Sustained effect of LACTIN-V (Lactobacillus crispatus CTV-05) on genital immunology following standard bacterial vaginosis treatment: Results from a randomised, placebo-controlled trial

Eric Armstrong
Anke Hemmerling
Steve Miller
Kerianne E. Burke
Sara J. Newmann

See next page for additional authors
Authors
Eric Armstrong, Anke Hemmerling, Steve Miller, Kerianne E. Burke, Sara J. Newmann, Sheldon R. Morris, Hilary Reno, Sanja Huibner, Maria Kulikova, Nico Nagelkerke, Bryan Coburn, Craig R. Cohen, and Rupert Kaul
Sustained effect of LACTIN-V (*Lactobacillus crispatus* CTV-05) on genital immunology following standard bacterial vaginosis treatment: results from a randomised, placebo-controlled trial

Eric Armstrong, Anke Hemmerling, Steve Miller, Kerianne E Burke, Sara J Newmann, Sheldon R Morris, Hilary Reno, Sanja Huibner, Maria Kulikova, Nico Nagelkerke, Bryan Coburn, Craig R Cohen, Rupert Kaul

Summary

**Background** Bacterial vaginosis might increase HIV risk by eliciting genital inflammation and epithelial barrier disruption, whereas vaginal *Lactobacillus crispatus* is associated with immune quiescence and HIV protection. We investigated the effect of a live biotherapeutic containing *L crispatus* CTV-05 (LACTIN-V) on genital immunology and key vaginal bacteria.

**Methods** This substudy included women aged 18–45 years who participated in the randomised, placebo-controlled, phase 2b trial of LACTIN-V to reduce bacterial vaginosis recurrence, conducted at four universities and hospitals in the USA. Women with negative results for sexually transmitted infection, pregnancy, and urinary tract infection were provided a 5-day course of vaginal metronidazole 0·75% gel. Those who met at least three of four clinical Amsel criteria for bacterial vaginosis and had a Nugent score of 4–10 from Gram staining were eligible. Participants in the LACTIN-V trial were randomly assigned (2:1) to receive either LACTIN-V or placebo, applied vaginally once per day for 5 days during the first week and then twice per week for 10 more weeks. Follow-up visits occurred 4, 8, 12, and 24 weeks after enrolment. Soluble immune factors and the absolute abundance of bacterial taxa were assayed by multiplex ELISA and quantitative PCR. The primary outcomes were vaginal levels of IL-1α and soluble E-cadherin at 24 weeks (ie, 13 weeks after treatment cessation).

**Findings** Between Feb 21, 2020 and March 18, 2021, we characterised genital immune parameters and the vaginal microbiota in a subset of 66 highly adherent participants who were randomly selected, with no exclusion criteria, from those who had attended all study follow-up visits (n=166) in the larger LACTIN-V clinical trial (n=228). 32 (48%) participants received LACTIN-V and 34 (52%) received placebo. LACTIN-V treatment was significantly associated with lower concentrations of the proinflammatory cytokine IL-1α (β coefficient 0·310, SE 0·149; p=0·042) and soluble E-cadherin (0·429, 0·199; p=0·035), a biomarker of epithelial barrier disruption.

**Interpretation** Vaginal administration of LACTIN-V following standard bacterial vaginosis therapy resulted in a sustained reduction in genital inflammation and a biomarker of epithelial integrity. The potential of LACTIN-V to reduce HIV susceptibility merits further investigation.

**Funding** Canadian Institutes of Health Research and the National Institutes of Health National Institute of Allergy and Infectious Diseases.

**Introduction** Bacterial vaginosis has been linked to adverse reproductive health outcomes among women and to increased risk of HIV acquisition.1,2 Bacterial vaginosis is characterised by diverse anaerobic bacteria3,4 and might elevate HIV risk by eliciting genital inflammation, which not only recruits HIV-susceptible CD4+ T cells to mucosal tissues but also causes epithelial barrier disruption with the cleavage of E-cadherin, a key component of epithelial cell-to-cell junctions,5 thereby increasing viral access to these target cells.6,7 Although standard antibiotic therapy for bacterial vaginosis rapidly reduces vaginal prototypic inflammatory cytokine IL-1α,8 treatment has been shown to increase vaginal concentrations of some chemoattractant immune factors linked to HIV risk, such as IFN-γ-induced protein (IP)-10,9,10 and so the immunonulogical benefits of treatment remain uncertain.

