spatial autocorrelation using the Moran's I statistic, where a *P* value of 0.05 was considered significant. Based on these analyses, no evidence of spatial autocorrelation could be confirmed. Nonetheless, a second approach using spatial modelling was attempted, where models were fit using a full Bayesian inference via an integrated nested Laplace approximation (INLA) algorithm in R [12]. In brief, INLA uses neighbouring structures to estimate the spatial autocorrelation of the dataset. We have modelled spatial effects using the Besag, York and Mollie's (BYM) model. Prior to the analyses, an adjacency matrix was computed to identify neighbouring areas. Uninformative priors were used for all parameters. Posterior modes of rates of change in the log of BSIs caused by each dominant ExPEC ST associated with an increase of 10 pets per habitant were inspected and respective 95% credible intervals were used for statistical inference. As results from the two approaches were in agreement, we selected results from overdispersed Poisson models for presentation. All analyses were carried out in R 4.1.2.

Results

From the 685 isolates available, 623 (91%) were typeable using the seven-SNP qPCR and sequencing. In total, 38 STs were identified, with the following five STs considered dominant: ST131 (*n* = 146 or 21.3% of included isolates), ST73 (*n* = 94 isolates, 13.7%), ST69 (*n* = 56 isolates, 8.2%), ST95 (*n* = 46 isolates, 6.7%), and ST1193 (*n* = 36 isolates, 5.3%). The majority of ST131 isolates belonged to clade C (*n* = 116), with 49 isolates identified as ST131-C1 and 67 isolates as ST131-C2.

Incidence of BSIs caused by ExPECs among Calgary residents was 48.8 cases per 100,000 resident-years (95%CI 45.2–52.6). Among dominant ST, ST131 had the highest incidence rate (10.4 per 100,000 resident-years [95%CI 8.8–12.3]) followed by ST73 (6.7 per 100,000 resident-years [95%CI 5.4–8.2]), ST69 (4 per 100,000 resident-years [95%CI 3.0–5.2]), ST95 (3.3 per 100,000 resident-years [95%CI 2.4–4.4]) and ST1193 (2.6 per 100,000 resident-years [95%CI 1.8–3.6]). When inspecting individual clades and subclades, incidence of BSIs caused by ST131-C1 and ST131-C2 among Calgary residents were 3.6 (95%CI 2.66–4.73) and 4.7 cases (95%CI 3.67–6.03) per 100,000 resident-years, respectively.

Number of companion animals per community ranged from 0 to 4.9 per 10 residents, with an average of 1.7 animals per 10 residents (95%CI 1.61–1.78). The number of dogs per 10 residents ranged from 0 to 3 within Calgary communities, with an average of 1.1 dogs per resident (95%CI 1.05–1.17) and mostly located in the south and southwest end of the city (results not shown). As for cats, there was an average of 0.6 cats per 10 residents in Calgary in 2016 (95%CI 0.54–0.62), ranging from 0 to 1.9 and most animals located in the central and adjacent communities (Fig. 1).

