ORIGINAL RESEARCH ARTICLE

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Mycoplasma genitalium presence, resistance and epidemiology in Greenland

Dionne C. Gesink[1]*, Gert Mulvad[2], Ruth Montgomery-Andersen[3], Upaluk Poppel[3], Stephan Montgomery-Andersen[3], Aka Binzer[3], Lee Vernich[1], Gillian Frosst[1], Flemming Stenz[4], Elizabeth Rink[5], Ove Rosing Olsen[6], Anders Koch[7] and Jørgen Skov Jensen[7]

[1]Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; [2]Centre for Primary Care Nuuk, Greenland; [3]Ilisimatusarfik-University of Greenland, Nuuk, Greenland; [4]National Board of Health, Nuuk, Greenland; [5]Department of Health and Human Development, Montana State University, Bozeman, Montana, USA; [6]Sisimiut Health Center, Sisimiut, Greenland; [7]Epidemiology Research (AK) and Microbiological Surveillance and Research (JSJ), Statens Serum Institut, Copenhagen, Denmark

Objectives. Greenland reports the highest rates of chlamydial infection and gonorrhea in the Arctic. Our objective was to determine the presence, and describe the basic epidemiology, of *Mycoplasma genitalium* for Greenland.

Study design. Cross-sectional study.

Methods. 314 residents from Nuuk and Sisimiut, between the ages of 15 and 65 years, participated in "Inuulluataarneq" (the Greenland Sexual Health Project) between July 2008 and November 2009. Participants provided self-collected samples for sexually transmitted infection (STI) testing and completed a sexual health survey. Descriptive statistics and logistic regression were used to summarize the basic characteristics of STI cases overall and *M. genitalium* and *Chlamydia trachomatis* specifically. Clinically relevant characteristics in each full model were gender (male or female), age (in years), age at sexual debut (in years), number of sexual partners in the past 3 months (continuous) and history of forced sex and community.

Results. The overall prevalence of STIs was 19.0%, specifically: 9.8% for *M. genitalium* and 9.4% for *C. trachomatis*; 100% of *M. genitalium*-positive cases carried macrolide resistance determinants. Being female [OR = 3.2; 95% confidence interval (CI): 1.1–9.8] and younger age (OR = 0.9; 95% CI: 0.9–1.0) were associated with *M. genitalium* positivity. Age was also associated with *C. trachomatis* (OR = 0.9; 95% CI: 0.8–0.9) and STI positivity overall (OR = 0.9; 95% CI: 0.9–0.9).

Conclusions. We observed a high prevalence of *M. genitalium* and macrolide resistance in this study. A better understanding of *M. genitalium* sequelae is needed to inform policy around testing, treatment, control and antibiotic use.

Keywords: *sexually transmitted diseases*; *Mycoplasma genitalium*; *antibiotic resistance*; *indigenous health*; *health services*

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Greenland reports the highest rates of infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the North American Arctic (1). *C. trachomatis* and *N. gonorrhoeae* are of concern because of the negative effect these infections have on fertility and pregnancy and the synergistic relationship they have with human immunodeficiency virus (HIV) (2–4). *Mycoplasma genitalium* is an emerging sexually transmitted infection (STI) (5–7) with symptoms similar to those for *C. trachomatis* and *N. gonorrhoeae.* The consequences of *M. genitalium* infection remain unclear (8), though it is thought that they may be similar to those for chlamydial infection, including increased risk for pelvic inflammatory disease (9,10), infertility (11,12) and preterm birth (13). Our objective was to determine if *M. genitalium* was present in Greenland and, if so,

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describe the epidemiology of those infected compared to those who were not.

Materials and methods

This project was approved by the Greenlandic ethics board, under the auspices of the Commission for Research in Greenland (KVUG), and the University of Toronto Research Ethics Board. Study subjects provided informed consent before participating in this study.

Sampling

Between July 2008 and November 2009, an iterative convenience sampling strategy was used to recruit participants into "Inuulluataarneq," the Greenland Sexual Health Project. In Nuuk (Greenland's capital and largest community, population approximately 17,000), participants were recruited between July and October 2008. Two recruitment strategies were used at the beginning of the study in Nuuk. First, a random sample of 1,110 Nuuk residents were selected from the 2005 Greenland population registry and contacted by phone about participating in the study; 395 of the 1,110 were contactable, of which 112 agreed to participate and showed up for their appointment, yielding a response rate of 42% for those individuals successfully contacted, but only 10% for those in the original random sample.

