

1 Vaginal gel component hydroxyethyl cellulose significantly enhances the infectivity of

2 *Chlamydia trachomatis* serovars D and E

3 **Running title:** Hydroxyethyl cellulose enhances chlamydial infectivity

4

5 Tímea Raffai^{a*}, Katalin Burián^{a*}, László Janovák^b, Anita Bogdanov^a, Johannes H. Hegemann^c,

6 Valéria Endrész^a, Dezső P. Virok^{a#}

7

8 ^a, Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged,

9 Hungary Address: H-6720 Szeged, Dóm sqr. 10., Hungary

10 ^b, Department of Physical Chemistry and Materials Science, University of Szeged, Szeged,

11 Hungary Address: H-6720 Szeged, Rerrich Béla sq. 1., Hungary

12 ^c, Institute of Functional Microbial Genomics, Heinrich-Heine-University of Düsseldorf,

13 Address: D-40225 Düsseldorf, Universitätsstraße 1., Germany

14 *: contributed equally

15

16

17 #: corresponding author

18 Address: Department of Medical Microbiology and Immunobiology, University of Szeged,

19 Szeged, Hungary

20 Address: H-6720 Szeged, Dóm sqr. 10., Hungary

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

22 Phone: +36-62-545541

23

24 **Abstract**

25 The transmission of the urogenital serovars of *Chlamydia trachomatis* can be significantly
26 influenced by vaginal gels. Hydroxyethyl cellulose is a commonly used gelling agent which
27 can be found in vaginal gels. Hydroxyethyl cellulose showed a concentration dependent
28 growth enhancing effect on *Chlamydia trachomatis* serovars D and E with a 26.1 fold
29 maximal increase *in vitro* and a 2.57 fold increase *in vivo*.

30

31 **Main text**

32 *Chlamydia trachomatis* (*C. trachomatis*) urogenital serovars D-K related infections
33 cause diseases such as urethritis, cervicitis and pelvic inflammatory disease, while serovars
34 L1-L2 are at the background of the less common disease lymphogranuloma venereum, a
35 sexually transmitted infection, with systemic rather than local manifestations. Among
36 urogenital *Chlamydia*, D and E serovars are highly prevalent (1)(2)(3). *C. trachomatis*
37 urogenital infections are globally among the most common sexually transmitted infections.
38 As an example, in 2016 1.598.354 *C. trachomatis* infections were reported in the US and the
39 number of reported infections steadily increased from 2000 to 2016, reaching 497.3 cases
40 per 100.000 population (4). The risk of *Chlamydia* transmission is greatly influenced by
41 components of the cervicovaginal microenvironment including vaginal lactobacilli and
42 indole-positive bacteria (5). Vaginal gels can be introduced into this microenvironment as
43 lubricants or therapeutic gels. Vaginal gels are present during sexual intercourse and due to
44 their spatial and temporal presence these gels may have a significant impact on the
45 acquisition of *Chlamydia* infection and other sexually transmitted diseases. A major
46 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 4×10^4 cells/well in 100 μ l 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 μ g/ml gentamycin, 1 μ g/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 1h 37°C, 5% CO₂. 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% CO₂ without centrifugation. After the infection, the cells were washed twice with PBS, and a culture medium containing 0.1 μ g/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

70 ChlamyCount immunofluorescent inclusion counting, as published earlier (12). Statistical
71 evaluation of qPCR data was performed as described previously (11). All reagents were
72 purchased from SIGMA, St. Louis, MO, USA, unless otherwise indicated.

73 To better mimic the cervicovaginal environment, we used the vaginal simulant to dilute HEC
74 and incubate *C. trachomatis* EBs. The pH of the vaginal simulant was adjusted to a pH4.2 or
75 pH7 to mimic the normal and elevated pH of the cervicovaginal tract. Figure 1A shows a HEC
76 concentration-dependent enhancement of chlamydial growth after the preincubation of *C.*
77 *trachomatis* EBs in pH4.2 vaginal fluid measured by qPCR 48 hours post infection. The *C.*
78 *trachomatis* serovar D maximum growth increase was 23.7 fold at the maximal 1.5% w/v
79 HEC concentration, and a noticeable, but non-significant growth enhancement tendency
80 could be detected up to a concentration of 0.188% w/v HEC. HEC at pH7 enhanced the
81 chlamydial growth significantly with a 13.8 fold growth increase at a concentration of 1.5%
82 w/v (Figure 1B). Interestingly, in the case of *C. trachomatis* serovar E, the maximum growth
83 increase (22.25 and 26.1 fold at pH 4.2 and pH7 respectively) was observed at the second
84 highest HEC concentration (0.75% w/v) at both pH 4.2 and pH7 indicating a different HEC-EB
85 interaction between the serovars (Figure 1A-B). To validate the qPCR results, we performed
86 the automatic *Chlamydia* inclusion counting using the ChlamyCount measuring system, at
87 pH4.2 or pH7 at 1.5% w/v and 0.75% w/v HEC concentrations for serovar D and serovar E
88 respectively. Inclusion counts showed similar, albeit lower growth enhancement than the
89 chlamydial genome measurements by qPCR with a 5.9-6.5 fold increase for serovar D and
90 5.95-6.05 fold increase for serovar E (Figure 1C). This difference is likely due to the fact, that
91 ChlamyCount measures the chlamydial inclusion number, while qPCR measures the bacterial
92 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 1×10^5 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 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$). 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

116 serotypes. Previous studies showed that their early interactions with epithelial cells are
117 different (16, 17), including the fact, that the centrifugation and dextrane pretreatment of
118 host epithelial cells increased the infection efficacy of urogenital *C. trachomatis* serovars but
119 had no impact on serovar L2. In addition, serovar E infection is heparin independent while
120 serovar L2 infection exhibits a strong heparin dependency (18). Since probably HEC
121 influences the early interactions between the EBs and the host cells, this effect may be
122 different between the lymphogranuloma venereum and urogenital serovars.

