Concomitant and productive genital infections by HSV-2 and HPV in two young women: a case report

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Abstract

Human papillomaviruses (HPVs), the most oncogenic virus known to humans, are often associated with Herpes Simplex Virus-2 (HSV-2) infections. The involvement of the latter in cervical cancer is controversial but its long-term infections might modulate the mucosal microenvironment in a way that favors carcinogenesis. We know little about coinfections between HSV-2 and HPVs, and studying the immunological and microbiological dynamics in the early stages of these infections may help identify or rule out potential interactions. We report two cases of concomitant productive, although asymptomatic, HSV-2 and HPV infections in young women (aged 20 and 25). The women were followed up for approximately a year, with clinical visits every two months and weekly self-samples. We performed quantitative analyses of their HSV-2 and HPV viral loads, immunological responses (IgG and IgM antibodies and local cytokines expression profiles), vaginal microbiota composition, as well as demographic and behavior data. We detect interactions between virus loads, immune response, and the vaginal microbiota, which improve our understanding of HSV-2 and HPVs' coinfections and calls for further investigation with larger cohorts.

Keywords: Human papillomavirus; Herpes simplex virus-2; co-infection; interaction; vaginal microbiota; qPCR.

Introduction

Herpes simplex virus (HSV-2) and human papillomavirus (HPV) are among the most common sexually transmitted infections (STIs) and are often found in coinfections [1]. Both viruses can cause genital infections. While HPV targets the basal layer of the epithelium, HSV-2 replicates in genital epithelial cells and establishes lifelong latency in the sacral ganglia [2]. Conversely, most HPV genital infections are cleared within 2 years, approximately 3.8% of them persist and can lead to carcinogenic lesions, making HPV the most oncogenic virus known to humans [3].

The high prevalence of coinfections between HSV-2 and HPV could result from sexual behavior but could also be caused by within-host interactions, especially because HSV-2 is a lytic virus. Frequent HSV-2 episodes could potentially modify the barrier effect of the cervical mucus and the stratified epithelium, or trigger an inflammatory immune response, both of which could enable HPV to access the basal layer. These facts have been fueling a long and controversial debate about the potential role of the interaction between these viruses in carcinogenesis [4].

Another potential component which could impact the possible interactions of HSV-2 and HPV is the vaginal microbiota. Certain profiles have been associated with HSV-2 and HPV infections [5–7]. Its diversity is limited to five main Community State Types (CSTs) [8] (see Supplementary Materials for details). The immunological and microbial microenvironment led by HSV-2 reactivations or HPV infections could favor the acquisition and/or persistence and reactivation of each other.

We currently lack quantitative studies analyzing HSV-2 and HPV coinfections, especially in the early stages of infections. This is unfortunate because a better understanding of the immune response and the microbiota dynamics could help better assess potential interactions between these viruses.

We report two cases of concomitant productive HSV-2 and HPV infections in women aged 20 and 25. These were enrolled in the PAPCLEAR longitudinal cohort, which was

designed to study HPV within-host kinetics (see [9] for details). Participants visited the clinic every two or four months. Samples were collected by a gynecologist or a midwife and participants also filled out questionnaires about their life and sexual behaviors. Furthermore, between visits, participants performed weekly or biweekly self-samples at home. 35 out of the 188 PAPCLEAR participants were found to be seropositive for HSV-2 at inclusion using a multiplex serology assay [10] (see Supplementary Materials for details). This prevalence of 18.6% is at the high end of the European estimates (95% confidence interval of 5.0 to 21.7% according to [1]). The presence of coinfections is also consistent with the relatively high prevalence of HPV infections at inclusion [11] and can be explained by the PAPCLEAR cohort having more sexual partners than the average of the population.

By analyzing all the clinic samples of the seropositive women by a quantitative Polymerase Chain Reaction (qPCR) assay, we detected two productive HSV-2 infections, which this case study presents in detail. In particular, we describe and analyze HSV-2 and HPV viral load dynamics, the immunological responses (circulating IgG and IgM antibodies and cytokines titration), the vaginal microbiota compositions, the vaginal pH, and data about demographic and sexual behavior.

Case report

Case A

The first case was a 20-year-old woman (Table 1). Although she reported a history of recurrent HSV-2 infections, she was asymptomatic during the follow-up. She was not vaccinated against HPV.

