Published in final edited form as:
J Med Primatol. 2011 August ; 40(4): 214–223. doi:10.1111/j.1600-0684.2011.00488.x.

Development of a pigtail macaque model of sexually transmitted infection/HIV coinfection using *Chlamydia trachomatis*, *Trichomonas vaginalis*, and SHIV$_{SF162P3}$

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Abstract

Background—Sexually transmitted infections (STIs) are associated with an increased risk of HIV infection. To model the interaction between STIs and HIV infection, we evaluated the capacity of the pigtail macaque model to sustain triple infection with *Trichomonas vaginalis*, *Chlamydia trachomatis*, and SHIV$_{SF162P3}$.

Methods—Seven SHIV$_{SF162P3}$-infected pigtail macaques were inoculated with *T. vaginalis* only (n = 2), *C. trachomatis* only (n = 1), both *T. vaginalis* and *C. trachomatis* (n = 2), or control media (no STI; n = 2). Infections were confirmed by culture and/or nucleic acid testing. Genital mucosa was visualized by colposcopy.

Results—Characteristic gynecologic signs were observed for both STIs, but not in control animals. Manifestations were most prominent at days 7–10 post-infection. STIs persisted between 4 and 6 weeks and were cleared with antibiotics.

Conclusions—These pilot studies demonstrate the first successful STI-SHIV triple infection of pigtail macaques, with clinical presentation of genital STI symptoms similar to those observed in humans.

Keywords

coinfection; genital; mucosal; non-human primate

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Introduction

Sexually transmitted infections (STIs) are highly prevalent in HIV-positive individuals and in populations at high risk of contracting HIV [1–4]. Additionally, multiple epidemiologic studies have associated STIs with an increased risk of HIV infection [2, 3, 5–8]. The precise underlying mechanism of this association, particularly in the case of non-ulcerative infections, remains unknown. A behavioral component is plausible; however, the recruitment of HIV target cells to the genital mucosa or a shift in the immune milieu of genital secretions because of STI-induced inflammation may also enhance susceptibility to HIV [6, 9, 10]. An animal model appropriate to analyze these susceptibility factors would greatly improve our understanding of the role of STIs in facilitating HIV infection.

In this current study, we describe the development of a pigtail macaque model of STI–HIV coinfection utilizing the bacterium *Chlamydia trachomatis* and the protozoan *Trichomonas vaginalis. Chlamydia trachomatis* is one of the most prevalent STIs worldwide [4] and is the most commonly reported bacterial STI in the United States. Incidence of *C. trachomatis* infection in the United States has risen significantly over the past several years and rates are highest in African Americans, particularly in reproductive-age women [1]. *Trichomonas vaginalis* is the most common non-viral STI, with approximately 170 million worldwide cases reported annually [4, 8]. Sutton et al., report a *T. vaginalis* prevalence rate of 3.1% among women in the United States, with African American women having the highest prevalence (13.3%) [1, 11]. *Chlamydia trachomatis* and *T. vaginalis* are commonly found to coinfect the female genital tract, and both are also associated with increased risk of HIV infection [5, 7, 8, 12]. Sorvillo et al., [7] suggests trichomoniasis may be a key component driving the increase in HIV incidence among African American women in the United States. We chose to model *T. vaginalis* and *C. trachomatis* infections in the setting of SHIV infection in the female pigtail macaque because of reproductive tract similarities to human females and our expertise in genital tract studies in this species [13–18]. Additionally, previous studies by Patton et al. with *C. trachomatis* and *T. vaginalis* have demonstrated the pig-tailed macaque is susceptible to infections with these pathogens, alone or in combination [19–24]. Our long-term goal is to evaluate the impact of these STIs on acquisition of SHIV.