In the absence of bacterial vaginosis, one of several species of *Lactobacillus* is typically predominant in the vaginal microbiome and has been linked to protection against HIV acquisition.11 For example, *Lactobacillus crispatus* predominance in the female genital tract has been linked to protection against HIV acquisition,12,13 most likely due to direct anti-inflammatory effects14,15 or the competitive exclusion of proinflammatory bacterial vaginosis-associated bacteria, or both.16 The standard of care for clinical bacterial vaginosis treatment involves...
oral or topical antibiotics targeting bacterial vaginosis-associated anaerobes, but recurrence rates are high and treatment does not always result in predominance of the protective *Lactobacillus* species. Although studies have investigated *Lactobacillus*-based probiotics or live biotherapeutics as alternative treatment strategies for bacterial vaginosis, none have linked probiotic use to reduced genital inflammation, which might be because of the use of probiotics containing *Lactobacillus* species that are not commonly predominant in the female genital tract. A phase 2 trial investigating the vaginal administration of a live biotherapeutic containing the *Lactobacillus crispatus* strain CTV-05 (LACTIN-V) following standard antibiotic treatment found that participants receiving LACTIN-V had lower rates of bacterial vaginosis recurrence than those receiving placebo, and these benefits were sustained for at least 3 months after treatment cessation.

We investigated whether LACTIN-V administration has a sustained effect on genital immunology, and the associations between immune alterations and vaginal microbiota.

**Methods**

**Study design and participants**

This substudy included women aged 18–45 years who participated in the randomised, placebo-controlled, phase 2b trial of LACTIN-V to reduce bacterial vaginosis recurrence, conducted at four universities and hospitals in the USA. The trial evaluated the ability of LACTIN-V to prevent bacterial vaginosis recurrence following standard antibiotic treatment. At entry screening, the participants’ medical history was obtained, and physical and pelvic examinations were performed. Women with negative results for sexually transmitted infection (STI), pregnancy, and urinary tract infection (appendix p 1) were provided a 5-day course of vaginal metronidazole 0·75% gel. Within 48 h of completing treatment, participants returned to the clinic and those who met at least three of four clinical Amsel criteria for bacterial vaginosis and had a Nugent score of 4–10 from Gram staining (done at screening before antibiotic treatment) were eligible for the trial. The LACTIN-V trial (NCT02766023) obtained written consent from all participants before enrolment. Study protocols for the larger trial were approved by the institutional review boards (IRBs) at University of California, San Francisco General Hospital (San Francisco, CA, USA; IRB 15-18143), Stroger Hospital of Cook County (Chicago, IL, USA), University of California San Diego Antiviral Research Center (San Diego, CA, USA; IRB: 160023X), and Washington University Infectious Disease Clinical Research Unit (St Louis, MO, USA). Immunology and microbiology performed at the University of Toronto (Toronto, ON, Canada) were reviewed and approved by the University of Toronto HIV Research Ethics Board (protocol #36947).
Articles

Procedures
Participants in the LACTIN-V trial were randomly assigned (2:1) to receive either LACTIN-V or placebo, which were applied vaginally once per day for 5 days during the first week and then twice per week for 10 more weeks. Participants returned for follow-up visits at 4, 8, 12, and 24 weeks after enrolment. Vaginal swabs were collected at all visits (appendix p 1).

For the soluble immune factor measurement, cervicovaginal fluid obtained from vaginal swabs was thawed and centrifuged at 4500 rpm for 30 min. Supernatant was removed for immune factor analysis and the bacterial pellet was left intact for quantification by qPCR (qPCR) analyses. The soluble immune factors IL-1α, IFN-α2A, IL-17A, IL-6, IFN-γ, IP-10, IL-8, macrophage-inflammatory protein (MIP)-1β, MIP-3α, monokine induced by IFN-γ (MIG), soluble E-cadherin, and matrix metalloproteinase (MMP)-9 were measured in duplicate with multiplex ELISA on the Meso Scale Discovery platform (Meso Scale Diagnostics, Rockville, MD, USA).

