

## Research



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# Herpes simplex virus-2 transmission probability estimates based on quantity of viral shedding

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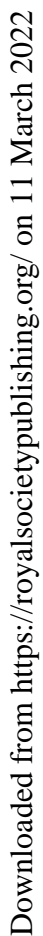
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Herpes simplex virus (HSV)-2 is periodically shed in the human genital tract, most often asymptomatically, and most sexual transmissions occur during asymptomatic shedding. It would be helpful to identify a genital viral load threshold necessary for transmission, as clinical interventions that maintain viral quantity below this level would be of high utility. However, because viral expansion, decay and re-expansion kinetics are extremely rapid during shedding episodes, it is impossible to directly measure genital viral load at the time of sexual activity. We developed a mathematical model based on reproducing shedding patterns in transmitting partners, and median number of sex acts prior to transmission in discordant couples, to estimate infectivity of single viral particles in the negative partner's genital tract. We then inferred probability estimates for transmission at different levels of genital tract viral load in the transmitting partner. We predict that transmission is unlikely at viral loads less than  $10^4$  HSV DNA copies. Moreover, most transmissions occur during prolonged episodes with high viral copy numbers. Many shedding episodes that result in transmission do not reach the threshold of clinical detection, because the ulcer remains very small, highlighting one reason why HSV-2 spreads so effectively within populations.

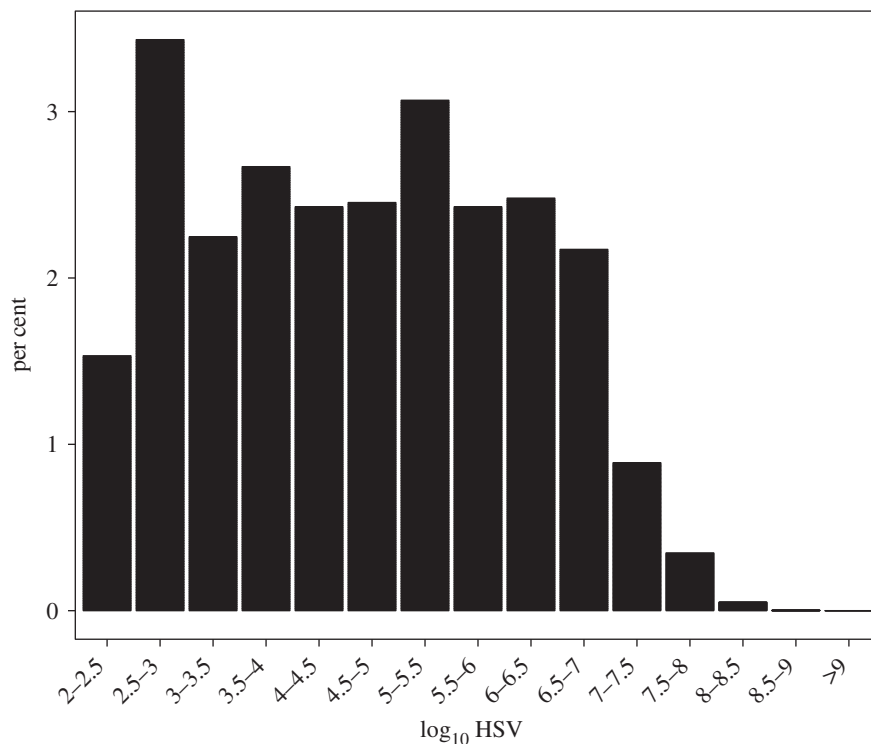
## 1. Introduction

Herpes simplex virus (HSV)-2 is a lifelong infection characterized by a genital shedding rate of 18%. HSV-2 shedding consists of frequent episodes (approx. 30 per year), which are heterogeneous in terms of duration (hours to weeks) and peak viral production ( $10^2$ – $10^9$  HSV DNA copies  $\text{ml}^{-1}$ ) [1–6]. Transmission to an uninfected partner occurs following coitus during genital shedding in an infected person. While many episodes are associated with visible lesions, and avoidance of sex during lesions offers some protection [7], most transmissions occur during asymptomatic reactivation [8,9], which accounts for approximately 80% of shedding. Accordingly, data from persons with newly acquired HSV-2 suggest that the number of coital acts prior to transmission is quite low [7]. Seroprevalence is therefore high (16% among US adults and more than 80% in certain regions within sub-Saharan Africa [10] and among men who have sex with men [11]).

Antiviral therapies or immunotherapies that more effectively maintain HSV-2 shedding below a certain level may be valuable for decreasing HSV-2 incidence. In murine models of infection, a threshold inoculum dose of HSV is needed to ensure infection [12]. Valaciclovir, dosed once daily, lowered the HSV-2 transmission rate in a clinical trial, but only by 50% [13]. Breakthrough shedding occurs in aciclovir- and valaciclovir-treated patients, explaining this intervention's incomplete efficacy [14]. Yet, there is room for optimism: for human immunodeficiency virus (HIV)-1, another viral sexually transmitted infection, whereas probability of transmission increases as a function of genital and plasma tract



We fitted our model in a bidirectional fashion given uncertainty regarding both biological and epidemiological parameters of



**Figure 2.** Frequency histogram of quantitative shedding. Data are from 531 study participants who submitted 14 685 genital swabs.

**Table 1.** Shedding model equations and parameter values.

$$S_0 = 1.67e5 \text{ cells per region}$$

$$\Delta S(i \dots 300) = [\lambda - (\beta_i \times S \times V_i) - (\beta_i \times S \times V_{\text{neu}}) - (\beta_e \times S \times V_e)] \Delta t$$

$$\Delta I(i \dots 300) = [(\beta_i \times S \times V_i) + (\beta_i \times S \times V_{\text{neu}}) + (\beta_e \times S \times V_{e,\text{adj}}) - (a \times I) - (f \times I \times E)] \Delta t$$

$$\Delta E(i \dots 300) = [(F(I) \times \theta \times E) - (\delta \times E)] \Delta t$$

$$\Delta V_{\text{neu}}(i \dots 300) = [\phi - (c \times V_{\text{neu}}) - (\beta_i \times S \times V_{\text{neu}})] \Delta t$$

$$\Delta V_i(i \dots 300) = [(p \times I) - (a \times V_i) - (\beta_i \times S \times V_i)] \Delta t$$

