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Is there a higher risk of exposure to *Coxiella burnetii* for pre-clinical veterinary students?

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ABSTRACT

Coxiella burnetii is globally distributed but evidence of zoonotic transmission in the Caribbean region is scarce. The bacterium presence is suspected on the Caribbean island of St. Kitts. The risk of exposure of veterinary students was reported in other regions of the world but is not documented in the Caribbean region. The present study aimed to evaluate the risk of exposure to *C. burnetii* for pre-clinical veterinary students (mostly coming from the U.S.) attending an island-based veterinary school.

A cross-sectional study was conducted to compare incoming and outgoing veterinary students' seroprevalence. Serology was performed using indirect immunofluorescence assay to test *Coxiella burnetii* Phase I and Phase II immunoglobulins M and G. Background data were gathered using a standardized questionnaire. A parallel study enrolled veterinary school employees in the same university.

Of the 98 participants (48 incoming and 50 outgoing students), 41 (41.8%, 95 %CI: 31.9–52.2) were seropositive to *C. burnetii*. There was no significant difference between the two groups (45.8% for incoming vs. 38.0% for outgoing students) ($p = 0.4$). No risk factors (demographic, animal handling practices or background) were significantly more reported in the seropositive group. In the employee study, the seroprevalence was high with 8/15 seropositives (53.3%, 95 %CI: 26.6–78.7).

Pre-clinical veterinary students do not have a higher risk of exposure to *C. burnetii* by attending the veterinary school in St. Kitts, but they are highly exposed before arrival on the island (seroprevalence of 45.8%). Most of these participants had experience with animals either through farming or previous veterinary technician employment. This indicates a high exposure in the U.S. young population aiming to become veterinarians. There is an urgent need to increase *C. burnetii* surveillance in animals and humans to apply relevant prevention and control measures, including recommendations for vaccination of students and professionals at risk.

1. Introduction

Coxiella burnetii is an obligate intracellular bacterium responsible for the zoonosis Q fever [1,2]. All mammalian species including humans and some bird species can be infected by the bacteria [3]. The infection of animals is usually asymptomatic or manifests as mild fever, with sporadic abortions in late pregnancies or moribund offspring [3,4]. In humans, most infections are also asymptomatic. Acute Q fever is characterized by an influenza-like illness. A more severe form with pneumonia or hepatitis may occur. Some individuals may also develop a chronic infection with risk of endocarditis [1,5]. During infection, the bacterium undergoes a phase of transition, leading to the presence of two serologically distinguishable phases. Phase I type is the bacterium's virulent form, and Phase II is avirulent [6]. The course of the disease is serologically divided into three stages: the onset with high Phase II immunoglobulins IgG and IgM, the acute phase with higher Phase II IgG, and a chronic phase characterized by high Phase I IgG [7].

Infections are usually sporadic, but major outbreaks in animals and

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humans have occurred in Australia [8] and The Netherlands [9]. Outside of these outbreaks, human Q fever is regarded as an occupational disease, with a higher risk of exposure in livestock farmers, abattoir workers, and veterinarians. Veterinary students are also at risk, with reported seroprevalence of 22% in Brazil [10], 35% in Iran [11], 20 to 30% in The Netherlands [12], up to 17% in Spain [13] and 17 to 58% in Slovakia [14].

Evidence of *C. burnetii* in the Caribbean is limited, with few reports from several islands, including Trinidad, Grenada, and Cuba [15–17]. In St. Kitts, several studies reported seropositive humans or animals [18–20]. Ross University School of Veterinary Medicine (RUSVM) delivers a pre-clinical Doctor of Veterinary Medicine (DVM) curriculum in St. Kitts (West Indies). The main objective of the study was to evaluate the risk of exposure to *C. burnetii* for students attending RUSVM.

2. Material and methods

2.1. Target population

At RUSVM, undergraduate students enter the school either in 1st semester or in a Veterinary Preparatory program. The curriculum is accelerated and one semester lasts four months (Spring, Summer and Fall semesters). After seven 'semesters' (~two and half years), the students complete their program with a three-semester clinical curriculum at an affiliated school, usually based in the U.S.

