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RESEARCH ARTICLE

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Disentangling serology to elucidate henipa- and filovirus transmission in Madagascar fruit bats

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Abstract

1. Bats are reservoirs for emerging human pathogens, including Hendra and Nipah henipaviruses and Ebola and Marburg filoviruses. These viruses demonstrate predictable patterns in seasonality and age structure across multiple systems; previous work suggests that they may circulate in Madagascar's endemic fruit bats, which are widely consumed as human food.
2. We aimed to (a) document the extent of henipa- and filovirus exposure among Malagasy fruit bats, (b) explore seasonality in seroprevalence and serostatus in these bat populations and (c) compare mechanistic hypotheses for possible transmission dynamics underlying these data.
3. To this end, we amassed and analysed a unique dataset documenting longitudinal serological henipa- and filovirus dynamics in three Madagascar fruit bat species.
4. We uncovered serological evidence of exposure to Hendra-/Nipah-related henipaviruses in *Eidolon dupreanum*, *Pteropus rufus* and *Rousettus madagascariensis*, to Cedar-related henipaviruses in *E. dupreanum* and *R. madagascariensis* and to Ebola-related filoviruses in *P. rufus* and *R. madagascariensis*. We demonstrated significant seasonality in population-level seroprevalence and individual serostatus for multiple viruses across these species, linked to the female reproductive calendar. An age-structured subset of the data highlighted evidence of waning maternal antibodies in neonates, increasing seroprevalence in young and decreasing seroprevalence late in life. Comparison of mechanistic epidemiological models fit to these data offered support for transmission hypotheses permitting waning antibodies but retained immunity in adult-age bats.
5. Our findings suggest that bats may seasonally modulate mechanisms of pathogen control, with consequences for population-level transmission. Additionally, we

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narrow the field of candidate transmission hypotheses by which bats are presumed to host and transmit potentially zoonotic viruses globally.

KEYWORDS
age–seroprevalence, filovirus, flying fox, force of infection, fruit bat, henipavirus, Madagascar, zoonosis

1 | INTRODUCTION

Bats have received much attention in recent years for their roles as reservoirs for several virulent, emerging human pathogens, including Hendra and Nipah henipaviruses, Ebola and Marburg filoviruses, and SARS coronavirus (Calisher, Childs, Field, Holmes, & Schountz, 2006; Munster et al., 2016; Olival et al., 2017). Despite their infamy, bat viruses are not well understood. Elucidation of viral transmission dynamics in bat hosts will be essential to preventing future cross-species emergence by facilitating predictions of viral shedding pulses thought to underpin spillover (Amman et al., 2012) and by highlighting intervention opportunities in enzootic disease cycles.

Serology often represents the most readily attainable empirical information for wildlife diseases; methods have been developed to infer dynamics underlying patterns of age-structured seroprevalence for immunizing infections and prevalence for persistent infections (Brook et al., 2017; Farrington, 1990; Grenfell & Anderson, 1985; Griffiths, 1974; Heisey, Joly, & Messier, 2006; Hens et al., 2010; Long et al., 2010; Muench, 1959; Pomeroy et al., 2015). Numerous studies have reported serological evidence of bat exposure to henipa- and filoviruses across the Old World (Epstein et al., 2008, 2013; Hayman et al., 2008, 2010; Iehlé et al., 2007; Leroy et al., 2005; Ogawa et al., 2015; Peel et al., 2012; Plowright et al., 2008; Taniguchi et al., 1999; Yuan et al., 2012), though only a few have attempted to use mechanistic models to infer transmission dynamics from serological data for any bat virus (*e.g.* for rabies: Blackwood, Streicker, Altizer, & Rohani, 2013; for henipavirus: Peel et al., 2018). The paucity of attempts to model such data may be attributable to the idiosyncratic landscape of chiropteran antibody responses. Experimental challenge trials with various bat species have demonstrated seroconversion after inoculation with Hendra (Williamson et al., 1998) and Nipah (Middleton et al., 2007) henipaviruses and with Marburg (Amman et al., 2014; Paweska et al., 2012, 2015; Schuh, Amman, Jones, et al., 2017; Schuh, Amman, Sealy, et al., 2017), Ebola and Sudan filoviruses (Jones et al., 2015; Paweska et al., 2016), though many studies (*e.g.* Halpin et al., 2011) report idiosyncratic antibody dynamics of seroconversion without demonstrable viral replication. Only a few studies have followed immunized bats for longer time horizons: in Marburg-immunized *Rousettus aegyptiacus*, antibody titres wane after inoculation and primary seroconversion, but subsequently re-challenged seronegative bats nonetheless remain protected from reinfection and primed to remount rapid antibody responses (Paweska et al., 2015; Schuh, Amman, Jones, et al., 2017; Schuh, Amman, Sealy, et al., 2017). The underlying immunological mechanisms for these responses remain unclear, but at least two pteropodid species were recently shown to maintain a constitutively expressed interferon complex (Zhou et al., 2016), offering an innate, non-antibody-mediated pathway for viral control.

The island of Madagascar is home to three endemic Old World Fruit Bat species, *Pteropus rufus*, *Eidolon dupreanum* and *Rousettus madagascariensis*, with respective Asian (Almeida, Giannini, Simmons, & Helgen, 2014), African (Shi et al., 2014) and pan-Indian Ocean (Goodman, Chan, Nowak, & Yoder, 2010) origins. All three species are widely consumed across the island as bushmeat (Golden, Bonds, Brashares, Rodolph Rasolofoniaina, & Kremen, 2014; Jenkins & Racey, 2008; Jenkins et al., 2011), offering abundant opportunities for zoonotic transmission. Previous work reports serological evidence of Hendra- and Nipah-related henipavirus spp. in *P. rufus* and *E. dupreanum* bats, as well as Tioman spp. virus in *R. madagascariensis* (Iehlé et al., 2007). To date, no filoviruses have been investigated in any Malagasy bat, although one early serosurvey of human communities in Madagascar highlights seropositivity to Ebola-related filoviruses (but not Marburg) in several localities across the island (Mathiot, Fontenille, Georges, & Coulanges, 1989). Recent modelling work has classed Madagascar within the "zoonotic niche" for both Ebola (Pigott et al., 2014) and Marburg virus disease (Pigott et al., 2015). These intriguing preliminary findings, combined with the extreme virulence and heavy public health cost of known bat-to-human henipa- and filovirus emergence events, motivated our study. We aimed to (a) document the extent of henipa- and filovirus spp. exposure among endemic Malagasy fruit bats, (b) explore patterns of seasonality in seroprevalence and serostatus in these populations and (c) compare mechanistic hypotheses for possible transmission dynamics underlying these data.

2 | MATERIALS AND METHODS

2.1 | Bat capture and sampling

We captured 740 Madagascar fruit bats (314 *Eidolon dupreanum*, 201 *Pteropus rufus*, 225 *Rousettus madagascariensis*) across four sites in 18 discrete sampling events between November 2013 and January 2016 using methods that have been previously described (Brook et al., 2015; Brook, Ranaivoson, Andriafidison, et al., 2019). Captured animals were measured, weighed, sexed, thumb-tagged and categorized by broad age/reproductive class. Between 0.03 and 1 ml of blood (no more than 1% of the animal's body mass) was collected from the brachial vein of each captured bat, centrifuged and stored separately

as serum and pelleted blood cell. A subset of adult bats (85 *P. rufus* and 90 *E. dupreanum*) were processed under anaesthesia using a halothane vaporizer (4% halothane in oxygen at 0.7 L/min), and a lower left premolar tooth was extracted from these individuals for ageing purposes. *R. madagascariensis* bats were deemed too small for tooth extraction and therefore not subject to anaesthesia or ageing.

Additionally, researchers at the Institut Pasteur of Madagascar (IPM) captured, sexed, weighed, measured and serum-sampled 440 *E. dupreanum* bats between November 2005 and July 2007 (Iehlé et al., 2007). We included measurement and serostatus data from these capture events in our Aim 1 and 2 analyses.