$$\Delta V_e(i \dots 300) = [(a \times V_i) - (c \times V_e)] \Delta t$$

$$\lambda = d(S - S_0)$$

$$F(I) = I/(I + r)$$

$$V_{e,\text{adj}} = V_e \text{ from six adjacent regions}$$

$$V_{e,\text{tot}} = V_{e1} + V_{e2} + \dots + V_{e300}$$

$$V_{i,\text{tot}} = V_{i1} + V_{i2} + \dots + V_{i300}$$

$$\beta_i = 6.6 \times 10^{-7} \text{ DNA copy days per cell}$$

$$\beta_e = 1.1 \times 10^{-12} \text{ DNA copy days per cell}$$

$$p = 10^5 \text{ DNA copies per cell per day}$$

$$\phi = 36 \text{ DNA copies per day}$$

$$c = 15.8 \text{ per day}$$

$$\theta = 7.2 \text{ per day}$$

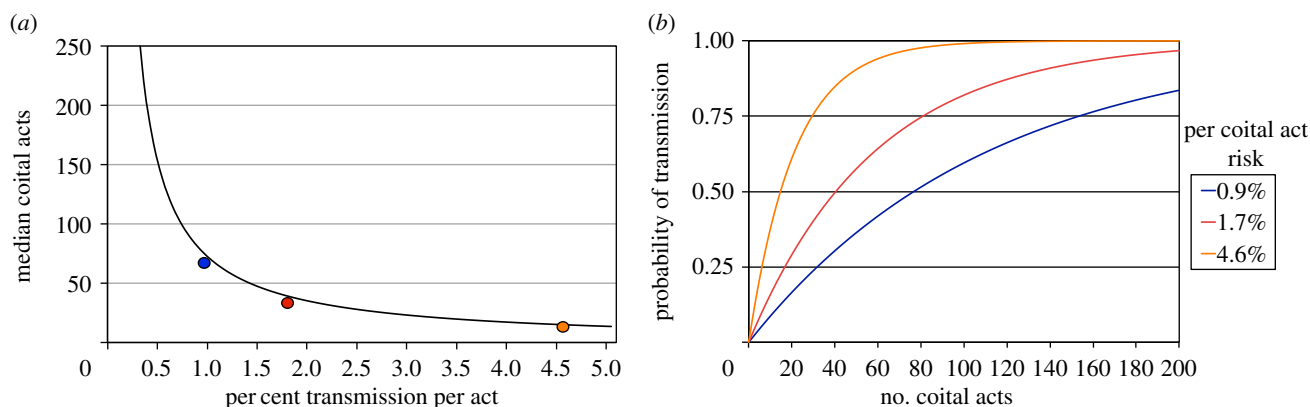
$$\delta = 10^{-2.6} \text{ per day}$$

$$r = 132 \text{ infected cells at which } \theta \text{ is half maximal}$$

$$\rho = \text{CD8}^+ \text{ T-cell regional co-dependence} = 0.3 \text{ (30\% overlap in T-cell values between regions)}$$

HSV-2 transmission: while per particle viral infectivity ( $\beta_e$ ) is relatively poorly defined and can only be estimated *in vivo* with mathematical modelling [19], per act transmission probability can also only be projected within a certain range from available epidemiological data [7], and may vary between

different populations. We therefore used estimates for the median number of acts prior to transmission to arrive at a value for viral infectivity, and then used an estimate of viral infectivity to estimate number of acts prior to transmission, to see if these processes produced consistent results.



**Figure 3.** Per coital act risk assessment. (a) Median number of acts prior to transmission as a function of per cent transmission per act. Coloured dots represent different estimates of transmission risk. Orange, red and blue are estimates for the median number of acts prior to transmission (red, median 40 acts; orange and blue, lower and upper IQR (15 and 75 acts, respectively) from a retrospective cohort study of acute infection [7]. (b) Probability of transmission following a given number of acts assuming differing per coital act risks. Line colours correlate with dot colours in (a).

The most rigorous available estimate for median acts prior to transmission is 40 (inter-quartile range (IQR): 15–75): these data are from a retrospective study in which 195 newly infected participants estimated the number of coital acts prior to acquisition in new, exclusive partnerships. Only 18% of couples used condoms during last coitus and only 13% of newly infected persons knew their partners had genital herpes. Participants had only one HSV-2-infected sexual partner in the time period leading up to transmission [7]. Because condom use is partly protective against HSV-2 transmission and acquisition [20,21], and most transmissions occur when the at-risk member of a relationship is unaware of their partner's HSV-2 infection [7], the conditions in the retrospective cohort study reflect those that drive the HSV-2 epidemic in the general population.

Given the cumulative probability distribution  $0.5 = (1 - p)^n$ , where 0.5 is total transmission likelihood such that 50% of people are infected after  $n$  coital acts given a per coital act transmission probability of  $p$ , we estimated a per coital act transmission likelihood of 0.017 (1.7%) assuming  $n = 40$  (red circle, figure 3a). If we assumed 15 and 75 median acts prior to transmission to establish reasonable bounds for the per coital act risk (based on the IQR in [7]), then the per coital act risk was 4.6% and 0.9%, respectively (orange and blue circles, figure 3a). Based on  $p_n = (1 - p)^n$ , where  $p_n$  is the transmission probability following  $n$  acts, a per coital act risk of 4.6% would lead to infection after a considerably lower number of acts than a per coital act risk of 0.9% (figure 3b). If the risk was less than 0.5%, more than 200 median sex acts were needed to transmit (figure 3a). Conversely, under the assumption that all shedding during sex results in transmission regardless of viral load, a median of 3.5 sex acts would result in transmission.

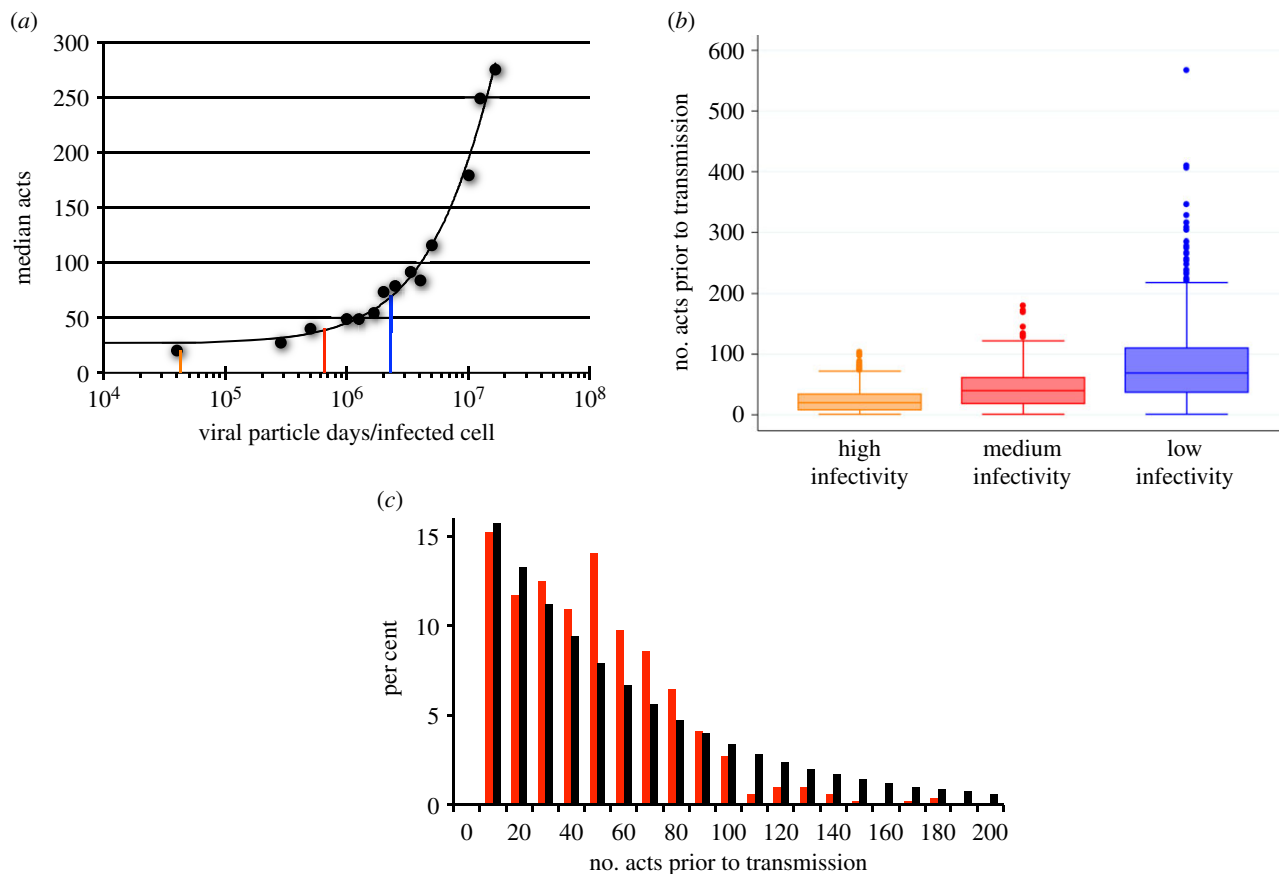
We next conducted 100 transmission simulations with 14 values (1400 simulations) of the cell-free infectivity parameter ( $\beta_i$ ). The inverse of the infectivity parameter ( $1/\beta_i$ ) can be interpreted as the number of cell-free viruses at steady state in the genital tract of an uninfected person that would result in an average of one infected cell over 24 h. Our model assumes that cell-free viral decay in the genital tract of the susceptible risk partner is rapid, and that probability of infection at each time step ( $dt = 0.001$  days) is less than the probability of infection over 24 h. Hence, estimated values of ( $1/\beta_i$ ) were less than viral loads required for transmission.

