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Using serosurveys to optimize surveillance for zoonotic pathogens
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Abstract:	Zoonotic pathogens pose a significant risk to human health, with spillover into human populations contributing to chronic disease, sporadic epidemics, and occasional pandemics. Despite the widely recognized burden of zoonotic spillover, our ability to identify which animal populations serve as primary reservoirs for these pathogens remains incomplete. This challenge is compounded when prevalence reaches detectable levels only at specific times of year. In these cases, statistical models designed to predict the timing of peak prevalence could guide field sampling for active infections. Here we develop a general model that leverages routinely collected serosurveillance data to optimize sampling for elusive pathogens. Using simulated data sets we show that our methodology reliably identifies times when pathogen prevalence is expected to peak. We then apply our method to two putative Ebolavirus reservoirs, straw-colored fruit bats (<i>Eidolon helvum</i>) and hammer-headed bats (<i>Hypsignathus monstrosus</i>) to predict when these species should be sampled to maximize the probability of detecting active infections. In addition to guiding future sampling of these species, our method yields predictions for the times of year that are most likely to produce future spillover events. The generality and simplicity of our methodology make it broadly applicable to a wide range of putative reservoir species where seasonal patterns of birth lead to predictable, but potentially short-lived, pulses of pathogen prevalence.
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<p>Data Availability</p> <p>Provide a Data Availability Statement in</p>	<p>All data used in this study was previously published and can be found online at at https://doi.org/10.5281/zenodo.8193102 from Pleydellet al. 2023. The data, R code and Mathematica Notebook used in this study can be found online at https://github.com/erinclancey/STOPPP-Model.</p>

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March 20, 2024

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Dear Editor,

We would like to submit our article, *Using serosurveys to optimize surveillance for zoonotic pathogens* for potential publication in *PLOS Neglected Tropical Diseases*. We believe our work, which predicts the timing of peak pathogen prevalence in reservoir populations with seasonal birth cycles, provides a valuable tool for confirming reservoir species and predicting spillover, and likely to be of broad interest to your readership. Specifically, we develop a general methodology that leverages routinely collected serosurveillance data to optimize sampling for elusive pathogens. We have tested the accuracy and utility of our methodology using simulated data and then applied it to systems with real-world importance, Ebola virus (EBOV; *Zaire ebolavirus*) in straw-colored fruit bats (*E. helvum*) and hammer-headed bats (*H. monstrosus*). Our method is simple enough for wide-reaching application to many field studies and pathogens, and therefore it is broadly applicable to a wide range of putative reservoir species where seasonal patterns of birth lead to predictable, but short-lived, pulses of pathogen prevalence.

We confirm that this work is original and has not been published, nor is it currently under consideration for publication elsewhere. We have no conflicts of interest to disclose. Please address all correspondence concerning this manuscript to me at erin.clancey@wsu.edu. Thank you for again considering our manuscript.

Sincerely,

Erin Clancey on behalf of all co-authors

Using serosurveys to optimize surveillance for zoonotic pathogens

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1 ABSTRACT

2 **Zoonotic pathogens pose a significant risk to human health, with spillover into human populations contributing to chronic**
3 **disease, sporadic epidemics, and occasional pandemics. Despite the widely recognized burden of zoonotic spillover, our**
4 **ability to identify which animal populations serve as primary reservoirs for these pathogens remains incomplete. This**
5 **challenge is compounded when prevalence reaches detectable levels only at specific times of year. In these cases, sta-**
6 **tistical models designed to predict the timing of peak prevalence could guide field sampling for active infections. Here**
7 **we develop a general model that leverages routinely collected serosurveillance data to optimize sampling for elusive**
8 **pathogens. Using simulated data sets we show that our methodology reliably identifies times when pathogen preva-**
9 **lence is expected to peak. We then apply our method to two putative *Ebolavirus* reservoirs, straw-colored fruit bats (*Ei-***
10 ***dolon helvum*) and hammer-headed bats (*Hypsipnathus monstrosus*) to predict when these species should be sampled**
11 **to maximize the probability of detecting active infections. In addition to guiding future sampling of these species, our**
12 **method yields predictions for the times of year that are most likely to produce future spillover events. The generality**
13 **and simplicity of our methodology make it broadly applicable to a wide range of putative reservoir species where sea-**
14 **sonal patterns of birth lead to predictable, but potentially short-lived, pulses of pathogen prevalence.**

15 AUTHOR SUMMARY

16 **Many deadly pathogens, such as Ebola, Lassa, and Nipah viruses, originate in wildlife and jump to human populations.**
17 **When this occurs, human health is at risk. At the extreme, this can lead to pandemics such as the West African Ebola**
18 **epidemic and the COVID-19 pandemic. Despite the widely recognized risk wildlife pathogens pose to humans, identi-**
19 **fying host species that serve as primary reservoirs for many pathogens remains challenging. Ebola is a notable exam-**
20 **ple of a pathogen with an unconfirmed wildlife reservoir. A key obstacle to confirming reservoir hosts is sampling ani-**
21 **mals with active infections. Often, disease prevalence fluctuates seasonally in wildlife populations and only reaches de-**
22 **etectable levels at certain times of year. In these cases, statistical models designed to predict the timing of peak preva-**
23 **lence could guide efficient field sampling for active infections. Therefore, we have developed a general model that uses**
24 **serological data to predict times of year when pathogen prevalence is likely to peak. We demonstrate with simulated**
25 **data that our method produces reliable predictions, and then apply our method to two hypothesized reservoirs for Ebola**
26 **virus, straw-colored fruit bats and hammer-headed bats. Our method can be broadly applied to a range of potential reser-**
27 **voir species where seasonal patterns of birth can lead to predictable pulses of peak pathogen prevalence. Overall, our**
28 **method can guide future sampling of reservoir populations and can also be used to make predictions for times of year**
29 **that future outbreaks in human populations are most likely to occur.**

30 INTRODUCTION

31 Spillover of zoonotic pathogens is a pervasive challenge [1], imposing a persistent burden on human health and creating con-
32 ditions ripe for the emergence of novel infectious disease [2]. One avenue to controlling the health impacts of spillover is to in-
33 crease surveillance within the human population, treating disease as it occurs and using public health measures to keep initial
34 events from expanding into epidemics or pandemics [3–5]. However, when surveillance and intervention systems fail, the results
35 can be catastrophic (e.g., West African Ebola epidemic; COVID-19 pandemic).

