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Sampling to elucidate the dynamics of infections in reservoir hosts

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The risk of zoonotic spillover from reservoir hosts, such as wildlife or domestic livestock, to people is shaped by the spatial and temporal distribution of infection in reservoir populations. Quantifying these distributions is a key challenge in epidemiology and disease ecology that requires researchers to make trade-offs between the extent and intensity of spatial versus temporal sampling. We discuss sampling methods that strengthen the reliability and validity of inferences about the dynamics of zoonotic pathogens in wildlife hosts.

This article is part of the theme issue 'Dynamic and integrative approaches to understanding pathogen spillover'.

1. Introduction

At any point in space and time, the risk of pathogen spillover from reservoir hosts to humans, or to other animals, is a function of the intensity of infection within reservoir host populations [1]. Spillover risk is then shaped by a series of processes including the release of infectious particles from reservoir hosts, response of pathogen survival to the environment, behaviours that affect exposure of recipient hosts, and biologically driven susceptibility of recipient hosts [1]. Here, we focus on the dynamics of infection in wildlife reservoirs that determine how pathogen intensity is distributed in space and time.

Quantification of these dynamics is necessary to predict and manage zoonotic transmission [2]. Emerging zoonoses often appear suddenly in novel recipient hosts, and sampling of reservoir hosts (hosts in which the pathogen is maintained [3]) is initiated before the dynamics of the zoonosis are fully characterized. Therefore, sampling often is opportunistic or haphazard and is guided by sparse information on, for example, the observed patterns of spillover and the natural history of the reservoir host. Moreover, information on the pathogen in reservoir hosts, which often tolerate infection with no apparent clinical symptoms or pathology [4], is difficult to obtain. Catching, restraining and sampling hosts, or testing excreta such as urine or faeces, is usually required [5,6], and can be logistically intensive, expensive and hazardous. Therefore, we address how sampling can be designed to minimize these challenges while maximizing information gain.

Design of any sampling strategy requires clear specification of objectives (table 1). The fundamental objective of sampling to inform management of spillover is to identify times and places at which the risk of spillover is elevated. If the reservoir hosts of an emerging pathogen are not well characterized, a wide variety of potential hosts must be sampled to determine whether and the extent to which they can be infected by the pathogen [7]. In other situations, the reservoir hosts are known and are subject to pathogen incursions from other locations (e.g. [8]). Here, we review sampling strategies for situations in which one or more potential reservoir species have been identified, and the pathogen may be endemic. We

Table 1. Illustrative goals and objectives motivating studies of pathogens in reservoir hosts.

goal	objectives	data needed	sampling approaches
identify distribution of reservoir hosts and how those hosts and populations are connected through space and time	estimate the spatial distribution and density of reservoir hosts estimate the spatial and temporal extents of connections between reservoir host units	spatially explicit presence and absence of marked or unmarked animals movements of known and instrumented individuals population-level genetics on individuals and groups	aerial or ground surveys that account for detection probability and use a robust design
identify times and places with high prevalence in reservoir hosts	determine coarse-resolution patterns of prevalence in reservoir hosts, variation in prevalence among reservoir host populations, and the spatial extent of infection estimate the spatial and temporal autocorrelation of infectious animals or populations	spatially and temporally replicated prevalence and seroprevalence within- and among-population prevalence (or seroprevalence if the refractory period is short relative to the host lifespan) in space and time and over life-history stages	probabilistic, spatially and temporally stratified sampling of reservoir host populations adaptive sampling of probabilistically selected, higher-prevalence sites
identify causes of high prevalence in reservoir hosts	estimate the infectious period, exogenous and endogenous covariates associated with infection, and shedding loads	pathogen status, load, immunity and demography of infected and uninfected hosts	probabilistic sampling of populations with high, moderate and low prevalence
identify patterns of transmission in the reservoir host	identify covariates associated with increased susceptibility or transmission estimate rates of change of prevalence to inform the temporal resolution of sampling	time-series of cases or seroconversions in space from same locations longitudinal sampling of individual infection status to identify change in infection state of individuals over time age-stratified prevalence or seroprevalence	longitudinal sampling of individuals and populations
estimate risk of spillover to recipient hosts across space and time to predict future events	investigate the pathogen's potential to persist in the environment describe when, where, and how reservoir and recipient host species interact	biotic and abiotic environmental attributes at small and large spatial extents and resolutions contacts among individuals multispecies (sero)surveys to identify high-prevalence hosts in areas with high prevalence comparative studies of exuded load per host across host species to understand variation in pathogen release among host species	probabilistic, spatially and temporally stratified sampling of reservoir host populations
explore interventions to reduce prevalence or magnitude of an epidemic, or eradicate infection from reservoir hosts	estimate rate of epidemic growth and reproductive number (R_0) estimate rates of effective vaccination or culling design effective implementation strategies (e.g. ring vaccination or culling, treatments at the infection front)	prevalence over time and duration in infection class age-stratified prevalence or seroprevalence and demographic data	longitudinal sampling during epidemics at invasion zones