Simulations with each value of  $1/\beta_i$  are denoted with circles in figure 4a. The model demonstrated a predictive ( $R^2 = 0.99$ ) quadratic relationship between median number of acts prior to transmission and infectivity ( $1/\beta_i$ ):  $y = -(2 \times 10^{-13}(1/\beta_i)^2) + (2 \times 10^{-5}(1/\beta_i)) + 25.661$ . As the number of viral particles required for transmission increased, median acts prior to transmission also increased (figure 4a), with a sharp inflection at  $1/\beta_i > 10^6$ : an intervention that decreases per particle infectivity 10-fold might therefore have a dramatic impact on transmission.

Based on the quadratic equation, we estimated that  $1/\beta_i = 7.2 \times 10^5$  and  $2.5 \times 10^6$  would result in 40 and 75 median acts (per coital act risk 1.7% and 0.9%) prior to transmission (red and blue lines in figure 4a). We labelled these  $1/\beta_i$  values as 'medium' and 'low' viral infectivity. While the quadratic equation was not predictive for  $1/\beta_i < 10^5$  (less than 26 median acts), in simulations with  $1/\beta_i = 4 \times 10^4$ , there were 15 median acts (per coital act risk 4.6%) prior to transmission (orange line, figure 4a): this value was labelled 'high' viral infectivity. Simulations with low, medium and high infectivity are used in the remainder of the article to estimate plausible ranges of viral load thresholds for transmission.

### 2.3. Use of per viral particle infectivity to estimate per coital act probability

Next, we compared our results generated from this transmission model with the transmission probability predicted in our spatial model of HSV-2 pathogenesis [18], which assumes that secondary ulcers form by viral seeding of adjacent regions from the primary ulcer according to parameter  $\beta_e$  in figure 1.  $\beta_e$  and  $\beta_i$  relate to a parallel biologic event (stochastic infection of a single epithelial cell by cell-free HSV-2). The estimated value for  $1/\beta_e$  is  $2.26 \times 10^5$  virus days per cell (range:  $2.15 \times 10^5$ – $3.46 \times 10^5$ ). Given this estimate of infectivity, the risk of HSV-2 transmission per sex act was 2.3% (median 30 acts prior to transmission). This per coital act estimate is 0.6% higher than our medium infectivity value (and falls between our low and high infectivity estimates), likely highlighting the fact that individual virions are exposed to greater hazards when passed from host to host ( $\beta_i$ ) than within a host to a new mucosal location ( $\beta_e$ ).



**Figure 4.** Model estimates for per particle infectivity. (a) Viral infectivity parameter  $1/\beta_t$  as a predictor of the median number of acts prior to transmission. Each circle represents results from 100 simulations at the given parameter. Smooth line is the fit of a quadratic equation ( $R^2 = 0.99$ ) to the data. Coloured lines represent values for  $1/\beta_t$  leading to the per coital act risk of equivalent colour in figure 3a. (b) Number of acts prior to transmission with assumptions of low, medium and high viral infectivity (500 simulations per infectivity). Boxes represent IQR and whiskers represent all values within 1.5 IQRs of the box. (c) A probability distribution function of acts prior to transmission in model simulations with medium viral infectivity (red histograms) is in relative accordance ( $R^2 = 0.88$ ) with per cent of transmission within each bar of the histogram (black histograms) predicted from the formula  $p_n = (1-p)^n$ , where  $p_n$  is the probability of transmission following  $n$  acts with per coital act risk of  $p = 0.017$ .

## 2.4. Multiple transmission simulations

Next, we conducted 500 transmission simulations assuming low, medium and high infectivity, and identified skewed distributions of the number of acts prior to transmission under each assumption (figure 4b). For medium infectivity, while the median number of acts before transmission was 40, there was considerable variability (IQR: 19–61, range: 1–180). Number of acts preceding transmission can be high within a minority of discordant couples despite a constant per coital act risk. This effect was most pronounced with decreasing viral infectivity, which substantially widened both the IQR and total range (figure 4b). We compared the distributions generated by the simulation model with our probability equation,  $p_n = (1-p)^n$ , and found a close correlation (figure 4c,  $R^2 = 0.88$ ), though the probability equation predicted a greater skew, with a slightly higher proportion of infections at lower and higher number of acts.

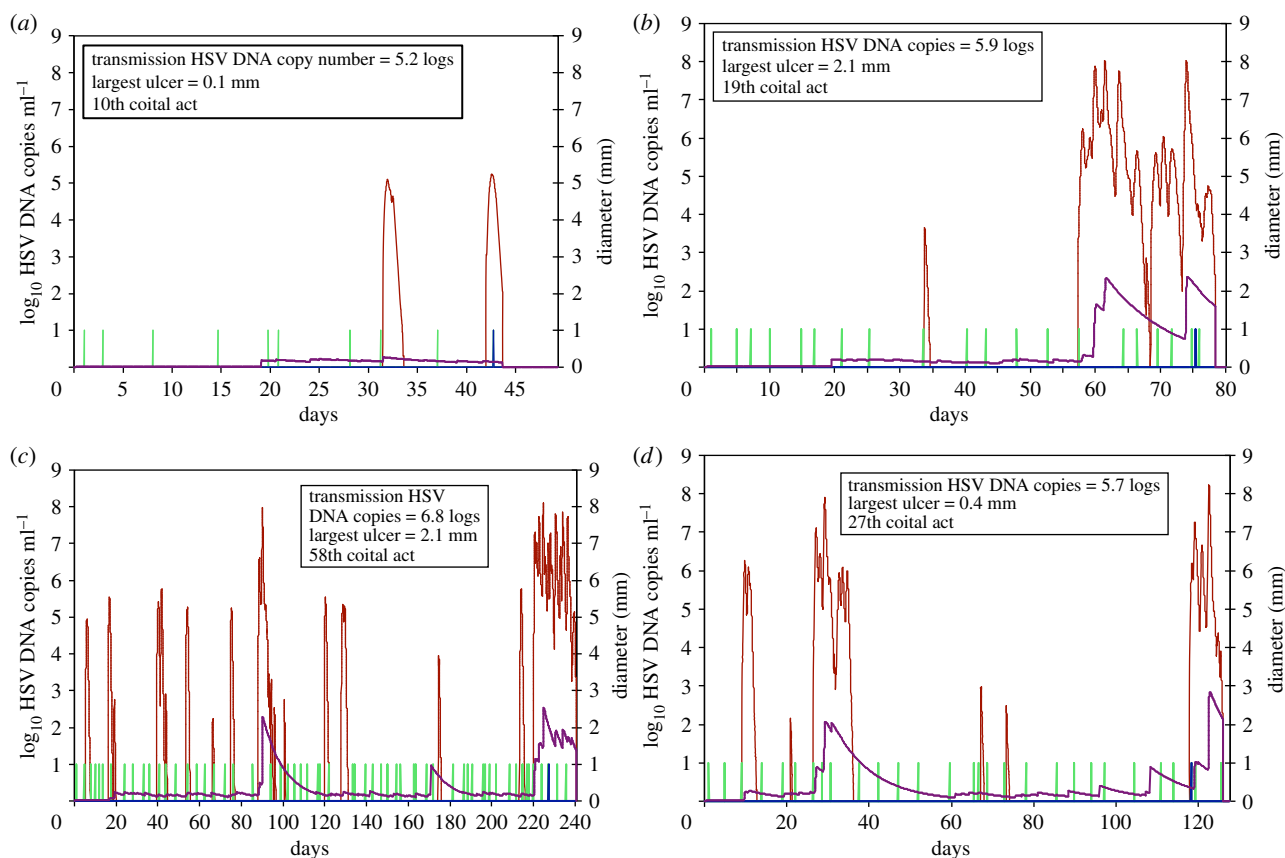
To demonstrate model output, we plotted four simulated transmissions and included: coital acts, genital tract viral load and lesion diameter. A fundamental feature of empirical and model generated shedding episodes is their diversity. While many episodes last less than 6 h and peak at less than  $10^4$  HSV DNA copies [2], episodes can also persist for more than 10 days with more than  $10^8$  HSV DNA copies. Episodes with higher copy number have more infected cells and, in

turn, larger ulcer diameter. In addition, their morphology is often notable for multiple peaks (figure 5a–d). In simulated transmissions, most coital acts occurred when shedding was absent. However, many coital acts that did not result in transmission occurred when HSV-2 DNA was present (figure 5a–d). This accounted for prolonged time before transmission during certain simulations.