2.2 | Ethics statement

All field work was carried out in accordance with guidelines posted by the American Veterinary Medical Association and under permit authorization from the Madagascar Ministry for Water and Forests (sampling permit #: 166/14/MEF/SG/DGF/DCB.SAP/SCB, 75/15/MEEMEF/SG/DGF/DCB.SAP/SCB, 92/16/MEEMEF/SG/DGF/DCB.SAP/SCB, 259/16/MEEF/SG/DGF/DSAP/SCB). All field protocols employed were pre-approved by the Princeton University Institutional Animal Care and Use Committee (IACUC Protocol # 1926), and every effort was made to minimize discomfort to animals.

2.3 | Sample processing and serological analysis

2.3.1 | Ageing

Tooth samples were exported and processed histologically at Matson's Laboratory (Missoula, Montana), following previously published protocols (Cool, Bennet, & Romaniuk, 1994; Divljan, Parry-Jones, & Wardle, 2006), to yield integer estimates of age via *cementum annuli* counts. Because fruit bats birth in annual pulses (Peel et al., 2014), we obtained more precise estimates of age by assuming a standard birth date for captured bats of a given species and adding the duration of time between capture and birth date to the integer estimate of age via *cementum annuli*. We computed ages for pups <1 year in the same way. In Madagascar, births are staggered among the three species, with the largest, *P. rufus*, birthing first, followed by *E. duprenaum* and *R. madagascariensis* (Andrianaivoarivelo, 2015), though the latter were not aged in our study. Assuming respective birth dates of October 1 and November 1, we computed age to the nearest day for 142 *P. rufus* and 109 *E. dupreanum*.

2.3.2 | Luminex-based serological assay

Serum samples were screened for antibodies against henipavirus and filovirus soluble glycoproteins (Hendra: HeV sG, HeV sF; Nipah: NiV sG, NiV sF; Cedar: CedPV sG, CedV sF; Ebola: EBOV sGp; and Marburg: MARV sGp) using a Luminex-based, Bio-Plex® (Bio-Rad, Inc.) assay that has been previously described (Bossart et al., 2007; Chowdhury et al., 2014; Hayman et al., 2008; Peel et al., 2012, 2013) (Supporting Information Text S1).

For the 2005–2007 Institut Pasteur subset of data, samples were screened for antibodies to NiV and HeV henipaviruses by standard enzyme-linked immunosorbent assay (ELISA). Only serostatus (no raw titres) were made available, and we accepted the original researchers' classification of individuals as seropositive or seronegative.

2.4 | Quantitative analysis

2.4.1 | Aim 1: Henipa- and filovirus spp. exposure

This investigation represents the first application of our Luminex assay to serum samples collected from Madagascar bats, meaning that no definitive positive or negative controls for any species examined were available. Instead, following previously published methods (Burroughs et al., 2016; Peel et al., 2013; Trang et al., 2015), we fit finite mixture models to the natural log of the mean fluorescence intensity (MFI) data to approximate a cut-off MFI value (and corresponding upper and lower confidence interval) for seropositivity for each species/antigen combination (Supporting Information Text S2; Tables S1 and S2; Figure S1).

Because our antigens were not originally obtained from Madagascar fruit bats, we required that each species/antigen data subset meet several additional criteria before further statistical analysis. For each data subset, we required that MFI values either (a) show correlation with an $R^2 > 40\%$ for associated soluble glycoproteins within the same viral genus (an indicator of reliable cross-reactivity among antibodies to related viruses; Supporting Information Figure S2), (b) have values >1,000 MFI for some individual(s) assayed (Gombos et al., 2013) or (c) result in >10% seroprevalence based on the mixture model cut-off (Supporting Information Text S3). We summarize all serological data, in conjunction with age and sampling data in Supporting Information Table S3.

2.4.2 | Aim 2: Seasonality in seroprevalence and serostatus

We next aimed to identify any seasonal trends in population-level seroprevalence or individual serostatus for antigens which met the criteria outlined under Aim 1. We restricted these analyses to adult-sized bats over 1 year in age from our own data, combined with *E. dupreanum* data from IPM (Iehlé et al., 2007). We analysed each species/antigen subset of our data separately for a total of seven independent analyses (see Results, Table 1). For each data subset, we fit a separate generalized additive model (GAM) in the binomial family, using a matrix of seropositive/seronegative counts by sampling event as the response variable and mid-date of sampling event as the smoothing predictor, with a random effect of site and year. All GAMs were fit via REML estimation, and we fixed the number of smoothing knots (k) at seven, as recommended by the package author (Wood, 2001). *R. madagascariensis* data were too sparse to permit model convergence at $k = 7$; in these cases, we fixed k at 6.

TABLE 1 Seroprevalence to henipa- and filovirus antigens in Madagascar fruit bats[a]

Species	Virus	*N*	Viral antigen assayed	Max MFI	MFI cut-off mean [lci[b], uci[c]]	Seroprevalence % (N pos)		
						At mean cut-off	At lci[b] cut-off	At uci[c] cut-off
Eidolon dupreanum	Cedar	314	CedPV-G	2436.3	166.46 [95.68, 374.55]	0.64 (2)	1.27 (4)	0.64 (2)
	Hendra/Nipah[d]	**314**	**NiV-G**	**6553**	**402.90 [225.50, 1506.48]**	**24.2 (76)**	**32.17 (101)**	**10.19 (32)**
Pteropus rufus	Hendra/Nipah	201	HeV-G	439.3	67.55 [61.29, 77.58]	5.47 (11)	6.97 (14)	3.48 (7)
	Ebola[d]	**201**	**EBOV-Gp**	**697.5**	**110.49 [90.58, 284.02]**	**10.4 (21)**	**12.94 (26)**	**4.48 (9)**
Rousettus madagascariensis	Cedar	225	CedV-F	623.8	75.75 [70.17, 84.00]	8.44 (19)	9.33 (21)	7.11 (16)
	Hendra/Nipah	225	HeV-F	437.3	77.46 [68.75, 94.77]	7.56 (17)	8.44 (19)	6.67 (15)
	Ebola	225	EBOV-Gp	5716	457.76 [358.52, 552.07]	8.44 (19)	12 (27)	6.67 (15)

[a]Seroprevalence here indicates evidence of pathogen exposure found in current (2013–2016) field studies; historical data from 2005 to 2007 is not included here. Results from species–virus combinations for which no seropositives were recovered (*E. dupreanum*: Marburg/Ebola, *P. rufus*: Cedar/Marburg, *R. madagascariensis*: Marburg) are shown in Supporting Information Table S2. [b]lci = lower confidence interval threshold for the MFI cut-off for seropositivity. This is a more lenient threshold than the mean. [c]uci = upper confidence interval threshold for the MFI cut-off for seropositivity. This is a stricter threshold than the mean. [d]Of these antigen/species combinations shown here, two (in bold) met more restrictive criteria for age–seroprevalence analyses. We report only results for NiV-G in *E. dupreanum* in the main text of the manuscript.

Once each model was fit, we used the predict.gam() function to obtain a predicted estimate of seroprevalence by sampling event, bounded by an upper and lower 95% confidence interval. We list the basic structural forms of all GAMs considered in Supporting Information Text S4 and summarize outputs from fitted binomial GAMs in Supporting Information Table S4 (Supporting Information Figure S3).

We next reformatted our data to examine seasonality within a calendar year, independent of year of study. We used binomial GAMs to test for seasonality in serostatus for adult bats of both sexes and all three species. We set a matrix of seropositive by seronegative counts per Julian day-of-year as our response variable, as computed from mean, lower and upper MFI thresholds for seropositivity, and modelled antigen type as the fixed predictor and day-of-year as the smoothing predictor. We used a "by" term to enable a separate smoother for each sex. All models included random effects of capture site and year. Because we investigated broad seasonal fluctuations, we restricted the number of smoothing knots (k) to four and used a cyclic cubic regression spline which forces the smoother to transition continuously from the end of 1 year to the beginning of the next (Supporting Information Text S4; Table S5; Figure S4).