do not cover well-described statistical approaches such as power and sample size analyses. We also simulate a theoretical wildlife disease to illustrate how spatial and temporal variability or synchrony in infection dynamics can inform sampling decisions and inferences about disease dynamics.

2. Processes driving the distribution and synchrony of pathogens in reservoir hosts

(a) Spatial and temporal distribution and intensity

The distribution and intensity of infection in reservoir hosts vary among individuals and among populations in space and time. This variation is driven by many within- and between-host factors, including host demography and movement, transmission rates, infectious periods and herd immunity. Below we describe the factors that influence spatial and temporal variation. In most instances, there is little *a priori* information to decipher the drivers of variation, and these knowledge gaps must be addressed with sampling (table 1).

When a pathogen first invades a population of hosts, transmission dynamics may be synchronized through the invasion process [9]. Such invasion dynamics may characterize West Nile virus and avian influenza H5N1 invasion in wild birds in the USA, or Zika virus invasion in marmosets (*Callithrix species*) in Brazil [10–12]. During this phase, the basic reproduction number, R_0, the average number of secondary infections generated over an individual's infectious period when infection is rare [13], is a powerful distillation of the efficiency of pathogen spread. Beyond the initial stage of pathogen invasion, however, R_0 does not capture all fundamental elements of pathogen dynamics that affect the distribution of infection in space and time. For example, a pathogen that produces an acute (short-lived) immunizing infection with high transmission and mortality rates might spread quickly through local host populations and then fade out, to be reintroduced after the pool of susceptible hosts is replenished. Such short-lived epidemics of *Yersinia pestis* in rats, for example, may explain the sporadic outbreaks of bubonic plague in humans in both fourteenth to sixteenth century Europe [14] and modern urban Madagascar [15]. By contrast, another pathogen with a comparable R_0, but a long infectious period and low transmission rate, may persist in the same population for long periods, eventually producing a spatially and temporally stable infection intensity as exemplified by *Mycobacterium bovis* in white-tailed deer (*Odocoileus virginianus*) in Michigan, USA [16].

Once a pathogen has established in the reservoir host populations, within-host factors (e.g. duration of infection within hosts) interact with the population dynamics, density and movement of reservoir hosts to determine the distribution of infection among hosts [17–19]. The intensity of infection within individuals is governed by immune responses and pathogen life history. Because individuals acquire immunity, they typically have higher pathogen loads during their first infection than during subsequent infections. For example, juvenile *Rousettus aegyptiacus* bats that excreted high levels of Marburg virus during their first infections were linked to spillover of the virus to humans in Uganda [20]. Similarly, pathogen burden and shedding rates can vary over the course of infection as a function of changes in the immune response, microbiome and pathogen distribution within the host tissues. Bank voles (*Clethrionomys glareolus*) infected with Puumala virus shed high titres of virus during the acute phase of infection and low titres during the chronic phase of infection [21]. Pathogen levels also can rise if host individuals are infected with multiple pathogens or are immunocompromised by physiological or environmental stress. Laboratory mice infected with both worms and bacteria, for example, shed more of both for longer than those infected with either pathogen in isolation [22], and bats (*Pteropus alecto*) are hypothesized to excrete zoonotic viruses during winter, when environmental stress drives reactivation of latent viruses [23–25], but not during summer, when food is abundant [26].

