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Influence of age and body condition on astrovirus infection of bats in Singapore: An evolutionary and epidemiological analysis

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ABSTRACT

Bats are unique mammals that are reservoirs of high levels of virus diversity. Although several of these viruses are zoonotic, the majority are not. Astroviruses, transmitted fecal-orally, are commonly detected in a wide diversity of bat species, are prevalent at high rates and are not thought to directly infect humans. These features make astroviruses useful in examining virus evolutionary history, epidemiology in the host, and temporal shedding trends. Our study screened for the presence of astroviruses in bats in Singapore, reconstructed the phylogenetic relations of the polymerase genes and tested for population characteristics associated with infection. Of the seven species screened, astroviruses were detected in *Rhinolophus lepidus* and *Eonycteris spelaea*. The *R. lepidus* sequences grouped with other *Rhinolophus* astrovirus sequences from China and Laos, while the *Eoncyteris* sequences formed a distinct clade with astroviruses from *Rousettus* spp. in Laos and *Pteropus giganteus* in Bangladesh, but not with other *E. spelaea* sequences. Longitudinal collections of *Eonycteris* feces demonstrated variable shedding. Juvenile status of bats was a risk factor for astroviruses. This study highlights the diversity of astroviruses in nectivorous and insectivorous bats in Singapore and provides a predictive framework for understanding astrovirus infection in these bats. It also suggests that in addition to host phylogenetic relatedness, host ecology, such as roosting behavior, may drive co-infections, virus maintenance and spillover.

1. Introduction

Bats are the second most speciose group of mammals behind rodents [1], their species diversity provides opportunity for virus diversity through co-evolution [2]. The intensive surveillance in bats for zoonotic viruses has provided biological samples that are screened for other virus families that may not be of human public health importance or pathogenic to the reservoir, but may be informative of evolutionary processes of bat viruses due to their high prevalence [3,4]. In addition to zoonotic viruses, such as Ebola and SARS-CoV, there are a number of commonly detected viruses that demonstrate varying degrees of host specificity at genus or species level [5–7].

The Astroviridae, a family of single stranded, positive sense non-enveloped RNA viruses [8], is a favorable candidate for studying evolutionary history because they infect a wide variety of species and recombine [9–11]. In addition, the phylogeny of the astrovirus RdRp segment indicates that cross-species transmission has driven the evolution of this virus family [12]. A number of these viruses have public health and economic importance [12,13]. Transmission is fecal-oral and diarrhea is the primary clinical symptom in most hosts, but astroviruses can also cause kidney-related ailments in chickens and humans and encephalitis in humans and cows [14–16]. Astrovirus outbreaks in commercial cow, pig, mink, turkey, duck, goose, chicken, and guinea fowl production facilities cause major economic losses [17–23]. In wildlife, avastroviruses have been detected in wild ducks, wading birds, passerines, pigeons, and penguins [24–27]. When infected, bats have been hypothesized notto become sick or display signs due to their unique immune response [28].

Singapore, a predominantly urban island that has lost the great majority of its original habitats and suffered a number of local species

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extinctions [29], harbors an estimated 26 resident bat species. Certain species of bats in Singapore exist in urbanized sites, while others reside in small forest and grassland fragments. As part of efforts to characterize the virus communities of Singapore bats, including the dynamics of infection, we caught and screened bats and then tested the impact of bat species, age class (juvenile vs. adult), sex, and body condition on the presence of astroviruses while also monitoring the temporal shedding of these viruses.

2. Materials and methods

2.1. Sample collection

Animal ethics approval for the study was given by the National University of Singapore - International Animal Care & Use Committee (Permit # B01/12) and bats were sampled with permission from the National Parks Board, Singapore (NP/RP11-011). Bats were trapped at seven different sites in Singapore from April 2011 through March 2014 as previously described [30]. Briefly, rectal and oral swabs were taken and age (classified as juvenile or adult), gender, forearm length, and weight were recorded from each individual. In addition, longitudinal fecal collections were taken from a colony of cave nectar bats (*Eonycteris spelaea*) from April 2011 until June 2015.