36 An alternative approach to managing the risk of spillover is preemptive, and focuses on stopping spillover before it occurs. For
37 instance, the risk of spillover could be managed by altering habitat availability for reservoir species [6, 7], changing human be-
38 havior to reduce contact with hosts [8, 9], or vaccinating reservoir species [10, 11]. For these preemptive approaches to work,
39 we must know which animal species serve as important reservoirs for a pathogen of interest. Recent progress in this direction
40 has been made by capitalizing on advances in machine learning that allow models to learn which suites of traits are associated
41 with suitability as a reservoir [2, 12]. For instance, Schmidt et al. [13] used boosted regression trees to predict which species are
42 most likely to serve as reservoirs for ebola viruses. Similar efforts have been used to predict reservoirs of SARS-CoV-2 [14], or-
43 thopoxviruses [15], betacoronaviruses [12], Nipah virus [16], and filoviruses outside of equatorial Africa [17]. Thus, we now have
44 tools in place to generate hypotheses for which species are likely to be reservoirs of any particular pathogen species.

45 Even with hypotheses for which species are likely to serve as a reservoirs in hand, testing and confirming that any individual
46 species serves as an important reservoir remains a significant challenge [12, 18–20]. Beyond the obvious complexities and lo-
47 gistical challenges associated with sampling wild animals in remote locations, verifying that an animal is a reservoir requires
48 capturing an animal with a detectable active infection [1]. Prevalence of some zoonotic pathogens is sufficiently high that screen-
49 ing reservoir animals for active shedding is straightforward (e.g., Lassa virus in *Mastomys natalensis* [21]), but more often it is ex-
50 tremely challenging for pathogens that generate short-lived acute infections concentrated at only certain times of the year [see
51 22–26]. In these cases, achieving even a modest chance of capturing an animal with a detectable active infection requires in-
52 tensive and temporally focused sampling during periods of peak prevalence [18]. To address different aspects of this problem,
53 several Bayesian approaches have been developed using serosurveillance data to predict incidence and prevalence in reservoir
54 populations. For example, Borremans et al. [27] used information about multiple antibodies over time, pathogen presence, and
55 demographic information to back-calculate the time since infection for individuals to estimate incidence of Morogoro virus in-
56 fection in multimammate mice (*M. natalensis*). Using a different approach, Pleydell et al. [28] fit an age-structured epidemi-
57 logical model specific to Ebola virus in straw-colored fruit bats (*Eidolon helvum*) to estimate the timing of peak prevalence in
58 the adult population. Although these methods are robust, adapting them quickly to other systems would be laborious and not
59 always feasible depending on data availability. Thus, a flexible method more easily tailored to different species that requires min-
60 imal data would aid empiricists developing surveillance sampling designs to target zoonotic pathogens.

61 Here we develop a general methodology that can be used to focus reservoir surveillance on periods of time that are most likely
62 to coincide with peak prevalence of a zoonotic pathogen (most often viral pathogens). Our method requires routine serosurveil-
63 lance data, knowledge of the rate at which detectable antibodies wane, and the rate at which individuals recover from infec-
64 tion. We test the accuracy and utility of our methodology using simulated data and then apply it to systems with real-world im-
65 portance, Ebola virus (EBOV; *Zaire ebolavirus*) in straw-colored fruit bats (*Eidolon helvum*) and hammer-headed bats (*Hypsig-
66 nathus monstrosus*) using previously published data. We believe this method is simple enough for wide-reaching application to
67 many field studies. Therefore our method provides a useful tool to guide the planning of field sampling and to study epidemi-
68 logical dynamics in reservoir populations when data on active infections are rare or absent.

69 METHODS

70 Mathematical foundation

71 Our approach to optimizing surveillance for zoonotic pathogens from serological surveillance data builds from a mathematical
72 model describing the ecology of the reservoir animal and the epidemiology of the pathogen. We illustrate our approach using
73 a model of a reservoir animal that reproduces seasonally and experiences both density independent and density dependent
74 mortality. We assume the pathogen can be adequately described by a modified SIR framework that takes into account both
75 short-term antibody mediated immunity and long-term immunity mediated by a T-cell response. This distinction is important
76 because we assume only the short-term antibody based response is detected by serology [29]. If we further assume individuals
77 encounter one another at random, the ecology and epidemiology of the system can be described using the following system of

78 differential equations:

$$\dot{S} = b(t)N - \beta SI - S(\mu + kN) + \omega_T R_T \quad (1a)$$

$$\dot{I} = \beta SI - \gamma I - I(\mu + kN) \quad (1b)$$

$$\dot{R}_A = \gamma I - R_A(\mu + kN + \omega_A) \quad (1c)$$

$$\dot{R}_T = \omega_A R_A - R_T(\mu + kN + \omega_T), \quad (1d)$$

79 where S is the number of susceptible individuals, I is the number of pathogen infected individuals, R_A is the number of individuals with antibodies detectable through serology, R_T is the number of individuals that are immune to pathogen but lack detectable antibodies, and $N = S + I + R_A + R_T$ is the total population size of the reservoir. All model parameters and their
80 biological interpretations are described in table (1).

Table 1: Model parameters and their biological interpretations. All rates are in days unless specified otherwise.