(b) Synchrony

The nature of the transmission process usually leads to synchrony in the distribution of infection among individuals at some spatial scale. Tobler's first law of geography states 'near things are more related than distant things' [27]. Accordingly, the correlation of values of a variable through space (spatial autocorrelation) and time (temporal autocorrelation) is usually positive. Understanding spatial synchrony of infection [28] can help inform sampling design.

Synchrony of infections within populations can arise through myriad processes that drive pathogen transmission (electronic supplementary material, table S1). These include processes that drive synchrony of animal populations (dispersal, social organization and Moran effects (correlation between population size or density that is linearly related to the correlation between their environments), figure 1), processes that drive synchrony of population-immunity (e.g. the strong and directional autocorrelation of invasion, the influx of susceptibles through birth, and synchronization of susceptibility through stress from environmental perturbations), and processes that drive synchrony of exposure, such as pathogen survival (e.g. the response of influenza survival to humidity [29] and of Hendra virus survival to temperature [30]), or behaviour (e.g. winter consumption of date-palm sap by humans in Bangladesh [31]). Synchrony of transmission commonly is seasonal, especially in temperate zones [32]. Efficient sampling of the distribution of infection requires estimating the spatial and temporal scales at which infection dynamics in the reservoir host are synchronized and the extent to which variability or trends in those dynamics are predictable [32].

The spatial extent of synchrony depends on the extent of the process driving synchrony. When host movement drives infection dynamics, spatial autocorrelation is driven by the rate of host movement relative to the infectious period of the pathogen [33]. For example, the more thoroughly mixed the contacts among populations, and the longer the infectious period, the lower the spatial variability in infection dynamics (figure 2*c*,*d*). However, acute pathogens in hosts with high connectivity may be as thoroughly mixed as chronic pathogens in hosts with low connectivity [33]. If movement rates of hosts are not high enough to ensure transmission to new subpopulations before recovery from infection, infection can become trapped in subgroups [35]. For example, the spread of measles, an acute disease, is limited in regions where walking is a more common mode of transportation than motorized vehicles [36]. By contrast, measles occurred in waves across the UK prior to vaccination [37] because great