In women, *C. trachomatis* infects the columnar epithelium of the endocervix and cervical transformation zone, with risk of the infection ascending into the upper reproductive tract [10, 25]. In contrast, *T. vaginalis* is tropic for the stratified epithelium of the vaginal mucosa and is rarely invasive; however, cervical inflammation may be observed [7, 26]. Both infections elicit an inflammatory response in the genital compartment, resulting in the trafficking of inflammatory and immune effector cells to the tissues and the upregulation of inflammatory cytokines [9, 10, 26–28]. Classical clinical/gynecologic presentation of *C. trachomatis* includes the presence of cervical mucosal erythema, mucopurulent discharge, friability, and edema. The presence of elevated numbers of polymorphonuclear cell infiltrates, as detected by microscopy, and in severe cases, epithelial erosion are also symptomatic of *C. trachomatis* infection [10, 29, 30]. Classic presentation of *T. vaginalis* infection includes erythematous, or ‘strawberry’ cervix and a foamy, yellow-green vaginal discharge [26, 31]. We aimed to replicate *C. trachomatis*– *T. vaginalis* coinfection in pigtail macaques as reported by Patton et al. [21, 22, 24] and demonstrate genital tract symptoms and manifestations similar to humans.

In this pilot study for model development, we have confirmed not only *C. trachomatis*–*T. vaginalis* dual coinfection, but also demonstrated STI-SHIV triple infections in macaques with an established $SHIV_{SF162P3}$ infection. Data from this novel and relevant triple coinfection model will be utilized in future studies to evaluate mechanisms of enhanced

susceptibility to HIV in SHIV-naïve animals and test biomedical HIV prevention strategies in the context of STIs.

Materials and methods

Macaques

Seven female, $SHIV_{SF162P3}$-positive pigtail macaques (*Macaca nemestrina*) were utilized in this study. All seven animals were of breeding age, with weights ranging from 6 to 10 kg, and were housed at the Centers for Disease Control and Prevention (CDC). The Institutional Animal Care and Use Committee of the CDC approved all procedures described in this study; procedures were in accordance with standards established in the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press, Washington, DC). Macaques were anesthetized with ketamine (10 mg/kg) prior to all procedures. After the study's conclusion, animals were humanely euthanized with an intravenous dose of pentobarbital (>100 mg/kg). One animal, 96Po17, was euthanized prior to the conclusion of the study because of bacterial hepatitis, a condition deemed unrelated to the study by the attending veterinarian.

The macaques were infected during previous titration studies of $SHIV_{SF162P3}$ stocks (unpublished data). $SHIV_{SF162P3}$ (SIV_{mac239} backbone with HIV-1 clade B envelope [32]) was provided by the NIH AIDS Research and Reference Reagent Program (NARRRP, catalog #6526) and propagated at CDC. Animals were infected using an established repeat low-dose (RLD), intravaginal challenge model [14, 15, 18]. With the exception of one animal, TD6, (challenged with 10 $TCID_{50}$) animals were inoculated with 50 $TCID_{50}$ of $SHIV_{SF162P3}$ virus stock. Median time frame between SHIV infection and STI inoculation was 278 days (approximately 40 weeks; range: 246–358 days).

SHIV RNA levels

To quantify plasma SHIV RNA levels, virions in 1-ml plasma aliquots were pelleted by high-speed centrifugation, and viral RNA was extracted using the NucliSens system, according to manufacturer's protocol. RNA levels were quantified using an internally controlled and normalized reverse-transcription real-time TaqMan© PCR (Applied Biosystems, Carlsbad, CA, USA) assay with a threshold sensitivity level of 50 copies per sample [33]. Plasma SHIV RNA levels were reported as copies per ml plasma. Secretions for genital SHIV RNA levels were collected from the endocervix and vaginal vault on polyester-tipped swabs, submerged in RNAlater (Applied Biosystems, Carslbad, CA, USA), and processed as previously reported [34]. Viral RNA extraction and quantitation proceeded as described above. Genital SHIV RNA levels were divided by 2 (to account for double swab collection) and evaluated as copies per swab (as much as 400 μl of genital secretions were collected per swab).