Parameter	Biological interpretation
$b(t)$	Seasonally fluctuating birth rate
μ	Density independent death rate
k	Density dependent death rate
β	Transmission rate
γ	Rate of recovery from infection
ω_A	Rate at which antibodies decay
ω_T	Rate at which T-cell immunity decays

81 If data on the abundance of each class are available, we could proceed directly from model (1). Unfortunately, this will not generally be the case, and data will more frequently come from serological testing of a random sample of n reservoir animals at various points in time. To calculate the probability that x animals will be seropositive within each sample of size n requires that we
82 make a change of variables (supplemental material, appendix 1) to express model (1) in terms of proportions:

$$\dot{s} = b(t) - s(b(t) - \iota\beta N) + \omega_T r_T \quad (2a)$$

$$\dot{i} = \iota(s\beta N - b(t) - \gamma) \quad (2b)$$

$$\dot{r}_A = \gamma\iota - r_A(\omega_A + b(t)) \quad (2c)$$

$$\dot{r}_T = r_A\omega_A - r_T(\omega_T + b(t)), \quad (2d)$$

83 where s , ι , r_A , and r_T are the proportion of reservoir animals in each class and N is the total population size of the reservoir animal.
84 With the model now written in terms of proportions, we can proceed to solve for the proportion of animals in the actively
85 infectious class, ι , as a function of the proportion of animals that carry antibodies, $r_A(t)$, using equation (2c):

$$\dot{\iota}(t) = \frac{\dot{r}_A(t) + r_A(t)(\omega_A + b(t))}{\gamma}, \quad (3)$$

90 where $\dot{\iota}(t)$ is the predicted proportion of the population that is actively infected at time t .

91 Equation (3) demonstrates that we can predict the proportion of the population that is actively infected at any point in time if

92 we can estimate four quantities: 1) the rate at which antibodies are produced following infection, γ ; 2) the rate at which antibod-
 93 ies wane over time, ω_A ; 3) a function describing the reservoir birth rate over time, $b(t)$; and 4) a function describing seropreva-
 94 lence over time, $r_A(t)$. We assume that the temporally constant parameters γ and ω_A are known or can be estimated using ex-
 95 perimental infections in the lab. In contrast, the seasonal pattern of birth $b(t)$ will generally not be known and may need to be
 96 estimated in some cases (supplemental material, appendix 2). If, however, animals live much longer than the lifespan of anti-
 97 bodies such that $b(t) \ll \omega_A$, birth can be safely ignored to a good approximation (figure S1). Finally, we assume that the sea-
 98 sonal pattern of seroprevalence, $r_A(t)$, will generally be unknown and will need to be estimated from serosurveys. In the next
 99 section, we outline how this can be accomplished using routinely collected serological data. All mathematical analyses were
 100 performed in Wolfram Mathematica 13.1 [30].

101 **Fitting the mathematical model to data**

102 Estimating a function that describes seasonal patterns of seroprevalence, $\hat{r}_A(t)$, is central to our approach and leverages data
 103 that is routinely collected across a wide range of systems. In general, we assume a sample of reservoir animals is captured at
 104 multiple times each year and tested for the presence of antibodies for a target pathogen to give the number of seropositive an-
 105 imals in a sample. Thus, data will consist of a sampling date (t), a sample size (n), and the number of animals within the sample
 106 that are seropositive (x). We take two approaches to fitting $\hat{r}_A(t)$, with the best approach largely dependent on the temporal res-
 107 olution of the data.

108 **Interpolation of temporally rich seroprevalence data**

109 If high-resolution seroprevalence data (e.g., weekly or monthly sampling) are available for a potential reservoir species, inter-
 110 polation provides an efficient method for fitting the function $r_A(t)$ to the data. We illustrate this approach by applying a kernel
 111 smoother to estimate the function, $\hat{r}_A(t)$. Specifically, we use the NadarayaWatson kernel regression estimate available in R [31]
 112 with a normal density as the smoothing kernel and a bandwidth of 90. The bandwidth must be increased in cases when data is
 113 sparse, thus we used the lowest value possible that accommodated all of our simulations (see simulated surveillance data
 114 section below). We then calculate the derivative of the interpolated function, $\dot{r}_A(t)$, by differencing the fitted values for $\hat{r}_A(t)$ per
 115 unit time (e.g. days, weeks, months etc.). As long as parameters γ and ω_A have been estimated independently, and $b(t)$ is neg-
 116 ligible (or estimated), this provides the information required for the frequency of infected individuals over time, $\dot{i}(t)$ to be pre-
 117 dicted using equation (3). Although computationally efficient and conceptually straightforward, we anticipate that this method
 118 will not perform well when data are sparse or highly clustered (i.e., when sampling effort is concentrated at specific times of year).

119 **Model fitting for sparse seroprevalence data**

120 In cases when sampling is sporadic and seroprevalence data are sparse, interpolation may not be feasible and an approach based
 121 on model-fitting may perform better. This approach uses an understanding of system specific biology to define a mathematical
 122 function describing how seroprevalence is expected to change over time. The limited seroprevalence data is then used to esti-
 123 mate the parameters that fine-tune the function $\hat{r}_A(t)$ (e.g., the timing of peaks). Here, we illustrate this approach for systems
 124 where seasonal birth pulses are thought to cause fluctuations in the prevalence of infection and concomitant fluctuations in
 125 seroprevalence.

126 In systems where seasonal birth pulses occur, we expect, in general, a subsequent increase in infected individuals followed by
 127 a downstream increase in individuals that have seroconverted. Qualitatively, this expectation can be modeled using a modified
 128 periodic Gaussian function e.g.,[32]:

$$r_A^*(t) = C_2 - C_1 \cdot e^{-a \cos^2(\pi f t - \phi)}. \quad (4)$$

129 Here $r_A^*(t)$ is a function specifying the predicted proportion of seropositive animals at time t , C_2 adjusts the average value of

130 seroprevalence over time, C_1 sets the amplitude of seasonal fluctuations in seroprevalence, a controls the shape of seasonal fluctuations, ϕ defines the phase shift, and f specifies the frequency. We assume f is determined by the natural history of the reservoir species and is known. For example, a reservoir species that reproduces either once or twice per year in a regular pattern would have values of $f = 1/365$ and $f = 2/365$, respectively, if the time units are given in days. In contrast, we expect C_1, C_2, a , and ϕ to be unknown and require estimation.

135 We used Bayesian inference to estimate the unknown parameters in equation (4) and estimate the uncertainty in our estimates
136 for i_{peak} using 95% credible intervals (CI). Specifically, the likelihood of observing a temporal sequence of seroprevalence values
137 is:

$$\mathcal{L}(\theta) = \prod_{i=1}^{\tau} \binom{n_i}{x_i} r_A^*(t_i)^{x_i} (1 - r_A^*(t_i))^{n_i - x_i}, \quad (5)$$

138 where the product is carried over τ total sampling time points and $\theta = \{C_1, C_2, a, \phi\}'$. For each time point i , n_i defines the number
139 of animals sampled at time point i , x_i defines the number of sampled animals found to be seropositive at time point i , and
140 t_i defines the time at which sample i was collected. Prior distributions for model parameters and details of the Bayesian estimation
141 procedure are given in supplemental material, appendix 3. Bayesian estimation was performed using rstan [33].