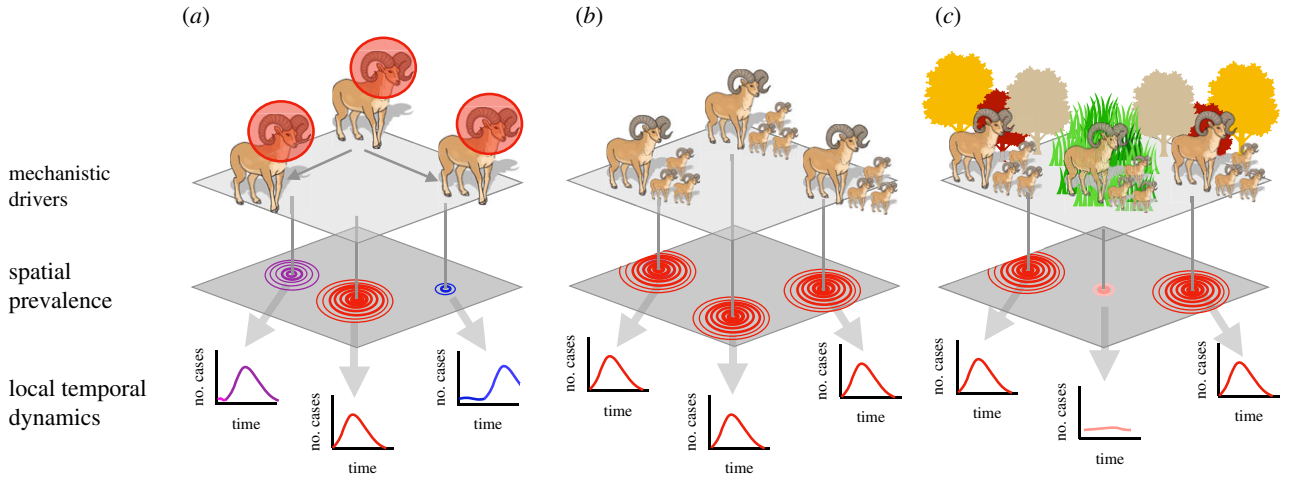

Figure 1. Mechanisms governing spatial and temporal patterns and structure of disease dynamics include dispersal of individuals, social organization and synchrony of host populations and Moran effects. For each mechanism, the color on the spatial prevalence panel indicates the time at which the local outbreak began, and the circles represent the extent of the pathogen in space. The prevalence curves in the local temporal dynamics panel show prevalence through time at the epicentre of each local outbreak. In (*a*), dispersal of infected hosts (bighorn sheep with red circles) produces spatial and temporal autocorrelation consistent with the movement patterns of the primary host. In (*b*), synchronous demographic or behavioural dynamics within the host species produce synchrony in spatial and temporal dynamics of prevalence within all host populations. In (*c*), Moran effects across populations create synchrony among populations experiencing similar environmental conditions. Here, we imagine that limited nutritional availability consistently increases host susceptibility to infection during autumn in forest populations. This leads to synchronous outbreaks in all forest populations that experience the nutrient deficit, without simultaneous outbreaks in populations in locations that are not forested. Cases are infected individuals.

