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Microclimates Might Limit Indirect Spillover of the Bat Borne Zoonotic Hendra Virus

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Abstract Infectious diseases are transmitted when susceptible hosts are exposed to pathogen particles that can replicate within them. Among factors that limit transmission, the environment is particularly important for indirectly transmitted parasites. To try and assess a pathogens' ability to be transmitted through the environment and mitigate risk, we need to quantify its decay where transmission occurs in space such as the microclimate harbouring the pathogen. Hendra virus, a *Henipavirus* from Australian Pteropid bats, spills-over to horses and humans, causing high mortality. While a vaccine is available, its limited uptake has reduced opportunities for adequate risk management to humans, hence the need to develop synergistic preventive measures, like disrupting its transmission pathways. Transmission likely occurs shortly after virus excretion in paddocks; however, no survival estimates to date have used real environmental conditions. Here, we recorded microclimate conditions and fitted models that predict temperatures and potential evaporation, which we used to simulate virus survival with a temperature-survival model and modification based on evaporation. Predicted survival was lower than previously estimated and likely to be even lower according to potential evaporation. Our results indicate that transmission should occur shortly after the virus is excreted, in a relatively direct way. When potential evaporation is low, and survival is more similar to temperature dependent estimates, transmission might be indirect because the virus can wait several hours until contact is made. We recommend restricting horses' access to trees during night time and reducing grass under trees to reduce virus survival.

Keywords Flying foxes · Spillover · Survival · Environmental transmission · Horses · Microclimates

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Introduction

Infectious diseases are transmitted when live particles of a pathogenic parasite are successfully introduced and are able to reproduce in the body of a susceptible host [1]. Factors that have the potential to reduce or increase the likelihood of transmission between individuals include, but are not limited to reservoir host immunity; excretion routes; the environment (temperature, humidity, wind speed) [2, 3]; presence of vector hosts; and the target organs of infection. The importance of these factors will differ depending on the mode of transmission of the parasite, vector, direct or indirect. For instance, the environment is particularly important for indirectly transmitted parasites [4], which can influence the magnitude of the transmission rate among individuals [5]. Consequently, the ability to be transmitted indirectly might depend on the predominant environmental conditions while the parasite awaits contact with a susceptible host. Similarly, to try and infer the most likely transmission route of a parasite in order to disrupt it and mitigate its impact, we need to understand the consequences of its interaction with the environment.

Accurate environmental data is essential to predict the decay of a parasite population. When microorganisms are excreted on to the ground, individuals can experience a wide array of micro-

climatic conditions. Microclimates differ from macroclimates in the spatial scale of their variability. Microclimates might change dramatically between adjacent small areas depending on the substrate, topography, and vegetation [6]. There are existing models to predict the air temperatures at different heights above ground that might result from interactions of solar radiation and wind with shade and type of soil [7]. However, these models are uninformative of the actual variability of structures that create the microclimates at a scale that is relevant for a microorganism's survival. Hence, we need a framework that allows us to understand and easily predict the range of conditions that occur in the structures where hosts and parasites are more likely to contact each other.

Hendra virus (HeV), a Paramyxovirus of the genus *Henipavirus*, was discovered in an outbreak involving the death of 14 horses and one human [8]. Its reservoir hosts are the four mainland Australian flying fox fruit bats (Chyroptera:Pteropodidae:*Pteropus*), although *Pteropus alecto* and *Pteropus conspicillatus* seem to be the most important reservoirs [9–11]. Transmission from flying foxes to horses is thought to occur after ingestion of urine contaminated feed. This hypothesis has been considered plausible given the ability of HeV to survive for up to 4 days in fruit juice and urine in laboratory conditions at 22 and 37 °C, although HeV is extremely sensitive to desiccation at these temperatures [12]. Even under the best virus survival scenario represented by air temperatures, spillover has not occurred in areas with the highest possible survival during certain seasons [11]. This suggests that survival as explained by air temperature is not important for spillover, and transmission is likely to be relatively direct. Regardless of these large-scale patterns, which might be driven by other temperature-related processes or heterogeneous HeV shedding patterns [13], more accurate estimates of HeV lifespan are necessary to better understand its transmission to horses. Therefore, we need to more closely investigate and determine the lifespan of HeV in the environment using accurate microclimatic measurements.

Here, we studied the microclimates where HeV is excreted and developed a series of models that predict these conditions using data from meteorological stations. We used the microclimate models' predictions to estimate survival reparameterising a published HeV survival model [11]. Because HeV is highly sensitive to desiccation, using a model of potential evaporation [12], we calculated the probability that survival is shorter than estimated with the temperature only model due to evaporation.

Methods

The Study System

Air and bare soil temperatures are not representative of the climatic conditions experienced by HeV excreted in horse paddocks, but to date, they are the only climatic variables used to model HeV survival [11, 14]. To estimate HeV survival in the conditions the virus experiences in paddocks, we sampled microclimates and generated a series of statistical models that predict microclimatic conditions using data from meteorological stations and physical characteristics of microclimates such as grass length and canopy openness.

