Vaginal gel component hydroxyethyl cellulose significantly enhances the infectivity of *Chlamydia trachomatis* serovars D and E

Running title: Hydroxyethyl cellulose enhances chlamydial infectivity

Tímea Raffai*, Katalin Burián*, László Janovákb, Anita Bogdanovc, Johannes H. Hegemannc;
Valéria Endrész, Dezső P. Virok*

a, Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary
Address: H-6720 Szeged, Dóm sqr. 10., Hungary

b, Department of Physical Chemistry and Materials Science, University of Szeged, Szeged, Hungary
Address: H-6720 Szeged, Rerrich Béla sq. 1., Hungary

c, Institute of Functional Microbial Genomics, Heinrich-Heine-University of Düsseldorf,
Address: D-40225 Düsseldorf, Universitätsstraße 1., Germany

*: contributed equally
#
: corresponding author

Address: Department of Medical Microbiology and Immunobiology, University of Szeged,
Szeged, Hungary
Address: H-6720 Szeged, Dóm sqr. 10., Hungary

email: virok.dezso.peter@med.u-szeged.hu

Phone: +36-62-545541
Abstract
The transmission of the urogenital serovars of *Chlamydia trachomatis* can be significantly influenced by vaginal gels. Hydroxyethyl cellulose is a commonly used gelling agent which can be found in vaginal gels. Hydroxyethyl cellulose showed a concentration dependent growth enhancing effect on *Chlamydia trachomatis* serovars D and E with a 26.1 fold maximal increase *in vitro* and a 2.57 fold increase *in vivo*.

Main text

*Chlamydia trachomatis* (*C. trachomatis*) urogenital serovars D-K related infections cause diseases such as urethritis, cervicitis and pelvic inflammatory disease, while serovars L1-L2 are at the background of the less common disease lymphogranuloma venereum, a sexually transmitted infection, with systemic rather than local manifestations. Among urogenital *Chlamydia*, D and E serovars are highly prevalent (1)(2)(3). *C. trachomatis* urogenital infections are globally among the most common sexually transmitted infections.

As an example, in 2016 1.598.354 *C. trachomatis* infections were reported in the US and the number of reported infections steadily increased from 2000 to 2016, reaching 497.3 cases per 100,000 population (4). The risk of *Chlamydia* transmission is greatly influenced by components of the cervicovaginal microenvironment including vaginal lactobacilli and indole-positive bacteria (5). Vaginal gels can be introduced into this microenvironment as lubricants or therapeutic gels. Vaginal gels are present during sexual intercourse and due to their spatial and temporal presence these gels may have a significant impact on the acquisition of *Chlamydia* infection and other sexually transmitted diseases. A major component of vaginal gels is the gelling agent itself. Hydroxyethyl cellulose (HEC) is a
commonly used gelling agent that can be found in lubricants and in therapeutic gels (6, 7).

To elucidate the potential impact of HEC on chlamydial transmission, we tested the effect of HEC on the growth of *C. trachomatis* D and E serovars.

HeLa 229 cells (ATCC) were placed into 96-well plates at a density of $4 \times 10^4$ cells/well in 100 µl of minimal essential medium (MEM) with Earle’s salts supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/l L-glutamine, 1x MEM vitamins, 1x non-essential amino acids, 0.005% Na-pyruvate, 25 µg/ml gentamycin, 1 µg/ml fungisone. The next day, the 90% confluent cells were infected with *C. trachomatis* D strain UW-3/CX (ATCC) and *C. trachomatis* serovar E strain DK20 (8). Before the infection, the chlamydial elementary bodies (EBs) were pre-incubated in HEC (European Pharmacopoeia 9.0 (9) quality, Molar Chemicals, Halásztelek, Hungary) dissolved in vaginal simulant buffer (NaCl 3.51 g/l; KOH 1.40 g/l; Ca(OH)$_2$ 0.222 g/l; bovine serum albumin 0.018 g/l; lactic acid 2.00 g/l; acetic acid 1.00 g/l; glycerol 0.16 g/l; urea 0.4 g/l; glucose 5.0 g/l) (10) and as a control, vaginal simulant buffer alone for 1 h 37°C, 5% CO$_2$. The HEC solutions were prepared by dissolving 30 mg of the HEC polymers in 1 ml of physiological salt solution (0.9% w/v NaCl), followed by 2-fold dilutions in the vaginal simulant (the applied HEC concentration range was 1.5 – 0.023% w/v). The pH of the vaginal simulant was adjusted to a pH 4.2 or pH 7.0.

The preincubated inocula were suspended in MEM supplemented with 0.5% w/v glucose, and the cells were infected at a multiplicity of infection of 8 for 60 min at 37°C, 5% CO$_2$ without centrifugation. After the infection, the cells were washed twice with PBS, and a culture medium containing 0.1 µg/ml cycloheximide was added. After a 48-hour incubation, the chlamydial genomic content was measured by quantitative PCR as described previously (11), the chlamydial inclusion count was measured by either standard manual or automatic
ChlamyCount immunofluorescent inclusion counting, as published earlier (12). Statistical evaluation of qPCR data was performed as described previously (11). All reagents were purchased from SIGMA, St. Louis, MO, USA, unless otherwise indicated.