During the same time period, information about the study was disseminated using a press release, interviews with local radio (KNR) and television (Nuuk TV) stations and through articles in the local newspaper (Sermitsiaq). Additionally, information about Inuulluataarneq was presented to the Greenland Medical Society, groups of students attending gymnasium and Ilinniarfissuaq (the Teachers Training College of Greenland) to raise awareness about the high rates of some STIs in Greenland. The purpose of these presentations was not to recruit additional participants but rather inform the community about the project. However, it resulted in many students approaching the research assistants following the presentations and expressing interest in being part of the study (n = 37).

In Sisimiut (second largest community, population approximately 6,000), residents were recruited in November 2009 during "Health Week," an annual health education and promotion event initiated by the Chief Surgeon and the Prevention Board for Sisimiut over 10 years ago. Non-essential services are suspended at the hospital for the week so that health resources can be used to promote education and health in the community. Inuulluataarneq (Greenland Sexual Health Project) was the central theme for health week in 2009. In addition to central activities at the community centre, the Greenland Sexual Health Project set up a satellite centre at the local high school on the last day of health week to facilitate student participation in the study.

In total, 314 Greenland residents between the ages of 15 and 65 years volunteered to participate in the study: 149 residents from Nuuk, and 165 residents from Sisimiut. Participants only participated once.

Activities

After informed consent was granted, each participant completed an interviewer-administered sexual health survey in either Greenlandic or Danish (14). The sexual health survey included questions on basic demographics, STI knowledge and sources of information, sexual partners, perceived risk, condom use, alcohol use, culture, identity and community (14). Sexual abuse and sexual assault were reported to be high in Greenland so we asked 1 question about forced sex. For us, forced sex meant any form of unwanted physical sexual contact ranging from sexual abuse to sexual assault at any age. However, recognizing the spectrum of forced sex, we left the wording of this question open so participants could apply their own definition to forced sex. If asked, we would elaborate based on our definition. None of the participants questioned how to interpret this question.

After the survey, participants provided a self-collected first-void urine sample (both men and women) collected in GeneLock™ (Sierra Molecular, Corp., Sonora, CA, USA) nucleic acid stabilization medium. Women also provided a vaginal swab specimen collected with a large flocked swab in Copan UTM (Copan, Brescia, Italy) for STI testing. Participants were not physically examined for STIs, nor asked about symptoms since this was an exploratory study to determine presence of the organism, not a study of symptomatic or asymptomatic infection.

Biologic samples were self-collected by participants in a lavatory and then passed to a research assistant who finished preparing the samples for storage and transport. Female participants were given a pictorial instruction sheet on how to self-sample using the vaginal swabs and the 2-inch insertion score was pointed out to ensure samples were collected properly. Both male and female participants were instructed to catch the "first burst" for their urine sample. All the biological samples were stored in a refrigerator at 5°C until they were packaged and mailed at ambient temperature to the Statens Serums Institut in Denmark. Samples were sent in weekly shipments from Nuuk and in 1 bulk shipment at the end of Health Week from Sisimiut to the Statens Serum Institut in Denmark.

Laboratory analysis

Urine samples and vaginal swabs were tested for *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae* and *Trichomonas vaginalis*. *M. genitalium* was detected with real-time PCR targeting the *MgPa*-gene (15) and confirmed by conventional PCR detecting the 16S rRNA gene (16). Macrolide resistance-mediating mutations in region V of the 23S rRNA-gene were detected by DNA

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sequencing of amplicons obtained directly from the clinical specimen (17). *C. trachomatis* was detected by a real-time PCR targeting the 16S rRNA gene, and all positive results were confirmed in a real-time PCR detecting the *C. trachomatis* cryptic plasmid (18) *N. gonorrhoeae* was detected by a porA pseudogene real-time PCR (19). *T. vaginalis* was detected by a conventional PCR with primers targeting a *T. vaginalis* specific repeat DNA fragment (20). Positive results were confirmed by repeating the assay after repeated DNA extraction of the original specimen. All assays had an internal control for detection of PCR-inhibition and suboptimal reaction conditions (21,22).

