Methods Miniature diaphragm-like cervical barriers were manufactured and provided by ReProtect, Inc. BufferGel, previously found ineffective in this model when used alone, was also provided by ReProtect, Inc. Twenty-four pigtailed macaques were randomly assigned to one of three study arms: cervical barrier device alone; cervical barrier with BufferGel; or no barrier device and no gel (control arm). Eight animals were enrolled in each arm. Each macaque underwent baseline exam, product administration if applicable, cervical challenge with C. trachomatis (E: 5×105 IFU), and weekly follow-up exams for 5 weeks. Chlamydia challenge occurred within 30 min of baseline exams. Cervical barrier devices were removed from all test macaques 18 h after insertion. Each exam included cervicovaginal colposcopy, vaginal pH and cervical swabs for chlamydia detection (culture and NAAT: GenProbe Aptima Combo2). Blood for serum antibody testing was collected at baseline and weeks 2 through 5 post-inoculation.

Results Detection of chlamydial infection is detailed in the Abstract O4-S2.04 table 1 below. Colposcopic exams were comparable across each of the three test arms of the study, and thus did not detect toxic effects of the gel or the cervical barrier. Baseline vaginal pH was somewhat higher in the No Product arm than in either of the test arms. After chlamydial challenge and throughout the duration of the study, mean vaginal pH remained lowest in the barrier with BufferGel arm, but were similar (within 1 pH unit) in all three study arms.

Conclusions In this pilot study, a cervical barrier used alone provided little or no protection, but the barrier used with BufferGel reduced transmission by 50% (p=0.08). These results should encourage further study of the ability of a cervical barrier combined with a microbicide to provide greater protection against sexually transmitted infections than either used alone.

Abstract O4-S2.05 Figure 1 Day 21 Post T. pallidum infection Epididymis.

Background Syphilis rates are again increasing in the US and globally and are associated with HIV transmission and rising rates of congenital syphilis. Humans serve as the only natural reservoir of the causative agent, Treponema pallidum, and no in vitro cultivation systems are available. Progress towards a vaccine and a better understanding of immune response to syphilis has been hampered over the last half century by lack of a murine model. We hypothesized that previous attempts to establish T. pallidum infection in mice were unsuccessful because of rapid clearance of the organism by an innate immune response. Myd-88 serves as a common signalling molecule stimulated by most toll-like receptors TLRs; pattern recognition receptors of the innate immune system found on most innate immune cells, for example, monocytes, macrophages, and dendritic cells) and is responsible for downstream cytokine responses. We utilised mice bred to be Myd-88 deficient to test this hypothesis and to ascertain whether this mouse strain could be used to study persistent syphilis infection, immune response mechanisms, and eventually vaccine candidates.

Methods T. pallidum, Nichols strain, was cultivated by intratesticular infection of New Zealand male rabbits housed at 62°C as per protocol. After 12 day incubation, T. pallidum was extracted and the concentration adjusted to 7×108 organisms/ml. Myd-88−/− mice and equally-aged C57BL/6 mice were inoculated intradermally, intraperitoneally, intravaginally or in testicles, and intrarectally each mouse receiving total dose of 1×108 organisms divided into the four body site aliquots). Mice were observed daily for signs or cutaneous disease and/or systemic illness and were sacrificed at day 10 and 21. DNA and RNA were extracted from skin, spleen, genitals, rectum, lymph nodes, spinal cord and blood for use in real-time quantitative PCR and RT-PCR, respectively. Corresponding tissue type were also evaluated by histopathology and immunohistochemical staining T. pallidum Ab, BioCare, Concord, California, USA). The experiment was repeated three times with variable number of mice per experiment.

Results A total of 18 Myd-88 mice and 19 C57BL/6 mice were infected. Myd-88−/− mice were more likely than B6 control mice to have detectable RNA at day 10 and 21 (day 10, 12/35 sites (35%) +RNA in Myd-88−/−; 3/35 sites (8.5%) +RNA in WT. Day 21, 11/40 sites (27.5%) +RNA in Myd-88−/−; 3/40 (7.5%) +RNA in B6). One experiment ongoing past 42 day post-infection reveals at day 42 sacrifice Myd-88−/− mice with 6/15 sites (40%) +RNA; B6 0/15 sites (0%). Histopathology revealed mild to moderate inflammation in MyD88−/− mice and demonstrable organisms by IHC Abstract O4-S2.05 figure 1).

Conclusion These preliminary experiments suggest that the immune recognition impairment caused by deleting Myd88 signalling protein results in productive and longer-lasting T. pallidum infection in this
Background Mycoplasma genitalium (MG) is a newly recognised pathogen associated with acute and persistent reproductive tract infection in men and women. Understanding of the disease mechanisms, persistence and immune avoidance of this organism is hampered by the lack of a suitable animal model.

Methods Female pigtail macaques (Macaca nemestrina) were inoculated cervically with ~109 genome equivalents (~108 cfu’s) of MG strain G37, then assessed at intervals over 8 weeks for the persistence of MG in lower tract specimens. Fallopian tube biopsies were collected via laparotomy at Weeks 4 and 8. Specimens were assessed for the presence of MG DNA by qPCR and for viable MG by growth in H broth and Vero cell co-cultures. Serum collected at intervals was evaluated by immunoblot and ELISA for reactivity to MG antigens. Finally the variable regions of the immunodominant surface antigens, MgpB and MgpC, were analysed by PCR cloning and sequencing to evaluate sequence variation during infection.

Results Of the five primates inoculated cervically with MG, three were infected throughout the 8 weeks of the study, one maintained infection for 4 weeks and one resisted infection. Recovery of viable MG from lower reproductive tract sites was improved by co-culture in Vero cells followed by qPCR to measure an increase in MG genomes during culture. Growth in H broth, as determined by colour change proved an unreliable indicator of the presence of viable MG in the specimen possibly due to the presence of primate microorganisms that inhibit the growth of MG. No viable MG or MG DNA was detected in upper tract tissues in any of the primates perhaps suggesting that longer infection times or repeated inoculations are needed to achieve ascension in this model. Analysis of mgpB variable regions B and G indicated that after 8 weeks of infection the predominant expressed sequence changed from that of the G37C inoculum to 1 to 5 novel sequences consistent with recombination between the expression site and the MgpFars. In contrast, no sequence variation was observed in the inoculum grown in vitro for a similar duration. Antibodies reactive with MG antigens, including the variable regions of MgpB and MgpC, were detected by immunoblot and ELISA in serum and cervical exudates.

Conclusions The cervical inoculation model of pigtail macaques results in long-term infection and can be used to study the persistence of MG, development of antibodies and antigenic variation.

**A PRIMATE MODEL OF MYCOPLASMA GENITALIUM CERVICAL INFECTION**

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**Background** Mycoplasma genitalium (MG) was detected by immunoblot and ELISA in serum and cervical tissues that were grown in vitro for a similar duration. Antibodies reactive with MG were detected in serum and cervical exudates of infected women. However, sequence variation was observed in the inoculum grown in vitro for a similar duration. MyD-88 deficient mice may hold the promise of serving as one of the first useful murine models to study immunopathogenesis of T. pallidum infection. Abstract O4-S2.05 figure 1: representative epididymis sections from Day 21 sacrifice. Formalin-fixed tissues were stained with H&E as well as T. pallidum-specific immunohistochemical stain (IHC).