142 Simulating surveillance data

143 To determine if our methods accurately predict the true peak prevalence of infection, i_{peak} , we applied each method to simulated
144 data sets. Specifically, we simulated a pathogen circulating in a wild animal population using model (1) with semi-annual
145 birth pulses using equation (S6). In general, this leads to two peaks in prevalence and seroprevalence each year, a pattern observed
146 in many bat species [e.g., 34–36]. Simulations focused on three different scenarios: low, medium, or high amplitude cycles in seroprevalence,
147 $r_A(t)$, and prevalence, $\iota(t)$, with the specific parameter values used provided in table (S2). We generated
148 100 replicate stochastic simulations for each scenario using the Gillespie algorithm with a tau leaping approximation [37]. Simulations
149 were initiated at the endemic disease equilibrium (supplemental material, appendix 1) and run for 10 years. We used
150 the last 394 days for analyses to include peaks occurring at the end of year 9 to beginning of year 10, and all days in year 10.
151 The two predicted peaks within the final 394 days, i_{peak} , were determined for each simulated data set by finding the time point
152 associated with the maximum value between days [0,170] and the time point associated with the maximum value between
153 days [170,360].

154 We applied our methodology to the simulated data for a range of possible field sampling designs. First, we analyzed the simulated
155 data sets assuming field sampling was performed at evenly spaced time intervals (daily, weekly, bi-weekly, monthly, bi-monthly)
156 over the 394 day study period. Second, we analyzed the simulated data sets assuming the number of sampling days
157 was fixed at 42 days, but the distribution of these days over the year differed (evenly spaced days, random days, 3-day clusters,
158 7-day clusters). Each of the nine sampling designs was applied to the low, medium, and high amplitude seroprevalence cycle
159 scenarios to yield 27 different combinations of epidemiological dynamics and sampling schemes (table S3). For each day of
160 sampling, we assumed $n = 20$ animals were captured at random and tested for antibodies to the focal pathogen to yield an
161 estimate for seroprevalence.

162 To evaluate the performance of our method, we compared the probability of detecting an actively infected animal (e.g., through
163 PCR, culture, or sequencing) when sampling was timed using our method with two benchmarks: 1) the best case scenario where
164 sampling was performed at the true peak and 2) the null solution where sampling was performed on a random day. In each
165 case, a sample of 20 animals was drawn at random and the number that were actively infected was recorded. Sampling was
166 repeated ten times for each case and the probability of detecting an actively infected animal calculated as the number of trials
167 in which at least one infected animal was found. Details on all simulations are given in supplemental material, appendix 4. All
168 simulations were performed in R [31].

169 **Study Populations and Surveillance Sampling**

170 African fruit bats are likely candidate reservoir hosts for Ebolaviruses evidenced by the presence of antibodies in many species
171 and viral RNA sequenced from several species, yet no replicating viral strain has been isolated from a wild bat population de-
172 spite extensive field sampling [see 22–25]. Many bat species have highly synchronous birth cycles [34] that can translate into
173 cycles of infection prevalence [32]. In addition, Ebolaviruses are cleared by their hosts and therefore viral shedding may only be
174 detected during a brief window [38]. Thus, predicting transmission cycles of Ebolaviruses in putative reservoir hosts would help
175 to optimize surveillance sampling and to understand spillover and the origins of Ebola virus disease in humans.

176 Two examples of frugivorous bat species with medium to high seroprevalence and the hypothesized potential to cause Ebolavirus
177 spillover events are straw-colored fruit bats (*Eidolon helvum*) and hammer-headed bats (*Hypsipathus monstrosus*) [38–40]. *E.*
178 *helvum* are common fruit bats that form large seasonal aggregations [38] and reproduce annually [41]. *H. monstrosus* form
179 large breeding aggregations [42], but unlike *E. helvum*, reproduce semi-annually [41]. Djomsi et al. [38] captured free-ranging
180 bats from a roosting site in Yaounde, Cameroon, and at a feeding site 40 km away near Obala, Cameroon. Samples were col-
181 lected at approximately monthly intervals between December 2018 and November 2019, with the largest inter-sampling in-
182 terval spanning two months. Whole blood samples and rectal and oral swabs preserved in RNA-later were collected from indi-
183 vidual bats. Bat species, *E. helvum* and *H. monstrosus*, were identified by molecular testing. Djomsi et al. [38] screened *E. helvum*
184 and *H. monstrosus* samples for antibodies to three *Ebolavirus* species using a Luminex-based serological assay previously adapted
185 for bats [see 38]. They also tested for active infections in *E. helvum* using a semi-nested PCR assay specific to Ebola virus (EBOV;
186 *Zaire ebolavirus*) targeting a 184 bp fragment on the VP35 gene [see 38]. For analyses in this study, we used the results from
187 the Res1GP.ZEBVkiss antigenic test, a test for on the glycoprotein of EBOV, following [28]. Specific details of all methods and
188 data are publicly available from [38] and [28].

189 To parameterize our models for *E. helvum* and *H. monstrosus*, we used values previously estimated values for the recovery rate
190 and rate of waning antibodies. Pleydell et al. [28] estimated the recovery rate ($\gamma = 1/1.5$ weeks) and rate of waning antibodies
191 ($\omega_A = 1/75$ weeks) for the *E. helvum* population in Cameroon, but did not estimate these value for *H. monstrosus*. For *H. mon-*
192 *strosus*, we used measurements from experimental studies in Egyptian fruit bats (*Rousettus aegyptiacus*) with Marburg virus
193 (MARV; *Marburg marburgvirus*) to approximate parameter values for the recovery rate ($\gamma = 1/1.43$ weeks) [43] and the rate of
194 waning antibodies ($\omega_A = 1/12.9$ weeks) [44].