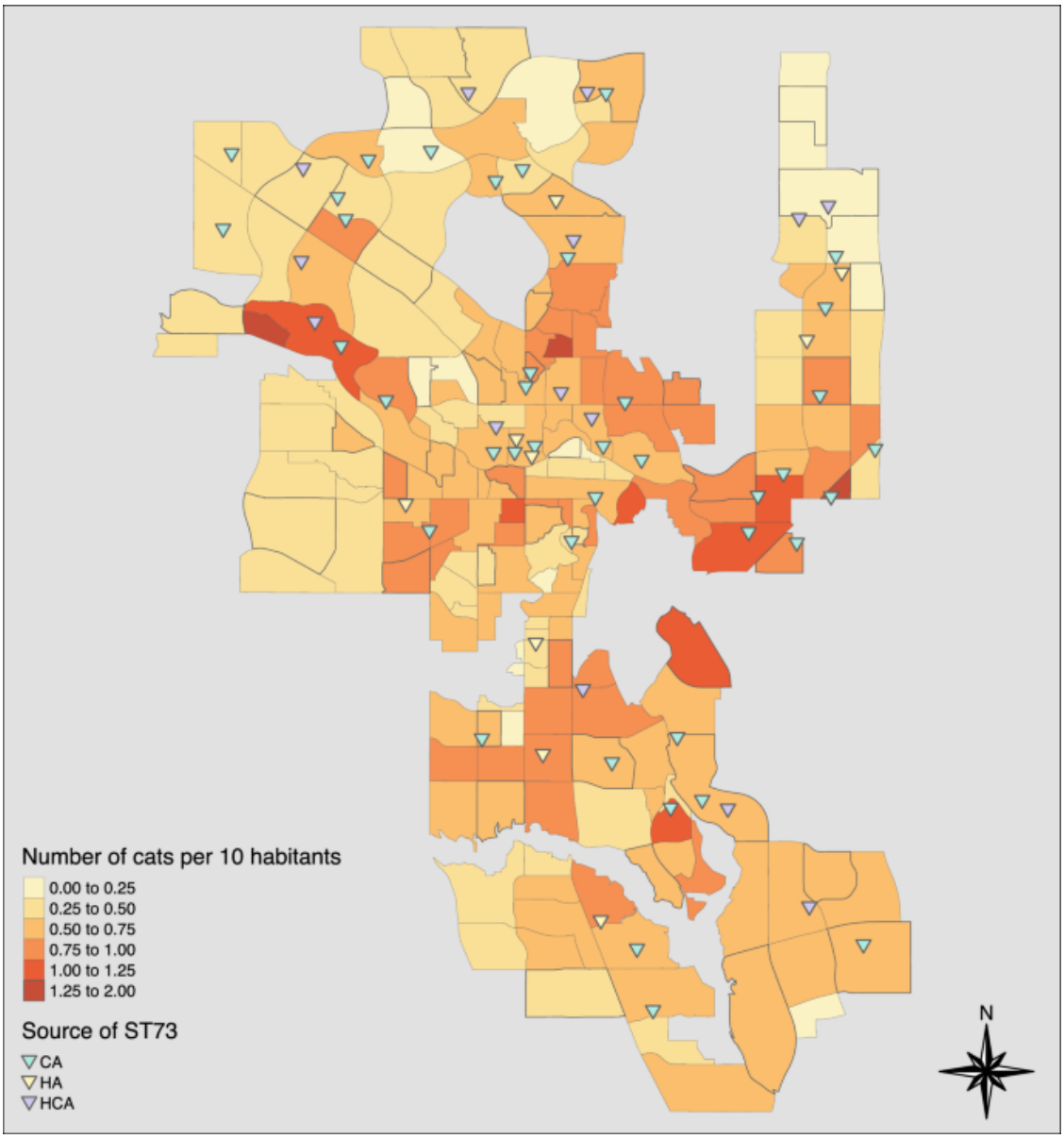

Fig. 1. Spatial distribution of the number of cats per 10 habitants in the Calgary region in 2016. Inverted triangles denote centroids of postal codes of individual cases of bloodstream infections (BSIs) caused by extraintestinal pathogenic *E. coli* (ExPEC) ST73 according to the bacteria source (green = community-acquired infection [CA]; yellow = hospital acquired infection [HA]; purple = healthcare-associated infection [HCA]). Non-residential areas and postal codes from outside Calgary were excluded. The incidence rate of BSIs caused by ExPEC ST73 was 3.6 times higher (95%CI 1.3–9.99) for every increase of 1 cat per 10 habitants in a community using overdispersed Poisson models (P = 0.01). No evidence of spatial autocorrelation could be confirmed using the Moran's I statistic

Pairwise correlations between proportion of elderly (>64 years old) or young females (20–34 years old) and number of dogs and cats per resident in communities yielded low, non-significant estimates, ranging from −0.43 for the correlation between dogs per 10 residents and proportion of young females in communities, to 0.12 for the correlation among cats per 10 residents and proportion of young females (Figs. 2 and 3).

