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Seasonal fluctuations of Astrovirus, but not Coronavirus, shedding in bats inhabiting human-modified tropical forests

Abstract

Emerging infectious diseases (EIDs) are considered a major threat to global health. Most EIDs appear to result from increased contact between wildlife and humans, especially when humans encroach into formerly pristine habitats. Habitat deterioration may also negatively affect the physiology and health of wildlife species, which may eventually lead to a higher susceptibility to infectious agents and/or increased shedding of the pathogens causing EIDs. Bats are known to host viruses closely related to important EIDs. Here, we tested in a paleotropical forest with ongoing logging and fragmentation, whether habitat disturbance influences the occurrence of astro- and coronaviruses in eight bat species. In contrast to our hypothesis, anthropogenic habitat disturbance was not associated with corona- and astrovirus detection rates in fecal samples. However, we found that bats infected with either astro- or coronaviruses were likely to be co-infected with the respective other virus. Additionally, we identified two more risk factors influencing astrovirus shedding. First, the detection rate of astroviruses was higher at the beginning of the rainy compared to the dry season. Second, there was a trend that individuals with a poor body condition had a higher probability of shedding astroviruses in their feces. The identification of risk factors for increased viral shedding that may potentially result in increased interspecies transmission is important to prevent viral spillovers from bats to other animals, including humans.
Seasonal fluctuations of Astrovirus, but not Coronavirus, shedding in bats inhabiting human-modified tropical forests

Introduction

Emerging infectious diseases (EIDs) are a critical threat to both human and animal health (Jones et al. 2008; Morse et al. 2012; Luis et al. 2013), the majority being caused by pathogens associated with wildlife species (Taylor et al. 2001; Jones et al. 2008; Morse et al. 2012; Wood et al. 2012). Anthropogenic encroachment of natural habitats is considered as one of the primary drivers promoting interspecies transmission of pathogens from wildlife reservoirs to humans (Brearley et al. 2013; Epstein and Field 2015). Involved processes include bushmeat consumption, deforestation, habitat fragmentation, agricultural land-use and urbanization (Calisher et al. 2006; Wood et al. 2012; Luis et al. 2013; Smith and Wang 2013; Pernet et al. 2014; Epstein and Field 2015; Schneeberger and Voigt 2016).

Southeast Asia is characterized by a combination of dense and increasing human population and associated anthropogenic activities such as modification of natural habitats by agricultural land-use, as well as a high biodiversity supporting pathogen diversity (Morse et al. 2012). Thus, Southeast Asia has been suggested as a hotspot for EIDs (Morse et al. 2012). Due to growing human populations and increased demand for natural resources in this region, ecosystems are deteriorating at unprecedented rates. Therefore, it is likely that cross-species transmissions will occur, which might potentially result in disease outbreaks with high morbidity and mortality (Li et al. 2010; Baker et al. 2013; Smith and Wang 2013). Thus, we need an improved understanding of the drivers of EIDs in order to prevent their emergence and mitigate potential outbreaks caused by these zoonotic pathogens (Morse et al. 2012).
The most fatal epidemics in the past decade, such as HIV/AIDS, severe acute respiratory syndrome (SARS), filoviruses (e.g. Ebola and Marburg virus) and influenza, are viral diseases that originated from wildlife species (Morse et al. 2012), mainly from bats (Calisher et al. 2006; Wood et al. 2012). The majority of bat-borne zoonotic viruses are ribonucleic acid (RNA) viruses (Smith and Wang 2013) that can be highly prevalent in bat populations (Wang et al. 2011). Two families of positive-sense single-stranded RNA viruses are of particular interest: Astroviridae and Coronaviridae. Whether Astroviruses (AstVs) and Coronaviruses (CoVs) cause acute or chronic infection in bats is still unclear (Chu et al. 2006; 2008; 2009; Dominguez et al. 2007; Shi 2010; Tang et al. 2006) and previous studies have reported no apparent clinical signs of disease in AstV or CoVs infected bats (Dominguez et al. 2007; Poon et al. 2005; Queen et al. 2015; Tang et al. 2006; Xiao et al. 2011). CoV are an important cause of diseases in humans and other animals, and have been found in more than 100 bat species in America, Africa, Europe, Australia and Asia (Woo et al. 2009; Ge et al. 2015). Bats carry CoVs related to those causing severe diseases in humans, e.g. SARS-CoV (Li et al. 2005) and Middle East Respiratory Syndrome-CoV (Corman et al. 2014). Studies on coronavirus ecology are particularly interesting because the transmission of CoVs between animals, including humans, is expected to continue (Ge et al. 2015). Although AstVs are not known to cause EIDs, they are a suitable model to understand the ecology of RNA viruses because they have typically high prevalence rates in bat populations (Chu et al. 2008; Queen et al. 2015; Young and Olival 2016).