## 2.5. Quantitative viral load and transmission

We recorded genital viral load during each simulated coital act during 1536 transmissions at varying levels of infectivity. At medium infectivity, the median  $\log_{10}$  genital viral load during 512 coital acts that resulted in transmission was 7.0 (IQR: 6.6–7.5, range: 4.2–8.3) and during 2777 coital acts without transmission was 4.5 (IQR: 3.1–5.7, range: 2.0–7.7). Therefore, 16% of coital acts during shedding resulted in transmission. Only three (0.6%) transmissions occurred with less than  $10^5$  HSV DNA copies and none occurred with less than  $10^4$  HSV DNA copies (figure 6a). Ninety-six per cent of transmissions occurred at more than  $10^6$  HSV DNA copies. At low infectivity, median  $\log_{10}$  genital viral load during 512 coital acts with transmission was higher: 7.3 (IQR: 6.8–7.6, range: 4.4–8.5) and only one (0.2%) transmission occurred at less than  $10^5$  HSV DNA (figure 6a). At





**Figure 5.** (a–d) Four model simulations demonstrating viral shedding and peak lesion diameter leading up to four transmissions. Non-transmission coital acts are denoted with green lines; transmission coital acts are denoted with blue lines; HSV DNA copies is shown with a red line and peak lesion diameter with a purple line.

higher infectivity, median genital viral load during coital acts with transmission was lower: 6.4 (IQR: 5.9–6.9, range: 3.7–8.1) and 2% of transmissions occurred at less than  $10^5$  HSV DNA (figure 6a).

For all three infectivity estimates, we divided HSV DNA copy number at coitus into strata separated by 0.1 log and measured per coital act risk of transmission within each stratum: there was a rise in transmission risk with increase in genital tract viral load (figure 6b). We fitted locally weighted scatterplot smoothing (lowess) smooth curves to the transmission estimates ( $R^2 = 0.98$ ) for each infectivity parameter, thus allowing estimates of transmission risk for each level of shedding. With medium infectivity, 50% per coital act transmission was predicted for 6.7 log HSV DNA copies (figure 6b).

## 2.6. Statistical risk models of transmission

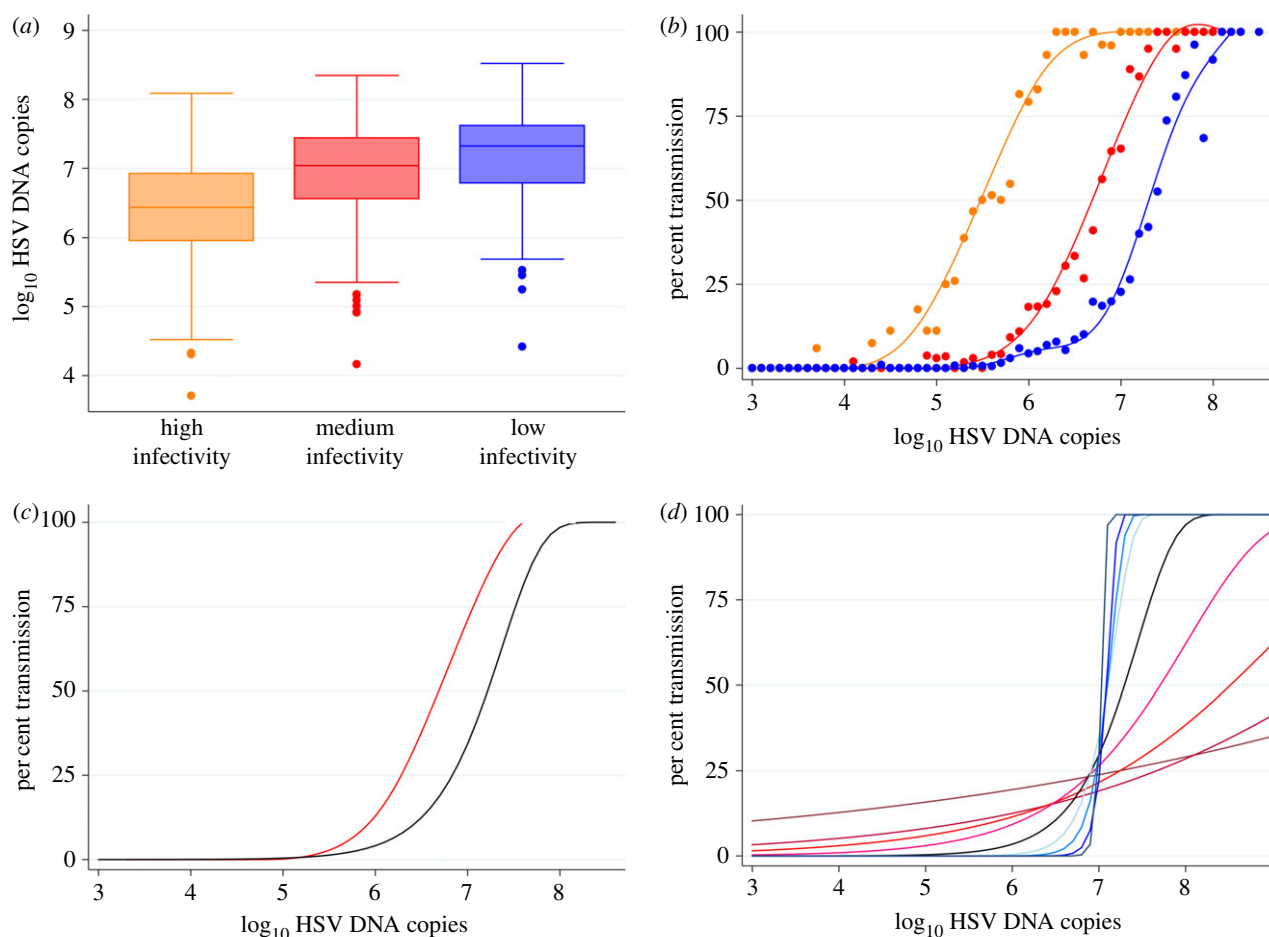
To reinforce our estimates of viral load transmission threshold, we constructed a statistical risk model (see Methods), which we fitted to previously obtained data on the number of sex acts prior to transmission [7]. The model assumes a transmission risk associated with each coital event. Viral load ( $V_e$ ) in the transmitting partner is randomly sampled from the distribution of shedding data (figure 2) for each event and is assumed to infect according to parameter  $\beta_t$ . Unlike the mechanistic mathematical model (figure 1), the statistical model ignores characteristics of shedding episodes (figure 5).

Because of variability introduced from randomly sampling viral load, the statistical model was simulated 1000 times to arrive at a maximum-likelihood infectivity value

( $1/\beta_t = 1.8 \times 10^6$ , range:  $1.6 \times 10^6$ – $2.1 \times 10^6$ ). This estimate was 2.5 times higher than the mathematical model value. The dose–response curve based on these data had a similar trajectory to the medium infectivity curve from the mathematical model (figure 6c), with a threshold viral load for 50% transmission probability of 7.3 log HSV DNA copies, which was two times higher than the mathematical model estimate (6.8 log HSV DNA copies).