2.2. RNA extraction, PCR, sequencing and phylogenetic analysis

Fecal samples were vortexed for 15 s and then centrifuged at 4000 rpm for 1 min. Oral and rectal swabs were vortexed and then pooled for each individual. Samples collected between 12 April 2011 to 2 March 2012 were pooled by date (5 individual samples per pool) to test for pooled prevalence. RNA extraction cDNA synthesis, PCR amplification, cloning and sequencing were performed as previously described [31,32]. 5′ and 3′ RACE was attempted to generate cDNA from the ends (Life Technologies, Carlsbad, CA), but neither approach was successful. RACE generally requires high amounts and high quality of target nucleic acids and these fecal samples may have relatively few astrovirus reads compared to the host and bacterial background. Over 700 global astrovirus *RdRp* sequences were downloaded from the NCBI GenBank and analyzed with 38 bat astrovirus sequences generated from this study (GenBank accession numbers MF983815-MF983852) using Geneious 7.1.7 software [33]. The final dataset was reduced to a total of 449 astrovirus sequences after the removal of short sequences and phylogenies were reconstructed using RAxML v8.0.14 [34] as previously described [30].

2.3. Epidemiological analysis

Individual prevalence from pools was estimated using a frequentist approach that assumed a fixed pool size and a perfect detection and a Bayesian approach with a Gibbs sampler model with AusVet EpiTools [35]. Details of the criteria used in the analysis are available in the supplementary materials. These were compared to individual prevalence rates on four dates within the sampling period. Multiple logistic regression (MLogR) was used to examine the effects of species, catch/roost location, sex, age, and bat body condition [36] on astrovirus infection. The scale of each predictor was determined by performing exploratory data analysis. A variance inflation factor (VIF) was calculated to assess multi-collinearity (inter-relatedness of the covariates). Hierarchical logistic models were tested to investigate significant predictors of astrovirus infection. Likelihood ratio tests (LRT) were generated to compare full, extended MLogR to more constrained models and assess whether the inclusion of interaction terms within a larger model were significant. Akaike Information Criterion (AIC) values were used to rank models and to determine the optimal set of predictors. Odds ratios were computed for the explanatory variables in the resulting model.

Forward and backward stepwise selection based on AIC and a 5% *p*-value cut-off were utilized as an aid to choose predictors for the final model. All covariates were considered for elimination. Based upon the statistical output of the stepwise processes, likelihood ratio tests outcomes, the variance inflation factor procedure, confounding assessments, cross tabulations of the outcome and variables, and prior knowledge regarding the subject, the final model was fit. The final model included 1) bat age and 2) bat species. The Pearson's chi-squared goodness-of-fit test (H_0: the model M_0 fits) was used to determine the overall fit of the final model. The *p*-value was computed at P: 0.27, indicating a well-fit model. All analyses were carried out using Stata version 12.1 [37].

3. Results

A total of 431 bats from 7 species were trapped and sampled (Table 1). Astroviruses were only detected in *Eonycteris spelaea* (30/169, 17.8%) and *Rhinolophus lepidus* (13/36, 36.1%), with an overall detection rate of 9.9% for the pooled oral-rectal swabs (Fig. 1). A total of 410 *E. spelaea* fecal samples were screened, with 184 (44.9%) astrovirus positives detected. However, the number of infected fecal samples varied by week with a high of 83% positive (2011-04-12) to a low of 0% positive (2012-01-31 and 2013-01-28) (Fig. 2). A total of 144

Table 1
Bat baseline descriptive characteristics by outcome group.