We performed qPCR for HSV-2 and HPV genotypes detected in the women upon screening. These values were normalized using the number of albumin copies in the samples (see Supplementary Materials for details about the methods used).

We detected a virus load of HSV-2 DNA ($1 \times 10^{-}2$ copies per cell) at the inclusion

visit. This was followed by an absence of detection for 100 days (Figure 1A). We then detected multiple samples with increasing HSV-2 virus loads between days of follow-up (DoF) 108 and 182, with a plateau starting around DoF 125. The virus loads at the plateau ranged from 1.2×10^1 to 2.3×10^3 HSV copies per cell. This 10-week episode contains samples in which HSV-2 was not detected which correspond to self-samples, that can have lower quality. Conversely, there were 5 self samples and 2 clinic samples negative for HSV-2 in a row after the inclusion visit so we conclude we have two independent reactivation events.

At inclusion, the cytology-based screening for precancerous and cancerous cervical lesions on Thinprep medium yielded a normal result. Given the persistence of the HPV infection, a second screening was performed 12 months later that also found a normal result.

Throughout the follow-up, the case was concomitantly infected with HPV31 with a viral load of approximately 2.4×10^4 copies per cell. The last two detections of HSV-2 viral DNA coincided with a new infection by HPV66. HPV31 and HPV66 infections persisted until the end of the follow-up. Furthermore, after HSV-2 clearance, HPV31 virus load steadily decreased by one order of magnitude in 150 days to approximately 2.7×10^4 copies per cell (Figure 1A).

We characterized vaginal microbiota communities using metabarcoding of the 16S RNA. We performed taxonomy and CSTs assignments with the speciateIT and Valencia software packages [12], respectively. At inclusion, the vaginal microbiota was mainly dominated by *Lactobacillus iners* with a minority of *Lactobacillus jensenii* (Figures 1C, and D). Strikingly, the increase in HSV-2 viral load from DoF 102 was accompanied by a switch from CST III to CST IV, with a more diverse profile dominated by facultative anaerobic species such as *Gardnerella vaginalis* and *Atopobium vaginae*. The microbial diversity, characterized by the Shannon index, was also higher during the reactivation (Figure 1B). We lack microbiota data between the two HSV-2 reactivation events but we

can detect elevated vaginal pH levels (above 4.5), which could indicate variations in CSTs (Figure 1H). The last two detections of HSV-2 DNA and the onset of HPV66 infection (at DoF 168 and 182) coincided with a shift to CST III with a dominance of *Lactobacillus iners* (Figures 1A, C, and D). The end of the reactivations and establishment of HPV66 infection, starting from DoF 207, was associated with a shift to CST I and a decrease in microbial diversity.

Note that the participant reported repetitive topical and gynecological mycosis treatment between the DoF 207 and 275 (Supplementary table 2).

To characterize the immune response, we measured circulating blood antibodies (IgM and IgG) with a multiplex serology assay targeting HSV-2, some HPV genotypes, and Varicella Zoster Virus (Human alphaherpesvirus 3, VZV) as a control. The threshold for seropositivity for each antibody type is shown with a dashed line in figure 1E. On DoF 164, we saw a strong increase in the titers of circulating anti-HSV-2 IgM, which was consistent with the second HSV-2 reactivation episode. This was followed by an increase in circulating anti-HSV-2 IgG titers from DoF 217 to 346.

From DoF 0 to 94, we observed a slight increase in anti-HPV31 IgM titers in response to HPV31 infection. Anti-HPV31 IgGs did not vary during the follow-up. Anti-HPV66 antibodies were not available in the multiplex panel used.

To study the local immune response, we collected cervicovaginal secretions using ophthalmic sponges and dosed five cytokines using MesoScale discovery (MSD) technology (see [13] and Supplementary Materials for details). The most striking pattern was an increase in IL-17A between days 94 and 164, i.e. during the second HSV-2 reactivation episode. We also observed a less intense decrease in IP-10 and MIP-3α and a slighter increase of the pro-inflammatory cytokine IL-1α during the same time period. In the fifth sample, all the cytokines measured increased simultaneously and reached their peak. Since the detailed follow-up does not point to any specific event, this could be attributed to an issue with this particular sample (Figure 1F).