We next adjusted the statistical model to include a new parameter  $\alpha$ , to account for the slope of the dose–response curve (figure 6d).  $\alpha < 1$  (less steep slope) would occur if confounding variables other than viral load (such as type of sex act, viral strain and host immunity) influence the probability of per coital act transmission.  $\alpha > 1$  (steeper curve) would only occur in the presence of cooperative binding between a virus and cellular receptors on the cell surface, such that attachment of HSV to a receptor promoted more binding to adjacent receptors. We demonstrated that values of  $\alpha$  greater than 0.2 displayed equivalent deviance from the transmission data (median  $\chi^2 = 24.9, 24.5, 24.2, 23.9, 25.8, 24.8, 24.3$  and 25.5 for  $\alpha = 0.2, 0.3, 0.5, 1, 2, 3, 5$  and 10, respectively), whereas  $\alpha = 0.1$  showed higher deviance ( $\chi^2 = 73.9$ ).

Best fit values for  $1/\beta_t$  generally increased with decreasing  $\alpha$ , thereby shifting the 50% per coital act viral load levels to the right for curves with lower slopes. Therefore, if more confounding variables are assumed to impact transmission, then transmission probability at lower viral loads will be higher, whereas transmission probability at higher viral loads will be lower (figure 6d). For the least steep dose–response curve that allowed model fit ( $\alpha = 0.2$ ), there was a 5% probability of HSV-2 transmission at  $10^4$  HSV DNA copies.



**Figure 6.** Genital tract viral load is a predictor of transmission. (a) Genital tract viral load at transmission during 512 shedding simulations with high (orange), medium (red) and low (blue) viral infectivity. (b) Proportion of simulated coital acts within different strata leading to transmission; lowess fit line represents a transmission probability curve for each degree of infectivity; colours represent infectivity values from figures 3, 4 and 6a. (c) A transmission probability curve generated from the statistical risk model (black line) approximates the medium infectivity dose–response curve of the mathematical model (red line from b). (d) Best fit dose–response curves from the statistical risk model with different assumptions of dose–response slopes dictated by parameter  $\alpha$ . The black line (from c) represents  $\alpha = 1$ , whereas values less than 1 (0.5, 0.3, 0.2, 0.1) are signified by darker shades of red and values more than 1 (2, 3, 5 and 10) are signified by darker shades of blue. Only the curve with  $\alpha = 0.1$  (darkest red) poorly reproduced the per coital act transmission data.

## 2.7. Episode characteristics and transmission

We next reverted to the simulation model (figure 1) to classify type of episodes associated with transmissions, by performing further analyses on the 512 simulated transmissions with an assumption of medium infectivity. We defined ‘coital episodes’ as episodes during which coital events but no transmission occurred and ‘transmission episodes’ as episodes associated with transmission. We allowed episodes to be counted multiple times to account for multiple coital acts during single episodes.

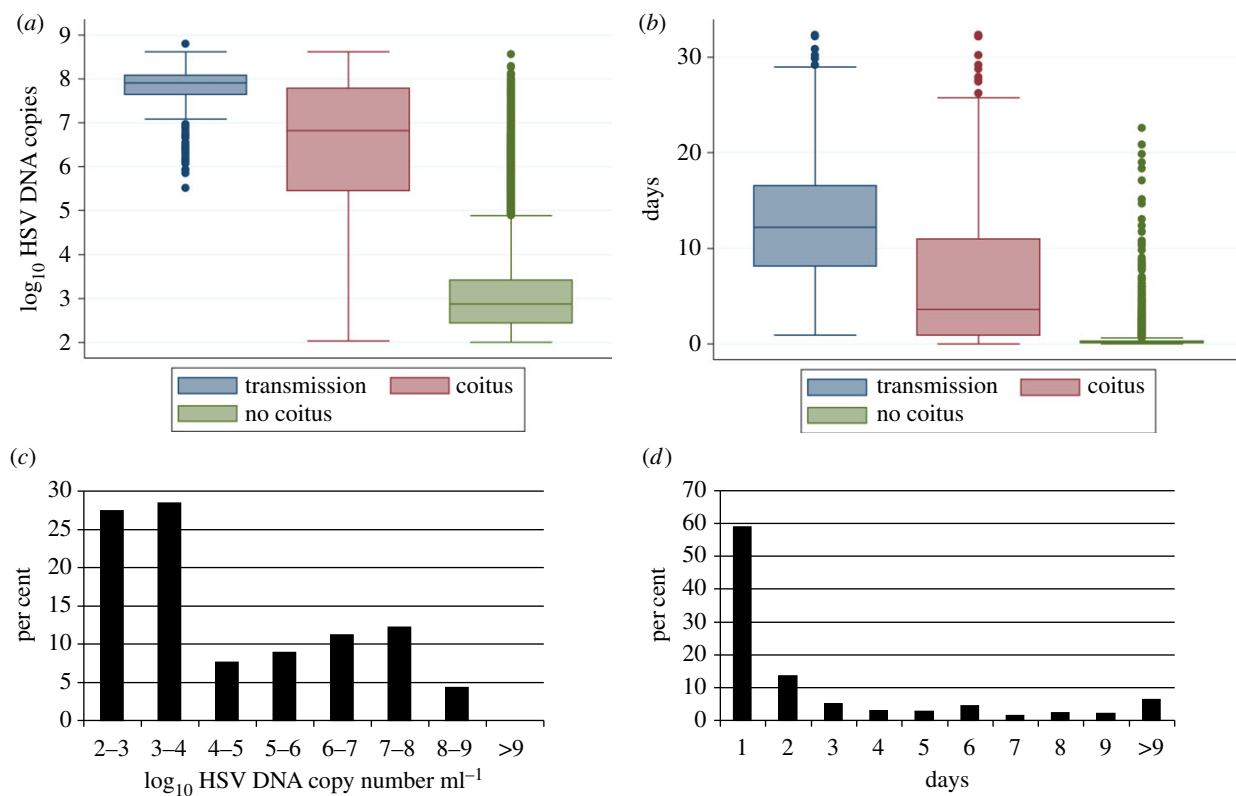
Viral loads in the genital tract fluctuated over hours and transmission did not usually occur at viral peaks within episodes: median peak  $\log_{10}$  viral load of 512 transmission episodes was 7.9 (IQR: 7.6–8.1, range: 5.5–8.8; figure 7a), whereas median viral load was 7.0 at the exact time of transmission (figure 6a). Median  $\log_{10}$  peak HSV DNA copy number of 2777 coital episodes was 6.8 (IQR: 5.4–7.8, range: 2.0–8.6; figure 7a). Median copy number of 15 780 non-coital episodes was 2.9 (IQR: 2.4–3.4, range: 2.0–8.6; figure 7a). Transmission episode duration was also longer than duration of coital and non-coital episodes (figure 7b).