Additionally, 17 unique *E. dupreanum* individuals (three female, 14 male) were captured twice across the duration of our study. Of the two *E. dupreanum* antigens that met criteria for statistical analysis (see Results, Table 1), only anti-NiV-G titres demonstrated substantial dynamism among recaptures. Data were too few for meaningful statistical analysis, but we nonetheless interpreted results anecdotally (Supporting Information Figure S5).

Finally, we used Gaussian GAMs to test for seasonality in mass:forearm residual for adult bats within a given year. The mass:forearm residual gives a crude measure of body condition by which to compare bat "health" within a given sex and species. Bats above the mass:forearm length regression line are "heavier" and those below the line "lighter" than predicted, suggestive of over- and under-nourished conditions—though we caution that we did not validate this inference by comparing measured "mass" with quantification of body lipid content (Pearce, O'Shea, & Wunder, 2008).

We first established a standardized mass:forearm residual for all adult bats in our dataset by (a) dividing the raw mass per individual by the mean mass of that particular species and sex, then (b) regressing standardized mass against forearm length and (c) calculating the residual from the species-specific linear model (Supporting Information Figure S6). We used "standard major axis" type 2 linear regression in this analysis since we anticipated variation and error in measurements for both x- and y-axes (Legendre, 2014). We then modelled these data with standardized mass:forearm residual as the response variable and Julian day-of-year as the smoothing predictor, including random effects of site and year and a cyclic cubic regression spline (Supporting Information Text S4; Table S6).

2.4.3 | Aim 3: Comparing mechanistic hypotheses

Finally, to recover the mechanistic underpinnings of our data, we fit a series of epidemiological models, encompassing a suite of bat virus transmission hypotheses, to longitudinal NiV-G age–seroprevalence data for *E. dupreanum* and to EBOV-Gp seroprevalence for *P. rufus*. For this final research aim, analyses were restricted to the 109 *E. dupreanum* and 142 *P. rufus* samples for which we possessed age estimates; for the purposes of model-fitting, we further subsampled age–seroprevalence data to include only those individuals captured at our longitudinally resampled Moramanga site. We evaluated each age–seroprevalence subsample for representativeness of the broader sampling event from which it was derived using bootstrapping techniques (Supporting Information Text S3) and ultimately fit models to serological data from 72 aged *E. dupreanum* and 123 aged *P. rufus* (Supporting Information Table S3).

All models were constructed using discrete-time, age-structured, matrix modelling techniques for epidemics (Klepac & Caswell, 2011; Klepac et al., 2009; Metcalf et al., 2012), assuming frequency-dependent transmission, homogeneous mixing and equilibrium structure across age classes (Supporting Information Text S5). We considered variations on five discrete model structures: (a) MSIR, (b) MSRIR, (c) MSIRS, (d) MSIRN and (e) MSIRNR. In all cases, we modelled the "M" (maternally immune; Supporting Information Figure S7) and "R" (recovered) classes as seropositive. The (a) MSIR (maternally immune, susceptible, infectious, recovered) model represents a classic paradigm in the dynamics of transmission for many perfectly immunizing infections, offering a null hypothesis against which to compare other dynamical structures (Bjornstad, Finkenstadt, & Grenfell, 2002; Metcalf, Bjørnstad, Grenfell, & Andreasen, 2009; Metcalf et al., 2012). The simplest extension, (b) MSRIR, allows bats to seroconvert directly into the R class without becoming demonstrably infectious, as has been shown in the experimental literature (Jones et al., 2015; Paweska et al., 2015). The (c) MSIRS model permits waning immunity and return of recovered individuals to susceptible status, offering one possible explanation for the intermittent pulses of bat viral excretion posited to underpin spillover events (Amman et al., 2012; Plowright et al., 2011, 2015, 2016). The (d) MSIRN model allows for antibody waning of seropositive bats from the R class into a seronegative but still immune class, "N," which could represent either non-antibody-mediated immunity or sub-seropositive antibody titres that still remain protective, again reflecting the experimental literature (Paweska et al., 2016; Schuh, Amman, Jones, et al., 2017; Schuh, Amman, Sealy, et al., 2017). The (e) MSIRNR model merely extends MSIRN to allow N-class bats to return to seropositivity after re-challenge and renewed contact with infectious individuals.

Other work has suggested that pulses in bat viral transmission may result from SILI-like (susceptible, infectious, latent, infectious) within-host dynamics. Optimization of a SILI model would require fine-scale recapture data documenting live virus infection across individual bats sampled longitudinally; lacking this, we instead approximated longitudinal serological variation in MSIRN/MSIRNR model forms, which allow for dynamic antibody titres post-initial seroconversion.

In all modelled epidemics, populations were jointly subjected to survival and epidemic transitions. Births were subsequently introduced into the population but restricted in duration to a 10-week, species-specific annual period. Births were distributed among the four or five epidemic states, according to parental effects: we assumed that S-class bats of reproductive age (≥2 years) produced susceptible offspring, while I- and R-class bats of reproductive age produced maternally immune offspring. We tested model forms both by which N-class dams produced S ("matSus")- and M-class ("matAB") offspring.

We controlled demographic rates under assumptions of stable age structure (annual adult survival = 0.793 for *E. dupreanum* and 0.511 for *P. rufus*; annual juvenile survival = 0.544; annual birth rate = 0.48 for both species; Brook, Ranaivoson, Andriafidison, et al., 2019). In keeping with previously developed multi-state matrix models for human diseases (Metcalf et al., 2011, 2012; Wesolowski et al., 2016), we modelled epidemic processes on a biweekly (14-day) time-scale, such that twenty-six survival–epidemic transitions were permitted across a given year. In all cases, we assumed homogenous mixing across age classes and a constant transmission coefficient (β) across the duration of the time series (though the force of infection, λ, nonetheless cycled annually in conjunction with changes in the infectious population). We fixed the recovery rate from infection at one biweek^{-1}, the average of rates approximated in the literature (Hayman, 2015; Paweska et al., 2012; Swanepoel et al., 1996) and optimized all other epidemic parameters, depending on the chosen model structure, by minimizing the negative log-likelihood of data of a specific age and biweek, given the model's output at that same age and time. For all models, we fit rates for waning of maternally inherited antibodies (ω) and transmission (β) held constant across age and time (Supporting Information Table S7). For MSIRS, MSIRN and MSIRNR models, we additionally fit a waning antibody rate for individuals exiting the R class (σ); for MSRIR models, a rate of direct seroconversion from S to R (ρ); and for MSIRNR models, a rate of antibody boosting (γ), by which bats returned to R from N. For MSIRN/R models, we explored variations in model structure under which N-class dams produced either maternally immune (-matAB) or susceptible young (-matSus). All seven models were re-fit six different times: to NiV-G/*E. dupreanum* and EBOV-Gp/*P. rufus* data at all three MFI thresholds for seropositivity, to yield 42 distinct sets of parameter estimations.

3 | RESULTS

3.1 | Aim 1: Henipa- and filovirus spp. exposure

In all, seven species/antigen combinations met criteria for further analysis, indicating the presence of reliable reactive antibodies to tested antigens in serum from species in question: NiV-G and CedPV-G in *E. dupreanum*, HeV-F and EBOV-Gp in *P. rufus*, and HeV-F, CedPV-G and EBOV-Gp in *R. madagascariensis* (Table 1 and

Supporting Information Table S2). These Luminex results indicate that all three Madagascar fruit bat species demonstrated antibody reactivity to Hendra and/or Nipah-related henipaviruses; the inclusion of *R. madagascariensis* represents an expansion on previous findings (Iehlé et al., 2007; Table 1). MFI values from the NiV-G/HeV-G and NiV-F/HeV-F Luminex assays were highly correlated (Supporting Information Figure S2), suggesting cross-reactivity against related Nipah/Hendra-like henipavirus antigens. For each species, we selected the Nipah/Hendra-like antigen that yielded the highest MFI per species for further ecological analysis: NiV-G for *E. dupreanum* and HeV-F for *P. rufus* and *R. madagascariensis* (Table 1).