At the University of Toronto, DNA was extracted from 175 μL of bacterial pellet using the Qiagen DNA Easy PowerSoil Kit (Qiagen, Valencia, CA, USA). Targeted qPCR was used to estimate the absolute abundance of individual bacterial species. At the University of California, San Francisco, DNA was extracted from 200 μL of vaginal sample using the EZ1 DNA Tissue Kit (Qiagen, Valencia, CA, USA) and eluted into 50 μL volume. The absolute abundance of L. crispatus and L. crispatus CTV-05 were estimated with qPCR. Details on microbiome analyses are provided in the appendix (p 1).

Outcomes
The primary outcomes were the vaginal IL-1α and soluble E-cadherin concentrations at 24 weeks (ie, 13 weeks after treatment cessation). Exploratory outcomes included vaginal concentrations of soluble immune factors IP-10, IL-6, IL-8, MIP-1β, MIP-3α, MIG, and MMP-9, and the absolute abundance of individual bacterial taxa including L. crispatus, L. crispatus strain CTV-05, Lactobacillus iners, Lactobacillus jensenii, Lactobacillus gasseri, Gardnerella vaginalis, Atopobium vaginae, Megasphaera species, and Prevotella species.

Statistical analysis
After visual inspection of distribution, soluble immune factor concentrations and bacteria copy numbers were normalised using log₁₀ transformation. Linear regression was used to evaluate the association between treatment group as an independent variable and both the concentrations of soluble immune factors and abundance of key vaginal bacteria taxa at 24 weeks as dependent variables. Soluble immune factors that were less than the lower limit of detection in more than 50% of participants were dichotomised as detectable and undetectable. Binary logistic regression was used to evaluate the association between treatment group and the detectability of soluble immune factors at 24 weeks. For all regression models that predicted concentrations and the detectability of soluble immune factors or abundance of vaginal bacteria taxa, baseline measurements of the dependent variable were included in the model to control for interindividual variability. To evaluate potential mediating effects of bacterial taxa on the relationship between LACTIN-V and soluble immune factors, we followed the framework by Baron and Kenny outlined in the appendix (p 1). Mann-Whitney U tests were performed to compare the change in soluble immune factors from baseline to 24 weeks between women with vaginal predominance of non-CTV-05 L. crispatus and those with predominance of L. crispatus CTV-05. All statistical tests were performed with SPSS (version 27.0.0.0) or GraphPad Prism (version 9.0.2).

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results
Between Feb 21, 2020 and March 18, 2021, we characterised genital immune parameters and the vaginal microbiota in a subset of 66 highly adherent participants who were randomly selected, with no exclusion criteria, from those who had attended all study follow-up visits (n=166) in the larger LACTIN-V clinical trial (n=228); 32 (48%) participants received LACTIN-V and 34 (52%) received placebo.

Baseline samples were missing from three participants who received placebo; immune and microbiota analyses (except for L. crispatus CTV-05 quantitation, which had been performed earlier) were not performed on these samples. Baseline participant characteristics and sexual behaviours, including recent sex and hormonal contraceptive use did not substantially differ between groups (table 1). All participants were women, with a median age of 33 years (IQR 27–38). As per eligibility criteria, none of the women tested positive for HIV, syphilis, Neisseria gonorrhoea, Chlamydia trachomatis, or Trichomonas vaginalis at baseline. All participants reported the application of at least 75% of LACTIN-V doses, which was confirmed with trypan blue staining of applicators.

LACTIN-V treatment was significantly associated with lower concentrations of the proinflammatory cytokine IL-1α (β coefficient 0.310, SE 0.149; p=0.042) and soluble E-cadherin (0.429, 0.199; p=0.035), a biomarker of epithelial barrier disruption (figure 1). Among the six additional immune factors that were included as exploratory endpoints, LACTIN-V was associated with elevated concentrations of IP-10 at 24 weeks (−0.380, 0.185; p=0.044; figure 1). IFN-α2A and IL-17A were undetectable in more than 50% of samples, so were dichotomised as detectable or undetectable. Detectability
of IFN-α2a or IL-17A was not significantly associated with the treatment group (appendix p 6).