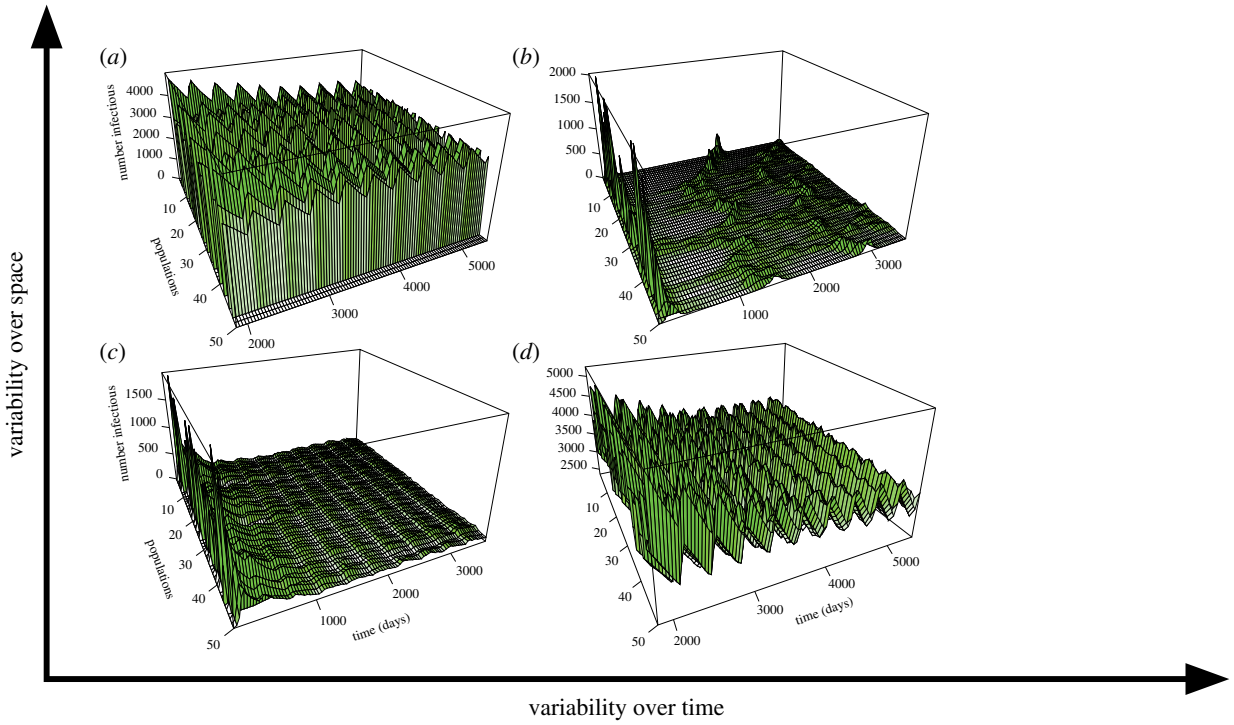

Figure 2. A simulation of pathogen dynamics in reservoir host populations with varying autocorrelation of prevalence in space (populations) and time (days). Prevalence falls along spatial and temporal gradients of variability. In (*a*), variability is high over space but low over time (e.g. chronic infections with highly variable prevalence over locations but stable prevalence over time such as hepatitis B in human populations) [34]. In (*b*), variability is high over space and time (e.g. acute pathogens such as canine distemper virus in carnivores at the extent of the USA). In (*c*), variability is low over space and time, as with chronic, endemic pathogens in highly connected populations, such as herpes simplex virus in human populations. In (*d*), variability is low over space but high over time, as in highly contagious infections with seasonal transmission such as influenza. Methods for the simulations are described in electronic supplementary material, Methods.

distances could be travelled by car or train within the two-week infectious period. High movement rates relative to infectious periods create synchronous dynamics across populations, maintenance of herd immunity and dampening of epidemics [38]. However, high synchrony also can lead to synchronous pathogen extinction across all infected populations. Understanding host movement relative to the pathogen's infectious period is critical to inform sampling.

Unpredictable spatial synchrony in transmission can be generated through synchronous environmental stochasticity

[39]. Stochastic perturbations in climate or extreme events such as hurricanes and fires have synchronized parasitic disease in grouse (*Lagopus lagopus scoticus*) [39,40] and have been hypothesized to cause pulses of viral shedding from bats (*Pteropus* species) [38,41]. Comparing the extent of synchronous infection dynamics with the extent of environmental variation can be useful for causal inference [42].

Truly decoupled dynamics across space or time, where dynamics vary chaotically among populations or depend on factors that are limited in space and time (figure 2*b*), also can occur. Isolation, for example on islands, may foster uncoupling from other populations, and reduce the likelihood of pathogen persistence, particularly if populations are small and infectious periods are short relative to host lifespans [33]. Complex transmission feedbacks within populations may drive spatial asynchrony. For example, longer infectious periods in older bighorn sheep (*Ovis canadensis*) may drive feedbacks that result in either high or low endemic states of pneumonia-causing *Mycoplasma ovipneumoniae* depending on the age-structure of the population at first infection [43]. These feedbacks could create dynamics that are relatively independent across space but have temporal trends. Thus, sampling multiple populations across space, with many sampling points through time, is essential to make inferences that can be generalized across all infected bighorn sheep populations [44,45].