$CD4^+$ T cell levels

Blood was obtained from the femoral vein using mononuclear cell preparation tubes (CPT) (Becton Dickinson Biosciences, San Jose, CA, USA), and peripheral blood mononuclear cells (PBMCs) were collected from mononuclear cell fractions after centrifugation. $CD4^+$ T cells were enumerated in freeze–thawed cells by flow cytometry using a FACS Calibur (Becton Dickinson Biosciences, San Jose, CA, USA) and a previously described protocol and antibody panel [35].

Trichomonas vaginalis culture, challenge, and detection

Trichomonas vaginalis strain Balt-42 was utilized in this study because it had previously been shown to enhance HIV infection in an *in vitro* model [36]. Cultures were propagated in

Diamond's media to high concentration and viability. Macaques were atraumatically inoculated with 6×10^6 viable trichomonads. Because the growth media contains antibiotics potentially disruptive to the vaginal microflora, just prior to challenge, trichomonads were resuspended in 1 ml of 37°C Roswell Park Memorial Institute (RPMI) media and drawn into a 3-cc syringe. Inoculums were then applied to the vaginal mucosa using a sized gastric feeding tube (similar to RLD virus challenge, [14, 15]). Control animals received mock inoculations of 1 ml plain RPMI.

To detect and monitor *T. vaginalis* infection, vaginal secretion samples were collected twice-weekly on cotton- tipped swabs and used to inoculate an InPouch© Trichomonas culture packet (BioMed Diagnostics, White City, OR, USA) [37, 38]. Cultures were incubated at 37°C and examined by microscopy every 24 hours post-collection for the presence of motile trichomonads. Cultures void of motile trichomonads after 72 hours were deemed negative [37, 38]. After completion of trichomoniasis analyses (14 weeks for TD6; 8 weeks for FH3 and 96Po78), animals were treated with metronidazole (35 mg/kg, once per day, for 3 days). Treatment was not administered to the euthanized animal. Test of cure was conducted 1 week posttreatment to confirm clearance of infection.

Chlamydia trachomatis culture, challenge, and detection

Two serovars of *C. trachomatis* were utilized. Serovar E (strain UWR109, clinical isolate) was obtained from the Chlamydia Laboratory at the University of Washington. Serovar D (strain D-LC, NCBI accession #CP002054) [39] was provided by Dr. Harlan Caldwell (NIH/NIAID Rocky Mountain Laboratories, MT). For both serovars, macaques were inoculated twice, at once-weekly intervals, with 1×10^6 inclusion-forming units (IFU) in 1 ml sucrose–phosphate–glutamate (SPG) media. Inocula were drawn into a 3-cc syringe and atraumatically applied to the surface of the ectocervix using a sized gastric feeding tube. Control animals received mock inoculations of 1 ml plain SPG media. *Chlamydia trachomatis* infection was detected and monitored using the APTIMA GenProbe system (San Diego, CA, USA) and confirmed with culture (ReadyCells© Chlamydia Detection System, Athens, OH, USA). After the 8-week study period of Chlamydia cervicitis, animals were cleared of *C. trachomatis* with azithromycin (14 mg/kg, once per day, for 7 days). Test of cure was conducted 1 week post-treatment via APTIMA testing and confirmed 3 weeks post-treatment by culture.

Characterization of genital tract inflammation

A pediatric speculum and colposcopy were utilized twice-weekly to visualize the genital tract mucosa [40]. Using a standard questionnaire, trained personnel documented the presence/severity of vaginal and cervical erythema, presence/severity, color, and consistency of vaginal and cervical discharge, and presence/severity of cervical erosion in each macaque. In the *C. trachomatis*- only- and *T. vaginalis* + *C. trachomatis*–infected macaques, colposcopic images of the cervix were captured using a Carl Zeiss colposcope (Model # LR66238).