We generated estimates of HeV survival in microclimates coupling a temperature virus survival model with the microclimate temperature predictions of two temperature models: one for minimum and another for maximum temperature. HeV survival in response to constant temperatures was estimated from laboratory data [11]. We modified the Martin et al. [11] model to make it more numerically stable using the same experimental data by Paul Selleck. While there are HeV experiments that account for the effect of more variables [12], Paul Selleck's experimental data encompasses a wider range of temperatures with intermediate measurements. The original model transformed the shape parameter κ of the Weibull distribution [5, 15] in a function of temperature, because the decay rate decreased with time, but such a decrease is temperature dependent, such that $\kappa(T) = \alpha_\kappa + \exp(\beta_\kappa T)$. One problem with this formulation is that it only makes sense if $\alpha \geq 0$. To fix this, we used the following alternative formulation: $\kappa(T) = \exp(\alpha_\kappa + \beta_\kappa T)$ hence the modified model

$$S(t,T) = exp\left[-exp\left(\alpha_\rho + \beta_\rho T\right)t^{exp(\alpha_\kappa+\beta_\kappa T)}\right]1\Big)$$

All our models were fit in a Bayesian inference framework using JAGS [16] for MCMC simulation and sampling. The temporal scale of the HeV survival data was modified to hours to match the scale of the recorded microclimate data.

We used these models to calculate the potential range of HeV survival 12 h after excretion, and the probability that the proportion of viable HeV in paddocks is greater than 0.01 24 h after excretion. We chose these time thresholds because (1) HeV survival was previously reported to be very low 12 h after excretion in soil conditions [11] and (2) to assess if there is potential for HeV accumulation. In order for accumulation to occur, the proportion of viable HeV has to be greater than sterility conditions (10^{-4}, [15]) once virus excretion is resumed (24 h later). With our analyses of potential evaporation, we calculated the probability that survival is lower than estimated with the survival model based on temperature alone.

Sampling of Microclimates

We measured temperature and relative humidity of ground level microhabitats occurring in horse paddocks with Hygrochron iButton© data loggers at 15-min intervals during day and night. We used this interval to maximise the likelihood of registering temperature and humidity extremes used for data analysis, while still being able to record data for a long

period. Loggers were deployed in four different paddocks up to 20 km north and south of Townsville, Queensland, Australia, which lies within the known distribution of spill-over of Hendra virus (Fig. 1). In each property, we selected at least three trees with different degrees of canopy openness and three grass/ground vegetation heights to cover the range of microclimates that exist in paddocks (Fig. 1). The effect of the tree on microclimates was assumed to be represented by its canopy openness and the effect of the grass by its height. No additional effects of tree or grass species were considered.

We measured canopy openness (percent of canopy area that allows direct transmission of light to the soil) with hemispheric photographs with a Vivitar 0.21× fisheye converter lens attached to a 14–42 mm Panasonic lens. Pictures were processed with the Gap Light Analyser (GLA, [17]). We took all photographs at dawn, dusk or on cloudy days so that the background was brighter than foliage and branches that make up the canopy structure. This was necessary because GLA requires high contrast images between foliage and sky to calculate the area open to direct sunlight. Because some *Eucalyptus* species have very light coloured bark, their canopy pictures had to be pre-processed manually to darken the bark.

We measured grass height with a measuring tape from the position of the data logger to the tip of the leaves that were covering the data logger. The long and medium grass treatments were enclosed with chicken wire and secured to the soil with turf pegs and plastic coated wire. This protected the grass from being eaten by horses and kept the microclimate intact.

Data was downloaded from data loggers every 21–42 days whenever possible. We measured grass length continuously and adjusted it where necessary if it had grown, been eaten or completely disintegrated after drying out. For those intervals where grass was growing/decreasing fast and continuously such that we did not have enough measured data, we interpolated the height linearly between the beginning and end of the data recording period. In cases where the grass was mowed we identified the date of mowing and registered the height for those dates. Then, we moved the logger to the nearest patch of grass with the correct height.

Analyses

We analysed all data with Bayesian models using a MCMC sampler for inference implemented in JAGS 4.0 [16] interfaced in R [18] with the R2jags package. We estimated parameters for the survival model in eq. 1 using flat priors with 10^{-4} precision for all parameters, 10 M iterations, a burn-in rate of 3 M and a thinning rate of 10 K.

From the recorded temperature and humidity data we only used the daily minimum and maximum temperatures and relative humidity to fit models. This was necessary as only minimum and maximum air temperatures were available from meteorological stations. We transformed relative humidity to absolute humidity and/or potential evaporation as described in the following subsection. Before fitting the microclimate models, we performed an exploratory analysis to identify the type of correlations and variance structures between and within explanatory (macroclimate and vegetation) and response variables (microclimate), and assessed visually the type of statistical distribution of the response variables. Macro climate data was obtained from the bureau of meteorology of Australia (http://www.bom.gov.au) from the closest meteorological station (Townsville Airport).