To better mimic the cervicovaginal environment, we used the vaginal simulant to dilute HEC and incubate C. trachomatis EBs. The pH of the vaginal simulant was adjusted to a pH4.2 or pH7 to mimic the normal and elevated pH of the cervicovaginal tract. Figure 1A shows a HEC concentration-dependent enhancement of chlamydial growth after the preincubation of C. trachomatis EBs in pH4.2 vaginal fluid measured by qPCR 48 hours post infection. The C. trachomatis serovar D maximum growth increase was 23.7 fold at the maximal 1.5% w/v HEC concentration, and a noticeable, but non-significant growth enhancement tendency could be detected up to a concentration of 0.188% w/v HEC. HEC at pH7 enhanced the chlamydial growth significantly with a 13.8 fold growth increase at a concentration of 1.5% w/v (Figure 1B). Interestingly, in the case of C. trachomatis serovar E, the maximum growth increase (22.25 and 26.1 fold at pH 4.2 and pH7 respectively) was observed at the second highest HEC concentration (0.75% w/v) at both pH 4.2 and pH7 indicating a different HEC-EB interaction between the serovars (Figure 1A-B). To validate the qPCR results, we performed the automatic Chlamydia inclusion counting using the ChlamyCount measuring system, at pH4.2 or pH7 at 1.5% w/v and 0.75% w/v HEC concentrations for serovar D and serovar E respectively. Inclusion counts showed similar, albeit lower growth enhancement than the chlamydial genome measurements by qPCR with a 5.9-6.5 fold increase for serovar D and 5.95-6.05 fold increase for serovar E (Figure 1C). This difference is likely due to the fact, that ChlamyCount measures the chlamydial inclusion number, while qPCR measures the bacterial genome content of the inclusions.
To monitor the effect of HEC in vivo, 6-8 week old female BALB/c mice were treated s.c. with 2.5 mg medroxyprogesterone acetate (Pfizer, Budapest, Hungary) 1 week before infection. Mice were inoculated intravaginally with $1 \times 10^5$ inclusion forming units (IFU) of *C. trachomatis* serovar D mixed with HEC (1.5% w/v) or without HEC and recoverable IFUs in cervicovaginal washing 3 days post infection were counted by using traditional immunofluorescence microscopy (12) (Figure 1D). All experiments were approved by the Animal Welfare Committee of the University of Szeged and conform to the Directive 2010/63/EU of the European Parliament. The in vivo data also showed that HEC significantly increased the growth of *C. trachomatis* serovar D in the mouse genital tract, with a 2.57 fold enhancement 3 days post infection. It is important to note, that the chlamydial EBs were not preincubated with HEC before the infection, indicating an immediate growth enhancing effect of HEC in vivo.

Interestingly, our results are different from those of Sater et al. (13), who used the lymphogranuloma venereum strain *C. trachomatis* L2 and showed a concentration- and pH-dependent inhibitory effect of HEC on chlamydial growth in vitro. However, there are important differences between the two studies, including the fact that we used a complex buffer which may mimic better the physicochemical properties of the vaginal fluid than the phosphate and acetate buffers used by Sater et al.. Moreover, we observed the growth enhancing effect at 1.5-0.75% w/v (15000-7500 μg/ml) HEC concentrations, the concentrations that are common in the vaginal gels (7)(14), while Sater et al. used significantly lower HEC concentrations (2-200 μg/ml). Instead of serovar L2, we also used the more prevalent urogenital serovars D and E. While *C. trachomatis* D and L2 have minor genetic differences (15), there are several phenotypic differences between the two
serotypes. Previous studies showed that their early interactions with epithelial cells are different (16, 17), including the fact, that the centrifugation and dextrane pretreatment of host epithelial cells increased the infection efficacy of urogenital C. trachomatis serovars but had no impact on serovar L2. In addition, serovar E infection is heparin independent while serovar L2 infection exhibits a strong heparin dependency (18). Since probably HEC influences the early interactions between the EBs and the host cells, this effect may be different between the lymphogranuloma venereum and urogenital serovars.

Altogether, our study shows that vaginal gel components, such as the gelling agent HEC have a significant growth enhancing effect on two prevalent C. trachomatis urogenital serovars. This enhancing effect was observed in vitro over a wide pH range, at lower concentrations, and also in vivo. Since the growth enhancement can theoretically lower the minimal number of bacteria required for infection transmission, these results suggest the need for testing current and future vaginal gels to determine their growth enhancing effects on C. trachomatis and on other sexually transmitted pathogens.

Funding
The study was supported by the EFOP-3.6.1-16-2016-00008 European Union – Hungary grant.

Conflict of interest
The authors declare that they have no competing interests.

References


**Figure Legend**

**Figure 1.** Impact of HEC on the growth of *C. trachomatis* serovars D and E in HeLa 229 cells in vitro at (A) pH4.2 and (B) pH7. Bacterial genome copy numbers were measured by direct qPCR (n=3). The qPCR data were validated by the ChlamyCount immunofluorescent automatic inclusion counting system (n=4). The images of the ChlamyCount processed wells and the counted inclusion numbers are shown (C). Recoverable *C. trachomatis* serovar D IFU in cervicovaginal swab samples 3 days post infection. Mice were infected intravaginally with *C. trachomatis* serovar D mixed with HEC (1.5% w/v) (n=7) or without HEC (n=5). Data are means +/- standard deviations. *: P < 0.05, **: P < 0.01 according to Student’s t-test.