Cervical infiltrate

Cervical cells were collected by inserting a cytobrush (Cooper Surgical, Trumbull, CT, USA) into the cervical os and gently rotating 360° twice. Samples were placed in RPMI 1640 supplemented with gentamicin and amphotericin B and processed within 4 hours of collection [41, 42]. Cells were eluted from the cyto-brush and enumerated by microscopy utilizing an Endtz-trypan stain to differentiate polymorphonuclear cells (PMNs), mononuclear cells, epithelial cells, and red blood cells [43]. Greater than or equal to 100 cells were enumerated from each sample, and each cell type was normalized to a percentage of the total population [41].

Mucosal IFN-gamma analyses

Endocervical secretions were collected on Merocel ophthalmic sponges (Medtronic, Jacksonville, FL, USA) and extracted using a protocol and buffer described by Castle et al. [44]. IFN-gamma levels were determined using the Monkey Cytokine 5-Plex Panel, according to manufacturer's protocol (Invitrogen, Cat # LPC0001, Carlsbad, CA, USA). Levels were normalized to collected secretion weight and evaluated as pg IFN-gamma per ml secretion.

Histopathology

Using standard biopsy forceps, vaginal and cervical pinch biopsies were collected pre-/post-STI inoculation and stored in 10% buffered formalin. Tissues were paraffin-embedded, thin-sectioned, H&E stained, and analyzed by a veterinary pathologist for epithelial integrity and infiltrate intensity using an Olympus BX41 light microscope. Biopsies were collected at baseline and then at 7 weeks post-inoculation from macaques FH3, 96Po25, 96Po58, and 96Po78. From macaques TD6 and 96Po26, biopsies were collected 4.5 weeks post-inoculation. Biopsies were collected from macaque 96Po17 at necropsy, 3.8 weeks post-inoculation.

Results

The median time from $SHIV_{SF162P3}$ infection to STI (or mock) inoculation was 278 days (40 weeks), and the time frame of STI infection follow-up ranged from 32 to 98 days (4.5–14 weeks) (Table 1). At the time of STI inoculation, $CD4^+$ T cells levels were within normal levels of SHIV-infected and uninfected animals in our colony; $CD4^+$ T cell levels did not change significantly throughout the course of the study (data not shown).

All STI-inoculated macaques (n = 5) were successfully infected with *T. vaginalis* alone (n = 2), *C. trachomatis* alone (n = 1), or *C. trachomatis* and *T. vaginalis* (n = 2). Two macaques were utilized as STI-negative controls, mock-infected as previously described. Colposcopy was used to visualize the cervicovaginal compartment and characterize the genital mucosa and discharge. In macaques infected with *T. vaginalis* only, characteristic manifestations of 'strawberry cervix' and green, foamy discharge were observed (Table 1). Similarly, characteristic gynecologic signs were observed in the macaque infected with *C. trachomatis* only, such as cervical erythema, blistering and erosion of the cervical mucosa, and mucopurulent discharge (Table 1). Signs were most overt in the dual STI-infected macaques. In addition to foamy vaginal discharge, increased severity of erythema and erosion, as well as mucosal edema, was also noted (Table 1). Manifestations were most prominent at days 7–10 post-inoculation, although erythema and discharge persisted to varying degrees in all STI-infected animals throughout the study course. Discharge persisted longer and was more consistently observed than mucosal erythema. These observations were compared with mock media-inoculated animals, in which normal genital mucosa and discharge were observed (Table 1).

Cervical cell infiltrate collected by cytobrush sampling was examined at different time points throughout the study in the *C. trachomatis*-only-, dual STI-infected animals, and mock media-inoculated animal. (Fig. 1). An increase in PMNs, relative to baseline, was observed in all STI-infected macaques, rising as high as 42% in one animal (FH3), and peaked at approximately 2–3 weeks post-infection. A similar increase was observed in the percentage of total leukocyte infiltrate (PMNs + mononuclear cells). Increased levels of cell infiltrate were observed only in STI-infected macaques. The number of animals in this pilot study was not powered to achieve statistical significance in these analyses.