3. Sampling the distribution of infection in reservoir hosts

(a) Basic sampling principles

Designing a sampling strategy that identifies how pathogens are distributed in space and time is challenging. In particular, trade-offs between the spatial extent of sampling and intensity of sampling are ubiquitous. Sampling intensity should be informed by temporal and spatial variability in prevalence (the proportion of infected individuals in a population) and by spatial and temporal autocorrelation. In general, more sampling is required in more variable and less autocorrelated systems. The observed autocorrelation depends on resolution of observation and units for sampling and analysis (e.g. individuals, populations or regions; days, months or years), which must reflect the objectives of the study (table 1). For example, canine distemper virus in wolves (*Canis lupus*) might appear patchily distributed at coarse resolution (e.g. counties within Montana), but synchronous at fine resolution (e.g. Yellowstone National Park [46]). If the resolution of data collection is coarse, one may infer that infection dynamics are spatially independent (figure 2*a*,*b*), whereas finer-resolution sampling within a more limited spatial extent may indicate spatial homogeneity (figure 2*c*,*d*). Neither choice is erroneous, as long as the inferences are aligned with the scale of investigation. Ultimately, however, trade-offs between spatial versus temporal sampling, and decisions on the resolution and units of analysis, are decided by the prioritization of objectives and resources available for sampling.

To draw valid statistical inferences from field data, theory generally prescribes sampling randomly in space and time [47]. However, for logistical reasons, probabilistic sampling (random selection of samples in which all units have equal probability of being selected) is rare in animal epidemiology. For example, social hosts, such as bats that roost in a common location, are not randomly distributed, and it is also challenging to randomly sample bats within these roosts [48]. Stratified random sampling or other generalized random stratified designs may be more feasible while achieving a spatially well-balanced random sample and valid inferences [49–52]. The stratification can be informed by knowledge of seasonal peaks in prevalence and concentrations of infection to focus efforts in high-risk places and times. Sampling also continues at times and in locations where risk of infection is thought to be low. Designs with unequal probability selection based on an auxiliary variable also can be considered in such cases.

Stratified random sampling is less feasible for pathogens for which prevalence responds to ephemeral environmental drivers, or to transient dynamics in the reservoir host. In these circumstances, one may wish to implement adaptive sampling, in which probabilistic sampling is complemented by more-intensive spatial and temporal sampling during an outbreak or spillover. Opportunistic sampling often is deployed following spillover in an effort to isolate pathogens or identify reservoir hosts [53]. However, if opportunistic sampling is accompanied by some probabilistic sampling, it typically provides more insight into the spatial and temporal dynamics of infection [54–58].

Although adaptive sampling designs are valuable for maximizing data collection around mortality events or spillover, or for sampling when there is little *a priori* information, simulations suggest that statistical power to detect temporal trends in infection dynamics is greater when populations are sampled repeatedly and consistently over a long period of time (e.g. monthly over a few years) than when a given population is sampled for a short period of time, albeit repeatedly [59] (box 1; e.g. daily or weekly over a few months). Alternative sampling designs [59,73,74] are robust to infrequent sampling, distant sites and large spatial extents. These include: rotating panels, which sample each site repeatedly but during temporal windows that do not fully overlap; augmented, serially alternating panels, which complement rotating panels with consistent sampling of one location; and partially augmented, serially alternating panels, in which infrequent sampling of a given location periodically is complemented with frequent sampling of the location (box 1 and table 2).

Another framework that recently has emerged from the disease ecology literature is model-guided fieldwork [75], where mathematical models of pathogen dynamics are developed *a priori* to guide field data collection. Modellers and biologists work together to incorporate multiple hypotheses and uncertainty about the structure of dynamics and then iterate between models and measurement. Such approaches can facilitate transdisciplinary research and lead to more robust inferences.

(b) Targeted approaches to increase information about prevalence

Prevalence is usually inferred from spatially and temporally explicit data on individual infection or exposure status. These data are usually information-weak because the outcome of every sample is binary (infected or not infected), and the outcomes may be subject to error. In addition to simply increasing the number of individuals sampled,

Box 1. Sampling to characterize pathogen prevalence in reservoir hosts.

To illustrate how sampling methods affect interpretation of pathogen dynamics, we used R [60] to simulate infection in a reservoir host as a random realization of a binomial point process [61] (figure 3); see the electronic supplementary material for more information and R code. The kernel-smoothed intensity of this process illustrates spatial and temporal concentrations of infection. We simulated sampling of this hypothetical host over space and time with different designs. Points and thick lines in figure 3 indicate the prevalence estimated by each sampling design. Although this simulation is a clear oversimplification of reality (e.g. no population structure, no underlying mechanistic model of infection dynamics), this serves as a heuristic tool to illustrate the variation in inference about prevalence estimates from different spatio-temporal sampling designs.