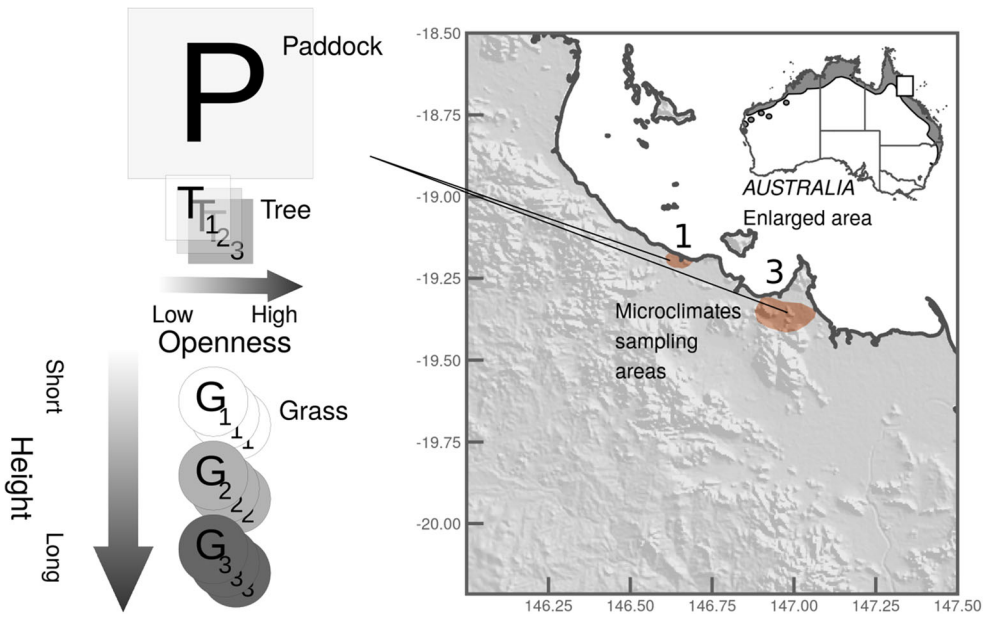

Fig. 1 Geographic location of the areas where microclimates sampling took place and the sampling design used in the four different paddocks. *P* paddock, *T* tree, *L* logger. The *number* in the map indicates the number of paddocks in each of the sampling areas

For each microclimatic variable, we fitted a series of models with combinations of all explanatory variables and their plausible interactions. In addition to these main effects, we included a random effect for paddocks. In all cases, we used poorly informative normal priors with 10^{-4} precision to let data dominate the posterior distributions. Each model was fit with three chains of 50 K iterations, a burn in of 10 K and a thinning rate of 30. To select models, we compared their convergence and deviance information criterion (DIC) and selected the model with the smallest DIC value. DIC is a fully Bayesian measure of goodness of fit, relatively equivalent to the Akaike information criterion used in frequentist statistics [19]. Then, we performed a posterior predictive check of the selected models, looking for a prediction rate as close as possible to 50%. These checks were only done with the temperature and humidity models, as there is currently no HeV survival data to validate predictions of survival in paddocks.

Analysis of Humidity Data

The recorded relative humidity data was transformed into absolute humidity and potential evaporation [20, 21]. Humidity at minimum temperature was analysed as absolute humidity, and humidity at maximum temperature was analysed as potential evaporation. Absolute humidity is the total mass of water per cubic metre of air, while potential evaporation is the difference between the observed absolute humidity and the maximum humidity that air can hold at a given temperature. The procedure followed to transform the relative humidity data into absolute humidity and potential evaporation is described in the supplementary materials.

Survival Simulations

We simulated HeV decay by coupling the survival model with the predictions of the temperature models. With these simulations, we generated a timeline of the expected amount of virus remnant 12 h after excretion, time lapsed until 0.1 of the virus was left and the probability that the surviving proportion is greater than 0.01 24 h after excretion. These simulations assume that excretion only occurs once in the night while temperature is at its minimum, to avoid having to cumulatively calculate several different survival curves. This approach represents the optimal scenario for virus survival because HeV decay is highest during the first moments after excretion [11].

To prepare data for simulation, we used a random subset of 2500 parameter combinations out of the nearly 4.66 billion possible combinations between the survival and temperature models' parameters. This combination results from the posterior parameter estimates for each model ($4002_{min} \times 4002_{max} \times 2910_{surv}$). A list of parameters and their values is given in supplementary information. For each simulation run we accounted for temperature changes over time with a cosine wave. We calculated the probability of observing a reduction in calculated survival rates based on the simulated potential evaporation per day. To indicate potential extent of virus survival reduction, we generated a timeline of potential evaporation in the soil. We ran these simulations in two contrasting scenarios, one with short grass and high canopy openness (5 cm and 77%) and another with long grass and low canopy openness (40 cm and 5%).

Results

Virus Survival Simulations

Virus surviving 12 h after excretion was higher in the scenario with long grass and low canopy openness compared with short grass and high canopy openness (40 cm grass—5% openness vs 5 cm—77.6%, Fig. 2). When we estimated the time elapsed until 90% of the virus lost viability (Fig. 3), a difference of up to 24 h (upper 95% credible interval) between scenarios was observed in winter when minimum soil temperature was10 °C (Fig. S1). The greatest difference between scenarios was seen in the probability that survival rates were greater than 0.01 24 h after excretion (Fig. 4). In short grass (*low* probability scenario), the probability that more than 1% of the virus is left is almost zero all year long, except for winter where it is nearly 0.15. However, in long grass, (*high* probability scenario), it rarely falls to 0.5, and mostly stays close to 1. But, survival rates are also likely to be lower than 10% of excreted virus.