Bat characteristics	Astrovirus + ve	Astrovirus − ve	Total	P-value*
	N = 43 (9.98%)	*N* = 388 (90.02%)	*N* = 431	
Bat species				< 0.001
Eonycteris spelaea	30 (69.8%)	139 (35.8%)	169 (39.2%)	
Cynopterus brachyotis	0 (0%)	144 (37.11%)	144 (33.4%)	
Penthetor lucasi	0 (0%)	79 (20.4%)	79 (18.3%)	
Rhinolophus lepidus	13 (30.2%)	23 (5.9%)	36 (8.4%)	
Macroglossus minimus	0 (0%)	1 (0.3%)	1 (0.2%)	
Myotis sp.	0 (0%)	1 (0.3%)	1 (0.2%)	
Pipstrelle stenopterus	0 (0%)	1 (0.3%)	1 (0.2%)	
Sex				0.17
Female	15 (34.9%)	179 (46.1%)	194 (45%)	
Male	28 (62.8%)	203 (52.6%)	231 (53.6%)	
Missing data[a]	1 (2.3%)	5 (1.3%)	6 (1.4%)	
Age				0.02
Juvenile	14 (31.8%)	81 (20.9%)	95 (22%)	
Sub-adult/adult	24 (54.6%)	299 (77.3%)	323 (74.9%)	
Missing data[a]	6 (13.6%)	7 (1.8%)	13 (3%)	
Roost location				< 0.001
Bukit Timah	13 (30.2%)	100 (25.8%)	113 (26.2%)	
Kent Ridge	1 (2.3%)	94 (24.2%)	95 (22%)	
Pulau Ubin	0 (0%)	3 (0.8%)	3 (0.7%)	
Rifle Range Road	29 (67.4%)	149 (38.4%)	178 (41.3%)	
Dairy Farm	0 (0%)	23 (5.9%)	23 (5.3%)	
Sian Tan Avenue	0 (0%)	1 (0.3%)	1 (0.2%)	
Telok Blangah	0 (0%)	18 (4.6%)	18 (4.2%)	
Body condition index				< 0.001
Mean	0.491	0.608	0.597	
Std. dev.	0.274	0.199	0.210	
Median	0.541	0.592	0.590	
Missing data[a]	5 (11.4%)	20 (5.2%)	25 (5.8%)	

In categorical variables, the first characterization under the variable heading is the reference category.
Information in parentheses (%).
* Categorical variables' p-value based on Chi-squared test (≥ 5 per cell) or Fisher's exact test (< 5 per cell). Continuous variable p-value based on Wald *Z*-test.
[a] Missing data for this variable; statistical analyses based on available data.

Fig. 1. Photographs of *Rhinolophus lepidus* (Blyth's horseshoe bat) and *Eonycteris spelaea* (Cave nectar bat).

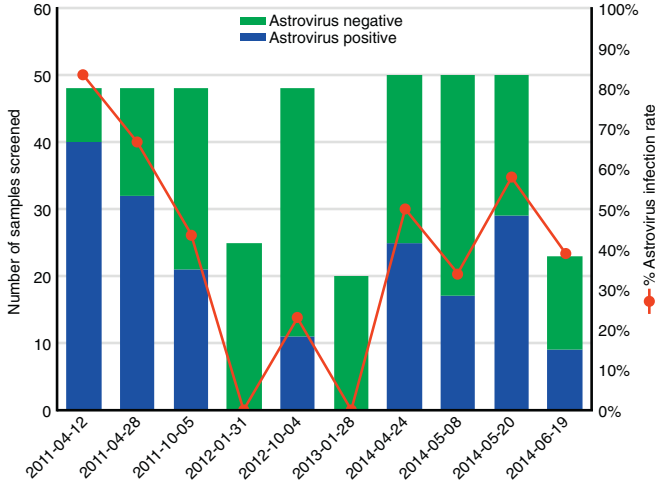

Fig. 2. Temporal variation in the infection rate of astrovirus detected in the feces collected from an *Eonycteris spelaea* colony using a hemi-nested PCR detecting the RNA-dependent reverse polymerase gene.