The recent outbreaks of fatal diseases among people highlight the need to gain a better understanding of the drivers of viral spill-overs from wildlife, especially from bats to humans (Drexler et al. 2013; Epstein and Field 2015; Meyer et al. 2016). It is therefore of utmost importance to broaden our understanding of the ecology of viral reservoirs for
emerging diseases. In tropical regions in particular, the effects of anthropogenic habitat
disturbance implemented by logging and fragmentation on viral shedding and host
physiology are of great scientific interest (Meyer et al. 2016). Besides habitat modification,
other factors should be considered when studying variation in viral shedding, for example
age, sex, nutritional and reproductive status, roosting ecology and temporal variation (Smith
and Wang 2013; Schneeberger and Voigt 2016). These factors are all associated with the
host’s immune function (e.g. Schneeberger et al. 2013) and/or are important for viral
transmission (e.g. Turmelle et al. 2010). While previous studies on virus ecology have been
conducted in bats (e.g. Plowright et al. 2008; Turmelle et al. 2010), data from large-scale
experimental areas where anthropogenic habitat modification is ongoing are scarce. Here,
we provide to the best of our knowledge the first data on viruses in insectivorous bats in
Borneo.

The objective of this research was to identify the relevance of habitat disturbance,
seasonal fluctuations, nutritional and reproductive status on viral detection rates using
AstVs and CoVs in paleotropical, insectivorous bats as model systems. We selected these
viruses because of their frequent detection in bats (AstVs) and their zoonotic potential
(CoVs). We focused on the eight most abundant species of insectivorous bats at our study
site in Borneo (Struebig et al. 2013). These species belong to families with relatively high
viral detection rates (Rhinolophidae, Hipposideridae and Vespertilionidae; Olival et al.
2015). Based on previous findings (Brearley et al. 2013), we predicted that logging and
fragmentation of forest habitat is associated with higher viral detection rates. Because lethal
sampling raises obvious ethical concerns, has little benefit over non-lethal sampling (Olival
et al. 2015; Young and Olival 2016), and both AstVs and CoVs can be easily detected in feces,
we used fecal samples collected from live bats for virus screening.
**Methods**

*Study site and species*

The study was conducted within the SAFE project area (Stability of Altered Forest Ecosystems, www.safeproject.net), a 7,200 ha landscape fragmentation experiment established in Sabah, Malaysian Borneo. The SAFE landscape comprises logged over dipterocarp rainforest, some of which is being converted to oil palm plantation, leaving behind a network of disturbed forest fragments, thus replicating a land-use transition common across much of Southeast Asia (Fitzherbert et al. 2008; Gaveau et al. 2014; Marlier et al. 2015). All sample locations were situated within 10 km of a research camp (approximately N4.73 E117.60, see www.safeproject.net for details). Prior to our study much of the landscape had been logged twice, and the forest allocated for conversion to oil palm had been heavily logged multiple times (Struebig et al. 2013). At the time of sampling, these areas were experiencing a final harvest before conversion and were at the early stages of fragmentation, with large areas devoid of tree cover. We sampled bats multiple times at various sites across this disturbance gradient: LFE, a twice-logged site at which logging operations ceased in the late 1990s; and sites B, C and F, which supported similar forest habitat to the LFE at the onset of the study, and then experienced heavy logging and fragmentation throughout the study period. These sites were located 2 to 10 km apart from each other, exceeding the mean home range size of foliage-roosting insectivorous bat species (Struebig et al. 2013). We categorized these sites into three disturbance levels at the time of sampling: relatively undisturbed prior to fragmentation (hereafter “Recovering forest”: LFE, B₁ and C₁, whereby subscript denotes the order of sampling), actively logged sites at the time of sampling (hereafter “Actively logged forest”: B₂, C₂ and F₁). After logging
had been completed, site F (i.e. F2) was categorized as “Fragmented” (for sites B and C logging was still underway at the end of sampling). Data collection took place in March to April (2014) and July to September (2014 and 2015, Table 1).

The landscape has a well described insectivorous bat fauna, which is known to have experienced a substantial shift in assemblage structure in response to past logging events (Struebig et al. 2013). We selected bat species of the families Vespertilionidae (subfamily: Kerivoulinae), Hipposideridae and Rhinolophidae, which were sufficiently abundant across the landscape to warrant sampling. Within the subfamily Kerivoulinae (woolly bats), we studied the following congeneric bats: Kerivoula intermedia, K. papillosa and K. hardwickii. Within the family Hipposideridae (leaf-nosed bats), we focused on the congeneric species Hipposideros cervinus and H. dyacorum, and within the family of Rhinolophidae (horseshoe bats) on Rhinolophus sedulus, R. trifoliatus and R. borneensis. All species are small, insectivorous bats with body masses ranging between 3 and 16 g (Payne et al. 1985). In 2011/2012, in all sites bat abundance was high, but species richness was lower in the repeatedly logged sites (B, C, F) compared to the twice logged site (LFE; Struebig et al. 2013).

Capturing of bats
In the morning hours, we set up a maximum of six harp traps (Bogor Zoology Museum, Bogor, Indonesia) along established forest trails, with a minimum distance of 30 - 100 meters between trapping locations. Harp traps have been successfully used before to study paleotropical bat assemblages, and result in higher capture rates and diversity estimates than other methods in paleotropical forests (Kunz et al. 2009; Meyer et al. 2016). Between
subsequent nights, we shifted traps, resulting in a total of 15 to 30 positions per plot and season. The total harp trap effort was 321 harp trap nights.