Microclimate Temperature Models

The microclimate minimum temperature model was the simplest because minimum temperature in the soil is highly correlated with air minimum temperature. Of all explanatory variables, air minimum temperature explained the most variance, followed by canopy openness. This model had a DIC = 35,554.9 and $R^2 = 0.856$. Despite this high correlation with air temperature, grass and openness had small negative effects on ground level temperatures and removing these variables increased the DIC (Table S1). Minimum temperatures in long grass tend to be less than one degree higher than in short grass and low canopy openness, with $P = 0.25$ of being higher. This temperature difference between minimum temperature scenarios results in immediate shorter half-life in long grass (Fig. S2).

Both variables tended to have a positive effect on temperature variance. The posterior predictive checks

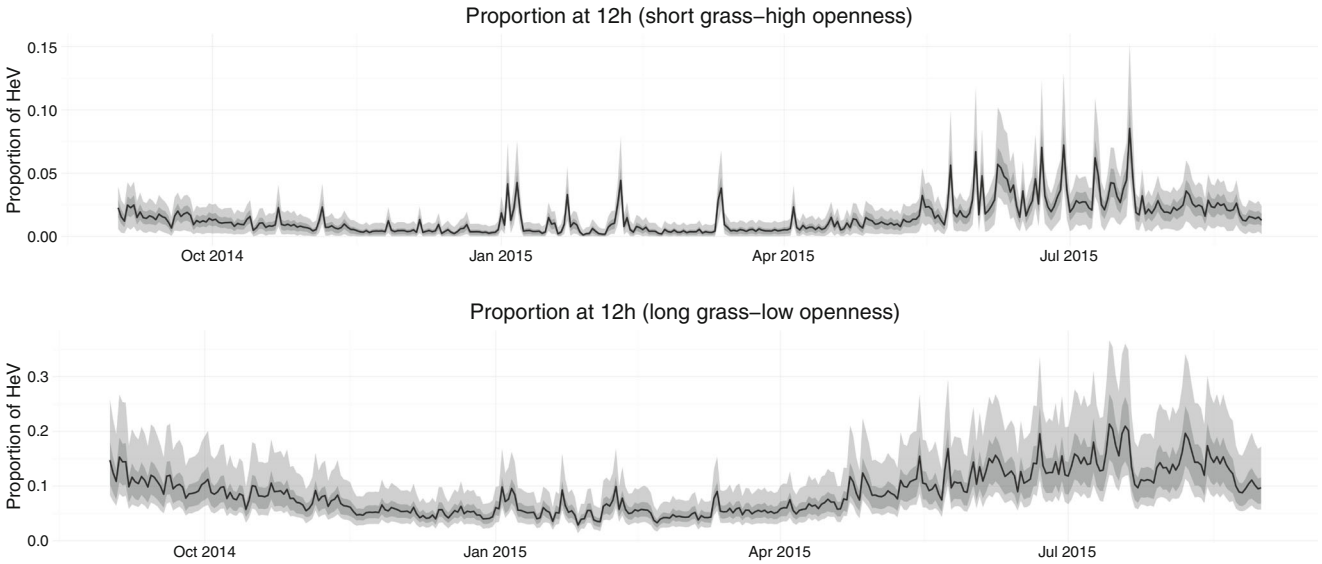

Fig. 2 Simulated survival between two contrasting scenarios. *Light grey ribbons* show the upper and lower 95% credibility intervals

resulted in prediction rates of 47.64%. Structure of the final model:

$$\begin{aligned}\mu_{\text{soilmin}} &= \alpha + \beta_1 \cdot \text{Temp}_{\text{airmin}} + \beta_2 \cdot \text{Grass} + \beta_3 \cdot \text{Open} + \gamma_{\text{property}};\\ \text{Temp}_{\text{soilmin}} &\sim \text{normal}(\mu_{\text{soilmin}}, \tau \cdot 1/(\text{Grass} + \text{Open}))\end{aligned} \quad (1)$$

Microclimate maximum temperature is more variable and consequently its model was more complex, including two interactions, between Openness and Sunshine and Grass and Openness. Its DIC = 68,818.8 and R^2 = 0.485 (0.479–0.487, 95% C.I.). In this model,