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

130

131 **Funding**

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

134

135 **Conflict of interest**

136 The authors declare that they have no competing interests.

137

138 **References**

- 139 1. Bošnjak Z, Džijan S, Pavlinić D, Perić M, Ružman N, Križan IR, Lauc G, Antolović-Požgain A,
140 Burazin J, Vuković D. 2012. Distribution of *Chlamydia trachomatis* serotypes in clinical
141 urogenital samples from north-eastern Croatia. *Curr Microbiol* 64:552–560.
- 142 2. Wikström E, Surcel H-M, Merikukka M, Ohman H, Namujju PB, Tasanen K, Tiitinen A,
143 Paavonen J, Lehtinen M. 2014. Changes over time in the *Chlamydia trachomatis* serotype
144 distribution in Finnish women. *Scand J Infect Dis* 46:397–400.
- 145 3. Gao X, Chen X-S, Yin Y-P, Zhong M-Y, Shi M-Q, Wei W-H, Chen Q, Peeling RW, Mabey D.
146 2007. Distribution study of *Chlamydia trachomatis* serovars among high-risk women in
147 China performed using PCR-restriction fragment length polymorphism genotyping. *J Clin*
148 *Microbiol* 45:1185–1189.
- 149 4. 2016 Sexually Transmitted Diseases Surveillance. Centers for Disease Control and
150 Prevention.
- 151 5. Aiyar A, Quayle AJ, Buckner LR, Sherchand SP, Chang TL, Zea AH, Martin DH, Belland RJ.
152 2014. Influence of the tryptophan-indole-IFN γ axis on human genital *Chlamydia*
153 *trachomatis* infection: role of vaginal co-infections. *Front Cell Infect Microbiol* 4:72.
- 154 6. Lai BE, Geonnotti AR, Desoto MG, Montefiori DC, Katz DF. 2010. Semi-solid gels function
155 as physical barriers to human immunodeficiency virus transport in vitro. *Antiviral Res*
156 88:143–151.
- 157 7. Mahalingam A, Simmons AP, Ugaonkar SR, Watson KM, Dezzutti CS, Rohan LC, Buckheit
158 RW, Kiser PF. 2011. Vaginal microbicide gel for delivery of IQP-0528, a pyrimidinedione

- 159 analog with a dual mechanism of action against HIV-1. Antimicrob Agents Chemother
160 55:1650–1660.
- 161 8. Eder T, Kobus S, Stallmann S, Stepanow S, Köhrer K, Hegemann JH, Rattei T. 2017.
162 Genome sequencing of Chlamydia trachomatis serovars E and F reveals substantial
163 genetic variation. Pathog Dis 75.
- 164 9. European Pharmacopoeia (Ph. Eur.) 9th Edition.
- 165 10. Owen DH, Katz DF. 1999. A vaginal fluid simulant. Contraception 59:91–95.
- 166 11. Eszik I, Lantos I, Önder K, Somogyvári F, Burián K, Endrész V, Virok DP. 2016. High
167 dynamic range detection of Chlamydia trachomatis growth by direct quantitative PCR of
168 the infected cells. J Microbiol Methods 120:15–22.
- 169 12. Bogdanov A, Endrész V, Urbán S, Lantos I, Deák J, Burián K, Önder K, Ayaydin F, Balázs P,
170 Virok DP. 2014. Application of DNA chip scanning technology for automatic detection of
171 Chlamydia trachomatis and Chlamydia pneumoniae inclusions. Antimicrob Agents
172 Chemother 58:405–413.
- 173 13. Sater AAA, Ojcius DM, Meyer MP. 2008. Susceptibility of Chlamydia trachomatis to the
174 excipient hydroxyethyl cellulose: pH and concentration dependence of antimicrobial
175 activity. Antimicrob Agents Chemother 52:2660–2662.
- 176 14. Gao Y, Yuan A, Chuchuen O, Ham A, Yang KH, Katz DF. 2015. Vaginal deployment and
177 tenofovir delivery by microbicide gels. Drug Deliv Transl Res 5:279–294.

- 178 15. Thomson NR, Holden MTG, Carder C, Lennard N, Lockey SJ, Marsh P, Skipp P, O'Connor
179 CD, Goodhead I, Norbertzack H, Harris B, Ormond D, Rance R, Quail MA, Parkhill J,
180 Stephens RS, Clarke IN. 2008. Chlamydia trachomatis: genome sequence analysis of
181 lymphogranuloma venereum isolates. Genome Res 18:161–171.
- 182 16. Davis CH, Wyrick PB. 1997. Differences in the association of Chlamydia trachomatis
183 serovar E and serovar L2 with epithelial cells in vitro may reflect biological differences in
184 vivo. Infect Immun 65:2914–2924.
- 185 17. Kuo CC, Grayston T. 1976. Interaction of Chlamydia trachomatis organisms and HeLa 229
186 cells. Infect Immun 13:1103–1109.
- 187 18. Hegemann J H, Moelleken, K. 2012. Chlamydial adhesion and adhesins Intracellular
188 Pathogens I: Chlamydiales. ASM press, Washington DC.

189
190 **Figure Legend**

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

199