2.2. Enrolment of participants

Inclusion criteria for the participants was the registration at RUSVM in the Veterinary Preparatory program or first semester for incoming group and in seventh semester for outgoing group. Fifty individuals were randomly selected in both groups based on the registration list of the Registrar's Office. Exclusion criteria for incoming students were their presence in St. Kitts for more than a month (based on air plane arrival) or a previous visit/vacation in St. Kitts.

2.3. Enrolment changes due to COVID-19 pandemic

Enrolment started in January 2020. Unfortunately, the teaching program at RUSVM was disrupted from mid-March 2020 onwards due to the COVID-19 pandemic. Online teaching was initiated and most of the students left St. Kitts to continue the curriculum from home. Face-to-face activities on island gradually resumed from September 2020, starting with 7th semester, until September 2021 (Veterinary Preparatory). To decrease temporal bias, incoming student enrolment was adapted from September 2020. Inclusion criteria were extended to registration in second or third semester at RUSVM. Exclusion criteria (less than a month in St. Kitts and no previous visit of St. Kitts) remained the same. We stopped enrolment in January 2021 without reaching the targeted sample size for incoming students (48 versus the targeted 50).

2.4. RUSVM employees

In parallel with the student investigation, we provided the opportunity for RUSVM employees that were in contact with livestock on campus to get tested for antibodies. Contact with livestock was defined as work with the RUSVM farming animals (cattle and sheep) at < 5 m for > 30 min at least once a month or with opened carcasses of farming animals at RUSVM during the last 6 months. Due to COVID-19 pandemic, exclusion criterion of being abroad for more than a month in the last six months was added. Department heads provided the list of employees who fit the above criteria. Forty-one employees fit our definition and were invited to join.

2.5. Questionnaire

Students responding positively to the study invitation email were scheduled an appointment. After signing a consent form, a standardized questionnaire was verbally administered to the participants and directly entered with Qualtrix® (an online survey platform). Questions included the possible associated factors to *C. burnetii* seropositivity as follows: demographic factors, living area, animal ownership, past veterinary/animal work, consumption of raw milk, and exposure to ticks. The outgoing students were also asked about their extra-curricular activities with animals during their time in St. Kitts (Appendix 1).

2.6. Sampling

A blood sample of maximum 8 mL was drawn by venipuncture by a certified nurse at Health Services of RUSVM. All blood collection tubes were centrifuged within 30 min to 3 h after collection and the serum was stored at −20 °C.

2.7. Serological testing

Two commercially available indirect immunofluorescent assays (IFA) were used to screen all samples for human IgG and IgM antibodies to *C. burnetii* (Focus Diagnostics Q Fever IFA IgG and IgM assays, Cypress, CA). Both immunoglobulin-specific assays consisted of slide wells in which each well contained 2 individual spots with *C. burnetii* (Nine Mile strain) Phase I and Phase II antigens, respectively. The human positive and negative control samples (Focus Diagnostics Q Fever ref. IF0211 and IF0213) served as the reference markers in identifying positive and negative results. The presence of bright green fluorescence of coccobacillary morphology and lack of background fluorescence were used to identify positive samples, while the total absence of fluorescence identified negative samples. All serum samples were initially screened at the manufacturer's recommended serum dilution (1:16) with the provided IgG Sample Diluent (Fig. 1A and B). Any serum sample found to be positive at the screening dilution for both IgG Phase I and II was further titrated in reconstituted phosphate buffered saline (PBS) using the manufacturer's recommended serum dilutions of 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024 (Fig. 1C). Any serum sample found to be positive for only one Phase of IgG was taken to end titer by threefold serial dilutions. Likewise, serum samples were screened for IgM antibodies at the manufacturer's recommended serum dilution (1:16) with IgM pretreatment diluent and subsequently diluted three- or six-fold to determine end titers (Fig. 1D). All IFAs and slide interpretation were consistently performed by the same two persons. Both were blinded for the study group. A random number of slide images ($N = 6$) were additionally sent to the German National Consiliary Laboratory of *Coxiella burnetii* (Stuttgart, Germany) for confirmation.