We checked traps at 1900 and 0700 of the following day. Bats were retrieved from harp traps and transported back to the camp in cloth bags for processing, unless they were stress sensitive, such as fruit bats (Pteropodidae), individuals of the species *H. cervinus*, juveniles, pregnant or lactating females of any species. These were released as soon as possible after capture. We identified species according to Kingston et al. (2006) and Struebig and Sujarno (2006). Juveniles were distinguished from adults by the epiphyseal closure of phalanges (Kunz and Anthony 1982). We classified the reproductive status of females (non-reproductive, pregnant, lactating or post-lactating) by abdominal palpation and visual inspection of the teats and the surrounding area.

We recorded body mass (g) by using a spring balance (Pesola balance, Switzerland), the length of forearm (mm) using a caliper (WihaWerkzeuge GmbH, Schonach, Germany), sex, age class and reproductive status. We marked all adult bats with a uniquely coded alloy forearm ring of 2.9 or 4.2 mm (Porzana Limited, East Sussex, UK), depending on the size of the bat, as described in Kunz and Weise (2009). We took measurements of all adult bats, but fecal or rectal swab samples (collected using a cotton bud (Copan Italia S.p.A., Brescia, Italy) moistened with RNA stabilization solution, RNAlater™ (Thermo Fisher, Waltham, USA), were limited to the eight focal species. We stored samples in a dry shipper at -80°C until further processing. All bats were released at the capture site within a maximum of 12 hours.

**Virus detection**

Fecal samples (pellets or swabs) were mixed with 500 µl of RNAlater RNA Stabilization Reagent (Qiagen, Hilden, Germany). For virus screening, 50 µl of fecal suspension was
extracted using the MagNAPure 96 DNA and Viral NA Small Volume Kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions. Elution volume was 100µl.

Virus screening for AstVs and CoVs was done using broadly reactive nested reverse transcription-PCR (RT-PCR) assays as described before (de Souza Luna et al. 2007; Chu et al. 2008; Annan et al. 2013).

Ethics statement

Our study and export of samples was authorized by the scientific committee of the SAFE project (Imperial College, London, UK) and the Sabah Biodiversity Center, Sabah, Malaysia (JKM/MBS.1000-2/2 (317); JKM/MBS.1000-2/3 JLD.2 (16); JKM/MBS.1000-2/2 JLD.3 (153)) and complies with the laws of Malaysia, Germany and UK.

Statistics

We used the statistical software R version 3.2.3 for all statistical analyses (R Development Core Team 2015). We conducted two-tailed tests and set the level of significance to \( \alpha = 0.05 \). We analyzed the data in two separate generalized linear mixed-effects models (GLMM) using the packages “lme4” and “car” in R (Fox and Weisberg 2011; Bates et al. 2014) to determine which predictor variables influence the occurrence of AstVs and CoVs. For these models AstV and CoV detection (presence or absence) were treated as dichotomous response variables. Errors were assumed to be binomially distributed, and a logit link function was applied. We included the following predictor variables in the statistical models: habitat type (recovering forest, actively logged forest and fragmented forest); season (dry season: March and April, characterized by a mean monthly precipitation of 77 mm, and beginning of the rainy season: July, August and September, characterized by
a mean monthly precipitation of 170 mm, personal communication from Prof. R. Walsh, Swansea University, UK; year (2014, 2015); species; sex and reproductive status (males, pregnant, lactating and non-reproducing females); AstV infection status (for the CoV model)/CoV infection status (for the AstV model) and body condition (body mass [g] divided by the forearm length [mm]). Also, we included plot identity (B, C, F and LFE) as a random variable to control for non-independence of sampling location. The estimated standard deviation of the random variable (plot) was <0.001 in the AstV and < 0.001 in the CoV model. If a non-continuous predictor variable with more than two categories had a significant effect on the occurrence of viruses, we used general linear hypotheses testing (GLHT) using the package “multcomp” (Hothorn et al. 2008) to compare between categories of the respective predictor variable. We randomly selected data points if we repeatedly sampled the same individual at different field seasons to achieve independence of data.

**Results**

Of 364 individual samples of eight species available, 17 (4.66 %) were positively tested for CoVs RNA and 78 (21.37 %) for AstV RNA. In samples of 15 individuals (4.1 %), we found both CoVs and AstVs. All of these individuals belonged to the species *Hipposideros cervinus*. Among the co-infected individuals, 7 originated from recovering forests and 8 from actively logged forests.

