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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

Vaginal Gel Component Hydroxyethyl Cellulose Significantly Enhances the Infectivity of *Chlamydia trachomatis* Serovars D and E

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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 that can be found in vaginal gels. Hydroxyethyl cellulose showed a concentration-dependent growth-enhancing effect on *C. trachomatis* serovars D and E, with a 26.1-fold maximal increase *in vitro* and a 2.57-fold increase *in vivo*.

KEYWORDS *Chlamydia trachomatis*, hydroxyethyl cellulose, infectivity, vaginal gel

Chlamydia trachomatis urogenital serovars D-K-related infections cause diseases such as urethritis, cervicitis, and pelvic inflammatory disease, while serovars L1 and 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*, serovars D and E are highly prevalent (1–3). *C. trachomatis* urogenital infections are globally among the most common sexually transmitted infections. For example, in 2016, 1,598,354 *C. trachomatis* infections were reported in the United States, and the number of reported infections steadily increased from 2000 to 2016, reaching 497.3 cases per 100,000 people (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* serovars D and E.

HeLa 229 cells (ATCC) were placed into 96-well plates at a density of 4×10^4 cells/well in 100 μl of minimal essential medium (MEM) with Earle's salts supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/liter L-glutamine, 1× MEM vitamins, 1× nonessential amino acids, 0.005% Na-pyruvate, 25 μg/ml gentamicin, and 1 μg/ml Fungizone. The next day, the 90% confluent cells were infected with *C. trachomatis* serovar D strain UW-3/CX (ATCC) and *C. trachomatis* serovar E strain DK20 (8). Before the infection, the chlamydial elementary bodies (EBs) were preincubated in HEC (European Pharmacopoeia 9.0 quality [9], Molar Chemicals, Halásztelek, Hungary) dissolved in vaginal simulant buffer [NaCl 3.51 g/liter, KOH 1.40 g/liter, $Ca(OH)_2$ 0.222 g/liter, bovine serum albumin 0.018 g/liter, lactic acid 2.00 g/liter, acetic acid 1.00 g/liter, glycerol 0.16 g/liter, urea 0.4 g/liter, glucose 5.0 g/liter] and vaginal simulant buffer

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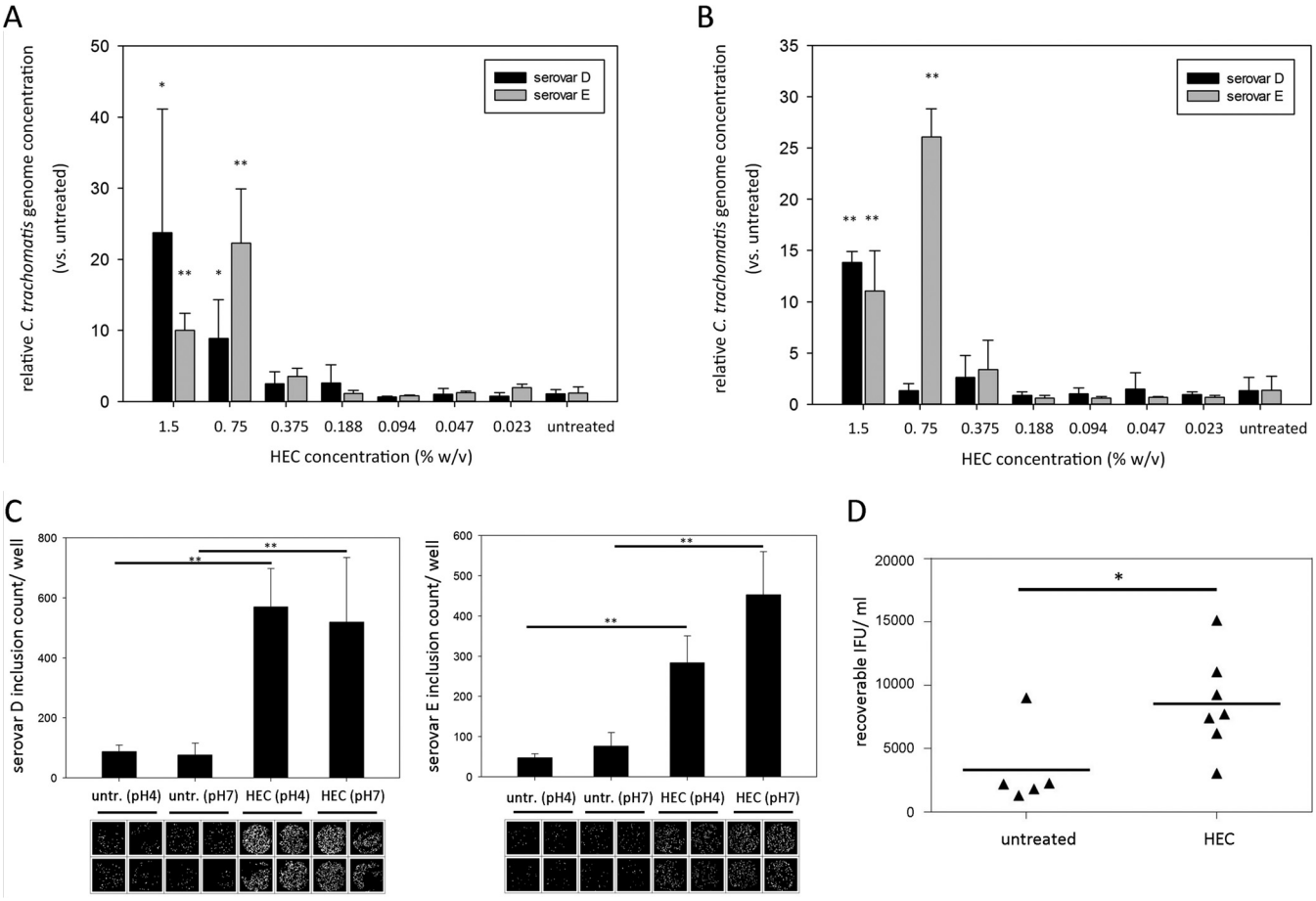