Cervical colposcopic images from a representative animal (FH3), which was dual STI-infected, are shown in Fig. 2A–C. Compared with baseline, vascularization and edema of the cervix visible by day 7 (post-administration of 1st *C. trachomatis* inoculums, Fig. 2B) and manifestations worsen by day 10 (post-administration of *T. vaginalis* and the 2nd *C. trachomatis* inoculums, Fig. 2C). Pronounced edema and discharge, severe erythema, and points of blistering erosion are visible (Fig. 2). These gynecologic signs are analogous to those documented in women for both *C. trachomatis* and *T. vaginalis* infections.

Plasma SHIV RNA levels were monitored throughout the study course, but did not fluctuate in response to STI inoculation/infection (Fig. 3). Genital SHIV RNA analysis was also undertaken, but conclusions were limited because of infrequent sample collection (data not shown).

Infections were observed a minimum of 32 days (4.6 weeks) to 98 days (14 weeks) before STI treatments were administered. One animal, 96Po17, was euthanized on day 32 of the study, prior to treatment, with a diagnosis of bacterial hepatitis; however, the attending veterinarian determined cause of death was unrelated to *T. vaginalis* infection. The remaining STIinfected macaques successfully cleared infections with the respective, appropriate treatment(s).

Infections with both *C. trachomatis* serovars D and E were tested and compared in this study, albeit with small animal numbers. Macaque 96Po58 was infected with serovar E only, whereas macaques 96Po78 and FH3 were both dual-infected with *T. vaginalis* and *C. trachomatis* serovars D and E, respectively (Table 1). More prolonged erythema and discharge were noted in the serovar D/Trichomonas-infected animal compared with the serovar E/Trichomonas-infected animal. Additionally, up to 500-fold higher levels of mucosal IFN-gamma were observed in the serovar D-infected animal between days 4 and 14 post- STI infection, compared with the serovar E-infected animal (data not shown). From the pinch biopsy sampling, increased vaginal histopathology (leukocyte infiltrate and epithelial sloughing) was also noted in the serovar D-infected animal (data not shown). Cervical histopathologic changes were not detected in any of the STI-infected animals (data not shown).

Discussion

These analyses are the first to demonstrate the triple infection of pigtail macaques with *C. trachomatis*, *T. vaginalis*, and SHIV. *Chlamydia trachomatis* and *T. vaginalis* infections in this study demonstrated characteristic profiles of erythema, discharge, inflammatory cervical infiltrate, and/or cervical mucosal disruption. Such gynecologic symptoms and manifestations are analogous to those documented in women diagnosed with Chlamydia cervicitis and/or trichomoniasis. [10, 26, 29, 31]. These pathogens were chosen because of their high prevalence and association with increased risk of HIV infection, as reported particularly in minority populations in the United States [1, 11]. Thus, we maintain this is a relevant model of STI coinfection which can later be adapted to study the effects of these infections on SHIV susceptibility.

Although *C. trachomatis* may cause cervical mucosal erosion, both *C. trachomatis* and *T. vaginalis* are considered non-ulcerative pathogens. However, their infections are characterized by increased cell infiltration to the genital mucosa [29, 31]. An advantage of a non-ulcerative model is that it will primarily address mechanistic issues related to lymphocyte and inflammatory cell infiltrates in the genital mucosa without the mechanical disruption of the epithelium, as has previously been modeled with herpes viral infections

[45]. Thus, this model will allow us to focus on the question of whether STI-induced lymphocyte infiltration increases susceptibility to HIV.

We aim to utilize *C. trachomatis* serovar D in the next phase of analyses utilizing SHIV-naïive animals. Our preliminary data (IFN-gamma levels, histopathology, persistence of erythema and discharge, and consistency in levels of PMN/leukocyte infiltrate) from these pilot studies suggest *C. trachomatis* serovar D may elicit a more heightened inflammatory response than serovar E. This cannot be concluded with certainty because comparisons were made between only two animals. However, these data are consistent with previous work characterizing the same serovar D strain, which yielded similar results in mice [39].