Follow-up

All participants were notified with their STI test results. We worked with the local STI clinics in both Nuuk and Sisimiut to have all STI-positive cases, and their sexual partners, treated for their infections. *M. genitalium* case medical records were reviewed for previous antibiotic use at follow-up for cases in Nuuk. We did not have the capacity or resources available to access medical records in Sisimiut. *M. genitalium* cases were treated with moxifloxacin 400 mg daily for 7 days. *C. trachomatis* cases were treated with azithromycin 1 g as a single dose. *N. gonorrhoeae* cases were treated with ciprofloxacin 500 mg as a single dose.

Statistical analysis

We summarized the characteristics of all cases testing positive for at least 1 of the 4 STIs and then stratified for *M. genitalium* and *C. trachomatis* cases only. Seventeen of the 314 subjects refused STI testing; therefore, analysis was conducted using data provided by the remaining 297 participants. We did not have sufficient numbers of cases to identify factors associated with *N. gonorrhoeae* or *T. vaginalis*. The exploratory survey completed by participants was quite broad (14). Low case counts (n = 29 for *M. genitalium* cases and n = 28 for *C. trachomatis* cases) caused us to focus our analysis on clinically relevant factors that might be associated with *M. genitalium* infection. Otherwise, the model became unstable. These factors included gender (male or female), age (in years), age at sexual debut (in years), number of sexual partners in the past 3 months (continuous), history of forced sex (yes or no) and community (Nuuk or Sisimiut).

The goal of our modelling was exploratory or predictive. That is, our intention was to determine which variables predicted STI, *M. genitalium* and *C. trachomatis* positivity. Basic descriptive statistics (mean, median and standard deviation for continuous variables, percentages for categorical and ordinal variables) were calculated for each variable to describe characteristics of the study sample, the STI-positive group and the STI-negative group. Crude and full model measures of association [odds ratios (OR) and 95% confidence intervals (CI)] were estimated for multivariate models of clinically relevant characteristics using logistic regression in SAS 9.0 (SAS Institute, Cary, North Carolina, USA). Again, clinically relevant characteristics in the full model were gender (male or female), age (in years), age at sexual debut (in years), number of sexual partners in the past 3 months (continuous), history of forced sex (yes or no) and community (Nuuk or Sisimiut). We focused our interpretation of the results on the full models (23), which reflected our conceptual model of predicting *M. genitalium* and *C. trachomatis* in a clinical setting.

We identified the most parsimonious predictive models for overall STI, *M. genitalium* and *C. trachomatis* positivity using backward elimination. The final models comprised those predictive variables considered statistically significant at the 95% confidence level.

Sensitivity analysis

Urine and vaginal specimens were used to detect *M. genitalium* in women, which could have biased the results to detect more *M. genitalium* in women than in men, who had only urine specimens analyzed. Therefore, we conducted a sensitivity analysis using the urine-based STI test results only to account for the fact that men only had urine tests and women had both urine and vaginal swab tests for STI testing.

Results

No important demographic differences were observed between Nuuk and Sisimiut participants (data not shown); so, the 2 samples were combined. More women participated in the study than men, over half were under 25 years of age, the average age of sexual debut was 15 years of age, and almost 30% had experienced forced sex (Table I). However, there were differences in the occurrence of STIs by community: overall STI positivity was 22% for Sisimiut and 16% for Nuuk; *M. genitalium* positivity was 13% for Sisimiut and 7% for Nuuk; and *C. trachomatis* positivity was 11% for Sisimiut and 8% for Nuuk.

The overall prevalence of having at least 1 STI was 19.0% in our study sample. Specifically: 9.8% (95% CI: 6.2–13.4%) for *M. genitalium*, 9.4% (95% CI: 6.0–13.0%) for *C. trachomatis*, 1.7% (95% CI: 0.05–3.3%) for *N. gonorrhoeae*, and 0.7% (95% CI: 0–1.8%) for *T. vaginalis*. The co-infection rate was low with 2% *M. genitalium – C. trachomatis* co-infection and less than 1% *C. trachomatis – N. gonorrhoeae* co-infection.