pools out of 221 (1105 individual samples) made from 19 sampling occasions were positive for astroviruses (65.16%) (Table 2). Minimum infection rates of individual samples throughout the sampling period ranged from 6%–20%. Frequentist analysis predicted individual sample prevalence ranged from 6.89%–36.9% across the sampling period with an estimated overall prevalence of 19.01% (95% Confidence Interval: 16.1%–22.2%), while the Bayesian analysis calculated an estimated mean prevalence of 23.75% (95% Credible Interval: 18.9%–34.0%) (Supplementary data 1). Both the frequentist confidence interval and the Bayesian credible interval produced similar values supporting the estimated prevalence.

The astroviruses phylogeny showed that the majority of bat astroviruses (indicated by grey branches) were basal to other mammalian astroviruses, but with a general lack of statistical support (Fig. 3). The 38 novel bat astroviruses sequences collected in Singapore (denoted by red branches in Fig. 3) fell into 2 groups. Two bat astrovirus sequences identified from *R. lepidus* formed a monophyletic clade (bootstrap [BS] = 100%) that clustered within other *Rhinolophus* astroviruses from China and Laos (BS < 60%). Similarly, 36 astroviruses sequences from *E. spelaea* formed a monophyletic lineage (BS = 69%) with two distinct sub-clades, and this lineage was closely related to other pteropodid bat astroviruses from Bangladesh and Laos. Furthermore, the clustering of all *E. spelaea* astrovirus sequences, despite being collected in different years, suggests sustained transmission in this host. The astroviruses from *R. lepidus* and *E. spelaea* from Singapore were genetically dissimilar (nucleotide sequence identity 54.0–57.2%) even though their sampling locations and collection periods were similar.

Interestingly, we revealed phylogenetically diverse astroviruses among *E. spelaea* bats, with at least two distinctive groups (Clade 1 and 2). Clade 1 (BS = 69%) is further composed of three smaller sub-clades (designated as a-c). Clade 1a (nucleotide identity 60.8–99.2%) includes 4 *E. spelaea* astroviruses from Singapore (BS = 100%) whereas the other comprises one *Pteropus giganteus* astrovirus from Bangladesh and a *Rousettus* sp. astrovirus from Laos. Clade 1b (BS = 65%; nucleotide identity 64.8–100%) is composed of *Rousettus* astroviruses from Laos. Clade 1c (nucleotide identity: 66.8–99.2%) includes astroviruses from *E. spelaea* (Singapore) and *Rousettus leschenaultia* (Laos). Both Singapore *E. spelaea* astrovirus clades were collected in the same sampling period. Clade 2 (BS = 51%; nucleotide identity 65.3–99.2%) contains two *E. spelaea* astroviruses, one *Rhinolophus* astrovirus and numerous *Rousettus* astroviruses, all from Laos. This suggests that *E. spelaea* astroviruses from Singapore are closely related to those of Laos *Rousettus spp.*, yet are distantly related to Laos *E. spelaea* astroviruses. Clade 2 further displays the close phylogenetic relationship between *E. spelaea* and *Rousettus* spp. astroviruses (BS = 61%), all occurring in Laos. It is likely that the co-roosting of these bat species facilitated the interspecies virus transmission.