Additionally, we document the first serological evidence of cross-reactivity with a third henipavirus, Cedar virus, in *E. dupreanum* and *R. madagascariensis*, although seroprevalences were low (anti-CedPV-G and anti-CedV-F seroprevalence = 1.27% and 9.33%, respectively). We also report the first serological evidence of filovirus exposure in any Madagascar wildlife. Samples from both *P. rufus* and *R. madagascariensis* tested seropositive to Ebola (EBOV-Gp) but not Marburg (MARV-Gp) virus antigen, while all *E. dupreanum* samples assayed seronegative to EBOV-Gp and MARV-Gp. We compiled individual serostatus by all three MFI cut-offs to compute seroprevalences for all species/antigen combinations across 33 discrete sampling events in our study (Supporting Information Table S3).

Because ages were unavailable for *R. madagascariensis*, and seroprevalences were low for HeV-F in *P. rufus* and CedPV-G in *E. dupreanum* (6.97% and 1.27%, respectively), we restricted mechanistic modelling of age–seroprevalence trends (Aim 3) to NiV-G in *E. dupreanum* and EBOV-Gp in *P. rufus* data only. Due to concerns over the lack of specificity and validation in our assay for EBOV-Gp in *P. rufus* (which met only one of our three criteria for analysis), we ultimately reported results for these fits in the Supporting Information only and reserved the main text of our manuscript for modelling of anti-NiV-G in *E. dupreanum* data, which met all three of criteria for analysis. This Luminex has been previously validated on samples from the sister species *E. helvum* (Hayman et al., 2008; Peel et al., 2018).

3.2 | Aim 2: Seasonality in seroprevalence and serostatus

Generalized additive modelling indicated significant seasonal trends for henipavirus seroprevalence (NiV-G) in the *E. dupreanum* time series and for ebolavirus spp. seroprevalence (EBOV-Gp) in the *P. rufus* time series (Figure 1; Supporting Information Figure S3; Table S4). Population-level seroprevalences appeared to increase across the gestation period for *E. dupreanum* anti-NiV-G and *R. madagascariensis* anti-EBOV-Gp data. Seasonal patterns were clearest in the 2005–2007 Institut Pasteur de Madagascar (IPM) subset of the *E. dupreanum* anti-NiV-G data, demonstrating biannual peaks in seroprevalence at the height of the wet season and the end of gestation.

Additional GAMs constructed using Julian day-of-year as a predictor indicated significant seasonality in seropositive status for female *E. dupreanum* and *R. madagascariensis* (Figure 2a–c; Supporting Information Table S5). Female *P. rufus* did not exhibit significant seasonality in serostatus, although the periodicity of the smoothing trend recovered from this model correlated with those from other species. Serostatus for certain antigens (NiV-G in *E. dupreanum*, EBOV-Gp in *P. rufus* and HeV-F in *R. madagascariensis*) tracked reproduction, increasing across gestation for females (time-lagged among the three species), then decreasing post-birth and through lactation and weaning.

Male bats did not exhibit significant seasonality in seroprevalence at the population level (Supporting Information Figure S4), though three of fourteen recaptured male and one of three recaptured female *E. dupreanum* demonstrated dynamic anti-NiV-G titres (Supporting Information Figure S5). Using the mean MFI cut-off, one adult male bat (unknown age), originally captured at the end of the dry season and recaptured at the close of the subsequent wet season, had transitioned from seronegative to seropositive (titres increased by >800 MFI). A second adult male (unknown age), caught first in the middle of the wet season, showed titres elevated by >700 MFI when recaught at the onset of the dry season but tested seropositive in both samplings. A third adult male (aged ~8.75 years), caught first in the middle of the dry season, showed *decreased* titres by ~200 MFI upon recapture a few months later into the dry season. Finally, a lactating female bat (unknown age) showed decreased titres by ~700 MFI after weaning her pup prior to recapture.

Seasonal smoothers incorporated into GAMs predicting annual variation in mass:forearm residual were significant for females of all three species and for *P. rufus* and *R. madagascariensis* males (but not for *E. duprenaum* males; Supporting Information Table S6). As with serostatus, seasonal periodicity in mass:forearm residual tracked reproduction for females—increasing across gestation, then declining post-birth and through lactation. For males, the seasonal smoother synchronously tracked the nutritional calendar: mass:forearm residual increased across Madagascar's fruit-abundant wet season, then declined through the nutrient-poor dry season. Female mass:forearm residuals were not corrected for pregnancy. The majority of female adult fruit bats give birth to one pup each year; Hayman et al. (2012) report that 96% of adult-age female *Eidolon helvum* give birth annually in Ghana. The gain in female mass:forearm residual across gestation exhibited in our data thus likely reflects a gain in foetal mass rather than improved body condition for the mother.

All told, these patterns suggest a significant seasonal component to serostatus for female Madagascar fruit bats, correlated with the reproductive calendar. Females are more likely to be seropositive during gestation (overlapping the dry Malagasy winter). No significant seasonal changes in male serostatus were observed in GAM-analysed population-level data; however, data from recaptured individuals suggest that antibody titres in male bats declined subtly across the dry season and increased again throughout the wet season when male bats were at peak body mass.