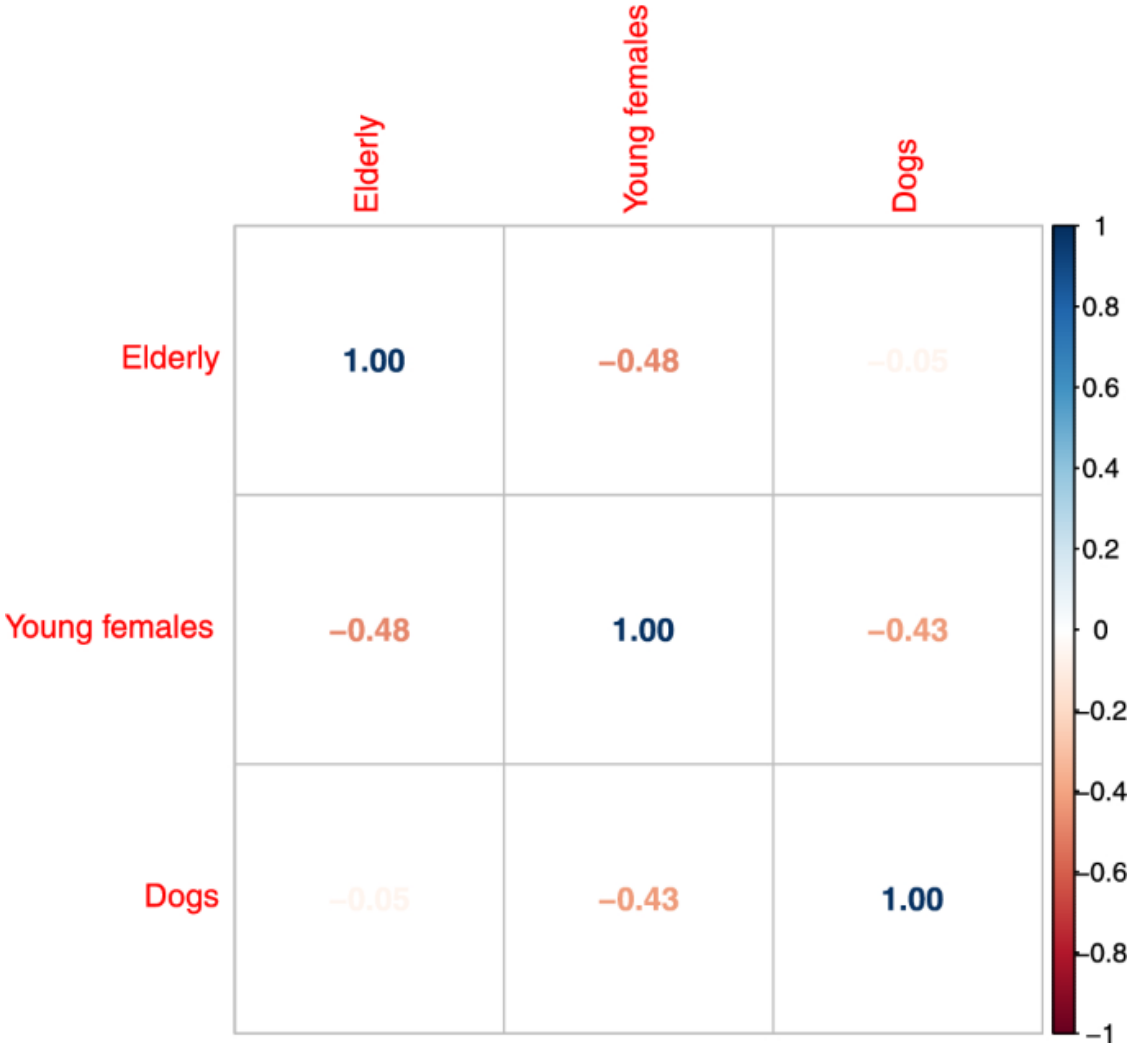

Fig. 2. Pairwise Pearson correlations between proportion of elderly residents (>64 year old) within communities (Elderly), proportion of young females (20–34 years old) within communities (Young females) and number of dogs per 10 residents within communities (Dogs)