We hypothesised that an altered abundance of specific vaginal bacteria were causing the sustained differences between treatment groups in genital immune parameters. LACTIN-V treatment was associated with a sustained increase in the abundance of \( L. crispatus \) (\( \beta = -2.085, \text{SE} = 0.649, p = 0.0021 \)) and \( L. gasseri \) 13 weeks after treatment cessation (\( -0.803, 0.346, p = 0.047 \); table 2). LACTIN-V treatment was also associated with a sustained reduction in the abundance of the bacterial vaginosis-associated genus \( Prevotella \) spp (1.033, 0.509; \( p = 0.047 \)) and borderline significance of reduced \( Megasphaera \) spp abundance (1.452, 0.731, \( p = 0.052 \)). \( L. crispatus \) was more likely to be detected 13 weeks after product cessation among participants who received LACTIN-V (68.8% vs placebo 31.2%; \( p = 0.0002 \)) and the \( L. crispatus \) strain CTV-05 was also present in those who received LACTIN-V (53.1% vs 2.9%; \( p = 0.0001 \); appendix p 2). Among participants with previous LACTIN-V treatment and a high abundance of \( L. crispatus \) (>1×10⁶ copies per mL) at 24 weeks, the \( L. crispatus \) strains present were predominantly non-CTV-05 in seven (41%) of 17 participants and predominantly CTV-05 in ten (59%; appendix p 4).

LACTIN-V administration was associated with the concentrations of soluble immune factors and abundances of multiple bacterial taxa. To explore potential causal pathways underpinning these relationships, we assessed the mediating effects of key bacterial taxa on the relationship between LACTIN-V treatment and concentrations of IL-1α, soluble E-cadherin, and IP-10 at 24 weeks. Change in IL-1α was positively associated with change in the abundance of \( Prevotella \) spp (\( 0.157, 0.029; p < 0.0001 \)) and \( Megasphaera \) spp (\( 0.085, 0.026; p = 0.0016 \); table 3). Change in soluble E-cadherin was negatively associated with change in \( L. crispatus \) (\( -0.128, 0.036; p = 0.0007 \)) and positively associated with change in \( Prevotella \) spp (\( 0.338, 0.038; p = 0.0001 \)) and \( Megasphaera \) spp (\( 0.157, 0.029; p = 0.0001 \)). Change in IP-10 was positively associated with change in \( L. crispatus \) (\( 0.108, 0.035; p = 0.0029 \)) and negatively associated with change in \( Prevotella \) spp (\( -0.161, 0.050; p = 0.0022 \)) and \( Megasphaera \) spp (\( -0.135, 0.029; p = 0.0001 \)).

Next, we assessed whether genital immune changes associated with LACTIN-V were mediated through treatment-elicited alterations in vaginal bacteria. When change in IL-1α was the dependent variable, there was a significant association with change in \( Prevotella \) spp (\( 0.167, 0.039; p = 0.0001 \); table 3) and \( Megasphaera \) spp (\( -0.075, 0.026; p = 0.0058 \)), but not with LACTIN-V treatment. Using change in soluble E-cadherin as the dependent variable, there were associations with change in \( L. crispatus \) (\( -0.114, 0.038; p = 0.0037 \)), \( Prevotella \) spp (\( -0.327, 0.038; p = 0.0001 \)), and \( Megasphaera \) spp.
Megasphaera predominance and seven (33%) showed non-CTV-05 L crispatus participants with high vaginal abundance of the CTV-05 strain of Prevotella change in (0·096, 0·037; p=0·011) and negatively associated with HIV acquisition in women) after successful bacterial chemoattractant chemokine linked to increased risk of treatment was associated with similarly sustained reductions in genital mucosal inflammation and a marker of epithelial barrier disruption. Echoing reductions in genital mucosal inflammation and a biomarker of epithelial barrier disruption. An important distinction between the three studies that have evaluated genital immunology showed little or no effect of Lactobacillus probiotics or live biotherapeutics, either alone or in combination with standard antibiotics. However, not all probiotics have been shown to reduce rates of bacterial vaginosis recurrence, and the present study is the first to show that application of an L crispatus-based live biotherapeutic after standard topical metronidazole therapy reduces concentrations of a proinflammatory genital cytokine and a marker of epithelial disruption. An important distinction between the present study and previous studies was the use of a live biotherapeutic comprised entirely of L crispatus, a species of Lactobacillus known to naturally predominate in the vagina, which has been associated with genital immune benefits and HIV protection among women.

In a longitudinal cohort study, vaginal predominance by L crispatus, but not by L iners, was associated with reduced risk of HIV acquisition compared with a bacterial vaginosis-type microbiota. Whether the HIV-protective effects of L crispatus are due to direct anti-inflammatory effects were primarily mediated by a reduced abundance of bacterial vaginosis-associated taxa, particularly Prevotella spp and Megasphaera spp, and to a lesser extent by an elevated abundance of L crispatus. Increases in vaginal IP-10 were specifically linked to abundance of non-CTV-05 L crispatus strains, rather than to the therapeutic CTV-05 strain.