2.8. Case definition

To define exposure, an individual with any immunoglobulin concentration equal or above the threshold of 1:64 was classified as seropositive. Low titers in IFA (< 1:64) are often unspecific to intracellular bacteria (such as *Mycoplasma* or *Legionella*). The decision was made to increase the threshold as per the manufacturer recommendation [21–23]. Cases of acute and chronic exposures were also defined and reported to participants. Acute exposure was suspected when Phase I and/or Phase II IgG were equal or superior to 1:256 and 1:512, respectively. A high phase I IgG equal or above 1:512 and superior to Phase II IgG would lead to suspicion of chronic infection. Finally, individuals with negative IgG and positive Phase II IgM (superior to 1:64) were suspected of developing an early infection [21,24,25].

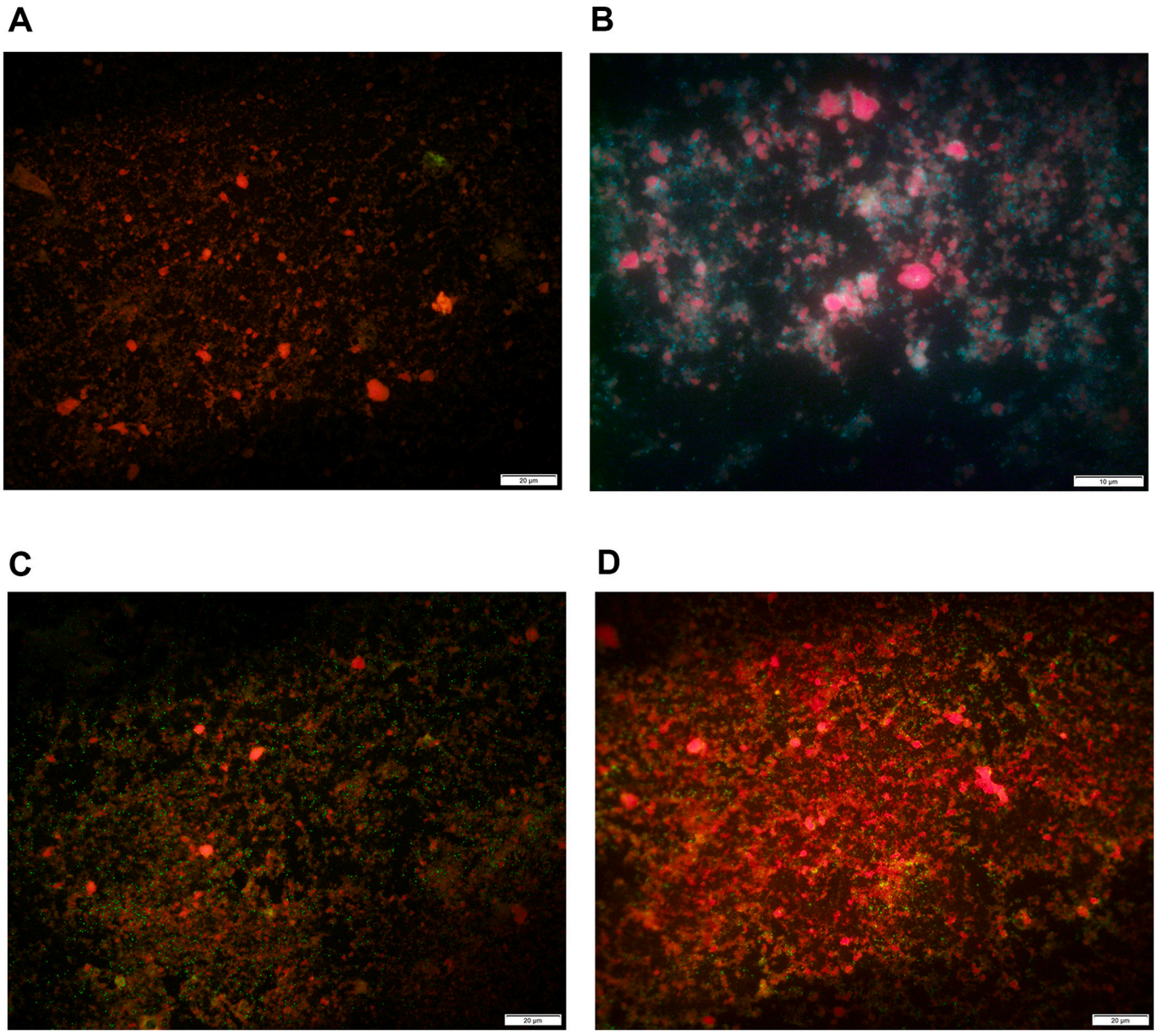

Fig. 1. Examples of results for the indirect immunofluorescent assay A) Negative; B) Positive for IgG screening at dilution 1:16; C) Positive for IgG dilution 1:64; D) Positive for IgM dilution 1:64.

2.9. Data analyses

Seroprevalence was expected to be 5% for incoming students and 20% for outgoing students, respectively. To detect a difference with a power of 80% and a significance of 5%, 50 individuals needed to be sampled in each group [26]. Data analysis was performed in R software. Confidence intervals of 95% (95% CI) were computed based on the binomial distribution. The main exposure variable (incoming/outgoing) was tested by univariate logistic regression. To test possible confounders, other risk factors (demographic, practices, background) were tested first using Fisher test and then with the incoming/outgoing variable forced in the logistic model. If $p < 0.1$, the variable was included in a multivariate model. Apart for the incoming/outgoing variable, variables to retain in the model were selected by a backward stepwise method ($p < 0.05$). For the sub-population of outgoing students, activities performed during their stay in St. Kitts were also tested using univariate logistic regression followed by multiple regression.