Overall, as age increased, the odds of STI positivity decreased, after adjusting for town (Nuuk or Sisimiut), gender, age at sexual debut, number of sexual partners in the past 3 months and history of forced sex (Table I). Age was the only statistically significant predictor of STI positivity. STI risk decreased by 10% per year, which translated to a 50% reduction in risk over 5 years. There was a slight suggestion that as the number of partners in the past

Table I. Characteristics of total study sample, STI cases and non-cases, Greenland, 2008–2009

Characteristic	Total	STI cases	STI non-cases	Odds ratio[a]	95% CI	Adjusted odds ratio[b]	95% CI
N	297	57	240				
Gender							
Males	37%	17%	83%	ref		ref	
Females	63%	20%	80%	1.2	0.7–2.2	1.2	0.6–2.5
Age (years)[c]							
15–19	31%	33%	67%				
20–24	24%	23%	77%				
25+	45%	8%	92%				
Mean (median, standard deviation) in years		21 (19, 7)	30 (25, 13)	0.9	0.9–1.0	0.9	0.9–0.9
Mean (median, standard deviation) age at sexual debut (years)		15 (15, 1)	15 (15, 2)	0.8	0.7–1.0	1.0	0.7–1.2
Mean (median, standard deviation) number of sexual partners past 3 months		2 (2, 2)	2 (1, 2)	1.2	1.0–1.3	1.0	0.9–1.2
History of forced sex							
No	73%	78%	81%	ref		ref	
Yes	27%	22%	19%	1.2	0.6–2.3	1.8	0.9–3.9

[a]Adjusted for town (Nuuk or Sisimiut).
[b]Adjusted for town, gender, age, age at sexual debut, number of sexual partners and history of forced sex.
[c]Missing responses: age at first sex (n = 19); partners past 3 months (n = 20); history forced sex (n = 3).

3 months increased, the odds of STI positivity increased, but this association was not statistically significant.

Stratified analysis for *M. genitalium* and *C. trachomatis* suggested that, in our study, slightly different groups were carrying the burden of these 2 infections. Based on the full model for *M. genitalium*, being female and being young were the 2 strongest predictors of *M. genitalium* positivity (Table II). Both gender (OR: 2.85, 95% CI: 1.02–7.93) and age (OR: 0.93, 95%CI: 0.88–0.98) were retained in the final, parsimonious model for *M. genitalium*. The full model for *C. trachomatis* positivity indicated that young age was the only significant predictor (Table III). After backward elimination, age was retained in the final, parsimonious model for *C. trachomatis* (OR: 0.86, 95% CI: 0.79–0.95).

Twenty-six of the 29 *M. genitalium*-positive cases provided samples with sufficient *M. genitalium* DNA to enable analysis for macrolide resistance. All 26 cases carried macrolide resistance determinants. Nine of the *M. genitalium* cases carried the A2059G mutation (*Escherichia coli* numbering); 17 carried the A2058G mutation, both of which are the most commonly detected mutations in other geographical areas. Looking back at the case history for the *M. genitalium*-positive patients from Nuuk, it was found that 70% of the *M. genitalium* cases had been treated with azithromycin within 3 years prior to the study (Table IV). Unfortunately, similar data for *C. trachomatis* or other STI cases were not collected, and thus no comparative figures can be given.

Sensitivity analysis

Of the 18 women testing positive for *M. genitalium*, 7 tested positive for *M. genitalium* by swab only, 5 tested positive by urine only and 6 tested positive by both swab and urine. Age and sex both dropped out of the final model when we did the sensitivity analysis using *M. genitalium* cases identified by urine test results only.

Discussion

We found a high prevalence of *M. genitalium* infection and macrolide resistance in our study sample. The prevalence of *M. genitalium* was comparable to the prevalence of *C. trachomatis*; however, there was very little *M. genitalium* - *C. trachomatis* co-infection, or *M. genitalium* co-infection with *N. gonorrhoeae* or *T. vaginalis*.