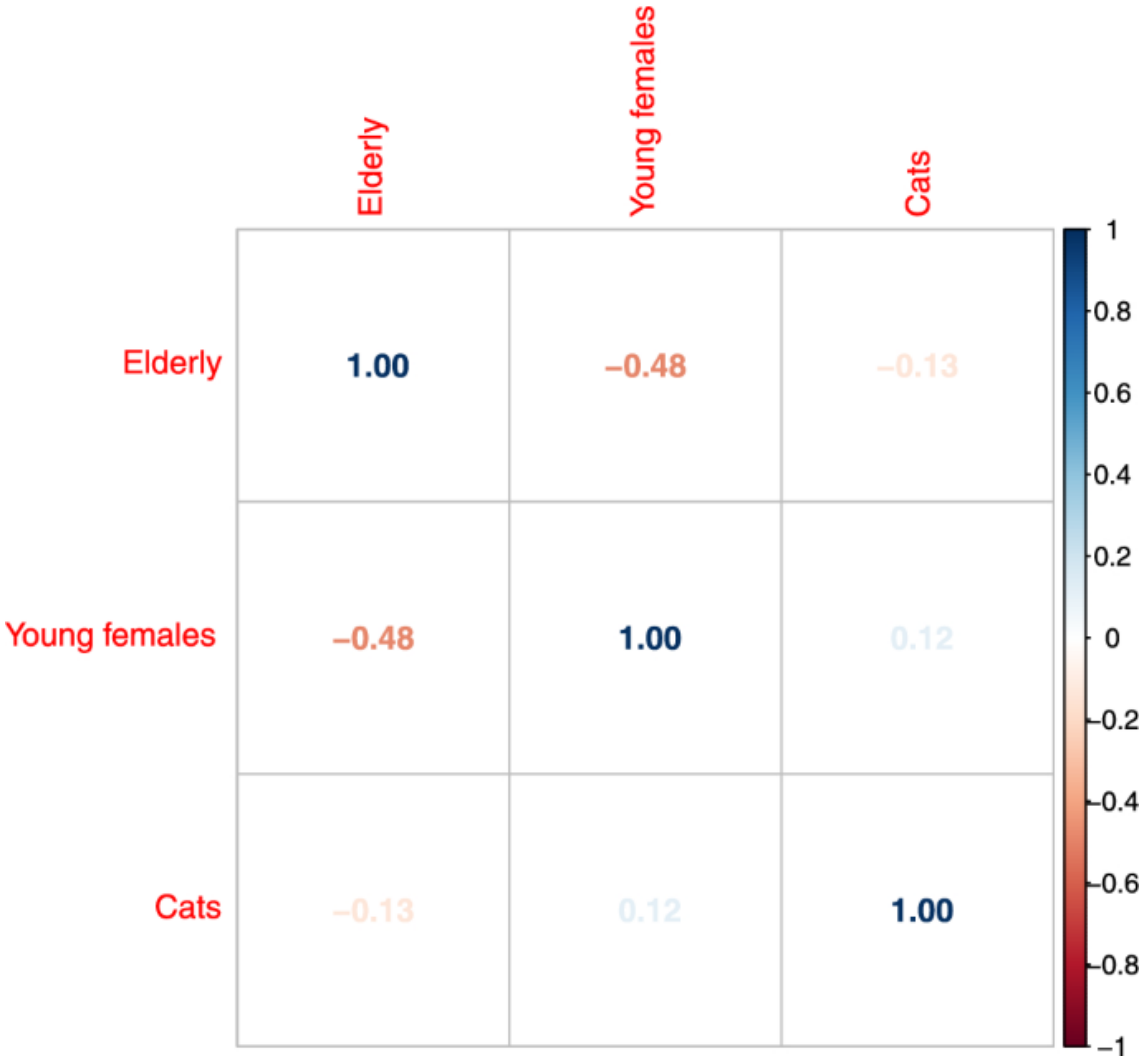

Fig. 3. Pairwise Pearson correlations between proportion of elderly residents (>64 year old) within communities (Elderly), proportion of young females (20–34 years old) within communities (Young females) and number of cats per 10 residents within communities (Cats)

