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

Running title: Hydroxyethyl cellulose enhances chlamydial infectivity

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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

47 commonly used gelling agent that can be found in lubricants and in therapeutic gels (6, 7).
48 To elucidate the potential impact of HEC on chlamydial transmission, we tested the effect of
49 HEC on the growth of *C. trachomatis* D and E serovars.

50 HeLa 229 cells (ATCC) were placed into 96-well plates at a density of 4 x 10^4 cells/well
51 in 100 µl of minimal essential medium (MEM) with Earle's salts supplemented with 10%
52 heat-inactivated fetal bovine serum, 2 mmol/l L-glutamine, 1x MEM vitamins, 1x non-
53 essential amino acids, 0.005% Na-pyruvate, 25 µg/ml gentamycin, 1 µg/ml fungisone. The
54 next day, the 90% confluent cells were infected with *C. trachomatis* D strain UW-3/CX (ATCC)
55 and *C. trachomatis* serovar E strain DK20 (8). Before the infection, the chlamydial
56 elementary bodies (EBs) were pre-incubated in HEC (European Pharmacopoeia 9.0 (9)
57 quality, Molar Chemicals, Halásztelek, Hungary) dissolved in vaginal simulant buffer (NaCl
58 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;
59 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,
60 vaginal simulant buffer alone for 1h 37°C, 5% CO_2. The HEC solutions were prepared by
61 dissolving 30 mg of the HEC polymers in 1 ml of physiological salt solution (0.9% w/v NaCl),
62 followed by 2-fold dilutions in the vaginal simulant (the applied HEC concentration range
63 was 1.5 – 0.023% w/v). The pH of the vaginal simulant was adjusted to a pH 4.2 or pH 7.0.
64 The preincubated inocula were suspended in MEM supplemented with 0.5% w/v glucose,
65 and the cells were infected at a multiplicity of infection of 8 for 60 min at 37°C, 5% CO_2
66 without centrifugation. After the infection, the cells were washed twice with PBS, and a
67 culture medium containing 0.1 µg/ml cycloheximide was added. After a 48-hour incubation,
68 the chlamydial genomic content was measured by quantitative PCR as described previously
69 (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.

93 To monitor the effect of HEC *in vivo*, 6-8 week old female BALB/c mice were treated s.c. with
94 2.5 mg medroxyprogesterone acetate (Pfizer, Budapest, Hungary) 1 week before infection.
95 Mice were inoculated intravaginally with $1x10^5$ inclusion forming units (IFU) of *C.*
96 *trachomatis* serovar D mixed with HEC (1.5% w/v) or without HEC and recoverable IFUs in
97 cervicovaginal washing 3 days post infection were counted by using traditional
98 immunofluorescence microscopy (12) (Figure 1D). All experiments were approved by the
99 Animal Welfare Committee of the University of Szeged and conform to the Directive
100 2010/63/EU of the European Parliament. The *in vivo* data also showed that HEC significantly
101 increased the growth of *C. trachomatis* serovar D in the mouse genital tract, with a 2.57 fold
102 enhancement 3 days post infection. It is important to note, that the chlamydial EBs were not
103 preincubated with HEC before the infection, indicating an immediate growth enhancing
104 effect of HEC *in vivo*.

105 Interestingly, our results are different from those of Sater et al. (13), who used the
106 lymphogranuloma venereum strain *C. trachomatis* L2 and showed a concentration- and pH-
107 dependent inhibitory effect of HEC on chlamydial growth *in vitro*. However, there are
108 important differences between the two studies, including the fact that we used a complex
109 buffer which may mimic better the physicochemical properties of the vaginal fluid than the
110 phosphate and acetate buffers used by Sater et al.. Moreover, we observed the growth
111 enhancing effect at 1.5-0.75% w/v (15000-7500 μg/ml) HEC concentrations, the
112 concentrations that are common in the vaginal gels (7)(14), while Sater et al. used
113 significantly lower HEC concentrations (2-200 μg/ml). Instead of serovar L2, we also used
114 the more prevalent urogenital serovars D and E. While *C. trachomatis* D and L2 have minor
115 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.

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Conflict of interest

The authors declare that they have no competing interests.

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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.