We collected demographic and behavioral information about the participants via questionnaires and interviews with the gynecologist or the midwife at the clinic. Case A reported high stress levels in a row, from DoF 63 to 94, before the second HSV-2 reactivation event. On the other hand, the reporting of a new sexual partner on DoF 164 and sexual intercourse on DoF 143 and 168 coincided with the acquisition of HPV66 (Figures 1G, J and K).

Case B

The second case was a 25-year-old woman (Table 1). Although she was seropositive for HSV-2, she did not report any past symptoms of the infection (Figure 2E). She was not vaccinated against HPV.

We detected an HPV52 infection at the inclusion which persisted until the DoF 154 with a relatively constant viral load (average of 1.2 copies per cell on average). We observed an HSV-2 reactivation at DoF 126 that was followed by two samples with increasing viral loads at DoF 154 and 217 (Figure 2A)). The exponential increase between the viral loads at these three visits, with $4.6 \times 10^{-}2$, $4.1 \times 10^{-}2$, and $42.4 \times 10^{-}2$ copies per cell respectively, suggests that they could belong to the same reactivation episode. Note that viral loads were lower than for case A for both viruses. Finally, the end of the HSV-2 reactivation event and of the HPV52 infection appeared to occur simultaneously (Figure 2A).

At inclusion, a trained pathologist diagnosed an ASC-US from the liquid cytology (Thinprep medium). Given that the HPV infection cleared, we did not perform a cytology 12 months later.

At inclusion, Case B had a vaginal microbiota dominated by *Lactobacillus iners* (i.e. CST III, Figures 2C and D) with rather elevated values of vaginal pH (Figure 2G). The HSV-2 reactivation occurred in a *Lactobacillus crispatus*-dominated CST, i.e. CST I, which was followed by a shift to CST V, but with a high abundance of *Gardnerella*

vaginalis (Figure 2A and C).

On the immunological side, the most striking patterns were a peak in anti-HV52 IgG on DoF 210 and IgM on DoF 273 that is right before and right after HPV52 clearance (Figure 2E). This pattern is unexpected for an acute infection but since the participant was infected at inclusion, we have no means to tell how long she had been infected for. The HSV-2 reactivation episode was also accompanied by a peak in anti-HSV-2 IgM titers on DoF 154, while IgG titers remained constant during the follow-up. This could be related to her low virus load not triggering a strong immune response (Figure 2E).

As for Case B, she reported elevated stress levels for three weeks in a row just before the HSV-2 reactivation episode (Figure 2F). She also reported a new partner on DoF 95 and 154, that is right before and right after the HSV-2 reactivation episode, and reported having sexual intercourse on DoF 77, before HSV-2 reactivation on DoF 126 (Figure 2I).

As for Case B, the women reported elevated stress levels for three weeks in a row, having sexual intercourse on DoF 77, and a new partner on DoF 95, that is right before HSV-2 reactivation on DoF 126. She also reported a new partner on DoF 154, right after the HSV-2 reactivation Figures 2F, I and J).

Discussion

Coinfections by HPV and HSV-2 are common and their potential impacts on public health are poorly known [1]. We reported a detailed follow-up of two cases of such productive coinfections in young women enrolled in the PAPCLEAR cohort [9]. The originality of this report, in addition to the coinfection itself, resides in the resolution of the longitudinal follow-up data and the stage of the HPV infections.

Our work highlights potential interactions between HSV-2 and HPVs. For instance, the end of the HSV-2 reactivation episode coincides with a decrease in HPV31 virus load in Case A and with a clearance of HPV52 in Case B. Moreover, in Case A, the decrease

in HPV31 could be linked to the acquisition of a new infection by HPV66 (although interactions between HPV genotypes are poorly known [14]).

The vaginal microbiota seemed to be more affected by HSV2 than by HPV infections. In Case A, the HSV-2 reactivation was clearly associated with a shift towards a CST IV with the domination of anaerobic bacteria including *Gardnerella vaginalis*. After the end of the reactivation, the microbiota returned to a *Lactobacillus*-dominated CST, which could be linked to mycosis treatments with imidazole and terbinafine. For Case B, we observed a similar pattern with an increase in the proportion of *Gardnerella vaginalis* bacteria in the vaginal microbiota during the HSV-2 reactivation episode. This is consistent with earlier work showing that HSV-2 is often associated with a dysbiotic microbiota [6], whereas the relationship between HPV infection and vaginal microbiota is more limited (but see [15]).