The prevalence of *M. genitalium* in our study sample was higher than the prevalence reported for the general population in Denmark (24), Japan (25), Russia (22), Vietnam (21) and Peru (13) and was higher than the prevalence reported for most studies involving STI clinic attendees (26) and the general adolescent population (6) in the United States. However, it was still lower than the prevalence reported for STI clinic attendees in Russia (27) and sex workers in Kenya (28), Ghana and Benin (29). The high proportion of *M. genitalium*-positive cases in our study compared to most previous studies may reflect widespread macrolide resistance among *M. genitalium* strains circulating in Greenland. Since 2009, most cases with *C. trachomatis*-negative

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Table II. Characteristics of *M. genitalium* (Mg) cases and non-cases, Greenland, 2008–2009

Characteristic	Total	Mg cases	Non-cases	Odds ratio[a]	95% CI	Full model odds ratio[b]	95% CI
N	297	29	268				
Gender							
Males	37%	4%	96%	ref		ref	
Females	63%	13%	87%	3.0	1.1–8.2	3.2	1.1–9.8
Age (years)[c]							
15–19	31%	12%	88%				
20–24	24%	15%	84%				
25 +	45%	5%	95%				
Mean (median, standard deviation) in years		22 (21, 6)	29 (24, 13)	0.9	0.9–1.0	0.9	0.9–1.0
Mean (median, standard deviation) age at sexual debut (years)		15 (15, 1)	15 (15, 2)	0.9	0.7–1.1	1.0	0.7–1.4
Mean (median, standard deviation) number of sexual partners past 3 months		2 (2, 2)	2 (1, 2)	1.1	1.0–1.4	1.2	0.9–1.4
History of forced sex							
No	73%	87%	91%	Ref			
Yes	27%	13%	9%	1.5	0.7–3.4	2.0	0.8–5.1

[a]Adjusted for town (Nuuk or Sisimiut).
[b]Full model included town, gender, age, age at sexual debut, number of sexual partners and history of forced sex.
[c]Missing responses: age at first sex (n = 19); partners past 3 months (n = 20); history forced sex (n = 3).

cervicitis or urethritis have been treated with tetracycline 500 mg b.i.d. for 10 days. Recommended syndromic treatment of urethritis and cervicitis is tetracycline 500 mg 4 times a day for 7 days (alternatively doxycycline 100 mg × 2 for 7 days). However, a significant number of *M. genitalium* infections may remain under-treated and become chronic.

We used an iterative convenience sampling strategy to recruit volunteer participants for our study. Convenience sampling limits our ability to make estimates of the

Table III. Characteristics of *C. trachomatis* (Ct) cases and non-cases, Greenland, 2008–2009

Characteristic	Total	Ct Cases	Non-cases	Odds ratio[a]	95% CI	Adjusted odds ratio[b]	95% CI
N	297	28	269				
Gender							
Males	37%	12%	88%	ref		ref	
Females	63%	8%	92%	0.6	0.3–1.4	0.6	0.2–1.4
Age (years)[c]							
15–19	31%	20%	80%				
20–24	24%	7%	93%				
25 +	45%	3%	96%				
Mean (median, standard deviation)		21 (18, 7)	30 (24, 13)	0.9	0.8–1.0	0.9	0.8–0.9
Mean (median, standard deviation) age at sexual debut (years)		15 (14, 1)	15 (15, 2)	0.8	0.6–1.1	0.9	0.6–1.3
Mean (median, standard deviation) number of sexual partners past 3 months		2 (2,2)	2 (1, 2)	1.1	0.9–1.3	0.9	0.7–1.2
History of forced sex							
No	73%	91%	90%	ref			
Yes	27%	9%	10%	0.9	0.4–2.2	1.6	0.5–4.7

[a]Adjusted for town (Nuuk or Sisimiut).
[b]Adjusted for town, gender, age, age at sexual debut, number of sexual partners and history of forced sex.
[c]Missing responses: Age at first sex (n = 19); partners past 3 months (n = 20); history forced sex (n = 3).