195 **RESULTS**

196 **Optimizing surveillance on simulated data**

197 **Interpolation of temporally rich seroprevalence data**

198 We begin our analyses by testing our methods on simulated surveillance data. Figure 1 shows an example of the true popula-
199 tion curves for $r_A(t)$ and $i(t)$ and the estimated curves, $\hat{r}_A(t)$ and $\hat{i}(t)$, that were fitted to simulated serological data using inter-
200 polation. We find that we can successfully estimate $\hat{r}_A(t)$ and predict prevalence pulses in populations with different epidemi-
201 ological dynamics (e.g., low, medium, and high amplitude dynamics in figure 1) using interpolation. When surveillance sam-
202 pling occurs at sufficient frequency and at even intervals across time, interpolation provides a good approximation to the true
203 epidemiological dynamics such that surveillance sampling can be optimized to detect active infections (figures 2 and 3a). The
204 accuracy of the predictions for the timing of peak prevalence in the population from interpolation for all sampling schemes are
205 given in table (S4). When the data requirements are met, this method can also be used retrospectively to understand epidemi-
206 ological dynamics when episodic shedding occurs randomly, for example, not necessarily coinciding with seasonal birth pulses
207 (figure S7). However, interpolation methods do not accurately predict the peak timing if serology data is sparse or sampling is
208 highly clustered in time (see figures 2 and 3a). In these cases, model fitting is a better option.

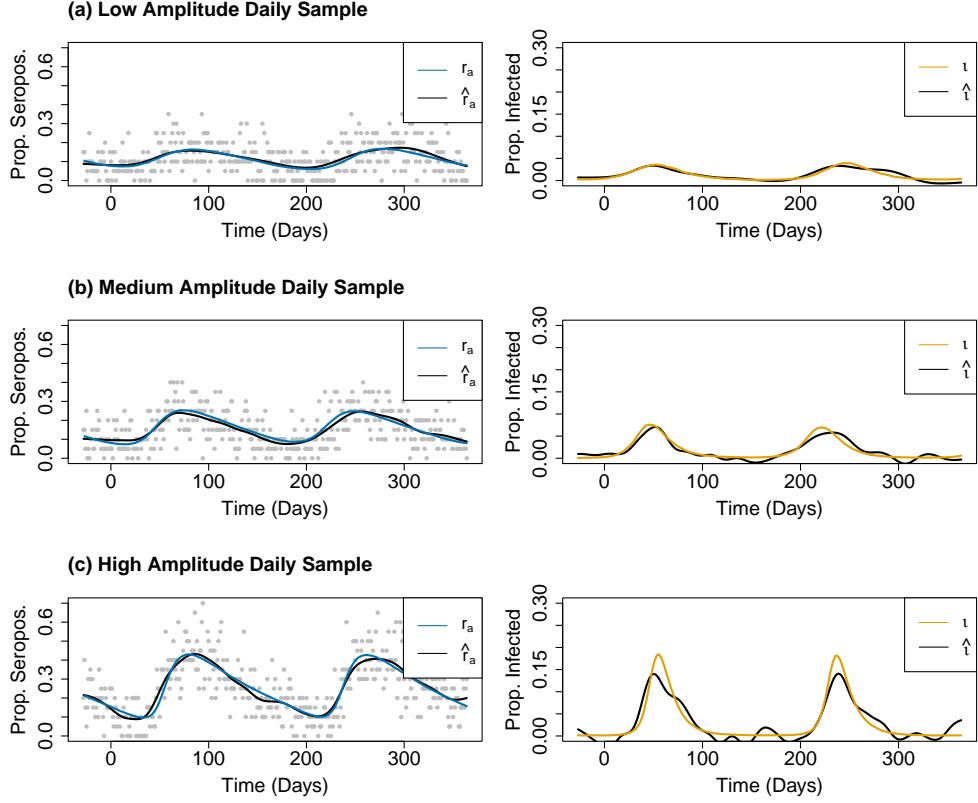


Figure 1: Results from interpolating serological data sampled daily for (a) low, (b) medium, and (c) high amplitude epidemic curves. Grey points represent the raw simulated data.

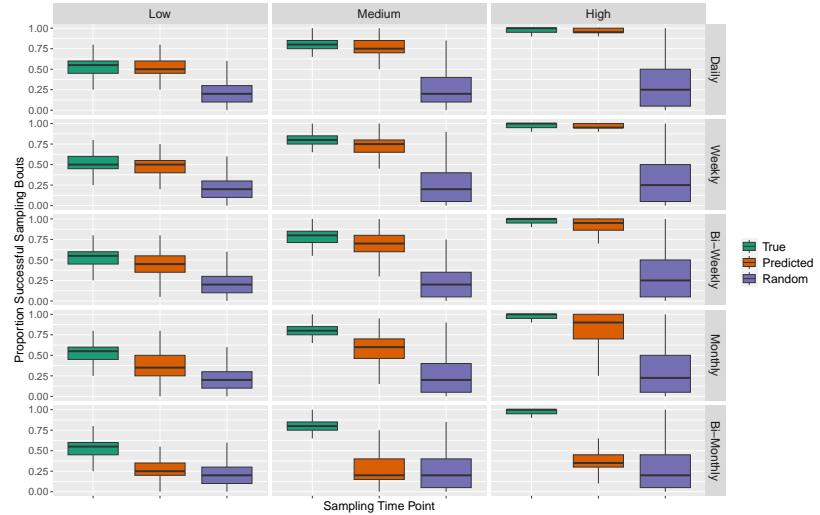


Figure 2: Proportion successful sampling bouts that occurred when the simulated population was sampled during the true peaks, i_{peak} , the interpolation prediction of peaks, \hat{i}_{peak} , and a random time point. The proportion of successful sampling bouts are shown for three different types of disease dynamics, where the amplitude of the cycles is low, medium, and high, and for five different sampling schemes, when sampling occurs daily, weekly, bi-weekly, monthly, and bi-monthly over 394 days.