The relative dearth of non-coital episodes with peak viral load between  $\log_{10}$  HSV DNA copy number 4 and 6, as well

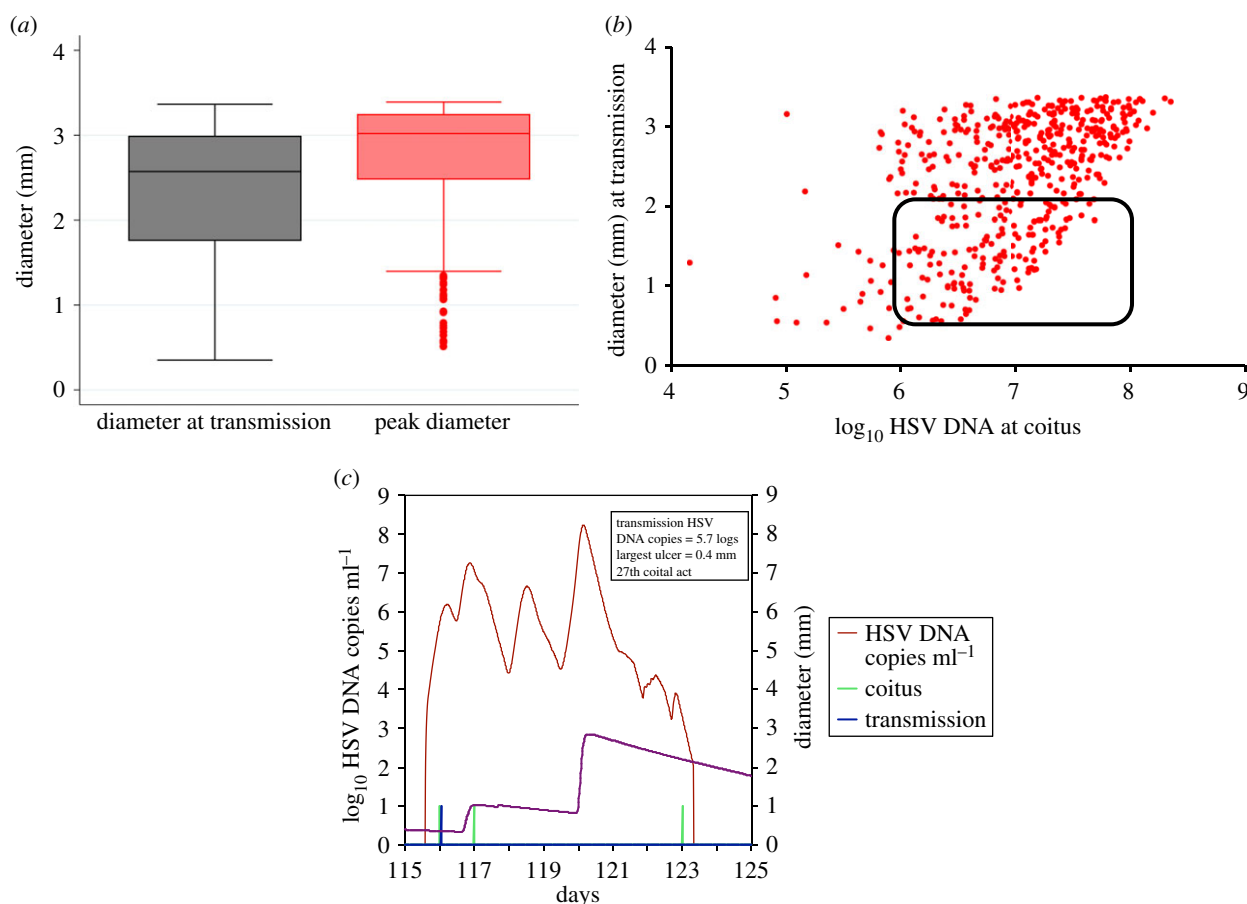
as duration between 0 and 10 days, demonstrates a fundamental feature of both empirical and simulated shedding data. While more than 50% of episodes are rapidly contained under  $10^4$  HSV DNA copies and within a day, once viral levels exceed these thresholds, viral loads more commonly reach peak levels exceeding  $10^6$  HSV DNA copies (figure 7c) and duration can take on a broad range of values (figure 7d). This occurs because there is a delay in  $CD8^+$  expansion to adequately control HSV-2 if immediate viral elimination is not achieved.

## 2.8. Transmission during asymptomatic periods with high viral quantities in the genital tract

We estimated lesion diameter with our spatial model by counting the number of missing cells owing to cell death within a single herpetic ulcer [19,22]. For the 512 simulated transmissions, the median diameter of the largest ulcer at the time of transmission was 2.6 mm (IQR: 1.8–3.0, range: 0.4–3.4; figure 8a); the median diameter of the largest ulcer during the entire course of a transmission episode was 3.0 mm (IQR: 2.5–3.2, range: 0.5–3.4; figure 8a). Therefore, simulated transmissions often occurred when the lesion had

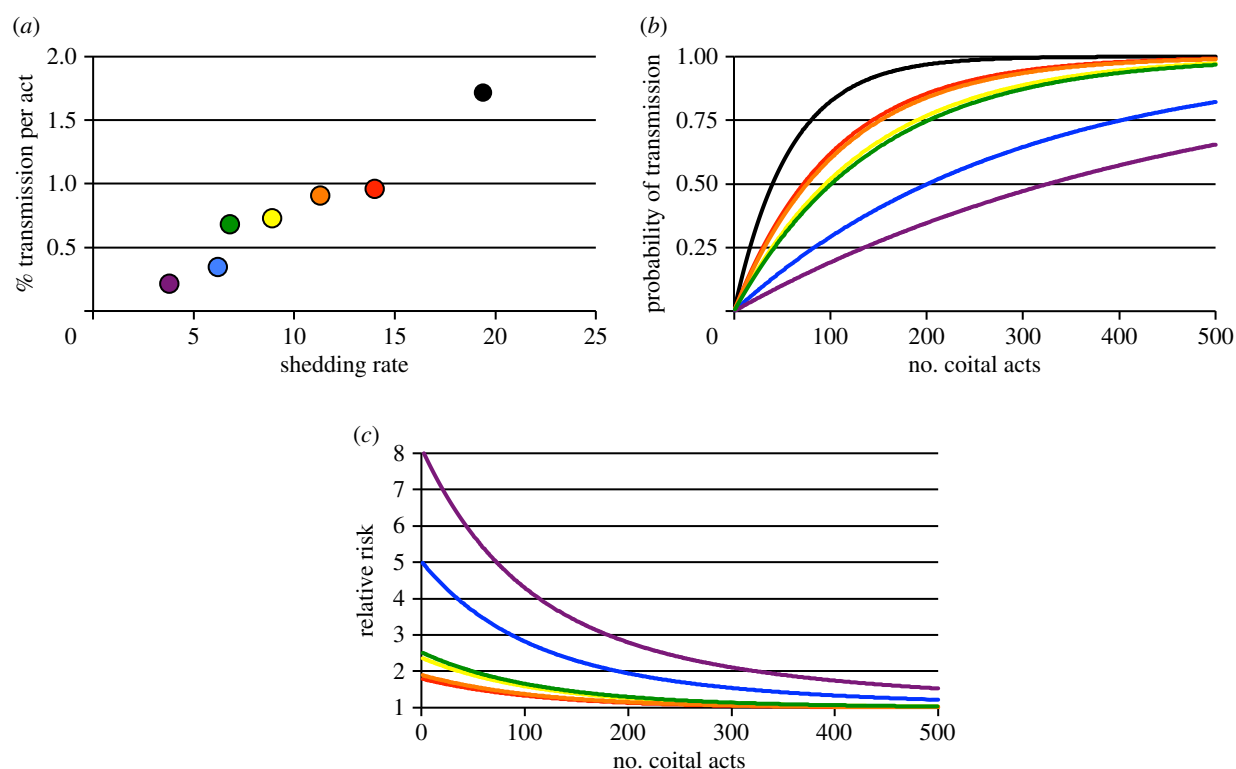


**Figure 7.** Severe shedding episodes as a predictor of transmission. All simulations assume medium infectivity. (a) Peak viral load and (b) duration of episodes during 512 transmission episodes, 2777 episodes with coitus but no transmissions and 15 780 episodes without coitus. Boxes represent IQR and whiskers represent all values within 1.5 IQRs of the box. Distribution of (c) peak viral loads and (d) episode durations in 1020 simulated episodes.



**Figure 8.** Many transmissions occur when viral load is high but lesions are not visible. Data from 512 transmission episodes. (a) Largest ulcer diameter at the time of transmission (black) and peak ulcer diameter during the entire transmission episode (red). Boxes represent IQR and whiskers represent all values within 1.5 IQRs of the box. (b) Largest ulcer diameter as a function of genital tract viral load at the time of coitus; the box represents areas where lesions may not be visible but high viral copy numbers are present. (c) Diagram of a simulated transmission with high viral load prior to development of a more than 1 mm diameter ulcer.