FIG 1 Impact of HEC on the growth of *C. trachomatis* serovars D and E in HeLa 229 cells *in vitro* at pH 4.2 (A) and pH 7 (B). Bacterial genome copy numbers were measured by direct qPCR ($n = 3$). The qPCR data were validated by the ChlamyCount immunofluorescent automatic inclusion counting system ($n = 4$). (C) The images of the ChlamyCount-processed wells and the counted inclusion numbers. (D) Recoverable *C. trachomatis* serovar D IFU in cervicovaginal swab samples 3 days postinfection. Mice were infected intravaginally with *C. trachomatis* serovar D mixed with HEC (1.5% wt/vol) ($n = 7$) or without HEC ($n = 5$). Data are means ± standard deviations. *, $P < 0.05$; **, $P < 0.01$, according to Student's *t* test.

alone as a control, for 1 h at 37°C, 5% CO_2 (10). The HEC solutions were prepared by dissolving 30 mg of the HEC polymers in 1 ml vaginal simulant, followed by 2-fold dilutions (the applied HEC concentration range was 1.5–0.023% wt/vol). The pH of the vaginal simulant was adjusted to a pH of 4.2 or 7.0. The preincubated inocula were suspended in MEM supplemented with 0.5% wt/vol 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 infection, the cells were washed twice with phosphate-buffered saline, and a culture medium containing 0.1 μg/ml cycloheximide was added. After 48 h incubation, the chlamydial genomic content was measured by quantitative PCR (qPCR) as described previously (11), and the chlamydial inclusion count was measured by 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), 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 4.2 or 7 to mimic the normal and elevated pHs of the cervicovaginal tract. Figure 1A shows HEC concentration-dependent enhancement of chlamydial growth after the preincubation of *C. trachomatis* EBs in pH 4.2 vaginal fluid measured by qPCR 48 h postinfection. The *C. trachomatis* serovar D maximum growth increase was 23.7-fold at the maximal 1.5% wt/vol HEC concentration, and a noticeable but nonsig-

nificant growth enhancement tendency could be detected up to a concentration of 0.188% wt/vol HEC. HEC at pH 7 enhanced the chlamydial growth significantly, with a 13.8-fold growth increase at a concentration of 1.5% wt/vol (Fig. 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 7, respectively) was observed at the second-highest HEC concentration (0.75% wt/vol) at pH 4.2 and 7, indicating a different HEC-EB interaction between the serovars (Fig. 1A and B). To validate the qPCR results, we performed the automatic *Chlamydia* inclusion counting using the ChlamyCount measuring system, at pH 4.2 or 7 at 1.5% wt/vol and 0.75% wt/vol HEC concentrations for serovar D and serovar E, respectively. Inclusion counts showed similar albeit less growth enhancement than the chlamydial genome measurements by qPCR, with a 5.9- to 6.5-fold increase for serovar D and a 5.95- to 6.05-fold increase for serovar E (Fig. 1C). This difference is likely due to the fact that ChlamyCount measures the chlamydial inclusion number, whereas qPCR measures the bacterial genome content of the inclusions.

To monitor the effect of HEC *in vivo*, 6- to 8-week-old female BALB/c mice were treated subcutaneously with 2.5 mg medroxyprogesterone acetate (Pfizer, Budapest, Hungary) 1 week before infection. Mice were inoculated intravaginally with 1×10^5 inclusion forming units (IFUs) of *C. trachomatis* serovar D mixed with HEC (1.5% wt/vol) or without HEC, and recoverable IFUs in cervicovaginal washing 3 days postinfection were counted by using traditional immunofluorescence microscopy (12) (Fig. 1D). All experiments were approved by the Animal Welfare Committee of the University of Szeged and conformed to 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 postinfection. It is important to note that the chlamydial EBs were not preincubated with HEC before infection, indicating an immediate growth-enhancing effect of HEC *in vivo*.

Interestingly, our results differ from those of Sater et al. (13), who used the lymphogranuloma venereum strain *C. trachomatis* L2 and showed concentration- and pH-dependent inhibitory effects 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 that may better mimic 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 0.75% to 1.5% wt/vol (7,500–15,000 μg/ml) HEC concentrations, which are common in the vaginal gels (7, 14), whereas 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 centrifugation and dextran 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, whereas serovar L2 infection exhibits a strong heparin dependency (18). Because HEC probably 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 range of pHs, at lower concentrations, and *in vivo*. Because 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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We declare that we have no competing interests.

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