Among the tested factors, origin location was used in different ways. We collected information about all world locations where the participant lived for over a year. As most participants previously lived in the U. S., only U.S. states were used for risk factor analysis. We grouped the states by population of cattle, sheep and/or goats (based on 2019 census, USDA, https://quickstats.nass.usda.gov/) and by the number of human Q fever cases reported in 2019 (CDC data, https://www.cdc.gov/qfever/stats/index.html). Each created variable was binary using the median as the threshold between high and low risk. Absence of data in a state was considered as no livestock population. A participant who lived in two states differing in risk was attributed with the high risk value.

2.10. Ethics statement

The ethic approvals were granted by (i) Interim Ethic Review Committee (IERC, Ministry of health, community development, gender affairs and social services, St. Kitts; protocol #: IERC-2020-01-037), (ii) the institutional review board of RUSVM) (IRB protocol #19–05-XP) and (iii) the Human Subjects Ethics Sub-Committee of City University of

Hong Kong (protocol #: 41005–20 Conan, application no H002441A).

3. Results

Out of the 98 enrolled participants (incoming and outgoing), 41 (41.8%, 95% CI: 31.9–52.2) were considered as seropositive to *C. burnetii*. The seropositivity of outgoing students was not significantly different from the seropositivity of incoming students ($p = 0.4$) (Table 1).

Seroprevalence in employees was high with 8 seropositives out of 15 sampled individuals (53.3%, 95% CI: 26.6–78.7).

Distribution of dilution by phase is presented in Table 2. Dilution was not performed until negativity, so only thresholds at 1:128 (Phase I) and 1:64 (Phase II) are described. There were more individuals positive to Ig Phase II ($n = 38$) than to Ig Phase I ($n = 26$, McNemar's test $p = 0.002$). There were no other differences between particular immunoglobulins (Table 2). None of the tested factors were significant during univariate and multivariable analysis ($p > 0.05$) (Table 3 and Appendix 2). Looking at the outgoing participants only, none of the tested exposure factors linked with their RUSVM, their student club or extra-curricular live animal activities were different between seropositive and seronegative individuals (Appendix 3).