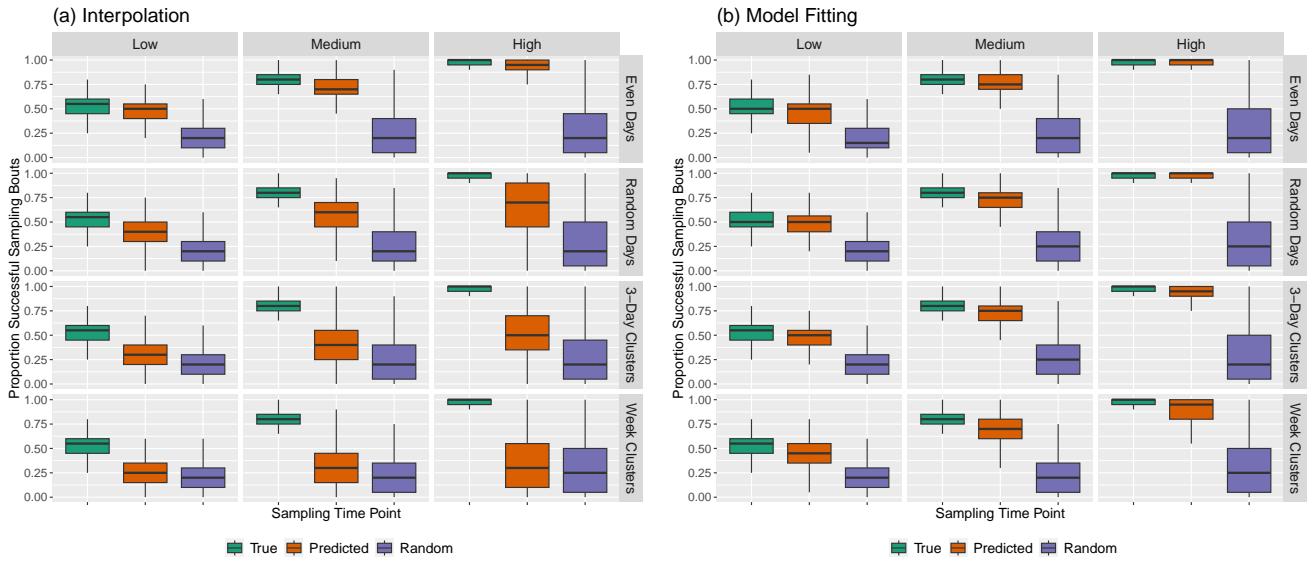


Figure 3: Proportion successful sampling bouts that occurred when the simulated population was sampled during the true peaks, t_{peak} , the predicted peaks, \hat{t}_{peak} , and a random time point when the predictions for $\hat{r}_A(t)$ were made by (a) interpolation or (b) model fitting. The proportion of successful sampling bouts are shown for three different types of disease dynamics, where the amplitude of the cycles is low, medium, and high, and for four different 42-day sampling schemes, when sampling occurs at even intervals, random days, 3-day clusters, and week clusters.

209 Model fitting for sparse seroprevalence data

210 Although estimating the function $\hat{r}_A(t)$ using model fitting is more computationally intensive than interpolation, our results
 211 show that this approach can accurately predict the timing of peak prevalence when interpolation would fail (figure 3a). Specif-
 212 ically, as seroprevalence data becomes less evenly distributed, we find that the model fitting approach continues to provide ac-
 213 curate guidance for sampling whereas the guidance provided by the interpolation approach degrades (figure 3b and 4). The
 214 accuracy of the predictions for the timing of peak viral shedding in the population from $r_A^*(t)$, the size of the 95% CI estimated
 215 via Bayesian inference, and the proportion of times the true population peak falls within the CIs are summarized in table (S5).

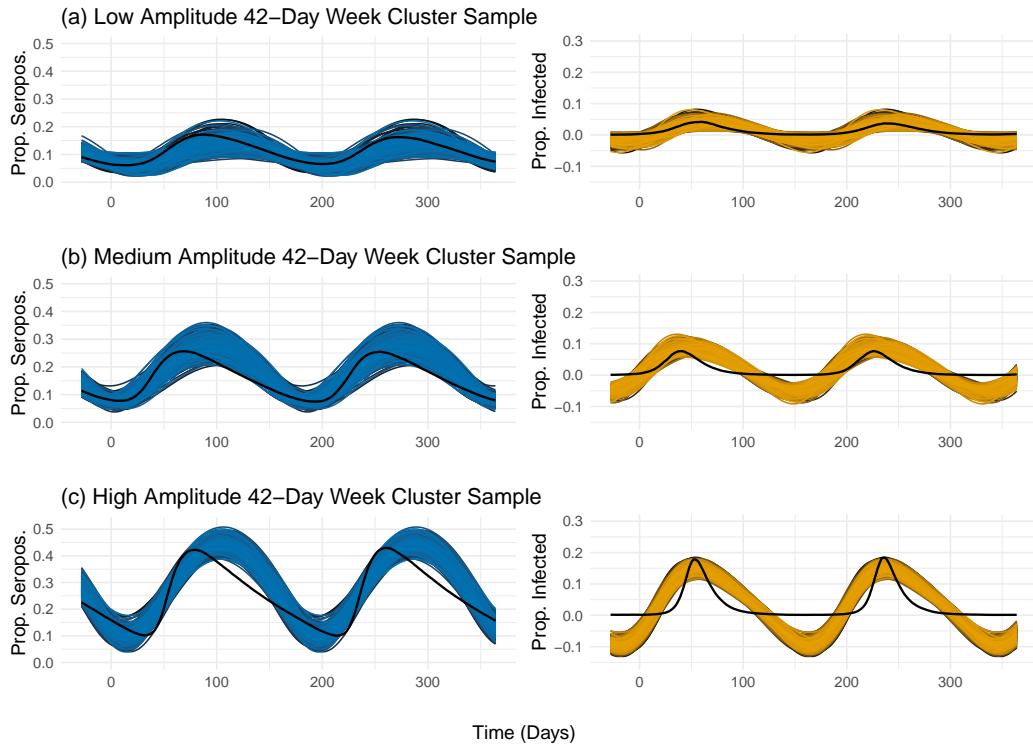


Figure 4: Results from model fitting with 42-day weekly clustered simulated serosurveillance data from (a) low, (b) medium, and (c) high amplitude epidemic curves. The blue and yellow lines represent the distribution of curves falling within the 95% CIs after Bayesian parameter estimation of $r_A^*(t)$ and predicting $i(t)$, respectively. The black lines represent the true population simulated dynamics.