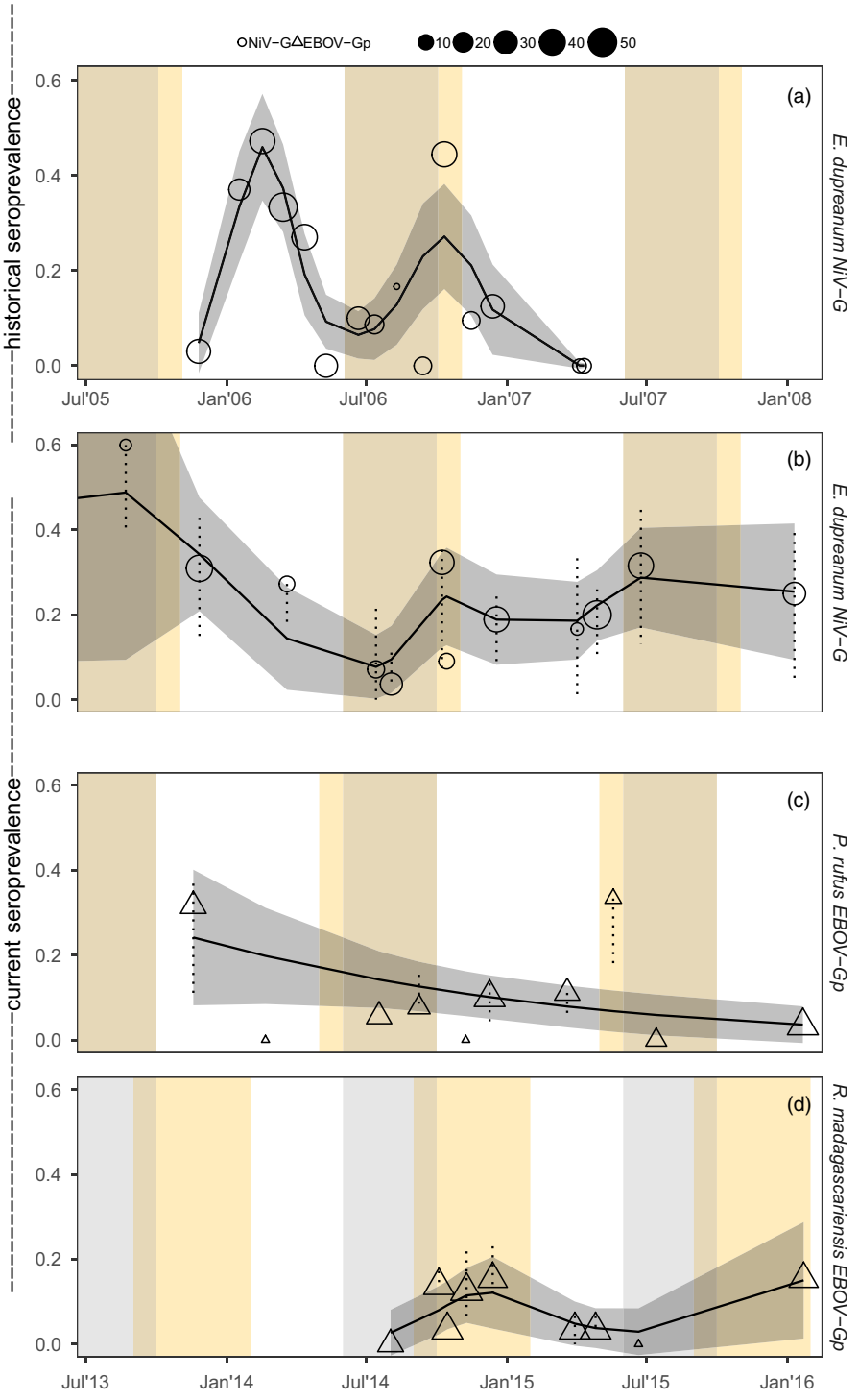

FIGURE 1 Seasonality in seroprevalence. (a) Predicted NiV-G seroprevalence by sampling date for *Eidolon dupreanum*, across range of historically sampled 2005–2007 data. The nutrient-poor Madagascar dry season is highlighted in grey vertical shading and the species-specific gestation period in yellow. Solid line and shaded 95% confidence intervals give the predicted seroprevalence from a significant binomial GAM construction of seropositive vs. seronegative by sampling date with random effects silenced for visualization purposes only. Data (with 95% exact binomial confidence intervals) are shown as open shapes in the background; shape size is correlated with sample size (as indicated in the legend). Analyses are repeated across the date range of the authors' current studies in (b), (c) and (d) for NiV-G in *E. dupreanum*, EBOV-Gp in *Pteropus rufus* and EBOV-Gp in *R. madagascariensis*, respectively. GAM constructions and results are summarized in Supporting Information Text S4 and Table S4. Seasonal smoothers by date (incorporating random effects) are significant for *E. dupreanum* and *P. rufus* data (panels a–c). Seasonal trends in seroprevalence for other species/antigen combinations in Table 1 are summarized in Supporting Information Figure S3

3.3 | Aim 3: Comparing mechanistic hypotheses

Teeth were processed histologically to yield integer estimates of fruit bat age (see Materials and Methods), producing species-specific age–frequency distributions for *E. dupreanum* and *P. rufus* (Figure 3). Adult mortality rates derived from exponential models fit to *E. dupreanum* data are compatible with assumptions of stable population structure, but age–frequencies recovered for *P. rufus* indicate that the species is likely in serious population decline. As such, we adopted juvenile mortality rates from *E. dupreanum* for epidemiological modelling of *P. rufus* data (Supporting Information Text S5). We combined age data with serological data amassed under Aim 1 to develop age–seroprevalence curves for NiV-G in *E. dupreanum* and EBOV-Gp in *P. rufus* (Supporting Information Text S5).

Composite age–seroprevalence data for *E. dupreanum* NiV-G demonstrated high seroprevalence in neonates, suggestive of inherited maternal antibodies (Supporting Information Text S5; Figure S7). This neonatal seroprevalence peak decreased rapidly

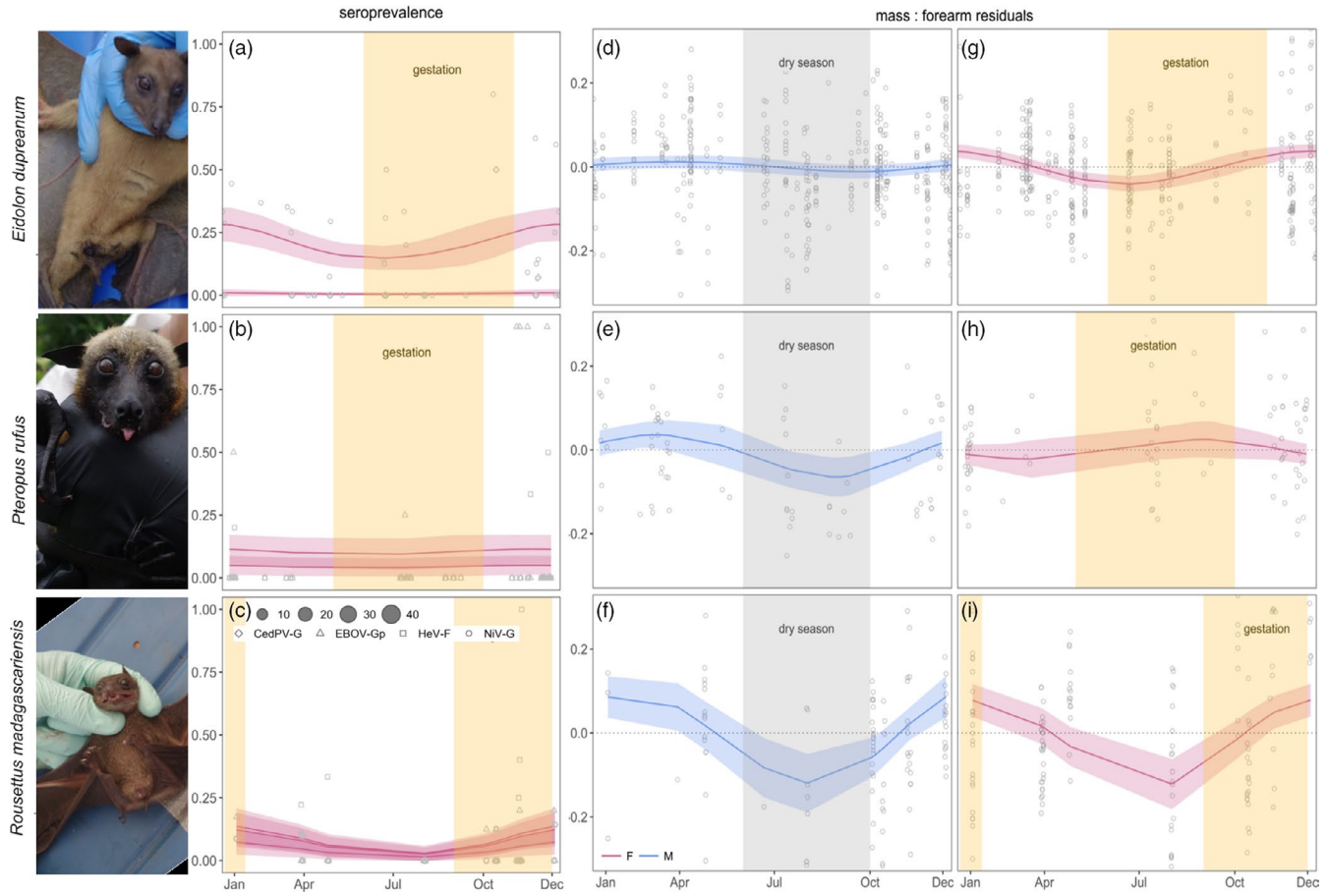

FIGURE 2 Seasonality in seroprevalence and body mass:forearm residual. Seasonal seroprevalence by discrete antigen in (a) female *Eidolon dupreanum*, (b) *Pteropus rufus* and (c) *R. madagascariensis* bats. Seasonal mass:forearm residual in, respectively, male and female (d, g) *E. dupreanum*, (e, h) *P. rufus* and (f, i) *R. madagascariensis* bats. The species-specific gestation period is highlighted in yellow shading on the female plots and the nutrient-poor Madagascar dry season in grey shading on the male plots. Solid lines (pink = female; blue = male) show the predicted seroprevalence for each antigen (a–c) and the predicted mass:forearm residual (d–i) from GAMs. Note that lines for seroprevalence for different antigens within a species (a–c) are indistinguishable; however, the top line for *E. dupreanum* (a) corresponds to anti-NiV-G seroprevalence, for *Pteropus rufus* (b) to anti-EBOV-Gp seroprevalence and for *R. madagascariensis* (c) to anti-HeV-F seroprevalence. Data for raw seroprevalence per sampling event (with 95% exact binomial confidence intervals) are shown as open shapes in the background (shape type corresponds to antigen, as indicated in legend). Raw mass:forearm residual data are shown, by month, in the background for each sampled individual (open circles) in d–i. Note that *E. dupreanum* data are combined with 2005–2007 sampling data from Institut Pasteur de Madagascar. Full GAM constructions are reported in Supporting Information Text S4 and results summarized in Supporting Information Table S5. The insignificant seasonal smoother for male serostatus and corresponding seroprevalence data are shown in Supporting Information Figure S4

following presumed waning of maternal immunity, then increased across early life, before tapering off once more in later age classes (Figure 4). When examined longitudinally, data demonstrated a decay in neonatal seroprevalence across the year, as pups' maternally inherited immunity waned following the birth pulse (Supporting Information Figures S8 and S9). The neonatal decline and early age increase in seroprevalence in our data replicates patterns previously reported for NiV-G exposure in African *E. helvum* (Peel et al., 2018), but our observed late-age seroprevalence decline contrasts with the late-age plateau of anti-NiV-G seroprevalence in the African system. We recovered similar age–seroprevalence patterns of EBOV-Gp exposure in *P. rufus* (Supporting Information Text S5; Figures S10–S12).

