Abstract P4-S3.05 Figure 1 Two-phyloptype population structure of global *Trichomonas vaginalis* isolates.

20°C, male samples were 100% reactive after 7 days storage, while female samples were 100% reactive after 6 days of storage. For samples stored at 30°C, 100% reactivity was obtained at 2 days storage for male samples and 4 days storage for female samples.

Conclusions This study shows urine samples from women infected with TV have a wide range of cell titres, with an average of ~2000 TV cells/ml. TV can be detected for 14 days when stored refrigerated or for about 1 week at 20°C. The use of sensitive, automated molecular tests such as the ATV assay for testing urine samples should facilitate screening for TV.

P4-S3.06 STUDY ON EARLY INFECTION OF THE LOW GENITAL TRACT OF FEMALE MICE BY DIFFERENT SEROTYPES *UREAPLASMA UREALYTICUM*

doi:10.1136/sextrans-2011-050108.521

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Objective To observe the early inflammatory response by animal model with low genital tract Uu infection for investigating the relationship between the serovars and their pathogenicity.

Methods Fifty 8–10-week-old, female BALB/c mice were randomly divided into five groups, namely, control group, serovar 1 group, serovar 3 group, serovar 4 group, and serovar 8 group, and then treated respectively with vaginal inoculation of liquid medium and Uu inoculae of serovars 1, 3, 4 and 8 after subcutaneous injection of oestradiol benzoate. The vulval symptoms and vaginal and cervical swabs were observed weekly after Uu inoculation. Four weeks later, all mice were sacrificed and their cervixes and vaginae were removed for histopathology.

Results There was a significant difference in the inflammation severity of cervical mucosa ($p < 0.001–0.05$) except vaginal mucosa among all groups. The inflammation severity of cervical mucosa in serovar 4 and 8 infection was superior to that in serovar 1 infection ($p < 0.01–0.05$), but there was no statistical difference among other groups ($p > 0.05$).

Conclusions The results suggest that Uu could colonise and infect the cervical columnar epithelium other than vaginal squamous epithelium. Uu-induced inflammation response is different among its serovars. Serovars 4 and 8 are more virulent compared with biovar 1 such as serovar 1.

P4-S3.07 POPULATION GENOMICS OF *TRICHOMONAS VAGINALIS* REVEALS A GLOBALLY DISTRIBUTED TWO-PHYLOTYPE POPULATION STRUCTURE

doi:10.1136/sextrans-2011-050108.522

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Objective *Trichomonas vaginalis*, the causative agent of human trichomoniasis, is the most prevalent non-viral sexually transmitted infection and has been associated with increased risk of HIV transmission, making detection and treatment a global health priority. In this study, we evaluate the population genomics of globally distributed clinical isolates to characterise genetic diversity and identify population structure.

Methods We use a panel of 21 microsatellite and three single copy gene markers to evaluate the population genomics of 18 clinical isolates collected from female patients attending New York City STD clinics in 2008, as well as 177 extant isolates collected from the USA, Mexico, Chile, Italy, Southern Africa, Papua New Guinea, and Australia. We use a panel of population genetic tools including Arlequin 3.11 and FSTAT to calculate expected heterozygosity (HE) and population differentiation (FST) statistics. To infer population structure, we use STRUCTURE 2.2, Network 4.516, and SeaView 4.2.4. We test for significance in clinical and demographic variables (χ^2 and t-tests) using JMP Genomics 5.0.

Results We detect significant genetic diversity within the species (HE=0.66) that is observed across global regions (range 0.52–0.67), and find that a two-phyloptype population structure is maintained globally with few instances in population differentiation defined by geographical origin. This two-phyloptype structure is further supported by minimum spanning networks, hierarchical clustering and phylogenies inferred from three single copy genes. These two phylogenies appear at nearly equal frequencies globally. Defining characteristics of the phylogenies include significantly different prevalence of *T vaginalis* virus infection (3% in phyloptype 1 and 73% of phyloptype 2 ($p < 0.0001$, χ^2)) see Abstract P4-S3.07 figure 1 and a significantly higher minimum inhibitory concentration of metronidazole in phyloptype 1 ($p = 0.0024$, χ^2 Wilcoxon Rank Sums).

Conclusions We detect clear population structure and high levels of genetic diversity from global *T vaginalis* isolates. This preliminary data

Abstract P4-S3.07 Figure 1 TVV infection status by phyloptype.

suggests that phylotypes differ phenotypically. An accurate survey of genetic diversity and population structure will facilitate responsible selection of drug and/or vaccine targets for future treatments, and will enable better understanding of virulence factors contributing to the wide range of severity of symptoms associated with trichomoniasis.

P4-S3.08 DEVELOPMENT OF A NOVEL CHIMERIC PROTEIN CONSTRUCT FOR IMPROVED DIAGNOSIS OF SYPHILIS

doi:10.1136/sextrans-2011-050108.523

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Objectives To develop a novel diagnostic protein in order to enhance early detection of syphilis infections and improve overall syphilis diagnosis.

Methods Using pooled serum samples from patients infected with syphilis, immunoreactive regions of two previously identified diagnostic protein candidates, Tp0326 and Tp0453, were elucidated. Focusing on these regions, a chimeric protein construct was created for expression in *Escherichia coli* and expression conditions were optimised to produce soluble protein expression. This Tp0326/Tp0453 chimeric construct was screened against serum samples from; patients with primary, secondary, latent, and neurosyphilis and uninfected individuals. These results were directly compared to the rapid plasma regain (RPR) test, and the microhemagglutination assay for *T pallidum* (MHA-TP).