**Figure 9.** Theoretical effects of antiviral therapy on transmission. (a) Simulations of different regimens decreased shedding rates to different degrees, which in turn correlated with decreases in the per coital act transmission percentage ( $R^2 = 0.94$ ). (b) Probability of transmission following a given number of sex acts assuming differing per coital act risks due to the impact of antiviral therapy on shedding. (c) Relative risk of transmission on treatment versus no treatment decreases following an increasing number of sex acts. Black, no drug; red, acidovir 400 mg daily; orange, valaciclovir 500 mg daily; yellow, acidovir 400 mg twice daily; green, valaciclovir 1000 mg daily; blue, valaciclovir 500 mg twice daily; purple, valaciclovir 1000 mg twice daily.

not yet, or had already, achieved its maximum diameter. Ulcers between 1 and 3 mm diameter are at the threshold of clinical detection: 30% and 76% of transmission episodes occurred when no lesion was wider than 1 or 2 mm. Twenty-four per cent of transmission episodes occurred with more than  $10^6$  HSV DNA copies present despite no ulcer wider than 2 mm (figure 8b). Many transmissions occurred at time points early during episodes prior to detectable lesions (figure 8c).

### 2.9. Effects of antiviral therapy

To forecast the effect of therapy on the risk of HSV-2 transmission, we simulated shedding on several dosing regimens of aciclovir and valaciclovir [14,23]. Shedding rate decreased on increasingly potent regimens with a dependent decrease in the per coital act transmission risk (figure 9a). High doses of valaciclovir were relatively protective against transmission, though the impact of partially protective regimens diminished over time as a function of both probability (figure 9b) and relative risk of transmission (figure 9c), provided that per coital act risk did not change over time after repeated exposure to virus.

## 3. Discussion

Our results identify  $10^4$  HSV DNA genomic copies as a conservative threshold below which coital transmission is unlikely to occur. This result is consistent across our mechanistic and statistical models, and across various assumptions of the per coital act transmission risk and transmission curve slope. We predict that an intervention which maintains genital viral load below this level will prevent most if not all transmissions.

Our model also highlights kinetic features of HSV-2 shedding which optimize transmission. Genital tract viral load is frequently high during the natural history of untreated disease. Inevitably, sexual acts sometimes occur when viral loads are substantial enough to support transmission. High viral loads are common despite an intense immune response that prevents viral replication from persisting in a single microenvironment for more than 24 h [18]. Even the addition of standard doses of antiviral therapy does not sufficiently suppress viral shedding to prevent all transmissions.

High-level shedding often occurs when lesions are likely to be at the threshold of clinical detection. Epidemiological studies document that asymptomatic shedding accounts for most transmissions [8,9,24]. Our model output suggests that episodes associated with sufficient viral loads for transmission ( $10^6$  HSV DNA copies), but not large diameter ulcers (more than 3 mm), can result in efficient transmission. Even during severe episodes that do ultimately lead to visible ulcers, extremely rapid HSV expansion allows high viral loads before lesions are visible. Therefore, while the discomfort and stigma associated with lesions may serve as a disincentive for coitus, most episodes do not attain this level of severity, and high viral quantities are typically present before lesions develop. On the other hand, virus may be absent when lesions are present as kinetics of tissue healing lag behind those of viral clearance.

We therefore propose a more nuanced view of asymptomatic shedding as a risk factor for transmission. We previously identified that the majority of shedding episodes are less than 12 h in duration and are asymptomatic [2]. Here, we identify that these brief episodes, while providing critical insights into the biology of latency and peripheral

immune control of viral replication, are unlikely to commonly result in transmission. In our simulations, the median episode associated with transmission was 12.1 days in duration with peak viral load of 7.9 log HSV DNA copies. While episodes lasting more than 8 days account for approximately 20% of total episodes in persons not undergoing antiviral therapy [1], a single 12 day episode has a net duration equivalent to 24 12-h episodes. Moreover, viral load during the course of prolonged episodes is typically 1000- to 10 000-fold higher than during rapidly extinguished episodes. Yet, prolonged episodes sometimes occur with barely appreciable symptoms, for all or most of the shedding period.

It is important to recognize that transmitting episode characteristics during antiviral therapy may differ from those off treatment. A detailed review of viral kinetics in 158 participants on twice daily aciclovir or famciclovir revealed that shedding episodes on therapy have shorter duration and lower viral load. Yet, a third of episodes were more than 3 days, and approximately 25% peaked at more than  $10^6$  HSV DNA copies [25]. Our analysis of transmission on therapy suggests a dose–response effect. High doses of available treatments may limit the probability of transmission. Unfortunately, for partially effective doses of treatment such as valaciclovir 500 mg daily, the relative benefit of therapy is likely to wane with time.

There are several limitations to our study. First, these *in silico* experiments cannot be validated clinically as sampling prior to coitus in numerous serodiscordant couples is not feasible. Next, our model considers HSV transmission in the absence of biomedical interventions. While therapies that lower genital viral load below  $10^4$  HSV DNA copies would be likely to have a favourable impact on incidence and disease severity, other interventions decrease transmission by exerting protective effects at all viral loads.

Other limitations are best interpreted in terms of the theoretical transmission curves in figure 6. These curves may not be equivalent between different populations. Most importantly, our model ignores gender effects: strikingly, quantitative shedding rates, and all features of episodes including their rate, viral production, and expansion and decay kinetics, are identical in women and men [1]. Yet, women have higher HSV-2 incidence in nearly all prospective trials [13,26]. Women have a wider surface area of mucosa than men that typically acquire HSV-2 in genital skin, in which a layer of anucleate cells must be breached for HSV-2 to access susceptible target cells. Consequently, women may have a lower threshold viral load for acquisition than men.

While the close bidirectional fit of our infectivity parameter is reassuring, the small discrepancy between our mathematical and statistical approaches highlights unavoidable uncertainty in our dose–response curves. The slight difference between these two techniques probably reflects the fact that our mathematical model's stochastic output shows very small deviation from the actual shedding data (figure 2) with repeated model simulations. The statistical risk model neglects analysis of episode features associated with transmission but has the strength of incorporating only empirical data rather than simulated output.

There is also uncertainty in the precise nature of transmission response curves based on potential biases in the available data. Our estimate for viral infectivity might be too high, primarily because our transmission cohort was defined

by acquisition and did not include partners who remain seronegative after many exposures to HSV [7]. On the other hand, there are several theoretical reasons why our estimate for infectivity might be too low. We derived the median number of coital acts prior to transmission from a retrospective study of transmission [7]: all primary infections were symptomatic, whereas most acquisitions in the community are asymptomatic. Asymptomatic acquisitions may tend to occur following lower viral loads, which would suggest higher viral infectivity (dose–response curve shifted left). Moreover, periodic use of condoms may make 40 median acts an overestimate for unprotected sex, in which case viral load threshold for transmission in the absence of condoms would also be lower. Finally, if partners are more likely to avoid coitus when lesions are present, then our estimates for viral load threshold may be too high as coitus would be less likely to occur at the highest viral loads.

Nevertheless, the data from the retrospective cohort study [7] are likely to be more representative of transmission dynamics in the general population than clinical trial data estimates, which reveal a far greater number of acts prior to transmission [13,26]. Trial participants are repeatedly counselled, are motivated to prevent transmission, and are aware their partners have HSV-2. Importantly, such couples have been together for a mean of 2 years and not transmitted, suggesting that they are 'survivors'. Therefore, the lower transmission rates in clinical trials do not mimic higher rates of spread in a population.

For these reasons, it is not possible to identify a precise threshold below which transmission is impossible to occur. However, even with assumptions of very high infectivity, very few transmissions occurred below  $10^4$  HSV DNA copies. Future trials of novel antivirals can be used to clinically validate this observation. Our previous work suggests that this level of shedding correlates with fewer than 10 infected cells within a plaque and is achieved within hours of plaque initiation [19,22]. Therefore, a successful intervention would need to have an early and potent effect to achieve this goal.