The three female and one male participants suspected of acute infection (2 incoming, 1 outgoing and 1 employee) had different backgrounds. While the employee lived exclusively in St. Kitts, the students lived in different states in the U.S. (Michigan, Minnesota, North Dakota, Ohio, Pennsylvania, Utah). Two had previous experience as veterinary technicians with ruminants and two had worked on farms. In St. Kitts, the incoming participants lived on campus and two others in two different island parishes. Species of animals owned by these four participants also varied among them (cattle, sheep, donkey, cat, pig, rabbit). All have owned a dog. None of the four have ever consumed raw milk and one reported to have been bitten by a tick.

The participants showing serological evidence of early infections were four incoming, two outgoing and one employee. Six out of seven had experience as veterinary technicians with pets and four indicated previous work on a cattle farm. None of the participants fit our definition of chronic infection.

4. Discussion

The seroprevalence in the population of students outgoing RUSVM was not significantly different from the seroprevalence of the incoming students. Therefore, studying pre-clinical veterinary sciences in St. Kitts doesn't increase the risk of being exposed to *C. burnetii*. These results differ from what was observed previously. In a study in the Netherlands, seroconversion was observed in 19% of veterinary students over 2 to 4 years of study [27]. Another study in the Netherlands showed that the risk of being seropositive to *C. burnetii* increased with the number of years spent at the university [12]. In Spain, students had a significantly higher seroprevalence at the end of the academic year compared to the beginning [13]. Several hypotheses could explain the difference with our results. First, RUSVM has a pre-clinical curriculum and therefore, students have less contact with live animals compared to during their subsequent clinical years. However, RUSVM students are exposed to live animals through different laboratory sessions that include manipulation of sheep and cattle, and through extra-curricular activities. Secondly, the prevalence of *C. burnetii* in livestock could be lower in St. Kitts compared to European countries. While the seroprevalence in the male sheep campus flock was found to be 26.3%, the prevalence of animal shedding is unknown [20]. Finally, the island environment differs with animals free-roaming in the community and general animal manipulations occurring outdoors, therefore preventing concentration of the bacteria in the environment. This, associated with the application of basic personal protective equipment may lower the transmission risk to students. Different studies, such as follow-up serology on RUSVM students during their clinical year, prevalence estimations in St. Kitts' livestock and assessing closed environments in veterinary schools could, test these hypotheses and inform veterinary schools worldwide on appropriate measures to protect students from exposure to *C. burnetii*.

Although the seroprevalence is not different between the two student groups, the incoming student one (43.5%) is higher than we expected. Two old studies (1960s–70s) detected seroprevalence of 5% in veterinary schools in the U.S. [10,28,]. Our results indicate that there is a high risk of exposure in this young population. We cannot generalize to the entire U.S. population, as veterinary students tend to have a history of more frequent and diverse contact with animals. Most study participants had indeed contact with animals through internships, veterinary technician work or farming prior to enrolment in the veterinary school. Still,

Table 1
Seroprevalence and 95% confidence interval of the incoming and outgoing students in St. Kitts (case definition: at least one immunoglobulin dilution equal or above 1:64). Seroprevalence by enrolment group is presented.

	Incoming		Outgoing
	1st/Vet prep no positive/N Seroprevalence (95% CI)	2nd - 3rd semester	7th semester
Spring 2020	10/23 43.5% (23.2–65.5)	Not tested	17/44 38.6% (24.4–54.4)
Summer 2020	Not on island	Not on island	Not tested
Fall 2020	Not on island	Not on island	2/6 33.3% (4.3–77.8)
Spring 2021	Not on island	12/25 48% (27.8–68.7)	Not tested
Positive (≥ 1:64)	22/48 45.8% (31.4–60.8)		19/50 38.0% (24.6–52.8)

95% CI: 95% Confidence interval (binomial distribution).
N: number of samples.

Table 2
Number and proportions of samples by phase and by positive dilution in the three groups (incoming, outgoing and employees).