On the immunological side, in both cases, HSV-2 reactivations were associated with an increase in the titers of anti-HSV-2 circulating IgMs. In Case A, where the virus load was higher, we also observed a strong increase in IgG as well. For HPVs, the decrease in HPV31 virus load coincided with an increase in anti-HPV31 IgMs. For Case A, the analysis of local cervical secretions detected an increase in IL-17A associated with the HSV-2 reactivation episode. Note that associations between IL-17A (and IFN-γ) and HSV-2 infections have been studied [16].

Finally, both cases reported high levels of stress as well as sexual intercourse before the HSV-2 reactivations.

The first limitation of our study resides in the use of self-samples, which are likely to be of lower quality than that collected by a healthcare professional at the clinic. This could explain the absence of detection of HSV-2 DNA between two clinic visits that yielded positive samples. Another interpretation is that HSV-2 reactivation events could be short lasting less than 24 hours [17] but in both cases, the temporal variation in HSV-2 viral load is consistent with an exponential growth, which would be unlikely

if all the events were independent.

Another limitation is that the cytokines were not available for Case B and that those available for Case A were chosen to maximize the detection of HPV-associated trends [13]. Different cytokines, such as IL-6, IL-8, IL-36-γ, IFN-β, TNF-$\alpha$and chemokines CCL2, CXCL9, CXCL10, could be better suited to capture HSV-2 reactivation events.

Finally, not all the analyses could be performed on all the samples for budgetary reasons. In particular, for the self-samples, we concentrated on those collected before and after clinic samples positive for HSV-2 DNA. This means that we could be missing short HSV-2 episodes or transient HSV infections. However, our minimal sampling window of two months remains smaller than the majority of studies, e.g. the control arm of the HPV vaccine trials [18].

As previously discussed, HSV-2 or HPV infections could modify the immunological and microbial microenvironment, for example through the occurrence of dysbioses in the vaginal microbiota [5–7]. Such changes could favor HPV persistence or HSV-2 reactivations, and, potentially, progression to cancer. Studying this co-factor role of HSV-2 could be particularly interesting in the specific case of less carcinogenic high-risk HPVs, such as the ones reported in this study. Although our follow-up is dense, its duration of less than a year is too limited to investigate long-term outcomes. To this end, longer prospective cohorts or larger cross-sectional cohorts could bring complementary insights to validate the trends detected in this case report. These studies should aim to include participants with frequent HSV-2 episodes, target more immunomodulatory cytokines, and HPV coinfections with high-risk genotypes.

List of abbreviations

- HSV-2: Herpes Simplex Virus-2
- HPV: Human papillomavirus

- STI: Sexually Transmitted Infection
- CST: Community State Type
- qPCR: quantitative PCRs

Ethics approval and consent to participate

The PAPCLEAR trial is promoted by the Centre Hospitalier Universitaire de Montpellier and has been approved by the Comité de Protection des Personnes (CPP) Sud Méditerranée I on 11 May 2016 (CPP number 16 42, reference number ID RCB 2016-A00712-49); by the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé on 12 July 2016 (reference number 16.504); and by the Commission Nationale Informatique et Libertés on 16 December 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016–488). This trial was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé on 20 July 2016 (reference 20160072000007). The ClinicalTrials.gov identifier is NCT02946346. All participants provided written informed consent.

Competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: TW serves on advisory boards for MSD (Merck) Sharp & Dohme.

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Availability of data and materials

Table 1, Fig. 1, Fig. 2, Supplementary Tables S1 and S2 have associated raw data. The data that support the findings of this study are available from the corresponding author upon request, and data are available in the Zenodo public repository (10.5281/zenodo.7038511).

Authors' contributions

V.T., V.F., I.G.B, J.R., M.S, C.L.M., N.Bo., S.A, and N.T. designed the study. C.B., V.B, M.R., C.L.M., N.Bo., and S.A. designed the experiments. C.B., V.B, S.Gra., S.Gro, M.R., and N.Be. performed experiments. C.B., V.B, S.Gra., S.Gro, M.R., M.B., C.G, C.L.M, and S.A. contributed to study design, patient recruitment and clinical data acquisition. I.B.U, N.T. performed the analyses. I.B.U, V.F., S.A. and N.T. wrote the manuscript. All authors approved the final version of the manuscript.