We report composite age–seroprevalence data for *E. dupreanum* NiV-G, combined with model outputs summarized across one age-structured equilibrium year, in Figure 4 (Supporting Information Figure S10). The right-hand panel in each subplot shows relative AIC within a given data subset; raw AIC scores are listed in Supporting Information Table S7. Compared to all other model structures, the MSIRN model most effectively recaptured data for both species under all putative MFI cut-offs when assuming that N-class mothers produced M-class young ("matAB"). Results for model specifications in which N-class mothers produced S-class pups are additionally reported in Supporting Information Figures S10–S12 and Table S7. Only MSIRN/R models effectively reproduced late-age declines in seroprevalence (with

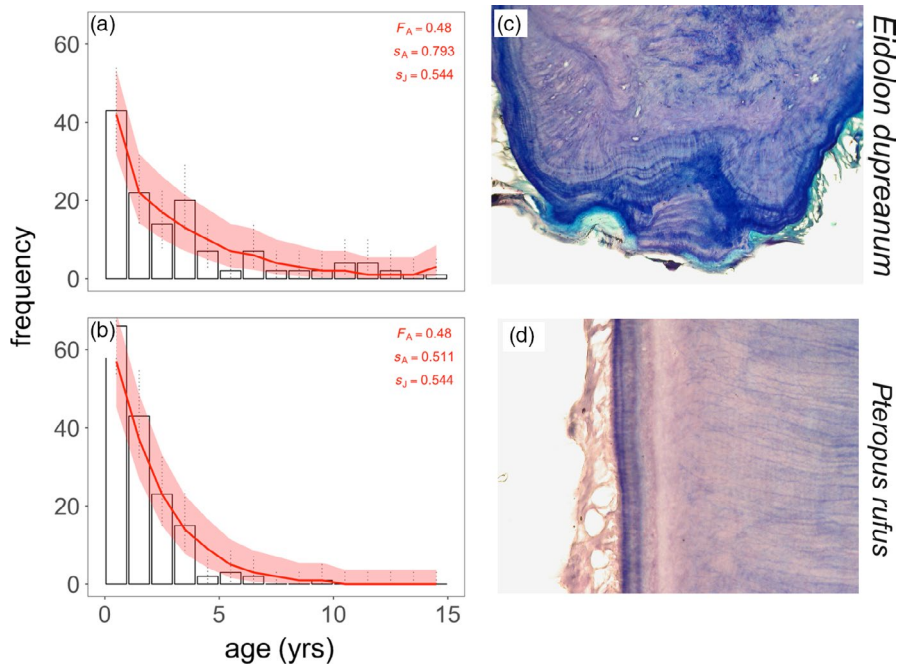

FIGURE 3 *Ageing Madagascar fruit bats via* cementum annuli. (a) Age–frequency distribution generated from *cementum annuli* counts of extracted *Eidolon dupreanum* teeth. Histogram is binned by year, with 95% exact binomial confidence intervals shown as dotted lines. The red curve is the predicted age–frequency distribution generated from the fit of a simple exponential model to age distribution >6 months, incorporating an annual adult survival rate of 0.793 and a juvenile annual survival rate of 0.544 (determined using Leslie matrix techniques to maintain a stable age distribution and constant population size; Supporting Information Text S5). Translucent shading shows 95% confidence intervals of the exponential fit by standard error. (b) Age–frequency distribution from *cementum annuli* counts of extracted *Pteropus rufus* teeth, with a fitted exponential model (red line) and 95% confidence intervals (red shading), incorporating an annual adult survival rate of 0.511 and a juvenile survival rate of 0.544 (constant population size was impossible for *P. rufus*, so we adopted the same rate as for *E. dupreanum;* Supporting Information Text S5). (c) Stained *cementum annuli* from a 14-year-old *E. dupreanum* sample. (d) Stained *cementum annuli* from a 2-year-old *P. rufus*

MSIRNR performing too poorly in AIC comparison for true consideration as a best fit model), while MSIRS predicted a late-age seroprevalence plateau.

Parameter estimates varied between the two best fit models: MSIRN-matAB and MSIRS. No empirical measurements of bat virus transmission (against which to compare β estimates) are available in the literature, but MSIRN models fit to the mean MFI cut-off for *E. dupreanum* NiV-G recovered optimized values for the rate of waning maternal immunity, ω (0.12 biweek^{-1}, corresponding to a maternal antibody duration of 4 months), and the rate of waning adult humoral immunity, σ (0.01 biweek^{-1}, corresponding to an adult antibody duration of 4 years), within the range previously reported in the literature for African *E. helvum* (6 months for maternal immunity and 4 years for adult humoral immunity) (Epstein et al., 2013; Peel et al., 2018). MSIRS models produced considerably higher optimized parameter values, indicating shorter durations of maternal antibodies (2 weeks) and adult humoral immunity (2 years). Such rapid rates of antibody waning were essential to avoid increasing seroprevalence with age but, arguably, less biologically defensible. AIC values, parameter estimates and confidence intervals for models fit to all three MFI cut-offs, as well as to the *P. rufus* EBOV-Gp data, are summarized in Supporting Information Table S7.

4 | DISCUSSION

We leveraged henipa- and filovirus serological data for three species of wild Malagasy fruit bat to evaluate support for contrasting mechanisms hypothesized to drive longitudinal, seasonal viral and immune dynamics in this system. Though Plowright et al. (2016) cautioned that "inference from serology alone is unlikely to differentiate among…proposed epidemiological scenarios" for mechanisms underpinning population-level patterns in bat virus data, the serological analysis methods employed here nonetheless narrow the range of plausible competing hypotheses considerably and simultaneously underscore critical knowledge gaps that could be addressed in future field studies. Our analysis of age-structured serological data highlights several key insights: (a) we expand globally on the known range of bat hosts for henipaviruses and filoviruses, (b) we demonstrate seasonal patterns in population-level seroprevalence and individual-level serostatus for Malagasy fruit bats, concomitant with the reproductive calendar, and (3) we use mechanistic models to reveal the critical role of waning humoral immunity and the potential for alternative immune processes in governing serological patterns witnessed in our data.