		Students			Employees
		Incoming students	Outgoing students	*p*-value*	
No of samples		48	50		15
Mean age		25.3	26.9		38.3
Phase I	≥ 1:16	28	26	0.5	10
		58.3%	52.0%		66.7%
	≥ 1:32	22	20	0.6	7
		45.8%	40.0%		46.7%
	≥ 1:64	14	12	0.6	5
		29.2%	24.0%		33.3%
	≥ 1:128	6	3	0.3	2
		12.5%	6.0%		13.3%
	IgG (≥ 1:64)	12	7	0.2	2
		25.0%	14%		13.3%
	IgM (≥ 1:64)	3	7	0.2	3
		6.2%	14%		20.0%
Phase II	≥ 1:16	38	36	0.4	13
		79.2%	72.0%		86.7%
	≥ 1:32	32	30	0.5	9
		66.7%	60%		60.0%
	≥ 1:64	22	16	0.2	8
		45.8%	32.0%		53.3%
	IgG (≥ 1:64)	18	14	0.3	7
		37.5%	28.0%		46.7%
	IgM (≥ 1:64)	6	6	0.9	4
		12.5%	12.0%		26.7%

* Comparison of positivity was performed between incoming and outgoing students.

Table 3
Exposure factors for *Coxiella burnetii* seropositivity in the student population.

		Seronegative $N = 57$	Seropositive $N = 41$	OR [95% CI]	p-value*
Age (median/IQR)		Median: 26 IQR: 24–28	Median: 25 IQR: 24–27	0.92 [0.79–1.07]	0.3
Sex	Female (ref)	52 91.2%	37 90.2%	1.06 [0.26–4.26]	0.9
	Male	5 8.8%	4 9.8%		
Time spent in St. Kitts (months)		Median: 26 IQR: 0.75–30	Median: 1 IQR: 0.75–30	0.89 [0.75–1.06]	0.2
Lived at least 1 year in a location					
	Rural	14 24.6%	15 36.6%	1.68 [0.69–4.13]	0.3
	Urban	44 77.2%	26 63.4%	0.49 [0.20–1.21]	0.1
	Peri-urban	47 82.5%	29 70.7%	0.54 [0.18–1.58]	0.3
Worked previously as a veterinary technician		50 87.7%	33 80.5%	0.55 [0.18–1.69]	0.3
Worked on a farm without being the owner		24 42.1%	17 41.5%	0.92 [0.40–2.11]	0.8
Handled animals during reproductive procedures		24 42.1%	22 53.7%	1.59 [0.71–3.57]	0.3
Animal living on the same property (any time)					
	Cattle	10 17.5%	10 24.4%	1.46 [0.54–3.95]	0.5
	Goat	7 12.3%	3 7.3%	0.54 [0.13–2.24]	0.4
	Sheep	3 5.3%	2 4.9%	0.89 [0.14–5.62]	0.9
	Horse	10 17.5%	8 19.5%	1.11 [0.39–3.13]	0.8
	Donkey	3 5.3%	3 7.3%	1.34 [0.25–7.09]	0.7
	Dog	56 98.2%	36 87.8%	0.14 0.01–1.27	0.08
	Cat	44 77.2%	33 80.5%	1.36 [0.49–3.79]	0.6
	Pig	8 14.0%	4 9.8%	0.61 [0.17–2.23]	0.5
	Rabbit	24 42.1%	11 26.8%	0.52 [0.22–1.25]	0.1
	Other mammals	23 40.4%	17 41.5%	1.00 [0.44–2.29]	1
	Chicken	11 19.3%	6 14.6%	0.67 [0.22–2.01]	0.5
	Other birds	14 24.6%	7 17.1%	0.62 [0.22–1.72]	0.4
Consumption of raw milk		3 5.3%	3 7.3%	1.21 [0.22–6.70]	0.8
Known exposure to ticks		6 10.5%	10 24.4%	2.75 [0.91–8.34]	0.07

* Model was built by logistic regression with the group (incoming/outgoing) forced as covariable in the model.

the seroprevalence observed is higher than previously reported in the veterinary profession (22.2%) [29]. Therefore, our results should urge to improve U.S. national surveillance and increase awareness of animal-related workers. These measures would help with early detection, risk communication, risk management, and prevention of severe outbreaks.