Results Screening results show high degrees of sensitivity and specificity for the Tp0326/Tp0453 chimeric construct, identifying all stages of syphilis infection from early primary to late latent.

Conclusion The Tp0326/Tp0453 chimera shows promise as a new diagnostic antigen for detecting all stages of syphilis infection and for development into point-of-care diagnostic test formats.

P4-S3.09 THE PREVALENCE OF TRICHOMONAS VAGINALIS VIRUS (TVV) IN GLOBALLY DISTRIBUTED TRICHOMONAS VAGINALIS ISOLATES

doi:10.1136/sextrans-2011-050108.524

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Objective *Trichomonas vaginalis*, a highly prevalent non-viral sexually transmitted infection, has been shown to be infected by a double-stranded RNA virus known as *T vaginalis* virus (TVV). The presence of this virus has been associated with increased trafficking of the immunogenic P270 to the surface of the parasite, and has therefore been hypothesised to be an important virulence factor in trichomoniasis. In the present study, we investigate the prevalence of TVV in globally distributed *T vaginalis* isolates and find an association between TVV infection and genetically distinct *T vaginalis* populations.

Methods 150 *T vaginalis* isolates from the USA, Mexico, Italy, Southern Africa, Papua New Guinea and Australia were screened for TVV infection by running total RNA extract on 1% agarose gels to detect the presence or absence of the diagnostic 4.5 kb dsRNA genome of the virus. The prevalence of TVV in genetically distinct *T vaginalis* phylotypes was compared using χ^2 tests.

Results TVV was found to be present in 37% of *T vaginalis* isolates. We find a difference in the prevalence of TVV infection between genetically distinct populations of parasites, with 3% of phylotype 1 isolates containing the virus vs 73% of phylotype 2 parasites (<0.001).

Conclusions TVV prevalence varies between *T vaginalis* phylotypes 1 and 2. This finding has implications suggesting that TVV is transmitted vertically among parasites, as more closely related *T vaginalis* strains are infected with TVV. Preliminary data also suggests that phylotype 2 parasites may show greater virulence, and further studies will be required in determining the role of TVV in this increased pathology.

P4-S3.10 A TRICHOMONAS VAGINALIS HUMAN VACCINE CANDIDATE: FREUND'S ADJUVANT VS ALUMINIUM HYDROXIDE ADJUVANT IN A BALB/c MOUSE VAGINAL INFECTION MODEL

doi:10.1136/sextrans-2011-050108.525

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Background *Trichomonas vaginalis* is an underreported STD known to increase HIV transmission. Development of a vaccine against *T vaginalis* could greatly affect transmission of *T vaginalis* and HIV as well as reducing their global prevalence. Herein, we aim to investigate the feasibility of developing a *T vaginalis* vaccine using the human-safe adjuvant Alhydrogel compared against an already established Freund's adjuvanted vaccine in a BALB/c vaginal infection mouse model.

Methods A prime-boost vaccination schedule was employed with live, whole cell *T vaginalis* (1×10^7 Tv/ml) is injected in 2–200 μ l aliquots 4 weeks apart and prior to vaginal infection challenge. Either Freund's, 0.5 mg Al/ml Alhydrogel, or 0.75 mg Al/ml Alhydrogel were applied as adjuvants. Additionally, 0.75 mg Al/ml Alhydrogel sham and non-vaccinated groups serve as controls. The BALB/c vaginal environment was modified to mimic humans by adjustment of vaginal flora and oestrogenisation prior to vaginal infection. The total IgG, IgG1 and IgG2a levels in serum were tested 3 weeks post each vaccine injection, then 2 and 4 weeks post-vaginal infection by ELISA. Groups were compared using Tukey's MCT (Post-Hoc one-way ANOVA; $\alpha < 0.05$).

Results Alhydrogel adjuvanted groups showed comparable total IgG and IgG1 levels to Freund's adjuvanted group. These levels for all adjuvanted groups were significantly different from control groups at all time points following boost-vaccination. IgG2a levels were not as consistent with large SD seen within Freund's adjuvanted mice. Alhydrogel adjuvanted groups were not significantly different from control groups at any time point for IgG2a levels suggesting no induction of IgG2. Controls and sham vaccines showed no Ig response. **Conclusions** Our data suggest that in line with expected Th2 skewed response from Alhydrogel adjuvant and Th1 skewed response from Freund's adjuvant there was a difference in IgG2a antibody production. Additionally, the Alhydrogel adjuvanted vaccines are otherwise similar to Freund's adjuvant in terms of total IgG and IgG1 levels suggesting the feasibility to pursue Alhydrogel as a vaccine candidate. Alhydrogel induces a significantly elevated immune response compared to natural infection antibody production (non-vaccinated control) and does not induce a nonspecific immune response.

Basic sciences poster session 4: Bacterial diversity

P4-S4.01 INVESTIGATION OF THE BACTERIAL DIVERSITY IN URINE OF URETHRITIS PATIENTS AND HEALTHY CONTROLS USING 454 HIGH-THROUGHPUT SEQUENCING

doi:10.1136/sextrans-2011-050108.526

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Background Non-gonococcal urethritis (NGU) is a common sexually transmitted disease in men but in 30–50% of NGU cases, no known organism is found. We have used 454 high throughput sequencing to analyse the micro flora in urine samples from cases of urethritis and from controls.