**Coronaviruses**

We detected CoVs in 16 out of 76 individuals (21.1 %) in *Hipposideros cervinus* and 1 out of 46 individuals in *Rhinolophus trifoliatus* (Table 2). For our statistical analyses, we considered only individuals of *H. cervinus* to avoid zero-inflation. The detection rate of CoVs in *H.*
*cervinus* did not significantly vary across habitat types (GLHT, recovering vs. actively logged forest: Estimate = -0.85, SE = 1.02, z = -0.83, p = 0.646; recovering vs. fragmented forest: Estimate = 25.77, SE = 5.32e+05, z = 0.0, p = 1.0; actively logged vs. fragmented forest: Estimate = -0.81, SE = 5.32e+05, z = 0.0, p = 1.0; see Table 3 for model output). The presence of CoV was significantly higher in individuals coinfected with AstVs (GLMM, Estimate = 3.57, SE = 1.33, z = 2.69, p = 0.007). No other predictor variable (body condition, reproductive status, season and year) influenced the occurrence of CoVs in *H. cervinus* (see Table 3 for model output).

**Astroviruses**

We detected AstVs in all eight study species with detection rates ranging between 10 % in *Kerivoula papillosa* (1 out of 10) and 55.6 % in *Rhinolophus sedulus* (10 out of 18, see Table 4). In total, 21.4 % of bats (78 out of 364) shed AstVs in their feces. The detection rate of AstVs did not vary significantly across habitat types (GLHT, recovering vs. actively logged forest: Estimate = 0.56, SE = 0.44, z = 1.25, p = 0.422; recovering vs. fragmented forest: Estimate = -0.25, SE = 0.52, z = -0.48, p = 0.88; actively logged vs. fragmented forest: Estimate = 0.54, SE = 0.54, z = -1.50, p = 0.291; see Table 5 for model output). The presence of AstVs was significantly lower in *H. dyacorum, Kerivoula hardwickii, K. intermedia, R. sedulus* and *R. trifoliatus* compared with *H. cervinus* (see Table 5). Although not significant, there was a trend that the detection rate of AstVs was higher in individuals with a poor body condition (log) compared to individuals with a better body condition (GLMM, Estimate = -2.3, SE = 1.3, z = -1.77, p = 0.077). The detection rate of AstVs did not vary with sex and reproductive status (see Table 5), and the presence of AstV was significantly higher in individuals co-infected with CoVs (GLMM, Estimate = 2.66, SE = 1.14, z = 2.33, p = 0.02).
Further, the abiotic factors season and year explained a significant proportion of variance in the likelihood of infection with AstVs. We detected AstVs more frequently during the beginning of the rainy season compared with the dry season (GLMM, Estimate = 2.57, SE = 0.51, z = 5.09, p < 0.001). In 2015, AstVs were less frequently detected than in 2014 (GLMM, Estimate = -1.65, SE = 0.51, z = -3.26, p = 0.001).

Discussion

The objective of our study was to compare viral detection rates in feces of insectivorous, forest-dwelling paleotropical bats among habitats of different levels of anthropogenic disturbance, with particular emphasis on CoVs and AstVs. In contrast to our hypothesis, the detection rate of CoVs and AstVs was approximately equal across sites with varying levels of forest disturbance. We provide evidence that abiotic factors, namely seasonal and annual fluctuations, are associated with AstV shedding in forest-dwelling bats, and that these factors may be more important drivers of disease dynamics in our study system than forest disturbance per se.

The detection rate of CoVs seems to be generally lower than the detection rate of AstVs in the focal species of our study site on Borneo. The detection rate of CoVs in our study was 4.7 % which is in the same range as in studies of other insectivorous bat species throughout the world: Gloza-Rausch et al. (2008) and Kemenesi et al. (2014) detected CoVs in 1.8 to 9.8 % of individual bats in Germany and Hungary, Pfefferle et al. (2009) detected CoVs in 9.8 % of individual bats in Africa and Corman et al. (2013) found 2.8 % of fecal samples from neotropic bats to be positive for CoVs RNA. The detection rate of AstVs was 21 %, similar to Fischer et al.‘s (2016) study on insectivorous bats in Germany (26 %), but higher than that reported by a study of bats in Hungary (7 %; Kemenesi et al. 2014).
However, the AstV detection rate was more than two-fold higher (46 %) in a study of nine bat species in Hong Kong applying the same RT-PCR assays, although the co-infection rate of individuals with CoVs and AstVs (4 %) was similar (6 %; Chu et al. 2008). We found that bats infected with either AstV or CoV were likely to be co-infected with the respective other virus. Although only marginally significant, AstV detection was more likely in individuals with a poor body condition suggesting that nutritional status and thus energy reserves are important for pathogen shedding. As mounting and maintaining an effective immune system is energetically costly (Schneeberger et al. 2013), animals in poor condition might lack an effective immune response and thus may acquire and transmit other pathogens that they lack defense against. In accordance, previously it has been shown that most microparasites in field vole (Microtus agrestis) interact positively (Telfer et al. 2010). Possibly, these bats may carry other pathogens that we did not test for.