The most predictive model for astrovirus infection included bat age and bat species: $\log(p/1 - p) = \beta_o + \beta_1(\text{Bat Age}) + \beta_2(\text{Bat Species})$. In the crude analysis, bat age was found to be a significant risk factor for astrovirus, (OR: 0.44, P: 0.02, 95% CI: 0.22–0.90) providing evidence that astroviruses were less likely to be detected in adults. Thus we estimate the odds of astrovirus infection to be reduced by 56% given an adult bat. Similarly, unadjusted bat species was a strong indicator of astrovirus, (OR: 2.62, P: 0.02, 95% CI: 1.19–5.75); thus, it is estimated that the odds of contracting astrovirus infection are ~2.6× greater among *Rhinolophus lepidus* bats as compared to the referent group *Eonycteris spelaea* bat. The five remaining species were automatically eliminated from the model, as none of them predicted astrovirus (astrovirus detection rate = 0%). However, adjusting for bat age in the larger model displayed more modest evidence for a bat age effect (OR: 0.59, P: 0.20, 95% CI: 0.26–1.34). Although, bat species did not appear to be an important confounder in the bat age/astrovirus relationship (determined by the adjusted bat age odds ratio lying between the 95% CI in the unadjusted model). Hence, the evidence in these data suggest, but are insufficient to conclude, that bat age has an independent effect on astrovirus outcome beyond that of bat species (Table 3).

4. Discussion

This is the first report of bat astroviruses from Singapore and the first report of astrovirus infection in *Rhinolophus lepidus*. Previous surveillance efforts have demonstrated variable rates of astrovirus infection from diverse bat species, including those tested here [38]. Astroviruses have been detected from ten other species of *Rhinolophus* bats in previous studies [39–43]. A recent study in Cambodia and Laos screened six members of the family Pteropodidae and astroviruses were only detected in *Eoncyteris spelaea* (3.8%) and *Rousettus spp.* (7.2%) [44]. For longitudinal monitoring, the volume of samples may hinder processing, making pooling a feasible option. However, if the number of positive samples is low, the sensitivity of the test may present false negatives and if all the pools are positive, as occurred on three sampling occasions in this study, one is unable to estimate individual prevalence. In cases where we screened individual samples, the individual detection rate was greater than the estimated prevalence. This may result from the pooling of the samples lowering the relative quantity of virus in comparison to the background material.

As Astroviruses replicate in the gastro-intestinal tract and are

Table 2
Pooled prevalence estimates using frequentist and Bayesian methods with a Gibbs sampler compared to individual prevalence rates.

	Collection date	Number of Pools	Number of positive pools	Minimum infection rate	Estimated individual prevalence (% CL: 2.5–97.5)	Standard error	Individual samples screened	Individual samples PCR positive	Individual sample prevalence
Frequentist estimation of pooled prevalence with fixed pool size and perfect test	12-Apr-11	35	15	8.6%	10.6% (0.059;0.170)	0.026	48	40	83.3%
	28-Apr-11	16	11	13.8%	20.8% (0.101;0.357)	0.059	48	32	66.7%
	12-May-11	10	6	12%	16.7% (0.059; 0.344)	0.064	–	–	–
	25-May-11	10	6	12%	16.7% (0.059; 0.344)	0.064	–	–	–
	13-Jun-11	10	3	6%	6.9% (0.014; 0.191)	0.039	–	–	–
	1-Jul-11	10	5	10%	12.9% (0.041; 0.285)	0.055	–	–	–
	14-Jul-11	10	10	20%	–	–	–	–	–
	27-Jul-11	10	10	20%	–	–	–	–	–
	8-Aug-11	10	8	16%	27.5% (0.111; 0.521)	0.092	–	–	–
	22-Aug-11	10	9	18%	36.9% (0.149; 0.698)	0.120	–	–	–
	9-Sep-11	10	10	20%	–	–	–	–	–
	21-Sep-11	10	9	18%	36.9% (0.149; 0.698)	0.120	–	–	–
	5-Oct-11	10	6	12%	16.7% (0.059; 0.344)	0.064	48	21	43.8%
	21-Oct-11	10	9	18%	36.9% (0.149; 0.698)	0.120	–	–	–
	2-Nov-11	10	4	8%	9.7% (0.0256; 0.235)	0.047	–	–	–
	30-Nov-11	10	6	12%	16.7% (0.059; 0.344)	0.064	–	–	–
	1-Jan-12	10	8	16%	27.5% (0.111; 0.521)	0.092	–	–	–
	31-Jan-12	10	5	10%	12.9% (0.041; 0.285)	0.055	25	0	0%
	2-Mar-12	10	4	8%	9.7% (0.0256; 0.235)	0.047	–	–	–
	Total	221	144	13%	19% (0.161; 0.222)	0.015	169	93	55%
Pooled prevalence with a Bayesian approach and a Gibbs sampler				Minimum Prevalence	Estimated Mean Prevalence	Standard deviation			
	Total	221	144	15.3%	23.8% (0.189; 0.340)	0.040			