216 Application to putative Ebolavirus reservoirs

217 In Cameroon, the fruit bat species *E. helvum* and *H. monstrosus* were shown to carry antibodies against EBOV but no active in-
 218 fections were detected in *E. helvum* [38]. Djomsi et al. [38] did not test the *H. monstrosus* population for active infections, how-
 219 ever, *H. monstrosus* is one of three bat species for which EBOV has been detected by real-time PCR and partially sequenced
 220 [45].

221 We used publicly available data from [28] to predict the peak period of active infection, \hat{i}_{peak} , using our methodology. This data
 222 set includes seroprevalence and the proportion of animals lactating for each species. First, we tested the assumption that *E.*
223 helvum reproduces annually and *H. monstrosus* reproduces semi-annually. Figures (S3) and (S5) demonstrate one annual birth
 224 pulse for *E. helvum* and two annual birth pulses for *H. monstrosus*, respectively. Next, we used results from serosurveys to pre-
 225 dict annual viral pulses, \hat{i}_{peaks} , in *E. helvum* by fitting the data to $r_A^*(t)$ for EBOV (figure 5). Figure (S4) shows the estimated sero-
 226 dynamics and predicted infection prevalence for this population. These results suggest this population has a high amplitude
 227 cycle relative to our simulated data, with an average amplitude of 0.56 and 95% CI equal to [0.51, 0.62], meaning that sampling
 228 this population at peak prevalence greatly optimizes sampling for active infections. The distribution of the timing of predicted
 229 peaks is given in figure (5) with the mode occurring at week 32 and 95% CI spanning weeks [31,33]. No samples contained in
 230 this dataset were collected during this predicted window of peak prevalence [28].

231 We used the same methodology to predict the period of peak prevalence for *H. monstrosus*. The estimated temporal patterns of
 232 seroprevalence and prevalence for this population are shown in figure (S6). These results suggest that this population has a low
 233 amplitude cycle, with an average amplitude of 0.053 and a 95% CI equal to [0.00, 0.14]. The estimates and 95% CIs for the two
 234 peaks are the first mode occurring on week 27 within the interval [20.00, 32.00] and the second mode occurring on week 1
 235 within the interval [46.00, 6.00] (weeks within a year are counted from 0 to 51) (figure 6). Djomsi et al. [38] collected sam-
 236 ples for this species during the predicted peak intervals, but the samples were not tested for active infections of EBOV.

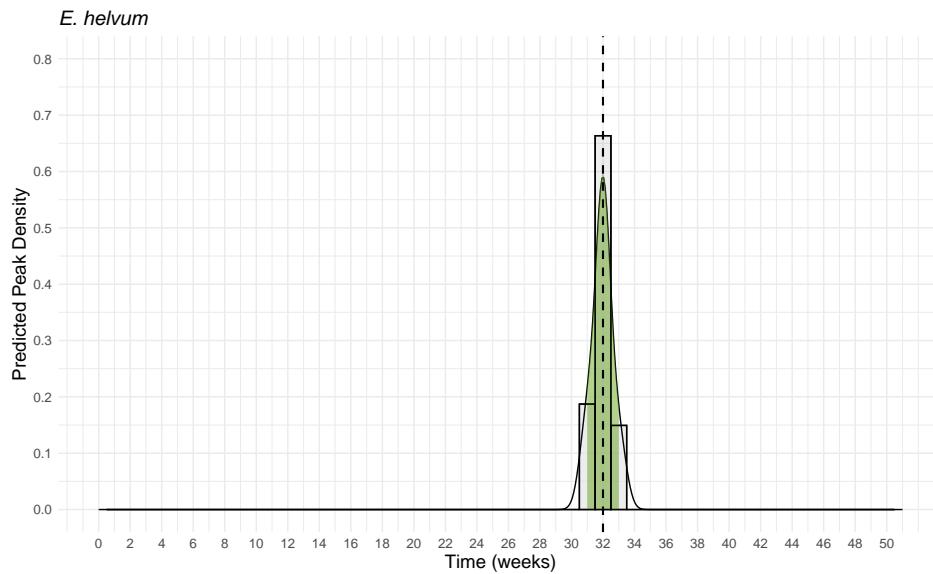


Figure 5: Distribution of weeks where the annual peak viral pulse was predicted for *E. helvum*. The vertical dashed line represents the mode and the green shaded area represents the 95% CI.

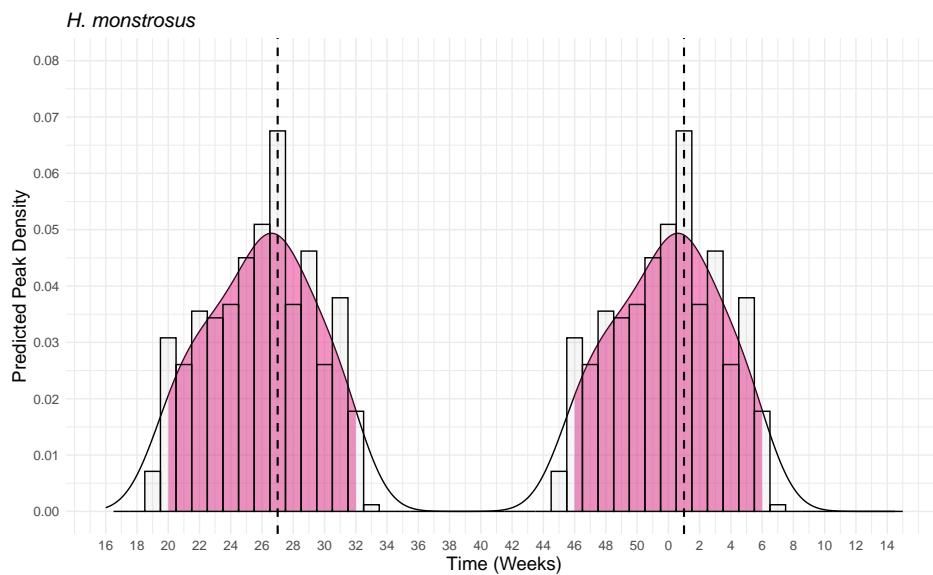


Figure 6: Distribution of weeks where the semi-annual peak viral pulses were predicted for *H. monstrosus*. The vertical dashed lines represent the modes and the pink shaded areas represent the 95% CIs for each pulse. Note the timescale on the x-axis begins at week 16 to accommodate the second peak that occurs at the end/beginning of each year.