This pilot study was conducted in a small number of SHIV-infected animals, but was necessary to develop methodologies and train personnel in new laboratory techniques and approaches. Because of limited availability and cost of SHIV-naïve female pigtail macaques, we chose to utilize SHIV-positive animals that had been used in previous virus titration studies to demonstrate that pigtail macaques may be triply infected with *C. trachomatis*, *T. vaginalis*, and $SHIV_{SF162P3}$. SHIV infection is a confounding factor, and it is possible these infections may have been less likely in SHIV-negative macaques. However, we contend this is unlikely because of the previous published work of Patton et al., indicating these infections are achievable in SHIV-negative/naïve macaques, with similar inoculation doses, both singly and as a dual coinfection [21, 23, 24]. It is also possible the gynecologic symptoms may be more pronounced in these animals than what might be observed in SHIV-negative macaques. Indeed, a strawberry cervix was observed in both *T. vaginalis*-only-infected macaques, whereas this is seen in <50% of *T. vaginalis*-infected women [46]. Also, despite these triple infections, the animals were clinically well, with localized infections, no alterations in $CD4^+$ or plasma SHIV RNA levels, and the STIs were easily treated with metronidazole or azithromycin.

Our model of STI followed by SHIV infection will incorporate a repeat low-dose intravaginal SHIV challenge modality which closely mimics mucosal HIV transmission [14–16]. This study design requires that STIs would need to persist throughout the period of repeated virus challenge. In these pilot analyses, infections were maintained for a minimum of 4.6 weeks and for as long as 14 weeks in one *T. vaginalis*-infected macaque (Table 1). This time frame, even at the minimum, would provide ample time for repeat virus challenges.

The development and future utilization of this model provides opportunities to better understand the role of STIs in HIV transmission. However, this model will also be valuable for areas of HIV prevention research. The efficacy of intervention strategies currently under investigation, such as vaginal rings, topical microbicides, and even oral PrEP modalities, could potentially be affected by the presence of STIs or STI-induced inflammation in the genital tract mucosa. This is a key consideration given the high prevalence of STIs in individuals at risk of HIV infection [2, 4]. The availability of an STI–HIV coinfection model will provide another platform for the rigorous evaluation of biomedical interventions safety and efficacy and enhance their ability to be translated into clinical practice.

Acknowledgments

We acknowledge Peter Augostini and Chen Cheng for their expertise and assistance with *T. vaginalis* culture and trichomonad analyses, respectively. We appreciate the input of Dr. Ronald Ballard on these STIs and study design. We also thank Carol Farshy for her assistance with *C. trachomatis* Gen-Probe APTIMA assays. We are grateful for the input of Drs. Katherine Paul and Lindsay Livingston in designing the macaque experiments and the assistance of Shanon Bachman in performing the procedures. Many thanks are given to Wei Luo, Debra Adams, Patricia Guenthner, and Dr. Ronald Otten for technical assistance and expertise in generating the $SHIV_{SF162P3}$ stock. We acknowledge Eileen Breding and Dr. Prachi Sharma of the Yerkes National Primate Center for their assistance with

histopathologic tissue analyses. We also thank Dr. James Smith for insightful scientific discussion of these experiments. This work was funded by the Centers for Disease Control and Prevention and partially supported by Interagency Agreement Y1-AI-0681-02 between CDC and the National Institutes of Health. T. H. was funded by a fellowship by the American Society for Microbiology (ASM)/CDC Program in Infectious Disease and Public Health Microbiology.