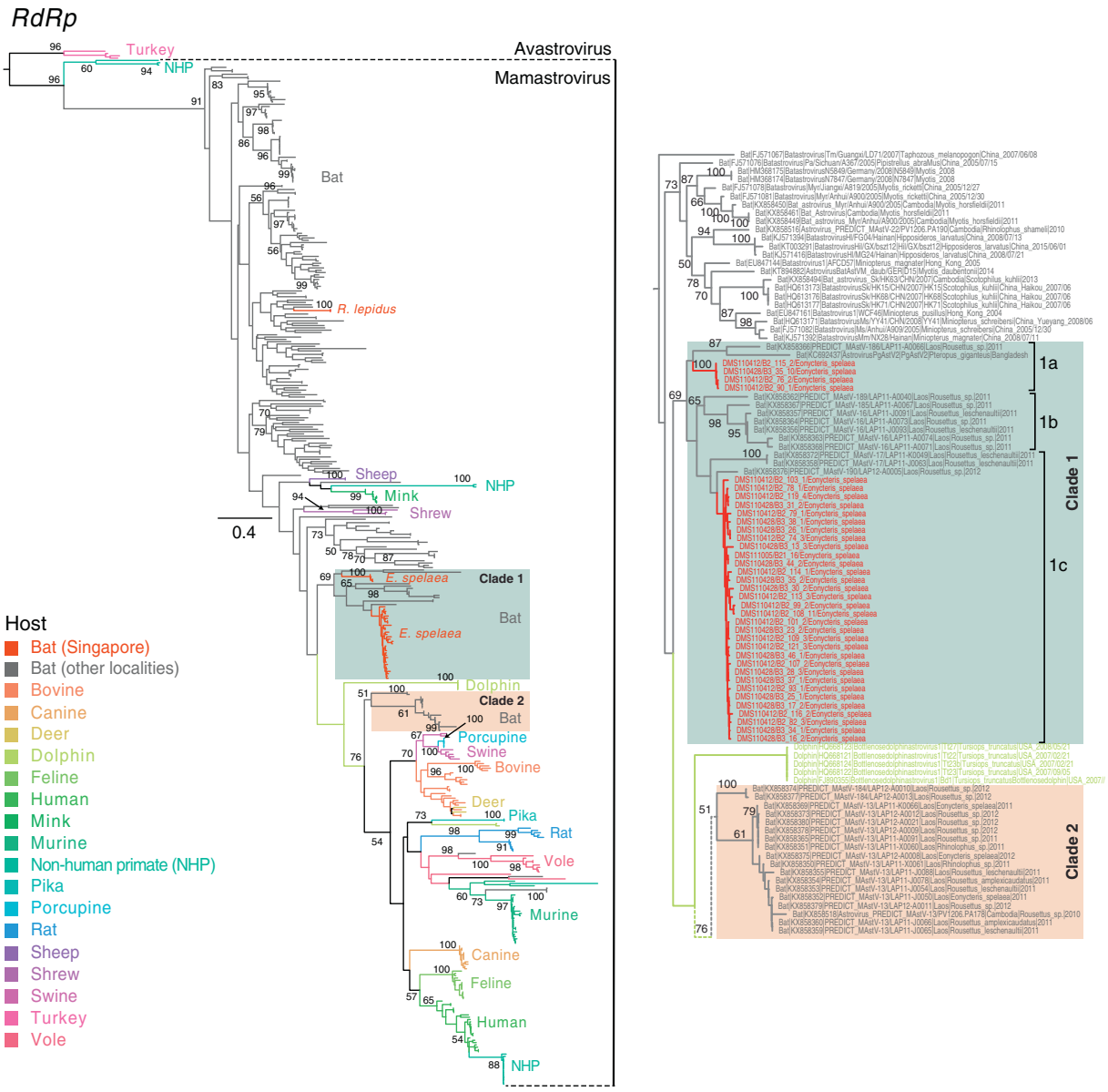

Fig. 3. Maximum likelihood phylogeny of the RNA-dependent RNA polymerase (RdRp) region of global astroviruses. Red branches denote novel bat astrovirus generated from this study, whereas the grey branches represent bat astrovirus collected from other geographical regions. The coloured branches denote different host species. Bootstrap values > 50% are indicated at major nodes. The scale bar represents the nucleotide substitutions per site. The insert on the right shows the details of astrovirus strains in Clade 1 and 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Risk of astrovirus infection according to bat age and bat species.

	Unadjusted			Adjusted		
	OR	95% CI	P	OR	95% CI	P
Bat age						
Juvenile	1 [Reference]			1 [Reference]		
Adult	0.46	0.22–0.90	0.02	0.59	0.26–1.34	0.20
Bat species[a]						
Eonycteris spelaea	1 [Reference]			1 [Reference]		
Rhinolophus lepidus	2.61	1.19–5.75	0.02	3.41	1.35–8.60	0.01

Abbreviations: OR, Odds Ratio; 95% CI, 95% Confidence Interval; P, P-value.
[a] Remaining bat species categories dropped due to perfect prediction of failure.

fecally-orally transmitted, the relative viral load may be higher in feces compared to swabs. This may explain our higher detection rate from *E. spelaea* feces, but as bat viruses may be shed differentially over time, we cannot exclude changes in viral load affecting our results. We found variable rates of shedding across our sampling period. In Singapore, temperatures are not variable month to month, but there are two monsoon seasons, which may be associated with bat borne virus shedding [43]. A study on *Myotis myotis* in Germany demonstrated distinct astrovirus amplification peaks that correlated with the formation of a suitable roost size and also a post-parturition period [45]. In the tropics, where there is lower seasonal variation and an absence of torpor, the reproductive behavior of bats may drive virus persistence [46]. This may not always be the case as astrovirus and coronavirus shedding in Borneo was not associated with reproductive status [43].