Numerous studies have demonstrated an association between pathogen prevalence and habitat disturbance. Brearley et al. (2013) revealed that across 19 studies, about half reported an increase in diseases prevalence associated with human-modified landscapes. Female Brazilian free-tailed bats (Tadarida brasiliensis) roosting under man-made bridges had a higher risk of infection with rabies virus than conspecifics roosting in natural caves (Turmelle et al. 2010). Bradley et al. (2008) and Gibbs et al. (2006) found that the antibody prevalence against West Nile virus in songbirds was positively related to increasing levels of urbanization. However, habitat disturbance was not associated with the detection rate of AstVs and CoVs in our study. In line with our findings in deer mice (Peromyscus maniculatus), disturbance (measured as vegetation cover) was not associated with Sin Nombre virus prevalence (Dearing et al. 2009). An explanation of these opposing patterns is that results vary with the type of disturbance and between specific host-pathogen
associations. In contrast to Dearing et al.’s study (2009), Langlois et al. (2001) and Mackelprang et al. (2001) reported a higher prevalence of Sin Nombre virus in deer mice from disturbed compared with undisturbed habitats, and related this association to higher encounter rates between hosts due to altered movement behavior and population densities in fragmented or disturbed compared with undisturbed areas. In these studies, disturbance was related to vehicle use by humans (Mackelprang et al. 2001) and landscape structure (Langlois et al. 2001). Additionally, the pattern was absent for *Litomosoides*, a haemoparasite in Jamaican fruit-eating bats (*Artibeus jamaicensis*), although the prevalence of trypanosomes was higher in fragmented habitats compared with continuous forests in the same host species (Cottontail et al. 2009).

Overall, human habitat disturbances are capable of changing the prevalence of pathogenic agents in ecosystems. However, the extent to which this occurs depends on the host-parasite system studied. Habitat disturbances may cause chronic stress (i.e. elevated plasma levels of glucocorticoid hormones) in some species, and consequently the disruption of homeostasis of individuals, e.g. immunosuppression (Sapolsky et al. 2000; Romero 2002; 2004; Suorsa et al. 2004; Wingfield 2005; Wikelski et al. 2006). Thus, a stress-induced immunosuppression may result in an increased susceptibility of individuals to acquire and shed viruses. However, such an effect on AstVs and CoV detection rate was absent in our study. It is possible that vulnerable individuals emigrated or deceased at the early onset of habitat disturbance and thus could not be sampled. The effect of habitat disturbance on viral detection rates may also work through other mechanisms at later time stages. In the long term, fragmentation of habitats leads to a reduced connectivity of remaining habitats (Brearley et al. 2013). This affects mobility and dispersal of some bat species and is known to result in reduced population genetic diversity (Struebig et al. 2011), which can make the
individuals more susceptible to catch and shed viruses in future generations (Brearley et al. 2013). However, we focused on very recent habitat disturbances, thus, the long-term effects of fragmentation may not yet have arisen. Further, the predicted increase in viral prevalences due to habitat disturbance might be outbalanced by higher rates of population turnover, ultimately resulting in no change or even a decrease in viral detection rates.

Logging and fragmentation of forest habitat might influence the invertebrate prey base of bats, which might lead to reduced fitness and force bats to forage further afield. When crossing open spaces, predation risk increases, resulting in an increased population turnover. Depending on the fraction of infected emigrating or deceased individuals, viral detection rates could increase, decline or remain stable during habitat logging and fragmentation.

Although the negative correlation between body condition and viral detection rates was marginally not significant, previous studies demonstrated an association. For example, Plowright et al. (2008) reported a correlation between nutritional stress and Hendra virus seroprevalence in little red flying foxes (Pteropus scapulatus). In support, Turmelle et al. (2010) found a relationship between low body mass and rabies virus infections in Brazilian free-tailed bats roosting under man-made bridges, although exclusively for females. These results indicate that nutritional stress can be associated with viral infections (Plowright et al. 2008), but could also suggest the lack of energetical investment on immune defense (see above). However, reported results are inconsistent. For example, Lau et al. (2010b) found a correlation between a high CoV detection rate and low body mass in Chinese horseshoe bats for one, but not for another CoV strain. In addition, Cottontail et al. (2009) did not find a relation between body mass in Jamaican fruit-eating bats and the prevalence of two haemoparasites.
Consistent with our findings, the prevalence of two haemoparasites did not vary in Jamaican fruit-eating bats between sex or reproductive status (Cottontail et al. 2009). In contrast, Plowright et al. (2008) identified reproductive stress as an important driver of Hendra virus antibody prevalence, reporting a higher prevalence in pregnant and lactating female little red flying foxes in comparison with non-reproductive females and males. In addition, lactating females of insectivorous temperate bat species were more likely to shed CoVs than non-reproducing individuals (Gloza-Rausch et al. 2008). Furthermore, the prevalence of CoVs in greater mouse-eared bats (*Myotis myotis*) peaks after parturition (Drexler et al. 2011). These studies demonstrate that reproduction is an important risk factor for viral diseases. Sex hormones can modulate immunocompetence (Alexander and Stimson 1988; Luis et al. 2013), and there is a trade-off between the reproductive and immune system for resources (Sheldon and Verhulst 1996), making reproducing individuals more susceptible to diseases and/or increase shedding. Additionally, reproductive females probably have more contact with susceptible offspring (Steece and Altenbach 1989; Gloza-Rausch et al. 2008; Turmelle et al. 2010) and mating and aggregation on maternal roosts may facilitate virus transmission (Lau et al. 2010b). In contrast to our study, these researchers sampled colony-roosting bats, whereas most of our study species live solitarily with their young or in relatively small colonies (up to 200 individuals). Thus, it seems likely that the increased contact rate between young and reproducing females in large aggregations of maternity roosts is the primary driver of higher viral prevalence in lactating females rather than the physiological trade-off between the investment in reproduction and immunity.