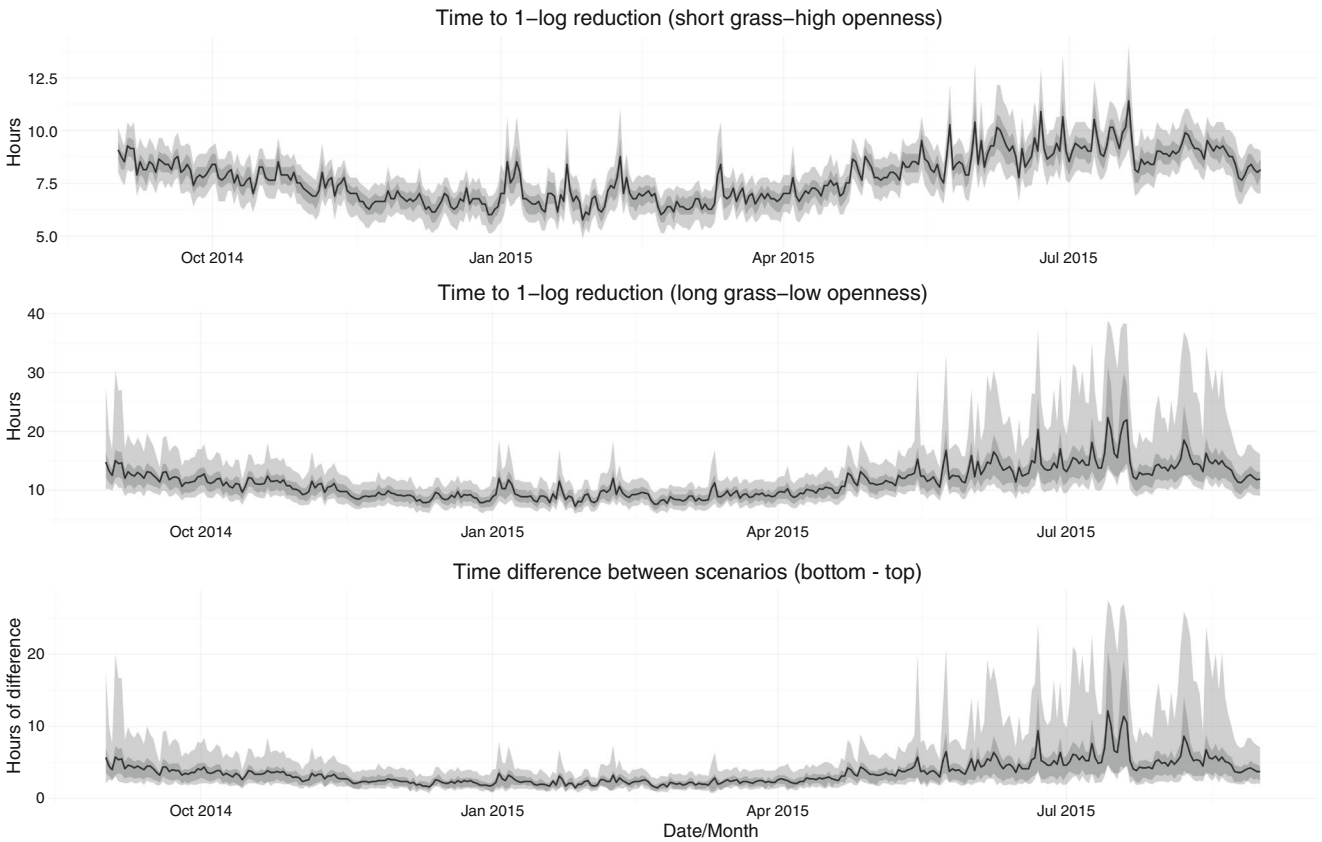

Fig. 3 Simulated timeline of hours spent until loss of viability of 90% of the free virus. *Bottom panel* shows the difference between scenarios; therefore, the greatest difference occurs in July/August where there is a maximum ca. 24-h difference (95% Cr. I.)

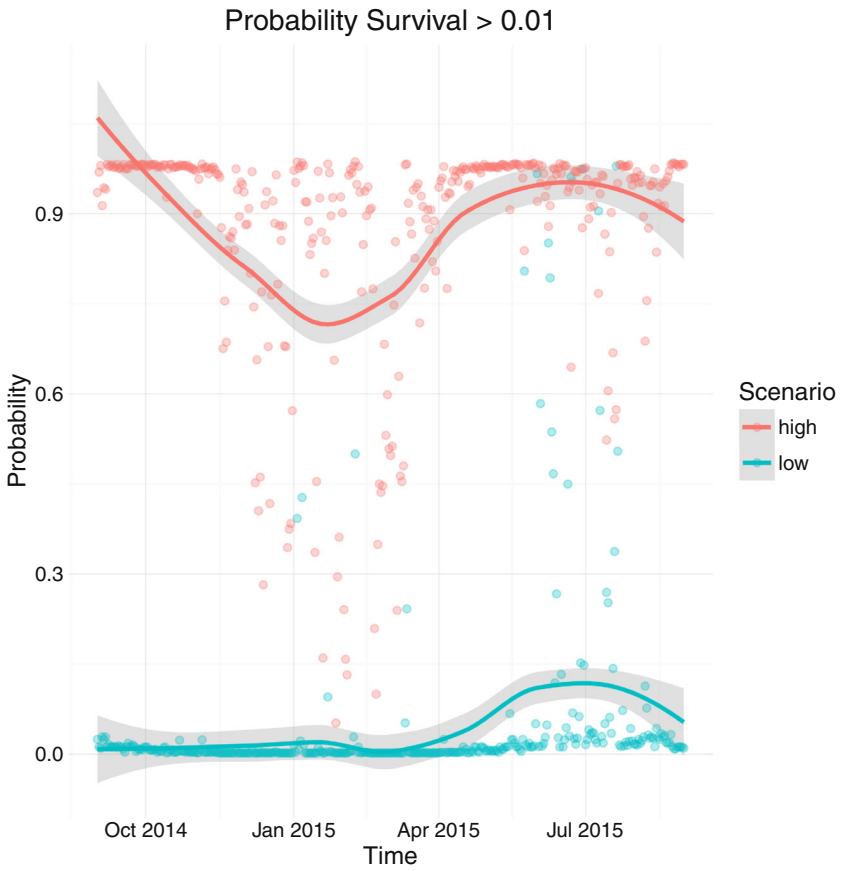

Fig. 4 Probability that survival rates 24 h after excretion are greater than 1%. *Lines* are the smoothed trend and *points* are the probability estimates in time. *Low* low grass/high canopy openness, *high* high grass/low canopy openness