We report many serological findings novel for the Madagascar ecosystem—including the first evidence of antibodies cross-reactive

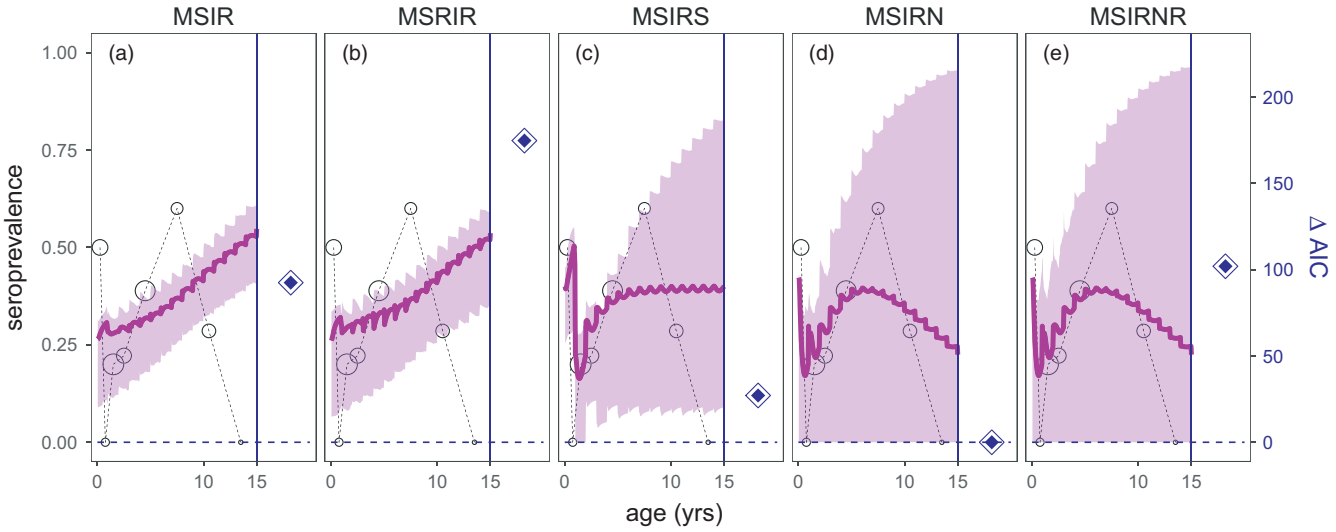

FIGURE 4 Model fits to age–seroprevalence data. Age–seroprevalence curves for *Eidolon dupreanum* NiV-G, using the mean MFI cut-off for seropositive status. Seroprevalence data (left *y*-axis) are shown as open circles, binned for 0–0.5 years, 0.5–1 years, 1–1.5 years, 1.5–3 years, and for 3-year increments increasing after that. Shape size corresponds to the number of bats sampled per bin (respective sample sizes, by age bin, are as follows: *N* = 10, 2, 20, 9, 18, 5, 7, 1). Solid purple lines indicate model outputs, and translucent shading highlights the 95% confidence interval derived from the Hessian matrix of the maximum likelihood of each model fit to the data. Panels are stratified into columns by model structure: (a) MSIR = maternally immune, susceptible, infectious, recovered; (b) MSRIR = maternally immune, susceptible, recovered via direct seroconversion, infectious, recovered; (c) MSIRS = maternally immune, susceptible, infectious, recovered, susceptible; (d) MSIRN = maternally immune, susceptible, infectious, recovered, non-antibody immune; (e) MSIRNR = maternally immune, susceptible, infectious, recovered, non-antibody immune; recovered). All MSIRN/R model outputs depicted assume that non-antibody immune dams produce maternally immune-class young. The right-hand *y*-axis (in navy) of each subplot shows ΔAIC for each model fit, relative to all other models in the figure (navy diamonds). The MSIRN model (d) offered the best fit to the data, corresponding to ΔAIC = 0. All parameter values, confidence intervals and raw AIC scores for each model fit are reported in Supporting Information Table S7. Model fits including MSIRN/R fits assuming N-class mothers produce susceptible young are shown in Supporting Information Figure S10, along with fits to seroprevalence data for *P. rufus* EBOV-Gp. Fits calculated using the lower and upper MFI thresholds for seropositivity are shown in Supporting Information Figures S11 and S12

with Cedar henipavirus (CedPV-G: *E. dupreanum* and CedV-F: *R. madagascariensis*) and Zaire ebolavirus antigens (*P. rufus* and *R. madagascariensis*) in any wild Malagasy host. The documentation of bat antibodies cross-reactive with Zaire ebolavirus (but not Marburg) antigen will interest the global public health community, as recent work classes Madagascar within the "zoonotic niche" of both Ebola (Pigott et al., 2014; Schmidt et al., 2017) and Marburg (Pigott et al., 2015) filoviruses. Ironically, Madagascar's inclusion in these risk maps has been largely derived from the species distribution of *Eidolon dupreanum* (Han et al., 2016; Pigott et al., 2014), the one Malagasy fruit bat for which we found no filovirus seropositive samples. This finding is not hugely surprising if we consider the relative rarity of Ebola seropositivity in *E. dupreanum*'s sister taxon, *E. helvum* (Olival & Hayman, 2014), which possesses a receptor-level substitution that makes it refractory to Ebola infection (Ng et al., 2015).

Given Madagascar's geographic isolation and the considerable phylogenetic distance separating its fruit bats from their nearest mainland relatives (Almeida et al., 2014; Goodman et al., 2010; Shi et al., 2014), it seems likely that some of the seropositives recovered in this study result from cross-reactivity of Malagasy bat antibodies to related, but distinct, antigens from those assayed here. To date, no henipaviruses or filoviruses have been identified (via live virus or RNA) in Madagascar. Detection and characterization of these viruses, together with description of the specificity, avidity and neutralization capacity of their antibodies, thus represents a critical research priority. The probable cross-reactivity of Malagasy bat antibodies derived from different—and potentially novel—henipa- and filovirus antigens adds considerable uncertainty to our tabulation of MFI thresholds for seropositivity.

The greatest challenge to our dynamical inference is the possibility that seropositive samples do not signify true circulating virus within any of our three species. In laboratory trials, for example, *R. aegyptiacus* bats are known to seroconvert upon contact with inoculated individuals without ever becoming detectably infectious (Jones et al., 2015; Paweska et al., 2015). While we attempted to explore these dynamics within a single bat population using our MSRIR model, it is possible that focal viruses circulate in species distinct from those studied here, resulting in seropositive samples via dead-end seroconversion from transient bat contact with an alternative reservoir. Although we cannot falsify this hypothesis, there are a few specifics of the Madagascar ecosystem that make such a scenario unlikely. In particular, all but one of the roosts surveyed in this study are largely single-species conglomerations: *P. rufus* is a tree-dwelling pteropodid which only roosts in single-species assemblages, while *E. dupreanum* predominantly inhabits cracks and crevasses with conspecifics (Goodman, 2011).

In cave environments, *E. dupreanum* and *R. madagascariensis* occasionally co-roost and roost with insectivorous bats (Cardiff, Ratrimomanarivo, Rembert, & Goodman, 2009), and all three fruit bat species contact at feeding sites. Nonetheless, given the relative rarity of these cross-species contacts, it is unlikely that the high seroprevalence recovered in our data for anti-NiV-G antibodies in *E. dupreanum* (24.2%) and anti-EBOV-Gp antibodies in *P. rufus* (10.2%) result from dead-end seroconversion alone. Previous work has investigated paramyxovirus spp. by PCR among insectivorous bats in Madagascar (Wilkinson et al., 2012, 2014), and no henipavirus spp. have been identified, further supporting our assumptions that Malagasy fruit bats maintain their own endemic viral transmission cycles.

The lack of specificity in our serological assay also permits the possibility that a given bat population might maintain active infections with multiple serologically indistinguishable viruses of the same family, which are nonetheless epidemiologically unique; serum from Ebola-infected humans, for example, will recognize all five known species of ebolavirus (MacNeil, Reed, & Rollin, 2011). An analysis like ours would consider serological evidence of any ebolavirus infection equivalently and model all seropositives as one population, though, in reality, each specimen could represent a distinct virus that maintains its own transmission cycle. Again, we cannot falsify this hypothesis, but recent molecular work supports a theory of single-bat, single-filovirus species interactions that runs counter to this claim (Ng et al., 2015). We observed vast differences in the range of MFI titres recovered for each antigen among our three bat species, recovering high MFI titres for EBOV-Gp in *R. madagascariensis* but only mid-range titres in *P. rufus* (Table 1). We also found that *E. dupreanum* serum reacted most strongly to the NiV-G antigen, while *P. rufus* and *R. madagascariensis* serum bound more tightly to the HeV-F antigen. Such differences could be attributable to cross-species variation in the robustness of the humoral immune response or could indicate that our tested antigens more closely align with the wild antigen from which one species' antibodies were derived vs. that of another. This species-specific variation in antibody binding to the same antigen challenge supports our decision to model each bat species–virus relationship independently, rather than allowing for significant interspecies transmission to govern viral dynamics in this system.