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References

[1] James, C. *et al.* Herpes simplex virus: global infection prevalence and incidence estimates, 2016. Bulletin of the World Health Organization (5 2020). 98(5):315. doi:10.2471/BLT.19.237149.

[2] Johnston, C. and Corey, L. Current concepts for genital herpes simplex virus infection: Diagnostics and pathogenesis of genital tract shedding. Clinical Microbiology Reviews (11 2015). 29(1):149–161. doi:10.1128/CMR.00043-15/ASSET/6D53576F-7DCC-43EA-8CC7-1927DF7812FC/ASSETS/GRAPHIC/ZCM0011625350003.JPEG.

[3] Schiffman, M. and Solomon, D. Cervical-Cancer Screening with Human Papillomavirus and Cytologic Cotesting. New England Journal of Medicine (12 2013). 369(24):2324–2331. doi:10.1056/NEJMCP1210379/SUPPL{_}FILE/NEJMCP1210379{_}DISCLOSURES.PDF.

[4] Cao, S. *et al.* Herpes simplex virus type 2 and the risk of cervical cancer: a meta-analysis of observational studies. Archives of Gynecology and Obstetrics (12 2014). 290(6):1059–1066. doi:10.1007/S00404-014-3365-7/FIGURES/3.

[5] Borgogna, J. *et al.* The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: a cross-sectional analysis HHS Public Access. BJOG (2020). 127(2):182–192. doi:10.1111/1471-0528.15981.

[6] Shannon, B. *et al.* Distinct Effects of the Cervicovaginal Microbiota and Herpes Simplex Type 2 Infection on Female Genital Tract Immunology. The Journal of Infectious Diseases (5 2017). 215(9):1366–1375. doi:10.1093/INFDIS/JIX088.

[7] France, M. *et al.* Towards a deeper understanding of the vaginal microbiota. Nature Microbiology 2022 7:3 (3 2022). 7(3):367–378. doi:10.1038/s41564-022-01083-2.

[8] Michael, F. *et al.* VALENCIA: A nearest centroid classification method for vaginal microbial communities based on composition. Microbiome (2 2020). doi:10.21203/RS.2.24139/V1.

[9] Murall, C. L. *et al.* Natural history, dynamics, and ecology of human papillomaviruses in genital infections of young women: Protocol of the PAPCLEAR cohort study. BMJ Open (6 2019). 9(6):25129. doi:10.1136/bmjopen-2018-025129.

[10] Waterboer, T. *et al.* Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clinical chemistry (10 2005). 51(10):1845–1853. doi:10.1373/CLINCHEM.2005.052381.

[11] Murall, C. L. *et al.* HPV cervical infections and serological status in vaccinated and unvaccinated women. Vaccine (12 2020). 38(51):8167–8174. doi:10.1016/J.VACCINE.2020.10.078.

[12] Ma, B. *et al.* A comprehensive non-redundant gene catalog reveals extensive within-community intraspecies diversity in the human vagina. Nature Communications (12 2020). 11(1). doi:10.1038/s41467-020-14677-3.

[13] Selinger, C. *et al.* Cytokine response following perturbation of the cervicovaginal milieu during HPV genital infection. Immunologic Research 2021 69:3 (4 2021). 69(3):255–263. doi:10.1007/S12026-021-09196-2.

[14] Chaturvedi, A. K. *et al.* Human Papillomavirus Infection with Multiple Types: Pattern of Coinfection and Risk of Cervical Disease. The Journal of Infectious Diseases (4 2011). 203(7):910–920. doi:10.1093/INFDIS/JIQ139.

[15] Brotman, R. M. *et al.* Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. Journal of Infectious Diseases (12 2014). 210(11):1723–1733. doi:10.1093/infdis/jiu330.

[16] Bagri, P. *et al.* Novel Role for Interleukin-17 in Enhancing Type 1 Helper T Cell Immunity in the Female Genital Tract following Mucosal Herpes Simplex Virus 2 Vaccination. Journal of Virology (12 2017). 91(23). doi:10.1128/JVI.01234-17.