Another source of uncertainty is that the relationship between viral inoculums and probability of infection may not be linear. A power law may apply whereby transmission probability increases more or less rapidly with increases in viral load, as has been shown in HIV [27]. If variables other than viral load, such as heterogeneity in availability of genital target cells owing to microbreaches in the epithelium or duration of coital exposure, are important in determining transmission probability, then the true transmission probability curve would appear more flat (figure 6d). Under this assumption, transmission at lower viral loads would be more likely while transmission at higher viral loads would be less certain. We lack sufficient empirical data to identify the true slope of the transmission curve and a wide range of slopes are statistically compatible with known distributions of transmission. However, in one study, patients who received valaciclovir daily transmitted to their partners despite the fact that viral levels were rarely more than  $10^5$  HSV DNA copies [13], a fact which at least indirectly supports the shallower slope of a power-law transmission probability curve with shape parameter less than 1.

Site of shedding in the transmitting partner may be a particularly important variable in determining transmission probability, which could lower the slope of the curve. Our dataset is not structured to discriminate transmission rates according to regional anatomic shedding or type of sexual

activity, though there is clearly a wide spatial dispersion of HSV-2 across the anogenital region [28]. Perianal shedding of virus, which predominates in many infected persons [29], may or may not promote transmission as readily as genital shedding. The assumption that all shedding can be summed and used as an overall measure of transmission risk is an oversimplification that may skew our results.

An important unanswered question is how HSV-2 infection impacts HIV-1 transmission dynamics. There is evidence that HSV enhances HIV-1 acquisition risk [30,31], but we are unable to accurately model this phenomenon based on spatial variability regarding anatomic sites of entry for HSV-2 and HIV-1. HSV-2 treatment appears to decrease HIV-1 viral load, but thus far this effect does not appear potent enough to lower HIV-1 viral loads below a threshold which would eliminate transmission.

In summary, HSV-2 shedding episodes are associated with highly effective transmission owing to their high frequency, rapid expansion to high-copy peaks and association with mild lesions. Our model suggests that quantitative shedding is an excellent surrogate endpoint in evaluating the effect that a biomedical intervention may have on transmission, and highlights successful strategies employed by the virus to facilitate frequent transmission.

## 4. Methods

### 4.1. Shedding model

Simulations were performed using C++ and R. The model consists of two components, shedding and transmission following coitus. The shedding model has been described elsewhere [18]. For this paper, it is used as a precise simulator of population-level shedding patterns.

Briefly, the stochastic model produces unique short-term shedding patterns with each run, though consistent trends are evident in simulations lasting a year. Its equations assume that HSV-2 DNA ( $V_{\text{neu}}$ ) is released from neurons into genital skin in a continuous steady rate ( $\psi$ ) across wide spatial gradients. Episodes are initiated periodically when a keratinocyte (S) is infected with neuron-derived HSV-2 according to rate  $\beta_i$ . HSV-2 replicates at rate  $p$  in infected cells (I). Cell-associated HSV-2 ( $V_i$ ) spreads within a single ulcer between keratinocytes with infectivity  $\beta_i$  leading to expansion in viral quantity and ulcer diameter.

$V_i$  converts to cell-free HSV ( $V_e$ ) when cells are lysed at rate  $a$ , or killed by CD8<sup>+</sup> T cells ( $E$ ) at rate  $f \times E$ . Containment of infected cells occurs owing to expansion of  $E$  at rate  $\theta$  within each region. CD8<sup>+</sup> T cells decay (rate =  $\delta$ ).  $V_e$  initiates new ulcer formation in adjacent regions of mucosa at rate  $\beta_e$ . The model assumes 300 microregions arranged in a hexagonal lattice and allows concurrent HSV expansion and containment within multiple regions.

Equations and parameters are in table 1 [32]. The model has been tested against a dataset of 1020 shedding episodes from 14 685 genital swabs performed by 531 study participants, and recapitulates population-level dynamics of HSV-2 shedding including rate of episodes, early HSV expansion rate, late decay rate, heterogeneity of episode duration and peak viral production, when simulated over many years [18]. Model output is consistent with clinical observations of widespread spatial spread of virus [33,34], and localized infiltrates of HSV-specific lymphocytes [35,36], suggesting focal areas of viral replication and immunity, dispersed in multiple genital tract regions.

The effect of antiviral medicines on shedding is determined by pharmacokinetic and pharmacodynamics equations, which

describe their impact on viral replication in neurons ( $\psi$ ) and epithelial cells ( $p$ ) [23].

### 4.2. Transmission equations

Coitus is simulated on average every 4 days with intervals between events drawn from a Poisson distribution. We measured the number of acts because the key probabilistic unit of sexual transmission is the single coital act.  $V_e$  are assumed to infect the seronegative sexual partner. We made the simplifying assumption that all  $V_e$  are transferred to the negative partner and that infection occurs when one or more cells are infected.

These assumptions are intended to reproduce transmission biology at the most simple scale possible. Several features of transmission are not included. In reality, only a percentage of HSV may be transferred to the negative partner during coitus; HSV may sometimes replicate in a few keratinocytes in the uninfected host before being cleared stochastically and not access neural ganglia to establish chronic infection; certain acts may predispose to transmission more than others. Because these concerns are not supported by data, we favour the simple set of assumptions described above.

### 4.3. Statistical model

This model used transmission risk =  $1 - e^{-((V_0/c)^{\alpha}\beta_t)}$  for each event, which was derived from the viral decay function ( $dV_e/dt = -cV_e$ ) and instantaneous risk ( $\beta_t \times V_e \times dt$ ). For each event, viral load ( $V_0$ ) in the transmitter was randomly sampled from the distribution of shedding data (figure 2). Infection was modelled stochastically by drawing a random uniform variable and comparing it with infection risk. Transmission occurred when the random variable was less than transmission risk. Coital events were simulated until infection occurred. The risk for each event was independent and risk did not depend on the time pattern of coital events [37].

To solve parameter  $\beta_t$ , we binned data from the previously mentioned study in which 195 subjects estimated the number of coital events with a partner before they developed HSV. Subjects were categorized in bins of 1, 2–4, 5–9, 10–19, 20–29, 30–49, 50–100 and 100+ acts [7]. To fit our model to these data, we simulated 195 infections, counted total events before transmission, binned the data into the equivalent categories and calculated deviance using the G-test for maximum-likelihood estimates.

We next incorporated parameter  $\alpha$  to characterize transmission curve slope. The formula for transmission probability ( $1 - e^{-((V_0^{\alpha}\beta_t)^{\alpha}/(\alpha^{\alpha}c^{\alpha}))}$ ) was derived using the viral decay equation,  $dV_e/dt = -cV_e$ , and instantaneous risk (hazard function),  $(V_e \times \beta_t)^{\alpha}dt$ , where  $t$  is time since coitus. Given a single viral transfer with initial viral load,  $V_0$ , the viral decay equation can be solved:  $V_e = V_0 \times e^{-ct}$ . The cumulative hazard  $(V_0 \times \beta_t)^{\alpha}/(\alpha \times c)$  was derived by integrating the hazard function (with the solved viral decay equation substituted) from  $t = 0 \rightarrow \infty$ , which was equivalent to overall survival probability of  $e^{-((V_0^{\alpha}\beta_t)^{\alpha}/(\alpha^{\alpha}c^{\alpha}))}$ .

While the risk for each coital event was independent when  $\alpha = 1$ , this was not the case for other  $\alpha$  values. To assess transmission probability of multiple coital events, we assumed coitus occurred as a Poisson process and time to event was drawn from an exponential distribution with a corresponding rate of 4 days. At each event, viral load was randomly sampled from the distribution of shedding data (figure 2). The risk was calculated for the time between each event by assessing one minus the survival function derived from integrating the hazard from  $t = 0 \rightarrow$  sampled next coital event time. We then recorded total events that occurred before transmission.  $\beta_t$  and  $\alpha$ -values that minimized deviance from the data were identified using maximum-likelihood.

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