237 DISCUSSION

238 We have developed a general methodology for predicting the timing of peak pathogen prevalence in seasonally fluctuating
 239 wildlife populations using temporally structured serological data. Our approach is motivated by the possibility that success-
 240 ful sampling of actively infected reservoir animals has been impeded by seasonal fluctuations in pathogen prevalence driven
 241 by seasonal birth cycles. By focusing the search for active infections on specific periods of time where infections are most likely
 242 to be discovered, our method may facilitate confirmation of long-suspected reservoir hosts. Thus, our method leverages rou-
 243 tinely collected serosurveillance data to extract information about the temporal pattern of active infection. When serosurveil-
 244 lance data is sufficiently rich for the temporal pattern of seroprevalence to be interpolated, our method is particularly straight-
 245 forward, computationally inexpensive, and accurate. Even when serosurveillance data is temporally sparse, our method can be

246 used to generate accurate predictions by first fitting a mathematical model to the serological data. This latter approach, how-
247 ever, is more computationally intensive and requires an additional assumption about the timing of the birth cycle.

248 Applying our methodology to two bat species in Cameroon, *E. helvum* and *H. monstrosus* [38], long hypothesized to harbor
249 EBOV, demonstrates its utility. Our results suggest the population of *E. helvum* has one prevalence peak occurring between
250 weeks 31-33 each year. The population of *H. monstrosus* has much wider semi-annual peak intervals spanning weeks 20 to 32
251 and 46 to 6 in the following year. If accurate, these interval estimates can be used to plan future surveillance surveys or predict
252 periods when a high proportion of infected animals may pose an increased threat of zoonotic spillover. Unfortunately, validating
253 our predictions will only be possible when animals with active infections have been captured from these populations. Nonethe-
254 less, evidence exists to support our interval estimates for each species. Our peak interval for *E. helvum* corresponds to the inter-
255 val (weeks 30-31) predicted by Pleydell et al. [28], who estimated an age-structured model to obtain the highest probability
256 predicted annual peak in the density of infectious adults. The semi-annual *H. monstrosus* intervals overlap the weeks in 2003
257 (week 5 and 22) in which Leroy et al. [45] captured bats PCR-positive for EBOV on the border between Gabon and the Republic
258 of the Congo.

259 Although the results from our simulated data are robust and empirical data are encouraging, limitations of our model may still
260 exist. First, the simulation testing assumed the mathematical model underlying our method accurately reflects the true biolog-
261 ical processes. If the assumptions of our relatively simple compartment model are violated in the wild, our testing may overes-
262 timate the performance of our method. For instance, the model we have studied here ignores age structure which may have
263 a significant impact on the relationship between seroprevalence and prevalence if sampling is not random with respect to age
264 class [e.g., 28, 34]. The method we present here also assumes seasonality is driven by fluctuations in birth rate rather than sea-
265 sonal changes in animal behavior that may influence contact rates and transmission [e.g., 42]. Even though we did not study
266 these alternative scenarios directly, instead choosing to focus on a simple but general scenario, it will often be possible to inte-
267 grate alternative biological assumptions by simply exchanging the underlying mechanistic model.

268 Next, a potential limitation specific to our model fitting method is that we assume the epidemiological cycles occur consistently
269 over time and the frequency can be specified using the number of birth pulses that occur annually for a particular species. In
270 reality, prevalence pulses can occur stochastically [e.g., 46, 47], annual patterns in some population include skip years [e.g., 28,
271 48] or episodic shedding can be hard to distinguish from transient epidemics [49]. If epidemiological cycles cannot be approx-
272 imated by a regular pattern, our model fitting method would not be appropriate. Our method also requires the rate of wan-
273 ing antibodies to either be known or estimated independently. Thus, our predicted peak intervals from model fitting are con-
274 ditioned on specific values for rate of waning antibodies. If including uncertainty for these estimates is desired, our likelihood
275 framework used in model fitting would easily accommodate a distribution for the rate of waning antibodies.

276 Last, our general method requires binary data describing whether an animal is seropositive or seronegative. Serological data
277 is prone to cross-reactivity [50] resulting in low specificity and variable sensitivity dependent on the immune dynamics of the
278 target species and pathogen, secondary antibody selection [51], and method of pathogen inactivation [52]. We assume reliable
279 thresholds will be used to determine seropositivity, but we do not provide a method to include the uncertainty from serological
280 data in our model.

281 Even in the face of these challenges, pathogen surveillance in wild animal populations is essential for identifying reservoir species,
282 collecting pathogen samples for genetic characterization, and predicting when spillover is most likely to occur. By leveraging
283 routinely collected serosurveys to optimize pathogen surveillance, the methodology we develop here has the potential to re-
284 duce the cost and labor associated with pathogen surveillance and increase our ability to successfully sample pathogens that
285 reach appreciable prevalence at only specific times of year. More broadly, this methodology can be used to identify times of year
286 when pathogen prevalence should peak, providing guidance for interventions aimed at reducing spillover risk.

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DATA AVAILABILITY

All data used in this study was previously published and can be found online at <https://doi.org/10.5281/zenodo.8193102> from [28]. The data, R code and Mathematica Notebook used in this study can be found online at <https://github.com/erinclancey/STOPPP-Model>.

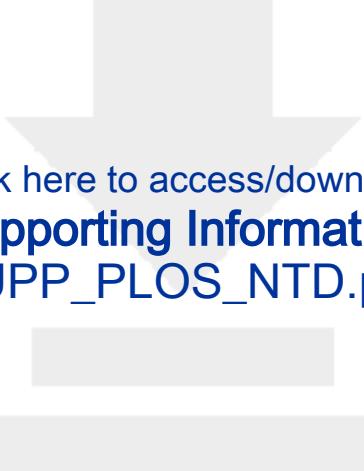
AUTHOR COMPETING INTERESTS

All authors declare we have no competing interests.

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