[17] Mark, K. E. *et al.* Rapidly Cleared Episodes of Herpes Simplex Virus Reactivation in Immunocompetent Adults. The Journal of infectious diseases (10 2008). 198(8):1141. doi:10.1086/591913.

[18] Kreimer, A. R. *et al.* Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. Journal of the National Cancer Institute (10 2011). 103(19):1444–1451. doi:10.1093/JNCI/DJR319.

Table

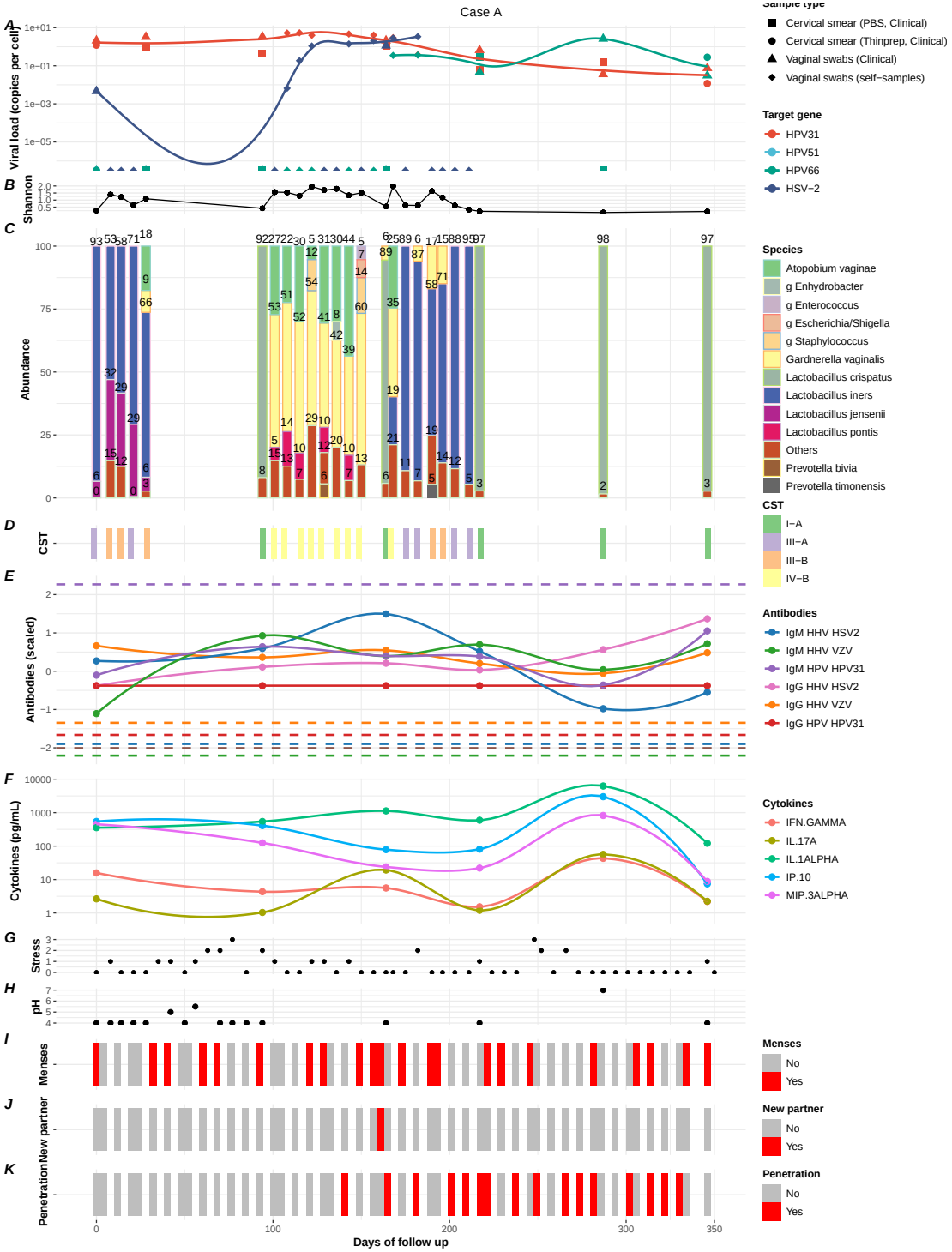
Table 1: **Key characteristics of two cases included in this report.** These variables were collected from the weekly or bi-weekly questionnaires, and the notes the gynaecologist, nurse, and midwife took during their visits every 4 or 8 weeks at the clinic.