We analyzed the astrovirus *RdRp* sequences from a broad range of hosts including bats and non-human primates [44,47,48]. Consistent with previous studies, our results indicate that bats harbor a genetically diverse group of astroviruses that are typically polyphyletic and predominantly situated in a basal position of the phylogenetic tree. Interestingly, we detected diverse lineages of bat astroviruses in our

samples that were collected from a small geographic area. We demonstrated that the astroviruses from *Rhinolophus lepidus* and *Eonycteris spelaea* in Singapore were phylogenetically distinct and clearly segregated into two distantly-related lineages. This is reflected by the low percentage (55.3%–56.9%) of nucleotide sequence identity between *Rhinolophus* and *Eonycteris RdRp* sequences. We also observed that the *E. spelaea* astroviruses from Singapore and Laos exhibited high levels of sequence variation and at least two clades were identified. Clade 1 is genetically more diverse than Clade 2, and further split into smaller sub-groups. This includes all *E. spelaea* astroviruses collected from Singapore, although these are not monophyletic. The majority of *E. spelaea* astroviruses detected in Singapore are more closely related with *Rousettus* astroviruses from Laos. As *Rousettus* and *Eonycteris* are commonly encountered in the same roosting environment, and there is evidence that *Rousettus* bat coronaviruses can infect other genera of bats [49], it is unsurprising that closely related astroviruses were detected in these species. In addition, we observed that some astroviruses of *E. spelaea* in Singapore are closely related with *Pteropus giganteus* astroviruses detected in Bangladesh. The close genetic relationship of these viruses from Bangladesh, Laos and Singapore indicates an important role for bats in the dispersal of astroviruses over a wide geographical range, consistent with previous observations for lineage D betacoronaviruses [30].