Our data suggests that the shedding of AstVs, but not CoVs, increases during the beginning of the rainy season (July to September) in Borneo, i.e. after the dry season. The
observation that seasonal fluctuations affect viral detection rates supports the findings of other studies (but see Hayman et al.’s study (2012) that did not find evidence for seasonal fluctuations in the prevalence of Lagos bat virus in *Eidolon helvum*). For example, Lau et al. (2010b) found the highest prevalence of two strains of CoVs in Chinese horseshoe bats in March to May, the beginning of the rainy season in this area. Furthermore, in Lyle’s flying foxes (*Pteropus lylei*), the main Nipah virus strain was most frequently detected in April to June compared with other months (Wacharapluesadee et al. 2010). In temperate climate zones, there can be differences in the prevalence of viral diseases as well. For example, in deer mice, the prevalence of Sin Nombre virus was higher in spring compared with fall (Safronetz et al. 2006), although results are inconsistent in this species (Lehmer et al. 2008; Dearing et al. 2009). Further, the prevalence of *Pseudogymnoascus destructans* in North-American bats can differ between December and March (Langwig et al. 2014). Often, seasonal fluctuations in viral infections are related to the reproductive cycle. For example, in older juvenile Egyptian fruit bats (*Rousettus aegyptiacus*) the prevalence of Marburg viruses peaks during the biannual birth seasons (Amman et al. 2012) and in big brown bats (*Eptesicus fuscus*) rabies virus transmission is highest when females form maternity colonies (George et al. 2011). However, in tropical ecosystems, there is more variety in the reproductive cycle among taxa: some bat species seem to breed year-round whereas others have annual or bi-annual birth peaks. Another factor possibly leading to stress-induced immunosuppression in animals and thus a higher susceptibility for contracting and shedding viruses are adverse weather conditions (Nelson et al. 1995; Brearley et al. 2013). Even in tropical latitudes, inclement weather, e.g. low precipitation, can act as a stressor, especially when they result in the lack of food resources and thus impact the health of bats (Smith and Wang 2013). Nutritional stress severely impairs the immune system which may result in an
increased susceptibility to pathogenic agents (Brearley et al. 2013). Indeed, although only marginally significant, low body condition, an indicator of chronic stress (Dickens and Romero 2013), was associated with an increased infection risk with AstVs in our study. In addition, during the beginning of the rainy season dry roost sites may be limited for foliage-roosting bats and thus lead to increased contact between individual bats and thus more opportunities for pathogen transmission. In our study the detection of AstVs in 2015 was lower than in 2014. Annual fluctuations were also reported for Sin Nombre virus infections in deer mice (Lehmer et al. 2008; Dearing et al. 2009).

Our results stress the complexity of virus ecology in bats. We suggest that seasonality may be the primary driver of viral infections, and should be considered in the prevention of outbreaks of zoonotic diseases, especially when considering future global climate changes. Our results suggest that abiotic factors, e.g. low precipitation, can lead directly to variation in viral shedding. In light of the evidence that Nipah virus emergence was also driven by extreme weather conditions, namely a severe drought caused by El Niño (Chua et al. 2002), our findings add strength to the hypothesis that inclement weather may increase the risk for spillovers of highly fatal viruses from wildlife to humans. However, in contrast to our hypothesis, habitat disturbance per se was not associated with higher viral detection rates, although habitat disturbance may become a major driver of zoonotic spillover events coupled with adverse abiotic conditions. Nevertheless, the conservation of natural habitats may avoid future outbreaks of emergent zoonotic diseases due to a decreased contact zone between wildlife and humans (Jones et al. 2008; Schneeberger and Voigt 2016). It is possible that stressful abiotic conditions during the dry season may have driven the variation in the viral detection rates in bats between the dry and the beginning of the rainy season. During the dry season, when food resources are limited, bats are in a poor
body condition (Seltmann et al., unpublished data) and might be less immunocompetent. Thus, individuals may become more susceptible to acquire viral infections in the subsequent rainy season. Data about the seroprevalence of viral infections (preferably by month), would shed more light on the infection dynamics of AstVs, e.g. when exactly individuals are more susceptible to shed AstVs. Additionally, monthly sampling could provide a better understanding of the bats’ reproductive cycle and its association with viral shedding.

Conclusion

By identifying the early rainy season and coinfection with other viruses as risk factors for increased viral shedding in bats, we contribute to means for predicting the emergence of infectious diseases. Risk mitigation agencies can take our findings into account, e.g. by limiting ecotourism or logging activities to low-risk times. Our results demonstrate the complexity of virus ecology in bats and underscore the intricacy of predicting viral incidence in natural habitats.