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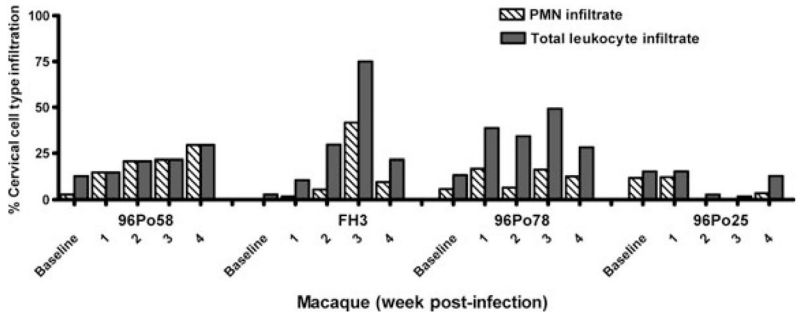

Fig. 1.

Relative increase in inflammatory cells in cervical cell collections of sexually transmitted infection (STI)-infected macaques. Analyses were conducted in *Chlamydia trachomatis*-only (96Po58)- and *C. trachomatis* + *Trichomonas vaginalis*–infected macaques (FH3 and 96Po78). 96Po25 received mock media inoculations (control). Cell populations were collected by cervical cytobrush sampling and enumerated by microscopy utilizing an Endtz-trypan differential stain. The graph longitudinally depicts the percent of cervical infiltrate cell types present at baseline and weeks 1–4 post-infection (week 1, relative to first *C. trachomatis* inoculum). An increase in the percentage of inflammatory cells consisting of polymorphonuclear (PMN) cells and/or total leukocyte infiltrate, relative to baseline, was observed in all three sexually transmitted infection (STI)-infected macaques over the course of the study.

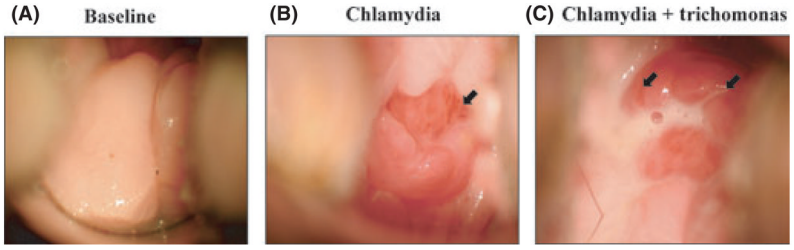

Fig. 2.

Cervical colposcopic images of a representative macaque (FH3) (10 × magnification). (A) Cervical mucosa (ectocervix) at baseline, characterized by normal, pink coloration, and presence of scant, clear normal menstrual-related discharge. (B) Cervical mucosa 7 days after administration of 1st *Chlamydia trachomatis* inoculum (1×10^6 IFU). Erythema, tissue vascularization (arrow), edema, and presence of mucopurulent discharge noted during visual inspection. (C) Cervical mucosa 4 days after administration of second *C. trachomatis* (1×10^6 IFU) and *Trichomonas vaginalis* (6×10^6 trichomonads) inoculums; 10 days post-infection, relative to 1st *C. trachomatis* inoculation. Findings include severe erythema and edema, areas of blistering erosion (arrows), and opaque, bubbly, mucopurulent discharge originating from the cervical os. IFU, inclusion-forming units.

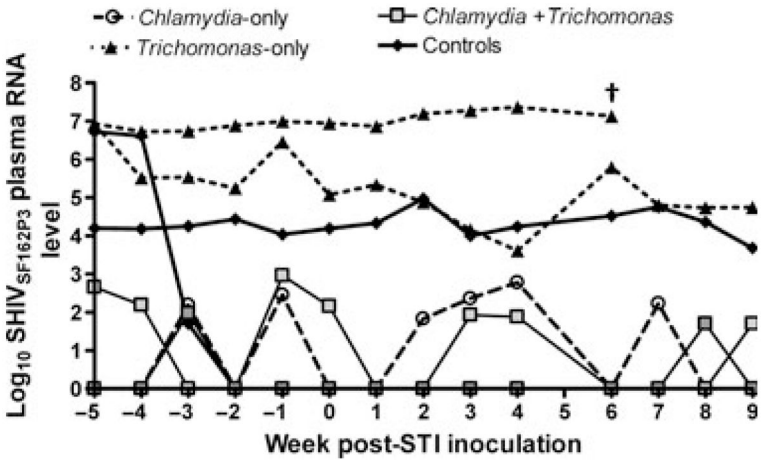

Fig. 3.