the variables that explained more variance in decreasing order were Openness, the interaction between Grass and Openness and Air maximum temperature. Both Grass and Openness on their own have positive effects on temperature but their interaction has a negative effect (Table S2). The posterior predictive checks show a 50.71% prediction. A timeline of predicted maximum and minimum ground temperatures of Townsville is shown in Fig. S1.

$$\begin{aligned}\mu_{\text{soilmax}} &= \alpha + \beta_1 \cdot \text{Temp}_{\text{airmax}} + \beta_2 \cdot \text{Grass} + \beta_3 \cdot \text{Open} + \beta_4 \cdot \text{Sun} \\ &+ \beta_5 \cdot \text{Open} \times \text{Sun} + \beta_6 \cdot \text{Grass} \times \text{Open} + \gamma_{\text{property}} \\ \text{Temp}_{\text{soilmax}} &\sim \text{normal}(\mu_{\text{soilmax}}, \tau \cdot 1/(\text{Grass} + \text{Open}))\end{aligned} \quad (2)$$

Maximum temperature in short grass was always higher than temperatures in long grass and low canopy openness. The temperature difference between these scenarios was close to 20 °C (Fig. S1).

Microclimate Evaporation and Humidity Models

Microclimate maximum humidity (humidity at minimum temperature) was modelled as Absolute humidity and its final model included four explanatory variables, Grass, Openness, Absolute humidity of air and the interaction between Absolute humidity of air and Grass. Of these, the variable explaining more deviance was Absolute humidity of air, followed by Grass. This model's DIC = −26,623.1 and R^2 = 0.755 (0.754–0.755). As determinants of microclimate absolute humidity, openness and grass have negative and positive effects, respectively, that is, higher openness decreases absolute humidity and longer grass increases it. The interaction between grass and air absolute humidity had a negative effect, probably due to the negative effect that grass has on temperature (see above results on minimum temperature) (Table S3).

$$\begin{aligned}\mu_{\text{Hsoil}_{\text{max}}} &= \alpha + \beta_1 \cdot A_{\text{Hair}_{\text{max}}} + \beta_2 \cdot \text{Open} + \beta_3 \cdot \text{Grass} + \beta_4 \cdot \text{Grass} \cdot A_{\text{Hair}_{\text{max}}} \\ &+ \gamma_{\text{property}} A_{\text{Hsoil}_{\text{max}}} \sim \text{normal}\left(\mu_{\text{Hsoil}_{\text{max}}}, \tau \cdot 1/(\text{Grass} + \text{Open})\right)\end{aligned} \quad (3)$$

Minimum microclimate humidity (humidity at maximum temperature) was modelled as potential evaporation. This model was more complex than the model of maximum microclimate humidity. It contained two interactions between Sunshine and Openness and between Grass and Air potential evaporation. The variables explaining more deviance were the interaction between Sunshine and Openness and Air temperature, which had a positive and negative effect respectively (Table S4). The

posterior predictive checks rendered 48.97% prediction, and the DIC = −3510.2, R^2 = 0.786.

$$\mu_{\text{potsoil}_{\text{min}}} = \alpha + \beta_1 \cdot \text{Temp}^2_{\text{soil}_{\text{max}}} + \beta_2 \cdot \text{Evap}_{\text{potair}_{\text{min}}} + \beta_3 \cdot \text{Temp}_{\text{air}_{\text{max}}} + \beta_4 \cdot \text{Sun} \times \text{Open} + \beta_5 \cdot \text{Grass} \times \text{Evap}_{\text{potair}_{\text{min}}} + \gamma_{\text{property}} \quad (4)$$

$$\text{Evap}_{\text{potsoil}_{\text{min}}} \sim \text{normal}\left(\mu_{\text{potsoil}_{\text{min}}}, \tau \cdot 1/(\text{Grass} + \text{Open})\right)$$

Evaporation, both at minimum and maximum temperature, is consistently higher in short grass/high openness compared with long grass/low openness (Fig. 5). The probability that evaporation is higher under short grass/high openness than evaporation in long grass/low openness is ≈1.

Virus Survival Model

The model converged with 10 M iterations, and we obtained the following half-life estimates for the experimental temperatures; HL_4 = 2.64 h (0.28–7.44, 95% credibility intervals), HL_{22} = 0.52 h (0.14–1.06), HL_{56} = 0.02 h (0.021–0.028, Fig. S11). Response to each of the fitted temperatures and the surface of the model are shown in Fig. S12. We compared estimates of this fitted model with that of Martin et al. (2015), and did not notice major differences. Although, when we fitted the model using the first eight data points for each temperature, the half life estimates were noticeably lower than those of Martin et al. 2015. As a result, we kept the model parameters estimated with the first five data points (results not shown).

