Period II. So HIV-related service capacity building should be enhanced in GHs while that related to syphilis in SHCs in Wuxi, China.

Disclosure of interest statement All authors declare no competing interests.

P08 - Chlamydia infections

P08.01 CHARACTERISING CD4$^+$ AND CD8$^+$ T-CELL RESPONSES BY INTRACELLULAR CYTOKINE STAINING IN WOMEN WITH AND WITHOUT *CHLAMYDIA TRACHOMATIS* REINFECTION

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Background *Chlamydia trachomatis* (CT) infection is the most prevalent bacterial sexually transmitted infection worldwide and, untreated, can lead to significant reproductive morbidity. Unfortunately, the prevalence remains high in the United States and no effective CT vaccine exists, in part because of an inadequate knowledge of immunological responses to CT infection in humans and, specifically, no correlates of protective immunity to guide vaccine studies. In animal studies, IFN-γ and/or TNF-α producing CD4 cells are known to mediate protection against *C. trachomatis*. The objective of this study was to characterise the T-cell mediated immune responses to *C. trachomatis* in humans with and without CT reinfection at a follow up clinic visit.

Methods In an ongoing study, peripheral blood mononuclear cells (PBMCs) are collected from CT-infected women at the time of treatment and stimulated *in vitro* with *C. trachomatis* antigens MOMP or PGP3, then fixed and permeabilized. The percentage of CD4$^+$ and CD8$^+$ T-cells expressing either IFN-γ or TNF-α is then assessed using intracellular cytokine staining (ICCS) and flow cytometry. Women return at 3- and 6-months for repeat genital chlamydia screening. Cytokine-specific ICCS responses are then compared in women with versus without subsequent CT reinfection at follow-up to assess for possible correlates of protection.

Results To date, we have performed ICCS on 44 women who have completed the study. Compared with women who had CT reinfection at a follow-up visit (n = 14), women without CT reinfection (n = 30) had higher baseline positive CD8$^+$ TNF-α responses (p = 0.042). There was no significant association of IFN-γ and/or TNF-α CD4 responses with CT reinfection risk.

Conclusions Our preliminary findings reveal that a TNF-α-producing CD8$^+$ T-cell response appears to correlate with a decreased risk for CT reinfection, suggesting a possible role in protective immunity. CD4 T-cell responses have not significantly differed in women with versus without CT reinfection.

Disclosure of interest statement Nothing to declare.

P08.02 *CHLAMYDIA TRACHOMATIS* CAUSES MITOCHONDRIAL DAMAGE IN KERATINOCYTES

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Introduction *Chlamydia trachomatis* causes two different sexually transmitted diseases: genital discharge (GD) and *lymphogranuloma venereum* (LGV). Apart from differences in tissue tropisms, little is known about differences in pathogenicity to the cells they infect, especially keratinocytes, the primary target of infection for LGV chlamydia.

Methods Human keratinocytes (HaCaT cells), one LGV (serovar L2) and one GD (serovar E) isolate were used for all experiments with uninfected cells as the negative control. Experiments were performed at 37 and 33°C. For transmission electron microscopy (TEM) cells grown on Thermanox coverslips within 24-well plates were infected (MOI = 0.25) and incubated for various time-points over 48 h. The diameter of 15 mitochondria were measured in uninfected and infected cells at 1, 36 and 48 h post-infection using image analysis software. For the MTT assay cells grown in 96-well plates were infected (MOI = 0.25) and incubated for various time-points over 48 h. Median mitochondrial diameter was compared using Kruskal-Wallis nonparametric ANOVA with Dunn's post-test. Percentage mitochondrial activity was compared using two-tailed paired T test. $P \leq 0.05$ was considered significant.

Results The GD isolate alone caused mitochondrial swelling, cristae rearrangement and a pale mitochondrial matrix at 1, 36 and 48 h post-infection at 37°C but not 33°C. Median mitochondrial diameter was 430 nm in uninfected cells; 477 nm, 470 nm and 1221 nm after 1, 36 and 48 h post-infection respectively for serovar E; and 343 nm, 420 nm and 392 nm after 1, 36 and 48 h respectively for L2. Mitochondrial diameter was significantly larger in serovar E-infected cells 48 h post-infection compared to uninfected cells ($P < 0.001$) or L2-infected cells ($P < 0.01$). The MTT assay indicated a significant ($P = 0.0373$) reduction in mitochondrial activity upon infection with serovar E, but not serovar L2 at 37°C.

Conclusion *C. trachomatis* of the GD biovar but not the LGV biovar causes transient mitochondrial swelling and a decrease in mitochondrial activity in infected keratinocytes at 37°C.

Disclosure of interest statement This study is supported by the National Research Foundation of South Africa. The authors have no conflict of interest to declare.

P08.03 MINING GENOME OF *CHLAMYDIA TRACHOMATIS* TO IDENTIFY VACCINE CANDIDATES

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Introduction *Chlamydia trachomatis*, an obligate intracellular parasite, is a major cause of genital infections in human. The pathogen is sexually transmitted and responsible for serious reproductive and other health problems. Despite the availability of antibiotic therapy, infection rates are increasing worldwide. This is mainly due to the asymptomatic nature of most infections and the lack of effective screening programs. Vaccination is therefore considered to provide the best means of controlling chlamydial infection. Attempts to vaccinate with whole-cell vaccine or with purified chlamydial proteins eliciting CD4$^+$ T cells and/or antibodies have failed as they provide only partial protection in animal models. Thus, identification of protective antigens that could be used either as an alternative to those already characterised or in combination with them is a high priority in chlamydial vaccine development.