Rapid bacterial vaginosis recurrence following standard antibiotic treatment has generated substantial interest in alternative treatment strategies and antibiotic adjuncts. Among these strategies is the use of non-ctv-05 Lactobacillus probiotics or live biotherapeutics, either alone or in combination with standard antibiotics. However, not all probiotics have been shown to reduce rates of bacterial vaginosis recurrence, and the present study is the first to show that application of an L crispatus-based live biotherapeutic after standard topical metronidazole therapy reduces concentrations of a proinflammatory genital cytokine and a marker of epithelial disruption. An important distinction between the present study and previous studies was the use of a live biotherapeutic comprised entirely of L crispatus, a species of Lactobacillus known to naturally predominate in the vagina, which has been associated with genital immune benefits and HIV protection among women.

In a longitudinal cohort study, vaginal predominance by L crispatus, but not by L iners, was associated with reduced risk of HIV acquisition compared with a bacterial vaginosis-type microbiota. Whether the HIV-protective effects of L crispatus are due to direct anti-inflammatory effects were primarily mediated by a reduced abundance of bacterial vaginosis-associated taxa, particularly Prevotella spp and Megasphaera spp, and to a lesser extent by an increased abundance of L crispatus. Increases in vaginal IP-10 were specifically linked to abundance of non-CTV-05 L crispatus strains, rather than to the therapeutic CTV-05 strain.

Finally, we investigated whether there was a differential immune effect of post-treatment vaginal colonisation with the CTV-05 strain of L crispatus. Of 21 selected participants with high vaginal abundance of L crispatus at 24 weeks, 14 (67%) showed CTV-05 strain predominance and seven (33%) showed non-CTV-05 strain predominance. Although predominance by CTV-05 or non-CTV-05 strains of L crispatus was non-significantly associated with similar reductions in IL-1α and soluble E-cadherin (p=0·74 for both), increases in vaginal IP-10 concentrations were significantly greater among participants with predominance of non-CTV-05 strains compared with predominance of the CTV-05 strain after LACTIN-V treatment (p=0·010; figure 2).
effects, the exclusion of bacterial vaginosis-associated bacteria, or other mechanisms is unclear. Vaginal predominance by *L crispatus* is associated with reduced vaginal concentrations of proinflammatory cytokines, including IL-1α, and our group has linked an increased *L crispatus* proportional abundance to reduced concentrations of genital proinflammatory cytokines and chemokines. Women with *L crispatus* predominance also exhibit fewer transitions to a bacterial vaginosis-type microbiota than women with *L iners* predominance, suggesting that *L crispatus* can competitively exclude inflammatory bacterial vaginosis-associated bacteria, mediated partly by the production of lactic acid and antimicrobial metabolites. In vitro models show that *L crispatus* is a key producer of lactic acid in the female genital tract, which might directly dampen the production of proinflammatory cytokines. Furthermore, addition of supernatant with *L crispatus* or *L crispatus* alone, or both, to epithelial cells decreased the production of proinflammatory cytokines relative to bacterial vaginosis-associated bacteria, and protected against epithelial barrier disruption in response to proinflammatory stimuli. Reduced genital inflammation following LACTIN-V could be mediated by the exclusion of bacterial vaginosis-associated bacteria, although our study showed that *L crispatus* might have direct but minor effects on concentrations of soluble E-cadherin and IP-10.

Observational studies have linked bacterial vaginosis with reduced vaginal concentrations of IP-10, suggesting that either the absence of bacterial vaginosis-associated bacteria or presence of *Lactobacillus* spp might elicit IP-10. Joag and colleagues showed that successful antibiotic bacterial vaginosis treatment resulting in an *L iners* predominant vaginal microbiome increased vaginal concentrations of several chemokines at 1 month, including IP-10 and MIG. In this study, we provide evidence that the reduced concentrations of soluble E-cadherin and elevated concentrations of IP-10 observed among participants receiving LACTIN-V were mediated partly by an elevated abundance of non-CTV-05 *L crispatus* strains. This finding suggests that *L crispatus* CTV-05 (LACTIN-V) might reduce genital inflammation and enhance epithelial barrier integrity in the same way as non-CTV-05 *L crispatus*, without eliciting an elevation in proinflammatory chemokines. Importantly, CTV-05 was detected in one participant who received placebo, probably because the CTV-05 strain found in LACTIN-V is derived from a naturally occurring vaginal strain of *L crispatus*.