Ethical approval

All applicable institutional and national guidelines for the care and use of animals were followed.
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detection of bat coronavirus from bats in the Philippines. Archives of Virology
157(12):2349-2355

rabies virus exposure in colonies of Brazilian free-tailed bats (Tadarida brasiliensis) at
natural and man-made roosts in Texas. Vector-Borne and Zoonotic Diseases 10:165-175

Wacharapluesadee S, Boongird K, Wanghongsa S, Ratanasetyuth N, Supavonwong P,
Saengsen D, Gongal GN, Hemachudha T (2010) A longitudinal study of the
prevalence of Nipah virus in Pteropus lylei bats in Thailand: evidence for seasonal
preference in disease transmission. Vector-Borne and Zoonotic Diseases 10:183-190


Young CCW & Olival KJ (2016) Optimizing Viral Discovery in Bats. Plos One 11(2); DOI: 10.1371/journal.pone.0149237 [Online February 11, 2016]
**Table 1.** Study sites, habitat type and sampling year

<table>
<thead>
<tr>
<th></th>
<th>Recovering forest</th>
<th>Actively logged forest</th>
<th>Fragmented forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFE</td>
<td>2014, 2015</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>2014, 2015</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>2014, 2015</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>2014, 2015</td>
<td>2015</td>
</tr>
</tbody>
</table>
## Table 2. Coronavirus detection rate (%) in relation to habitat type. Numbers in brackets indicate positively tested individuals in relation to total individuals of a species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recovering forest</th>
<th>Actively logged forest</th>
<th>Fragmented forest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hipposideros cervinus</em></td>
<td>16.3 (7 of 43)</td>
<td>36 (9 of 25)</td>
<td>0 (0 of 8)</td>
</tr>
<tr>
<td><em>Hipposideros dyacorum</em></td>
<td>0 (0 of 3)</td>
<td>0 (0 of 8)</td>
<td>0 (0 of 23)</td>
</tr>
<tr>
<td><em>Kerivoula hardwickii</em></td>
<td>0 (0 of 51)</td>
<td>0 (0 of 12)</td>
<td>0 (0 of 9)</td>
</tr>
<tr>
<td><em>Kerivoula intermedia</em></td>
<td>0 (0 of 44)</td>
<td>0 (0 of 23)</td>
<td>0 (0 of 22)</td>
</tr>
<tr>
<td><em>Kerivoula papillosa</em></td>
<td>0 (0 of 7)</td>
<td>0 (0 of 1)</td>
<td>0 (0 of 2)</td>
</tr>
<tr>
<td><em>Rhinolophus borneensis</em></td>
<td>0 (0 of 6)</td>
<td>0 (0 of 7)</td>
<td>0 (0 of 7)</td>
</tr>
<tr>
<td><em>Rhinolophus sedulus</em></td>
<td>0 (0 of 13)</td>
<td>0 (0 of 5)</td>
<td>NA</td>
</tr>
<tr>
<td><em>Rhinolophus trifoliatus</em></td>
<td>5.3 (1 of 19)</td>
<td>0 (0 of 17)</td>
<td>0 (0 of 10)</td>
</tr>
</tbody>
</table>
Table 3. Generalized linear mixed-effects model of risk factors for coronavirus detection in *Hipposideros cervinus* (SE = Standard error, body condition = body mass [g]/forearm length [mm], N = 75)