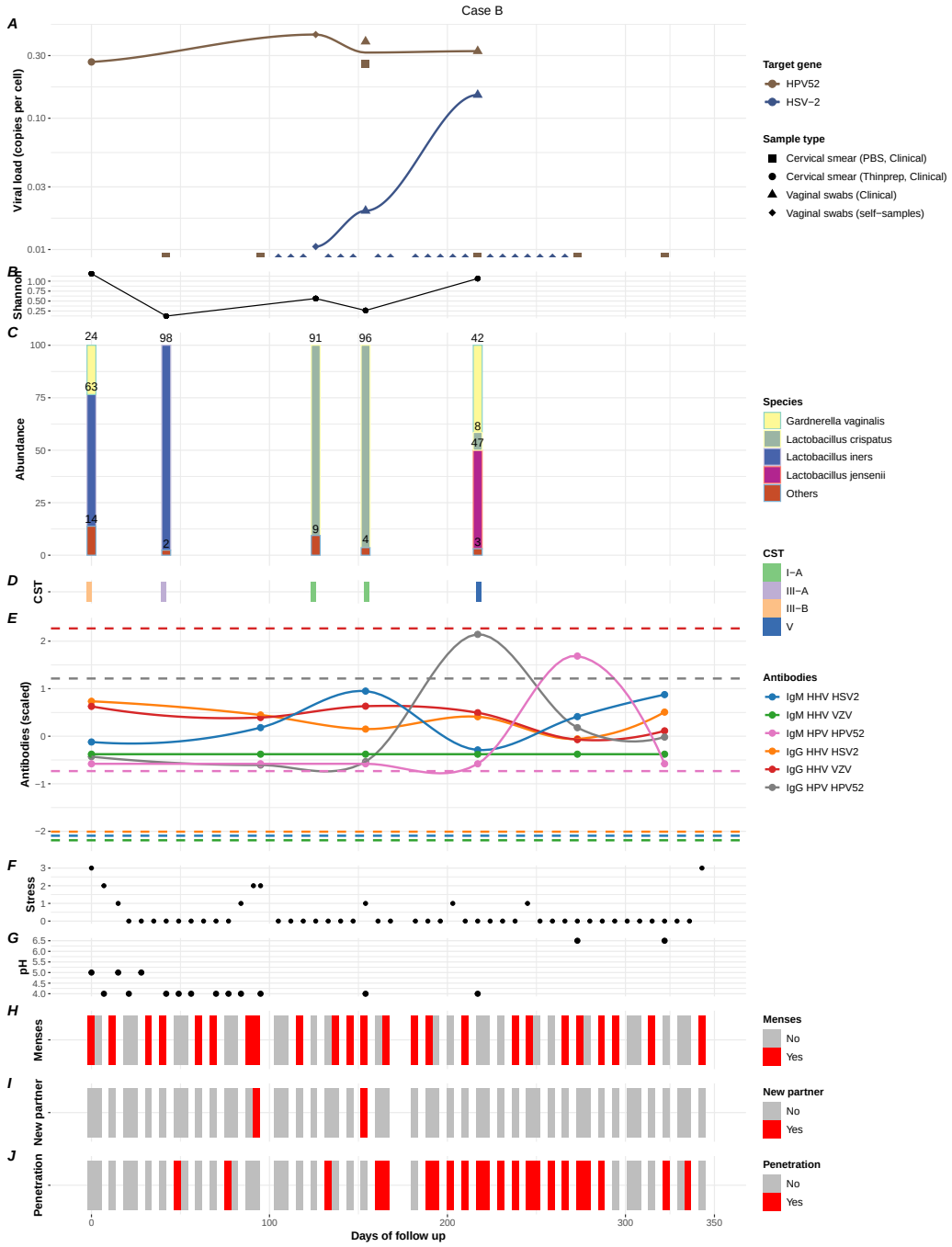
	Case A	**Case B**
Age	20	25
Follow-up duration	346 days	351 days
Contraception method	Preservative	Intrauterine device (UID)
Regular menses	Yes	Yes
BMI	20.7	33.24
The number of total life-time sexual partners	5	25
HPV vaccine status	Not-vaccinated	Not-vaccinated
HSV-2 symptoms	Asymptomatic	Asymptomatic
HSV-2 history	Yes	No
Other infections during the follow-up	Gynecological mycosis	Anginis
Medication usage during follow-up	betalactamines, imidazole derivative	betalactamines