There are several limitations of our study. First, STIs were routinely assessed at screening, but afterwards were only tested if women presented with symptoms. Therefore, we were unable to control for the presence of asymptomatic STIs during the study, which might affect genital immunology at follow-up visits. However, we would expect a low incidence of STIs because all participants were negative for STIs at trial screening, diagnostics were performed in the context of any genital symptoms, and the overall 6-month incidence of STIs is low in sexually active women in the USA. Second, the stage of the menstrual cycle was not recorded at each visit, except for menstruation. Although no study visits occurred during menstruation, this limitation reduced our ability to control for fluctuations in sex hormones, a

---

**Figure 2: Change in soluble immune factors from baseline to 24 weeks**  
Horizontal line indicates median. For participants with high *Lactobacillus crispatus* abundance (>1x10⁶ copies per mL), the change in vaginal IL-1α (A), soluble E-cadherin (B), and IP-10 (C) was compared between groups with sustained predominance by the *L crispatus* non-CTV-05 strain versus the LACTIN-V L crispatus CTV-05 strain.
potential determinant of genital immunology. Third, targeted qPCR rather than 16S rRNA gene sequencing or metagenomic sequencing was used to show the association of cervicovaginal IP-10 concentrations with non-CTV-05 *L. crispatus* strains, limiting our ability to evaluate the vaginal microbiota and identify contributions by other microbes. Fourth, the study population consisted only of US American women, albeit a heterogeneous cohort in terms of ethnicity. Finally, the immunology analyses involved a subset of participants who had attended all trial visits in a larger clinical trial, which had strict eligibility criteria.\(^{26}\) Therefore, although we believe that these results reflect a causal relationship between LACTIN-V and genital immunology, they should be viewed as hypothesis generating. To ensure generalisability and definitively show causation, future studies are needed.

Strong links between the vaginal microbiota and HIV risk suggest that promoting vaginal predominance by *L. crispatus* might reduce HIV risk. Treatment with LACTIN-V following standard antibiotics has been shown to reduce bacterial vaginosis recurrence compared with placebo, even at 3 months after the last dose.\(^{29}\) In this study, we show that LACTIN-V treatment was also associated with sustained reductions in biomarkers of genital inflammation and epithelial barrier damage, through the promotion of increased *L. crispatus* abundance and decreased abundance of *Prevotella* species and *Megasphaera* species. Additionally, LACTIN-V was associated with elevated concentrations of the pro-inflammatory chemokine IP-10, although this finding appeared to be driven by non-CTV-05 *L. crispatus* rather than *L. crispatus* CTV-05. Future work is needed to determine whether LACTIN-V administration following standard antibiotics can reduce HIV acquisition among women at high risk.

**Contributors**

RK, EA, CRC, and AH contributed to the study design. AH, SM, SRM, KEB, SJN, HR, and CRC contributed to the study execution. EA and SH completed the immune assays. EA, BC, and NN contributed to the data analysis. EA, RK, CRC, AH, and BC were involved in the study interpretation. All authors reviewed the manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication. EA and RK have accessed and verified all the data in the study.

**Declaration of interests**

CRC is chair of the scientific advisory board for Osel. BC reports grants from Western Foundation, Canadian Institutes of Health Research, Canadian Cancer Society, McLaughlin Foundation, National Cancer Institute, and Cystic Fibrosis Foundation; and material receipts for research from Nabiyyota. SM reports grants from Canadian Institutes of Health Research (#PJT-156123). All other authors declare no competing interests.

**Data sharing**

Data requests should be sent to the corresponding author. The full clinical trial protocol is available at https://www.clinicaltrials.gov/ct2/show/NCT02766023.

**Acknowledgments**

This study is funded by the Canadian Institutes of Health Research and the NIH National Institute of Allergy and Infectious Diseases. We thank all study participants for involvement in this study. We thank Marie-Christine Perry and Rachel Liu for technical assistance with qPCR assays. We also thank Emily Crawford and Gloria Castañeda for organising sample shipping and logistics.

**References**