<table>
<thead>
<tr>
<th>Effects</th>
<th>Estimate</th>
<th>SE</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Habitat type</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recovering forest</td>
<td>reference</td>
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<td></td>
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</tr>
<tr>
<td>actively logged forest</td>
<td>0.85</td>
<td>1.02</td>
<td>0.83</td>
<td>0.405</td>
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<tr>
<td>fragmented forest</td>
<td>-25.77</td>
<td>5.325e+05</td>
<td>0.00</td>
<td>1.0</td>
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<tr>
<td><strong>Individual characteristics</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>body condition (log)</td>
<td>7.32</td>
<td>4.36</td>
<td>1.68</td>
<td>0.093</td>
</tr>
<tr>
<td>Reproductive condition:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating females</td>
<td>reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pregnant females</td>
<td>-11.02</td>
<td>7.48e+06</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>non-reproducing females</td>
<td>17.89</td>
<td>7.69e+03</td>
<td>0.00</td>
<td>0.998</td>
</tr>
<tr>
<td>males</td>
<td>18.94</td>
<td>7.692e+03</td>
<td>0.00</td>
<td>0.998</td>
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<tr>
<td><strong>Astrovirus infection status</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>negative</td>
<td>reference</td>
<td></td>
<td></td>
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<tr>
<td>positive</td>
<td>3.57</td>
<td>1.33</td>
<td>2.69</td>
<td>0.007**</td>
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<tr>
<td><strong>Abiotic factors</strong></td>
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<td></td>
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<tr>
<td>Season: dry season</td>
<td>reference</td>
<td></td>
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<td>------------------</td>
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<tr>
<td>Season: beginning of rainy season</td>
<td>-0.3</td>
<td>1.33</td>
<td>-0.23</td>
<td>0.819</td>
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<tr>
<td>Year: 2014</td>
<td>reference</td>
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<tr>
<td>2015</td>
<td>1.64</td>
<td>1.41</td>
<td>1.16</td>
<td>0.245</td>
</tr>
</tbody>
</table>
Table 4. Astrovirus detection rate (%) in relation to habitat type. Numbers in brackets indicate positively tested individuals in relation to total individuals of a species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recovering forest</th>
<th>Actively logged forest</th>
<th>Fragmented forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hipposideros cervinus</td>
<td>44.2 (19 of 43)</td>
<td>64 (16 of 25)</td>
<td>25 (2 of 8)</td>
</tr>
<tr>
<td>Hipposideros dyacorum</td>
<td>0 (0 of 3)</td>
<td>62.5 (5 of 8)</td>
<td>13 (3 of 23)</td>
</tr>
<tr>
<td>Kerivoula hardwickii</td>
<td>2.0 (1 of 51)</td>
<td>41.7 (5 of 12)</td>
<td>0 (0 of 9)</td>
</tr>
<tr>
<td>Kerivoula intermedia</td>
<td>11.4 (5 of 44)</td>
<td>17.4 (4 of 23)</td>
<td>0.1 (2 of 22)</td>
</tr>
<tr>
<td>Kerivoula papillosa</td>
<td>14.3 (1 of 7)</td>
<td>0 (0 of 1)</td>
<td>0 (0 of 2)</td>
</tr>
<tr>
<td>Rhinolophus borneensis</td>
<td>16.7 (1 of 6)</td>
<td>42.9 (3 of 7)</td>
<td>42.9 (3 of 7)</td>
</tr>
<tr>
<td>Rhinolophus sedulus</td>
<td>23.1 (3 of 13)</td>
<td>0 (0 of 5)</td>
<td>NA</td>
</tr>
<tr>
<td>Rhinolophus trifoliatus</td>
<td>10.5 (2 of 19)</td>
<td>11.7 (2 of 17)</td>
<td>10 (1 of 10)</td>
</tr>
</tbody>
</table>
Table 5. Generalized linear mixed-effects model of risk factors for astrovirus detection
(SE = Standard error, body condition = body mass [g]/forearm length [mm], N = 364)

<table>
<thead>
<tr>
<th>Effects</th>
<th>Estimate</th>
<th>SE</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Habitat type</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovering forest reference</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>actively logged forest</td>
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<td>0.45</td>
<td>-1.25</td>
<td>0.211</td>
</tr>
<tr>
<td>fragmented forest</td>
<td>0.25</td>
<td>0.52</td>
<td>0.48</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Individual characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species: <em>Hipposideros cervinus</em> reference</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Hipposideros dyacorum</em></td>
<td>-2.15</td>
<td>0.72</td>
<td>-3.00</td>
<td>0.003**</td>
</tr>
<tr>
<td><em>Kerivoula hardwickii</em></td>
<td>-3.98</td>
<td>1.01</td>
<td>-3.94</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td><em>Kerivoula intermedia</em></td>
<td>-3.32</td>
<td>0.95</td>
<td>-3.48</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td><em>Kerivoula papillosa</em></td>
<td>-23.22</td>
<td>724.08</td>
<td>-0.03</td>
<td>0.974</td>
</tr>
<tr>
<td><em>Rhinolophus borneensis</em></td>
<td>0.89</td>
<td>0.71</td>
<td>-1.26</td>
<td>0.209</td>
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<tr>
<td><em>Rhinolophus sedulus</em></td>
<td>-1.97</td>
<td>0.88</td>
<td>-2.24</td>
<td>0.025*</td>
</tr>
<tr>
<td><em>Rhinolophus trifoliatus</em></td>
<td>-2.9</td>
<td>0.76</td>
<td>-3.82</td>
<td>&lt;0.001***</td>
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<tr>
<td>body condition (log)</td>
<td>-2.30</td>
<td>1.3</td>
<td>-1.77</td>
<td>0.077</td>
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<tr>
<td><strong>Sex: female</strong> reference</td>
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<tr>
<td>male</td>
<td>-2.97</td>
<td>190.15</td>
<td>-0.02</td>
<td>0.988</td>
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<tr>
<td>Reproductive status: Lactating females</td>
<td>reference</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pregnant females</td>
<td>1.62</td>
<td>1.3</td>
<td>1.25</td>
<td>0.213</td>
</tr>
<tr>
<td>non-reproducing females</td>
<td>1.36</td>
<td>0.88</td>
<td>1.54</td>
<td>0.124</td>
</tr>
<tr>
<td>males</td>
<td>4.56</td>
<td>190.15</td>
<td>0.02</td>
<td>0.98</td>
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</table>

<table>
<thead>
<tr>
<th>Coronavirus infection status: negative</th>
<th>reference</th>
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<tr>
<td>positiv</td>
<td>2.66</td>
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</tbody>
</table>

Abiotic factors

<table>
<thead>
<tr>
<th>Season: dry season</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season: beginning of rainy season</td>
<td>2.67</td>
</tr>
</tbody>
</table>

| Year: 2014 | reference |
| Year: 2015 | -1.51 | 0.50 | -3.01 | <0.003** |