No impact of sexually transmitted infections on plasma $SHIV_{SF162P3}$ RNA levels. No differences in plasma SHIV RNA levels (*y*-axis) was observed in response to *Chlamydia trachomatis* (96Po58), *Trichomonas vaginalis* (TD6 and 96Po17), or *C. trachomatis* + *T. vaginalis infection* (FH3 and 96Po78) (Controls: 96Po25 and 96Po26) (*x*-axis). Threshold sensitivity level = 50 copies per sample. SHIV RNA levels reported at copies per ml plasma. †Animal euthanized for reasons unrelated sexually transmitted infection (STI) inoculation/infection.

J Med Primatol. Author manuscript; available in PMC 2012 July 23.

Table 1

Study overview: time frame of infection and gynecologic sign presentation

Experiment Arm/Macaque	Time (days) between SHIV+ and STI inoculation	STI infection time frame (days)	Gynecologic sign presentation[1]		
			Cervical	Vaginal	Time frame[2]
Trichomonas vaginalis only					
TD6	358	98[3]	Erythematous mucosa; 'strawberry cervix'	Yellow-green, foamy discharge	*Initial*: 4 dpi *Peak*: 7 dpi *Persist*: until treatment (gradual decrease in severity)
96Po17	278	32[4]	Erythematous mucosa; 'strawberry cervix'	Yellow-green, foamy discharge	*Initial*: 4 dpi *Peak*: 7 dpi *Persist*: n/a[4]
Chlamydia trachomatis only					
96Po58 (E)	281	56[3]	Erythematous mucosa with punctate erosion; thick, semi-transparent purulent discharge	Mild-to-moderate clear discharge	*Initial*: 7 dpi *Peak*: 10 dpi *Persist*: until treatment; persistent erythema, gradual decrease in discharge
T. vaginalis + C. trachomatis					
FH3 (E)	291[5]	56[3]	Edematous and severely erythematous mucosa with blistering erosion; thick, opaque bubbling discharge originating from os	Moderate mucosal erythema; moderate semi-transparent, foamy discharge	*Initial*: 7 dpi *Peak*: 10 dpi *Persist*: until treatment; persistent erythema, gradual decrease in discharge
96Po78 (D)	277[5]	56[3]	Edematous and severely erythematous mucosa with blistering erosion; thick, opaque bubbling discharge originating from os	Severe mucosal erythema; severe, milky, foamy discharge	*Initial*: 4 dpi *Peak*: 10 dpi *Persist*: until treatment; both erythema and discharge consistent until treatment
Controls					
96Po25	263[6]	n/a	Pink (normal) mucosa; normal, menstrual cycle-related discharge	Pink-to-pale pink (normal) mucosa; scant, clear discharge (normal)	n/a
96Po26	246[6]	n/a	Pink (normal) mucosa; normal, menstrual cycle-related discharge	Pink (normal) mucosa; scant, clear discharge (normal)	n/a
	Median = 278	Median = 56			

dpi, days post-infection; STI, sexually transmitted infection.

[1] Signs most predominant at 7–10 dpi.

[2] Time frame—assessments limited by biweekly access to animals.

[3] Appropriate treatment (*T. vaginalis*—metronidazole, *C. trachomatis*—azithromycin) administered to resolve infection.

[4] Animal euthanized.

[5] With respect to 1st *C. trachomatis* inoculation (2nd *C. trachomatis* + *T. vaginalis* given 1 week later).

[6] Between SHIV+ and mock inoculation.