No association between the number of dogs per 10 residents and IR of BSIs caused by ExPECs was detected, regardless of ST and / or subclades (Table 1). Conversely, we detected a significant association between number of cats per resident and IR of BSIs caused by ST73. The IR of BSIs caused by ST73 was 3.6 times higher (95%CI 1.3–9.99) for every increase of 1 cat per 10 habitants in a community. That is, communities with more cats per residents had a higher incidence of BSIs caused by ST73 than communities with a lower number of cats per resident (Fig. 1). This association was dependent on the source of isolates. Whereas IR of BSIs caused by CA ST73 was strongly associated with number of cats per community (incidence rate ratio = 4.6 [95%CI 1.3–15.9]), no association involving IR of BSIs caused by either HA or HCA ST73 and number of cats was detected. As expected, incidence rate of BSIs caused by any ExPEC ST was 2 times higher (95%CI 1.17–3.39) for every increase of 1 cat per 10 habitants in a community, most likely driven by the increased incidence of CA ST73, as for other STs, no associations were detected between number of cats in a community and IRs (Table 1).

Table 1 Incidence rate ratios (IRR) and respective 95% confidence intervals (95%CI) associated with an increase of 1 dog or cat per 10 habitants for each dominant extraintestinal pathogenic *E. coli* sequence type (ST) included in the present study

Factor	ST	IRR (95%CI)	*P*
Cats	ST131	2.04 [0.83; 5.00]	0.12
	ST131-C1	1.27 [0.33; 4.97]	0.73
	ST131-C2	2.02 [0.43; 9.41]	0.37
	ST69	0.97 [0.26; 3.67]	0.97
	ST1193	0.75 [0.11; 5.26]	0.77
	ST95	2.66 [0.68; 10.45]	0.16
	ST73	3.60 [1.30; 9.99]	0.01*
	Any ST	2.00 [1.17; 3.39]	0.01*
Dogs	ST131	1.06 [0.60; 1.86]	0.84
	ST131-C1	0.79 [0.36; 1.76]	0.57
	ST131-C2	1.22 [0.46; 3.24]	0.69
	ST69	0.48 [0.22; 1.04]	0.06
	ST1193	0.48 [0.16; 1.50]	0.21
	ST95	0.83 [0.33; 2.04]	0.68
	ST73	1.32 [0.65; 2.68]	0.44
	Any ST	0.93 [0.66; 1.30]	0.65

*A P-value of 0.05 or less indicates that the respective IRR was different than 1 at the 5% significance level.

Discussion

Here we aimed to identify associations between number of companion animals in communities and IR of BSIs caused by ExPECs in the Calgary region using an ecological study design. Our study led to 2 main findings. First, no association was evident between number of dogs in a community and IR of any dominant ExPEC ST. Second, there was a clear link between the number of cats in a community and IR of BSIs caused by ExPEC ST73.

There is building evidence that companion animals behave as spillover hosts rather than reservoirs of fluoroquinolone-resistant ExPEC STs causing clinical infections in humans. Genomic analysis demonstrated that most dog and cat fluoroquinolone-resistant isolates belonged to phylogenetic groups other than B2, which harbours the human-derived pandemic lineages ST131 and ST1193 [6]. Furthermore, prevalence of fluoroquinolone-resistant STs causing clinical infections in companion animals did not increase significantly over a 5-year study interval [6], whereas the same has been increasing in the human population. Accordingly, our population-based assessment is in agreement with previous findings, as no associations were detected involving human-associated ExPEC ST131 and ST1193 and number of companion animals in communities.