Temperature has an effect on both shape and rate parameters of the Weibull distribution, κ and ρ respectively. These parameters control how decay changes with time (κ) and how steep decay is (ρ). In the case of HeV, both parameters are controlled by temperature. Given parameter estimates, decay rate decreases with time ($\kappa < 1$). The effect of temperature on this parameter indicates that at higher temperatures, immediate rates of decay are higher. That is, at higher temperatures, greater proportions of HeV die immediately after excretion because $\kappa(56)$ = 0.44 (0.21–1.02, 95% Cr. I.), $\kappa(22)$ = 0.53 (0.3–0.89) and $\kappa(4)$ = 0.58 (0.36–0.83) (Table S5).

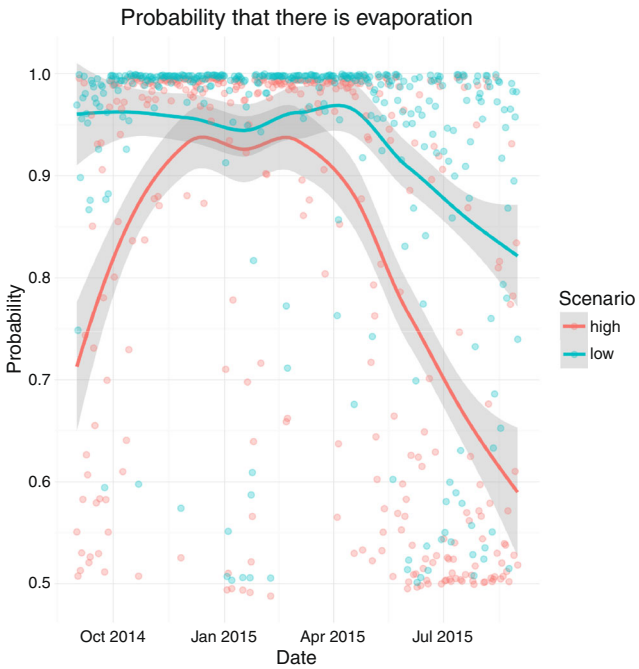

Fig. 5 Smoothed probability that evaporation occurs in two contrasting scenarios. Assuming that evaporation as measured drives death by desiccation, the occurrence of evaporation can be interpreted as probability that survival is lower than predicted with the temperature conditions of simulations. *Low* low grass/high canopy openness, *high* high grass/low canopy openness

Sampling of Microclimates

We recorded temperature, relative humidity data, grass height and canopy opennes data from October 2014 to August 2015. During this period, we had to relocate the data loggers around the same tree to keep the appropriate grass height and canopy openness level on several occasions, especially in two of the four properties due to grass mowing and horses knocking off or burrowing the data loggers. The effect of grass growing, dying or being eaten, and canopy openness changing between seasons was also controlled with these changes.

Discussion

Survival rates of HeV were consistently higher in long grass and low openness compared with short grass and high openness. Differences were consistent among all monitored thresholds, survival 12 h after excretion, time elapsed until 90% of the virus lost viability and probability that survival rates were greater than 0.01 24 h after excretion. Overall survival estimates based on temperatures suggest that potential for virus accumulation in successive days is higher in long grass during winter, and evaporation increases the disparity between long and short grass all year round. These estimates, based on the most likely microclimatic conditions experienced by HeV excreted in paddocks, can be used to make informed decisions for active preventive management of HeV spillover in paddocks. Furthermore, our microclimate temperature and humidity models can be used for improving understanding of the effects of microclimates on parasite transmission and their survival in the environment in general.

Studies of bat movements indicate that bats return to feed in the same tree on consecutive nights. Therefore, there is potential for an infected bat to excrete virus repeatedly in the same tree for the length of its infectious period [22]. Previous estimates of HeV survival predicted an ability to survive for much longer than 24 h, suggesting great potential for accumulation

if viable HeV was excreted on successive days [14]. Here, we demonstrate that under the real temperature conditions experienced by the virus in the environment, survival for more than 24 h is feasible under very specific circumstances; long grass/low openness and no evaporation. Although accumulation would occur at much lower levels than the mentioned study [14] and likely at biologically insignificant levels. This is because the necessary temperature, humidity, grass length and canopy cover conditions for significant virus accumulation over a period of days leading to an increase in spillover risk might not be generally present [11].

The effect of humidity on survival and transmission of viruses seems to differ among them. For instance, higher absolute humidity decreases influenza transmission and survival in the air [20, 23], while Gumboro virus survival increases with relative humidity [21]. Hendra virus is highly sensitive to desiccation [12], which is the process of loss of humidity and is influenced by the potential evaporation in the air [21, 24]. Based on our current knowledge of HeV survival capacity, we cannot predict the exact evaporation dependent rates of decay. Fogarty et al. [12] calculated a 1200 times reduction of half-life between survival with and without desiccation at 37 °C; therefore, we infer that decay should increase monotonically with potential evaporation. As a result, the predicted survival rates at 12 h shown in Fig. 2 could decrease in a magnitude proportional to what is shown in Fig. S3 (potential evaporation).