Methods We used "Reverse Vaccinology" approach using available bioinformatics tools to identify chlamydial proteins eliciting humoral and/or cell-mediated immunity and selected effective antigen vaccine combinations.
Results Reverse vaccinology technique has helped us to identify putative antigens that could serve as vaccine candidates for different strains of *C. trachomatis*, prevalent in India. This approach has led to the identification of novel and highly conserved protein antigens that are either secreted or expressed on the bacterial surface and demonstrate protection *in silico*. Notably, each of these identified putative antigens, can be produced as a soluble recombinant protein in *Escherichia coli*, a property that is a considerable asset for the commercial production of a vaccine. The Reverse vaccinology approach thus could help reduce the time and cost required for the identification of novel and suitable antigen candidates and subsequent vaccine production.
Conclusion The successful integration of genome screening method, coupled with the use of various bioinformatics tools has facilitated us to identify better vaccine candidates.
Disclosure of interest statement No conflict interest.

P08.04 PATHOGENICITY OF PLASMID POSITIVE AND NEGATIVE *CHLAMYDIA TRACHOMATIS* IN A MACAQUE MODEL OF OCULAR AND GENITAL TRACT DISEASES

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Introduction Previous studies demonstrated that a *Chlamydia trachomatis* plasmid negative ocular strain (A/P-) resulted in attenuation of infection and pathology, paired with immune stimulation that produced protective immunity in a monkey ocular model. Expanding upon these findings, we assessed a plasmid negative genital strain (D/P-) in the pigtailed macaque model of chlamydial reproductive tract infection. Infection and pathology were compared to a plasmid positive genital strain (D/P+).
Methods Groups of six macaques were cervically challenged with *C. trachomatis* D/P+ or D/P-. All animals were followed for infection, circulating antibody, local immunity, and tissue inflammation. Upon spontaneous clearance of cervical infection, each animal underwent repeated challenges with matched strains to drive upper reproductive tract disease. The same strains were similarly compared in the macaque ocular infection model.
Results Similar rates and duration of chlamydial genital infection were documented in both the P+ and P- challenged macaques. Likewise serum and local antibodies were similar. Tissue inflammation graded by gross observation during laparoscopic procedures and by tissue histology yielded no discernible patterns to disease pathology between P+ and P- strains.

Because A/P+ and A/P- ocular strains in macaques exhibited dramatic differences in infectivity and pathology in the eye, we ocularly challenged animals with D/P+ and D/P-. Unexpectedly, no differences in infectivity or pathology were observed between the D/P+ and D/P- strains; each produced similar infection kinetics with ocular disease characterised by conjunctival hyperemia and follicle formation.
Conclusions Unlike the ocular strain, the plasmid negative genital strain is not attenuated in either genital or ocular macaque infection models. This suggests that genetic determinants unrelated to the plasmid play a dominant role in the pathogenesis of urogenital strains.
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P08.05 *CHLAMYDIA TRACHOMATIS* AND *TRICHOMONAS VAGINALIS* CO-INFECTION IN THE MACAQUE MODEL

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Introduction We have used the pigtailed macaque model to individually study chlamydia (bacteria) and trichomonal (parasite) infections. To increase utility of the model, we explored infection potential for both chlamydia and trichomoniasis, when delivered simultaneously to macaques. This co-infection model will be useful for testing the efficacy of developing multi-purpose technologies.
Methods Twelve female Macaca nemestrina were challenged by cervical inoculation with *C. trachomatis* E (CT: 5E6 IFU in 0.5 mL volume), immediately followed by vaginal exposure to *T. vaginalis* ATCC 50148 (TV: 5E5 trichomonads in 0.5 mL volume). For five weeks, infection status, chlamydia-specific serum antibody, and tissue responses were followed. Nucleic acid amplification tests were employed to detect each organism; cervical swabs were also cultured for chlamydia detection. Cervicovaginal tissues were monitored by colposcopy. Each animal completed a five day regimen of oral azithromycin plus metronidazole followed by test of clearance.
Results Nine of twelve macaques tested positive for infection with both pathogens on at least three weekly visits. One animal was positive for chlamydial infection only (TV negative) for two weeks. These ten animals developed chlamydial IgG antibody titers of at least 1:16. The two remaining animals had limited, self-clearing infections; CT positive for one week, plus TV positive for one or two weeks. Neither of these macaques developed serum antibody to CT. During five weeks of secretion sampling, chlamydia positivity ranged from 1 to 5 weeks (median 5 weeks) and TV positivity ranged 0–5 weeks (median 5 weeks) as well. Colposcopy revealed blisters on the cervix of three co-infected animals. Neither infection could be correlated to changes in vaginal pH, exudate or polymorphonuclear cell presence.
Conclusion We have demonstrated that co-infection with *Chlamydia trachomatis* and *Trichomonas vaginalis* is achievable in the pigtailed macaque model. Infections are individually detectable and concurrent treatments are effective in clearing both organisms.
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