Conversely, our analysis revealed important associations between IR of BSIs caused by ExPEC ST73 and number of cats per resident in communities. ST73 isolates causing BSIs are largely susceptible to fluroquinolones and have moderate to high resistance rates against beta-lactams [7]. In Australia, ST73 is the most prevalent fluroquinolone-susceptible phylogenetic B2 ExPEC causing UTIs in cats, and cat and human ST73 isolates were intermingled in phylogenetic analysis, which suggests bi-directional transmission of isolates between species

[9]. Our findings provide a measure of directionality, as the outcome was measured in humans and the primary exposure was the number of cats per resident in a community. If transmissions occurred exclusively from humans to cats, most likely no association would have been detected, as observed for fluoroquinolone-resistant ExPEC STs. Unfortunately, we did not have access to household pet data from cases, which prevented us from further exploring the role of cats as risk factors for BSIs caused by ExPEC ST73 at the patient-level. Furthermore, no data is available on the distribution of ExPEC STs causing UTIs in cats nor on the presence of ST73 among commensal faecal bacteria from cats in Calgary; such studies are currently underway. Nevertheless, our findings warrant further investigation and support the role of cats as important reservoirs of ST73 causing BSIs in humans.

We recognize that community-level factors other than number of cats are associated with BSIs caused by ExPEC ST73. Of note, BSIs caused by any of the dominant STs studied are known to have a higher incidence among the elderly in Calgary [7]. Further, ST73 is common among young females in the same population. Such age and gender factors could distort associations between number of cats and BSIs caused by ST73. Nevertheless, we believe our findings are internally valid for at least two reasons. First, we explored pairwise correlations between proportion of elderly (>64 years old) or young females (20–34 years old) and number of dogs and cats per resident in communities. Comparisons involving cats yielded low, non-significant estimates, suggesting that no correlation truly existed between number of cats and age-gender distribution of communities. Such correlations, in theory, were necessary to distort associations reported. Secondly, associations detected were exclusive for cats and ST73. If the number of cats per resident was linked to factors such as age-gender distribution of communities (e.g. communities with increased proportion of elderly having more cats per resident), significant associations between number of cats and remaining STs that are also affected by age and gender were expected.

Additionally, we cannot rule out the role of cats as potential indicators of environmental exposure to ST73 rather than agents necessarily implicated in the transmission of ExPECs to humans. Potential environment contamination and salmon diet have been suggested as sources of ExPEC ST73 to whales [13]. Likewise, cats and humans living in same communities might have become colonized by ExPEC ST73 due to exposure to common sources (e.g. salmon diet).

As limitations, our main exposure variable (the number of dogs and cats per resident in communities) could be underestimated. In short, the number of resident and companion animals per community were obtained from the 2016 City of Calgary Census [11]. In Calgary, pet census data in tied to licensing, and under city bylaws owners are required to pay to renew their pet's licence yearly. To date, there is no estimate about the compliance rate of this licencing program. Thus, the number of companion animals per resident in a community could potentially be underestimated if residents did not renew their licenses in 2016. Yet, we have no reason to suspect that compliance was tied to community, which means that our findings would not suffer from low compliance of pet owners even if the number of companion animals per community were severely underestimated. Finally, ecological studies, although very useful for hypothesis generation, are not regarded as optimal for hypothesis testing because of unmeasured and uncontrolled confounding that may occur when making patient-

level inferences from aggregated data. Therefore, our findings should be confirmed by case–control and molecular studies in the same population.

In conclusion, our ecological study demonstrated an association between number of cats in a community and IR of BSIs caused by ExPEC ST73, which suggests that cats may be important reservoirs of ST73 to humans. For other STs as well as for dogs, no associations were detected. We recommend follow up molecular studies using a case–control design at the household-level to further verify the role of cats in the epidemiology of BSIs caused by ExPEC ST73.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

All procedures performed involving human participants were in accordance with the ethical standards of the institutional and national research committees.

Ethical approval

Ethics approval for this study was obtained through the University of Calgary Conjoint Health Research Ethics Board (REB16-2457).

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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