Additional factors that can further decrease HeV survival in microclimates are UV and dust [25, 26]. To date, the effects of UV on HeV have not been tested, but it is an effective germicide, and enveloped viruses like HeV are usually highly sensitive to it [25]. Desiccation, exposure to UV radiation and dust, are likely to change the HeV response to temperature, accelerating the rate of decay in time through cumulative damage, a known phenomenon in populations of free-living microorganisms [15]. In support of this, some of the HeV survival experiments of Fogarty et al. [12], appear to show concave or convex non log-linear survival responses between substrates, which are indicative of cumulative damage. Consequently, temperature-dependent survival, as estimated here, is more likely to be the best case survival scenario and an overestimate. This means that the poor contribution of HeV survival towards predicting its spillover [11] could be caused by its susceptibility to the conditions that occur in microclimates, and the stronger effect that temperature has on other processes that influence HeV spillover risk like bat densities and *Eucalyptus* phenology [27, 28].

Previous attempts to quantify HeV survival rates in microclimates assumed that soil temperatures were up to 5 °C lower than air temperature [14]. Alternatively, broad-scale temperatures of types of bare soil were used under different levels of shade [11]. None of these studies have used the specific conditions that occur where HeV is excreted as we have done here. Our results, not surprisingly, differ; on one side Scanlan et al. [14] overestimate survival, because their microclimatic conditions represent a better survival scenario than air temperatures. On the other, Martin et al. [11] underestimate survival in microclimates because some of the temperatures used correspond to bare soil, sand or rock, which represent the worst temperature-dependent survival scenario. Our data suggests that HeV survival rates in most cases are probably lower than under air temperatures and higher than estimated at bare soil temperature.

We used our models with macroclimatic data from other regions to predict microclimatic conditions; however, these predictions have not been validated. While temperature estimates seem reasonable given our current knowledge, there are other complexities we did not account for. For instance, sunshine hours is used to represent solar radiation; however, radiation changes latitudinally, hence the effect of sunshine hours would be different farther south than in Townsville. In addition, topographic shading can result in further microclimatic differences, even in areas with similar macroclimatic conditions [29]. These extrapolated predictions generated from Brisbane and Cairns (supplementary materials), and the resulting survival rates do not differ greatly from Townsville. These simulations show a similar pattern of lower survival than with air temperatures and higher than bare soil [11]. For the reasons mentioned above, these simulations still need to be validated and their purpose is to show what survival might be like.

Martin et al.'s [11] conclusion that virus transmission is likely to follow relatively direct routes is supported. This is because we found that evaporation and the consequent probability of survival reduction are relatively high at all times. However, we found that virus survival under the optimum microclimate temperature conditions experienced by HeV excreted in paddocks can be as long as 20 h, assuming that a 90% decay is required to significantly reduce risk to horses. In this case, 20 h is certainly long enough for indirect contact to occur. Determining the length of time for optimal survival for spillover depends mainly on the minimum infectious dose and the amount of virus excreted by flying foxes. Consequently, indirect transmission during the night is more likely because HeV loads are higher, and during the day evaporation and UV radiation will very likely further increase temperature dependent decay, making virus transmission during the day and virus accumulation on successive days of excretion even less probable.

The experimental data used here was generated under tightly controlled laboratory conditions. What the data actually represents is the decay of a HeV population in EMEM culture medium [14] under constant temperatures. The first step taken to run these simulations was transforming the temperature into a function of time. We currently ignore how HeV actually

responds to varying temperatures over time; the cummulative damage of different temperatures through time could result in different decay rates compared with constant temperatures. One feature of the HeV survival data that indicates that decay under varying temperatures would be different is the lack of log-linearity. Log-linearity generally results from immediate response to an external factor like temperature when is there is no effect of the time of exposure [30] . In the case of HeV, its decay slows down with time. Consequently, there is an effect of the time of exposure to temperature, and the only way to assess the effect of exposure to varying temperatures is experimental validation.

The modification of the Weibull survival model with respect to Martin et al. [11], resulted in shorter half-life estimates, especially at 4 °C. However, when survival simulations were performed, the difference between models given the range of termperatures used was negligible. Similarly, Scanlan et al. [31], argue that the recording error in Scanlan et al. [14], had no sensible effect on survival estimates due to the prevailing temperatures used in simulation of HeV decay. However, as with any empirical model, extreme care should be taken when they are used under conditions that are outside the range used to fit them. Therefore, we recommend that when the microclimate temperature models are validated, thorough comparisons of model predictions should also take place.

We identified scenarios where small changes in conditions could lead to large changes in the amount of virus available to horses. These changes are caused by long grass and low canopy openness. Therefore, the reduction of pasture under trees can be an effective preventive strategy for indirect HeV transmission. However, restricting access to trees during the night is likely to reduce risk more effectively because transmission can be relatively direct. Further manipulation of microclimates might involve increasing canopy openness when feasible to increase the radiation that reaches the soil. These measures will likely reduce the probability that horses contact viable viruses either by increasing the decay rate of the virus or reducing the likelihood of horses feeding on Hendra virus contaminated areas of the paddock. We recommend that further research focuses on validating the predictions we produced for the northern and southern extremes of the distribution of HeV spillover, and that more accurate estimates of HeV survival in response to evaporation are obtained under laboratory conditions.

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