Female serostatus for both henipavirus and filovirus spp. varied seasonally in our data, tracking reproduction for *E. dupreanum* and *R. madagascariensis*; female bats showed elevated antibody titres during reproduction, consistent with previous work (Baker et al., 2014). This pattern suggests that viral control is one of many costs to which resource-limited hosts must allocate energy and that male and female bats do so differently while facing distinct metabolic demands. While higher serotitres in reproductive females may seem counterintuitive if viewed as increased investment in immunity, recent research suggests that bats may control viral infections primarily via innate immune pathways (Zhou et al., 2016), which are more metabolically costly than adaptive immunity (Raberg et al., 2002). It is possible then that female bats trade off innate immunity with less metabolically demanding means of viral control (i.e. antibodies) during reproductive periods (Brook & Dobson, 2015) or that contact rates with infectious individuals are elevated during these seasons, resulting in antibody-boosting effects (*e.g.* Paweska et al., 2015; Schuh, Amman, Jones, et al., 2017; Schuh, Amman, Sealy, et al., 2017). Alternatively, elevated antibody titres might be independent of both exposure and metabolic trade-offs; for example, production of the milk protein prolactin (typically elevated in late pregnancy and early lactation for mammals) is known to stimulate antibody production and facilitate maternal antibody transfer to young (Spangelo, Hall, Ross, & Goldstein, 1987).

Males, with fewer reproductive constraints, demonstrate no clear shifts in seasonal serostatus at the population level. Nonetheless, recapture data suggest that male antibody titres subtly track seasonal peaks and troughs in body mass, increasing during the fruit-abundant wet season and declining during the dry season. Understanding seasonal trade-offs in bat immune investment will be critical to enhancing our capacity for predicting seasonal pulses in viral transmission and informing possible zoonotic risk. Paired field studies, tracking viral excretion in conjunction with individual serostatus, will be essential to elucidating these dynamics in the future.

One of the largest questions arising from our investigation addresses the extent to which seropositive status correlates with infectiousness and immunity. Previous work highlights notable seasonality in spillover of both Hendra (Plowright et al., 2015) and Ebola viruses (Schmidt et al., 2017), although the mechanistic contributions of bat demography versus physiology remain unclear. In our models, the force of infection varied seasonally as a result of birth pulse-mediated cycles in the infectious population (Supporting Information Figure S13). Seasonal fluctuations in the magnitude of transmission—which could emerge from changes in host contact rates (Ferrari et al., 2008; Grenfell, Bjornstad, & Finkenstadt, 2002), variation in within-host immunological susceptibility (Dowell, 2001) or periodicity in viral shedding (Plowright et al., 2015)—might further modulate seasonality in FOI. Several studies have highlighted the possible role that latent infections and viral recrudescence could play in bat virus transmission (Plowright et al., 2016; Rahman et al., 2011), but data from longitudinally resampled individuals were too few to allow for evaluation of any such model in our study. If future field work is able to demonstrate a role for seasonal transmission independent of demography, then the extent to which observed seasonality in serotitre could serve as a biomarker for an individual bat's infectiousness or susceptibility will be critical to resolving predictive power from cross-sectional serological data.

We fit age-structured, epidemic models to age–seroprevalence data and recovered strong support for models incorporating waning humoral immunity (i.e. MSIRS, MSIRN). This result is consistent with previous experimental findings, which demonstrate rapidly declining antibody titres in Marburg-infected *R. aegyptiacus*, after inoculation and seroconversion (Paweska et al., 2015; Schuh, Amman, Jones, et al., 2017; Schuh, Amman, Sealy, et al., 2017). In our system, we can eliminate the hypothesis by which simple SIR dynamics incorporating a seasonal birth pulse might drive seasonality in viral shedding (Plowright et al., 2016); such a model yields a pattern of

monotonically increasing seroprevalence with age at odds with our data. In our analysis, the MSIRN model consistently outperformed all other tested models in fits to the data, when constructed such that N-class dams produced M-class offspring. We hypothesize that N-class serotitres could be dynamic: N-class females may exhibit seasonally elevated seropositive titres during reproduction (consistent with findings from Aim 2), then subsequently reduce titres to seronegative levels post-gestation.

In a few cases in our analysis, the late-age seroprevalence plateau predicted by the MSIRS model was statistically indistinguishable from the decline predicted by MSIRN, likely due to low sample sizes among older age individuals. More extensive sampling will be needed to parse whether the seroprevalence decline witnessed in our dataset holds. Recent modelling of age–seroprevalence trends for NiV-G in *E. helvum* suggests that in the African system at least, seroprevalence plateaus at older ages, consistent with MSIRS dynamics (Peel et al., 2018), though it is possible that late-age susceptibles in this system were captured during low titre periods in seasonally dynamic N-class individuals. With our present data, we are unable to adequately distinguish between MSIRS and MSIRN hypotheses—and unable to assess the plausibility of an MSILI hypothesis—but both the experimental literature and our findings under Aim 2 suggest that the dynamics of humoral immunity post-initial seroconversion are likely more complex than a complete return to the susceptible class would assume. Field and laboratory studies tracking viral pathogenesis in individual bats are greatly needed to enable construction of accurate within-host models and to reduce our reliance on difficult-to-obtain wildlife age data (Borremans, Hens, Beutels, Leirs, & Reijniers, 2016; Pepin et al., 2017). More nuanced within-host models might, for example, incorporate multiple classes for seropositive bats, differentiated by MFI value: R-class individuals could have extremely high MFIs, while N-class individuals might have titres at some intermediate level. At present, there is a trade-off in selecting an MFI cut-off conservative enough to limit potential for false positives and lenient enough not to miss true seropositives with dynamic titres. If, in the future, chiropteran immunologists successfully develop assays capable of distinguishing N-class bats (e.g. some marker of cell-mediated immunity), construction of age–N-prevalence curves would be illuminating. We would expect MSIRN dynamics to yield patterns of monotonically increasing N-prevalence with age, while MSIRS and MSIRNR assumptions would yield age–N-prevalence plateaus.

Finally, we note that late-age declines in seroprevalence can be recaptured under assumptions of infection-induced mortality (*e.g.* Williams, Gouws, Wilkinson, & Karim, 2001). Preliminary experimentation with such model forms indicated that they were unable to more effectively recapture our data than those simpler model constructions investigated here, making this added complexity statistically unjustifiable. To date, no study has yet demonstrated any clinical signature of infection-induced morbidity or mortality in bats naturally or experimentally infected with henipa- or filoviruses (Amman et al., 2012; Jones et al., 2015; Paweska et al., 2012; Schuh, Amman, Jones, et al., 2017; Schuh, Amman, Sealy, et al., 2017; Williamson, Hooper, Selleck, Westbury, & Slocombe, 2000; Williamson et al., 1998). Although we chose not to explore models of this form at this time, we caution that we should remain cognizant of these possible mechanisms in the future.

Although no known bat-borne zoonoses have been documented in Madagascar, our work confirms a history of exposure to potentially zoonotic henipaviruses and filoviruses in several widespread, endemic fruit bat species. These species are widely consumed throughout Madagascar, and the majority of bat hunting—and corresponding bat–human contact—is concentrated during the resource-poor winter, overlapping with bat gestation and elevated anti-viral seroprevalence in our data (Golden et al., 2014; Jenkins & Racey, 2008; Jenkins et al., 2011). If seasonal changes in serostatus are revealed to have any bearing on viral transmission, insights from our modelling will offer a predictive framework to safeguard public health.

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AUTHORS' CONTRIBUTIONS

C.E.B., J.-M.H., C.J.M. and A.P.D. conceived the ideas and designed methodology. C.E.B. and H.C.R. collected the field data. C.C.B., A.A.C. and J.L.N.W. designed the Luminex serological platform. L.G. carried out the serological assays. C.E.B. analysed all data with input from A.J.P., C.J.M. and A.P.D. C.C.B., A.A.C., J.-M.H. and J.L.N.W. contributed materials and reagents. C.E.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Raw data used in all analyses described in this manuscript are available for public access in the following Dryad Digital Repository: https://doi.org/10.5061/dryad.61tc3hd (Brook, Ranaivoson, Broder, et al., 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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