None of the tested risk factors showed a significant difference between seronegative and seropositive participants. While the power of the exposure analysis may be too low for this study and there is a certain recall bias, some hypothesis can be posed. First, most of the participants worked previously as veterinary technicians (90%). The high seroprevalence may be particular to our target population and may not be applicable to the general population. Second, four factors were more frequent in seropositives: known exposure to ticks, residence location in rural areas, reproductive procedures (particularly in cattle) and working as a cattle veterinary technician. These factors are known to be risk factors of *C. burnetti* exposure. Risk factors on the American continent include living on a farm [29,30]. In the Netherlands, the veterinary student seropositivity was associated with the number of years lived on farms [12]. Interestingly, the factors "owning a dog" and "work on an equine farm" were more frequent in seronegatives. Finally, no difference in geographical origin of the participants could be observed. This would indicate that the infection is endemic in most of the U.S. territory. It is possible that the state differences in CDC-reported human case result from better surveillance in some states compared to others.

The employee population was not added to our exposure factor analyses because of the high disparity in their demographics, country origin, and professional background compared to a homogenous student population. We also observed high seroprevalence in this population but due to the low sample size no conclusions can be made about potential risk factors. However, this confirms the risk of exposure to *C. burnetii* in the veterinary and related professions [1]. A larger seroprevalence study could be conducted in veterinary school workers to identify potential risk activities. Awareness of animal workers should be raised and regular testing recommended. Moreover, the provision of vaccination should be considered for staff working in veterinary and agricultural schools where the employees are in regular contact with animals.

In total, four participants were suspected to be in the acute form of the disease. These participants were encouraged to visit the RUSVM Health Services or their personal doctors. One of the study limitations is the absence of questions related to current or recent symptoms. The detection of acute disease followed by appropriate treatment could reduce the risk of progression to chronic disease. Therefore, awareness campaigns for veterinary students, veterinary-related workers, and human practitioners to increase the testing of *C. burnetii* in case of flu-like symptoms is recommended.

Other limitations of our study include the lag between incoming participant arrival and sampling and the IFA interpretation. First, the incoming students tested in the month after arrival could have been exposed at arrival on St. Kitts. Indeed, IgM can be detected within two weeks after exposure [31,32]. However, this hypothesis is unlikely as the comparisons between group by phase and immunoglobulin were not significant. The IgG seroprevalence would be low if there was exposure during this first month [33]. Also, the incoming students have no direct animal contact during the first month of the curriculum. Moreover, the 2nd cohort of incoming participants arrived in St. Kitts while COVID-19 restrictions were still in place and were therefore quarantined in their accommodation for two weeks.

The second limitation is the IFA test, that can be prone to subjective interpretation. We implemented several safeguards to decrease this bias: the high cut-off of 1:64 (also avoiding cross-reactions with Rickettsia), consistent interpretation by a singular person, and a random confirmatory reading by the German National Consiliary Laboratory of *Coxiella burnetii* (no discrepancy was observed between both laboratories). Therefore, we believe the bias of IFA interpretation has been minimized.

In conclusion, our study does not indicate an increased risk of exposure to *C. burnetii* for students attending RUSVM. However, we report a high seroprevalence in the incoming student population, indicating a high risk of exposure in the U.S. young population aiming to become veterinarians. This highlights the need for urgent and necessary measures to increase surveillance of *C. burnetii* in the human and animal populations in the U.S. Measures of prevention and control, and awareness of the animal-worker population should be improved, and vaccination of animals and humans should be considered at the farm, veterinary school, and national levels.

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CRediT authorship contribution statement

Anne Conan: Conceptualization, Methodology, Supervision, Formal analysis, Funding acquisition, Writing – original draft. **Christa Gallagher:** Supervision, Funding acquisition, Writing – review & editing. **Nicole Erskine:** Investigation. **Marshalette Smith-Anthony:** Methodology, Funding acquisition, Investigation. **Silvia Marchi:** Investigation. **Ioannis Magouras:** Writing – review & editing. **Ananda Müller:** Writing – review & editing. **Anne A.M.J. Becker:** Methodology, Supervision, Formal analysis, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2023.100485.

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