Figure captions

Figure 1: **Dynamics of immunological, microbial, and behavioral variables of productive HSV-2 and HPV coinfection in Case A. (A:)** Per cell viral loads (log). The color indicates the virus genotype (or species) and the shape the type of sample. **(B:)** Shannon diversity index. **(C:)** Abundance and diversity of the main bacterial species found in the vaginal microbiota communities. Colors indicate different species, numbers above the bars indicate the percentage of reads attributed to that species. "g" refers to bacteria for which we can only identify the genera level and not the precise species. Only species with a normalized abundance greater than 5% are shown. **(D:)** Community State Types (CST) dynamics. **(E:)** Circulating IgG and IgM antibody titers. Colors indicate different antibodies and corresponding dashed lines indicate the threshold values for each antibody. **(F)** Cytokines local concentrations. Colors show different cytokines. **(G)** Self-reported stress levels. **(H)** Vaginal pH measures. **(I)** Self-reported menstruation within the last week. **(J)** Self-reported occurrence of a new sexual partner during the last week. **(K)** self-reported sexual intercourse with vaginal penetration during the last week. Red bars indicate a yes answer to the question. In panels A, E, and F lines were obtained by fitting a spline curve. The data is shown per day of follow-up (DoF).

Figure 2: **Dynamics of immunological, microbial, and behavioral variables of productive HSV-2 and HPV coinfection in Case B.** See Figure 1 for details about the figure contents.


Case A
A
Viral load (copies per cell)
B
Shannon
C
Abundance
D
CST
E
Antibodies (scaled)
F
Cytokines (pg/mL)
G
Stress
H
pH
I
Menses
J
New partner
K
Penetration
Days of follow up
Sample type
Cervical smear (PBS, Clinical)
Cervical smear (Thinprep, Clinical)
Vaginal swabs (Clinical)
Vaginal swabs (self−samples)
Target gene
HPV31
HPV51
HPV66
HSV−2
Species
Atopobium vaginae
g Enhydrobacter
g Enterococcus
g Escherichia/Shigella
g Staphylococcus
Gardnerella vaginalis
Lactobacillus crispatus
Lactobacillus iners
Lactobacillus jensenii
Lactobacillus pontis
Others
Prevotella bivia
Prevotella timonensis
CST
I−A
III−A
III−B
IV−B
Antibodies
IgM HHV HSV2
IgM HHV VZV
IgM HPV HPV31
IgG HHV HSV2
IgG HHV VZV
IgG HPV HPV31
Cytokines
IFN.GAMMA
IL.17A
IL.1ALPHA
IP.10
MIP.3ALPHA
Menses
No
Yes
New partner
No
Yes
Penetration
No
Yes


Case B
A
Viral load (copies per cell)
0.30
0.10
0.03
0.01
Target gene
HPV52
HSV−2
Sample type
Cervical smear (PBS, Clinical)
Cervical smear (Thinprep, Clinical)
Vaginal swabs (Clinical)
Vaginal swabs (self−samples)
B
Shannon
1.00
0.75
0.50
0.25
C
Abundance
100
75
50
25
0
24
98
91
96
42
63
14
2
9
4
8
47
3
Species
Gardnerella vaginalis
Lactobacillus crispatus
Lactobacillus iners
Lactobacillus jensenii
Others
D
CST
CST
I−A
III−A
III−B
V
E
Antibodies (scaled)
2
1
0
−1
−2
Antibodies
IgM HHV HSV2
IgM HHV VZV
IgM HPV HPV52
IgG HHV HSV2
IgG HHV VZV
IgG HPV HPV52
F
Stress
3
2
1
0
G
pH
6.5
6.0
5.5
5.0
4.5
4.0
H
Menses
Menses
No
Yes
I
New partner
New partner
No
Yes
J
Penetration
Penetration
No
Yes
0
100
200
300
350
Days of follow up