Clade 2 is composed of astroviruses from several bat species from Cambodia and Laos that are most closely related to astroviruses from other mammalian species. Notably, *E. spelaea* astroviruses from Singapore (Clade 1) are only distantly related to *E. spelaea* astroviruses from Laos (Clade 2), indicating these astroviruses have arisen from different origins. Specifically, the Clade 2 bat astroviruses are most closely related to viruses from other mammals, indicating that the may have arisen by interspecies transmission from a non-bat host. This is in contrast to previously observed relationships of coronaviruses and astroviruses, where bats generally harbor virus lineages that are ancestral to viruses found in other mammalian hosts [50–52]. However, this could also reflect sampling biases. Furthermore, some terrestrial mammalian astroviruses in Clade 2 were monophyletic (e.g. dogs and rats) that may reflect host restrictions, while others (e.g. those from humans and non-human primates and swine) were not monophyletic, indicating that the interspecies transmission among these hosts is less restricted. More sequence data from different host species are necessary to better understand the underlying factors influencing the cross-species transmission and persistence of the virus.

The epidemiological models indicate that bat age is the most predictive characteristic beyond that of species, displaying an overall viral burden bias towards juveniles. The overall effect was largely driven by the larger *E. spelaea* sample size, especially the larger juvenile cohort, however, the proportion of juveniles positive for astrovirus in both species tested were not significantly different. Astroviruses predominantly infect juveniles [53–57] and the later an animal in life is infected, the shorter the period of detection, possibly reflecting a reduced period of viral shedding [58]. Our results did not yield a significant effect of bat gender on astrovirus infection, and while bat body condition and roost location were significant in the crude analyses, these two variables were not included in our main model due to extreme collinearity with other predictors. This was mirrored in a study on insectivorous bats in Borneo, where poor body condition was slightly significant for astrovirus detection while gender was not [35].

Bats have several traits that make them ideal reservoirs, including being long lived, the ability to fly, and living in large roosts [59]. Contact rates drive infectious disease transmission and larger colony sizes may play a role in transmission [60,61] through periodic introductions of immunologically naïve individuals [46]. Differences in roosting behaviors between the three pteropodid bats in Singapore may be a reason why astroviruses were only detected in *E. spelaea*. This species roosts in large colonies and the study site has over 3000 individuals, while *Cynopterus* bat species roost in small colonies, one study observing that 87.5% of colonies contained fewer than 15 individuals [62]. The *Penthetor lucasi* colony in Singapore roost in a single-species, artificial cave and unless the founding individuals introduced astroviruses, the colony may be uninfected.

The developed model provides a predictive framework for understanding the burden of astrovirus in Singapore bats. Periodic sampling of tagged bats may help to determine if reproductive status plays a role in female bats' infection status, and further, if there are periods within the life cycle that coincide with higher pulses of virus [63]. Dichotomizing female sex by reproduction status not only in the study design phase, but also in the study analysis phase, may shed light on the effects of pregnancy and lactation, which may be currently hidden within the female variable. For example, Heideman and Utzurrum [64] showed that there are two distinct periods of births in *E. spelaea* – February to mid-May and from July to mid-October. Sampling of adults within these two periods as well as outside would be useful for the detection patterns in astrovirus infection. Serological testing would have revealed if older bats had been exposed to astrovirus at a younger age. Performing a longitudinal study with both PCR testing and serological surveys would help to characterize the distribution of astrovirus infection as well as determine temporal patterns of immunity in the bats.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2017.10.001.

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