Table IV. Azithromycin prescription in 3 years prior to study for *M. genitalium* cases

Case	After study (post 30.09.08)	During study (03.08.08–30.09.08)	1 year prior (03.08.07–08)	2 years prior (03.08.06–07)	3 years prior (03.08.05–06)
1	X				
2	X		XX		
3	XX	X	X		
4	X	XX			
5	X		X		
6	XX				X
7	X			X	
8	X	X		X	
9	X				X
10		X	XX		
11	XX				

population prevalence; therefore, we have limited our discussion to prevalence within our study. Many of our participants were motivated to participate because they believed STIs were a problem in Greenland and wanted to be part of the solution. Many others participated because they wanted STI testing without having to go to the clinic. Consequently, our sample prevalence may be biased and is not generalizable to the larger Greenlandic population. However, our goal was to determine if *M. genitalium* was present in the population and, if so, to begin to understand the epidemiology of those with the infection, making the use of convenience sampling less of a methodological limitation.

Age was the single most predictive factor of STI positivity. This was likely because each STI had slightly different risk profiles, but age was common to them all. Consistent with previous work indicating that risk factors for *C. trachomatis*, *N. gonorrhoeae* and *M. genitalium* differ (30,31), we also found different demographic characteristics associated with *M. genitalium* and *C. trachomatis* positivity. *C. trachomatis* cases had greater odds of being very young (15–19 years) and *M. genitalium* cases had greater odds of being young (15–24 years) and female.

Contrary to our expectation, the number of sexual partners in the past 3 months was not associated with STI, *M. genitalium* or *C. trachomatis* positivity. This could be because "number of sex partners" is measuring something completely different in Greenland compared to Europe and North America. That is, in North America, having sex is generally perceived as a risky behaviour, except under the strictest conditions. In Greenland, sex is not regarded as a risky behaviour but rather a part of life and abstinence is perceived as unrealistic (14). An alternative explanation could be that the median number of life-time sexual partners in this study was much higher than that usually seen in general population samples in Europe and North America, and thus, this effect was already saturated.

The high prevalence of macrolide-resistant *M. genitalium* in Greenland could be the result of widespread use of azithromycin either as a 1-g single-dose for treatment of chlamydia or treatment for other infections (17,32). Although this hypothesis operates at the community level, in Nuuk, we found that 70% of the *M. genitalium* cases had been treated with azithromycin within 3 years prior to the study, and 55% within the past year (Table IV). We did not test whether *M. genitalium* cases had more azithromycin treatments than non-cases because those data were beyond the scope of this project, but the high occurrence of prior azithromycin use among cases suggests that this hypothesis should be explored in greater depth. After macrolide resistance was detected, all cases were successfully treated with moxifloxacin 400 mg once daily for 7 days.

The presence and high prevalence of *M. genitalium* resistance to standard treatment for *C. trachomatis*-negative cervicitis or urethritis has lead to important public health and policy discussions for Greenland's public health and medical communities, including what current pragmatic recommendations can be made and what should the public health message be to the society. Possible courses of action being discussed include: do nothing, make *M. genitalium* testing available, or change the treatment strategy for non-gonococcal urethritis and cervicitis.

The epidemiology of *M. genitalium* is not yet fully understood. The long-term consequences of *M. genitalium* remain unclear, but there is evidence that they may be the same as those of *C. trachomatis* infection and almost as common (9–13,26,27,29,33,34). At the same time, the presence of *M. genitalium* does not necessarily lead to disease. There is an asymptomatic carrier state for *M. genitalium* of unknown clinical relevance, just as there is for *C. trachomatis*. That is, for

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some people, the infection is carried asymptomatically with no disease, while for others, infection becomes associated with urethritis or cervicitis or even ascending to the upper genital tract. How long individuals may be asymptomatically colonized before a clinical condition develops is still unknown. Asymptomatic infection was beyond the scope of this investigation but will be an important addition to future investigations.

One of our motivations for studying *M. genitalium* is the frustration many providers experience trying to handle *C. trachomatis*-negative cervicitis or urethritis treatment failures. Not knowing the cause of an infection may lead to inappropriate treatment and case management with high costs to fertility and antibiotic resistance (26). There is an urgency to better understand the pathology and severity of sequelae due to symptomatic and asymptomatic *M. genitalium* infection given the potential consequences of this bacterial STI on fertility and HIV transmission.

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***Dionne C. Gesink**
Dalla Lana School of Public Health
University of Toronto
155 College Street, 6th Floor
Toronto, Ontario M5T 3M7
Canada
Email: dionne.gesink@utoronto.ca

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