In opportunistic or haphazard designs, nearby populations are sampled following a spillover event (A). For example, the emergence of severe acute respiratory syndrome in 2002 in Guangdong Province, China was followed rapidly by surveys of mammals in wet markets [62]. Similarly, the emergence of Hendra virus in Brisbane, Australia in 1994 was followed by surveys of wild and domestic animals through Queensland [63]. Although important for isolating virus and identifying reservoir hosts, opportunistic or haphazard sampling may overestimate prevalence, may not capture both spatial and temporal variation in infection dynamics, and may limit the validity of inferences about the population [47]. Repeated (i.e. longitudinal) sampling of single or a few populations (B, C) is common in disease ecology [64–67]. However, longitudinal sampling across a broad number of spatially replicated populations is rare during epidemiological surveillance of wildlife [68]. Opportunistic sampling of multiple populations may be complemented with repeated sampling of one population (e.g. [69]). Longitudinal sampling often is conducted at regular intervals (e.g. every four months), and such designs can capture or consistently fail to detect temporal peaks in viral shedding from reservoirs.

Because pathogen transmission processes are temporally dynamic, even coarse-resolution spatial sampling must be temporally explicit. Estimates of prevalence from one population at one point in time may be misleading if disease dynamics are sufficiently rapid that prevalence changes substantially over the study period. For example, a cross-sectional sample of a travelling wave epidemic could bias estimates of spatial variance if different populations are at troughs and peaks of prevalence. Nevertheless, estimates of prevalence that are based on pooling of samples in space or time frequently are reported in the literature [70].

Random sampling can reduce bias that results from sampling at regular intervals (D). Although sampling with random designs may reduce bias in estimates of spatial and temporal infection dynamics, it may not capture temporal trends within a given location. Furthermore, random samples may be clustered in time and space. Additionally, random selection of sampling locations may have less statistical power than intentional selection of sampling locations [71]. Moreover, truly random sampling may not make sense for certain taxa, such as central-place foragers (e.g. many bats), which are most easily sampled at locations that are not randomly distributed (e.g. roosts) [48]. Stratified random sampling in space and time [72] may be more effective. For example, in E, two samples are drawn from each region. Another alternative to random sampling is adaptive sampling (F), in which random sampling is augmented by more-intensive sampling in the spillover region. This design reduces bias associated with longitudinal surveys while capitalizing on opportunistic sampling following spillover (e.g. virus isolation).

Panels F through I illustrate designs from the sampling literature [59,73,74]. Rotating panels (G) sample each site a finite number of times; as sampling of each site ceases, sampling of another site begins. Although rotating panels can help infer fine-resolution temporal dynamics efficiently over space, they also can restrict broader longitudinal analyses and may change the state of the epidemiological system if sampling of a given site occurs too frequently [73]. Serially alternating sampling is similar to rotating sampling but increases the interval between samples of each site. Both the rotating and the serially alternating designs can be augmented with longitudinal sampling of single or multiple sites (e.g. H). The partially augmented, serially alternating design replaces longitudinal sampling of one site with sampling of multiple sites at consecutive intervals (I). Prior simulations suggested that the power of augmented, serially alternating and partially augmented, serially alternating sampling to detect temporal trends is greater than that of rotating sampling [59]. However, given that these designs include replicated sampling over time per multiple sites, their implementation can require ample sampling effort and therefore resources in terms of personnel, time and funding.

the information content of wildlife field samples can be augmented in several ways.

Information on ages of sampled animals is useful because age–seroprevalence or age–prevalence curves can be used to estimate transmission rates [17]. Seroconversion of juveniles provides clear evidence of ongoing pathogen transmission within a population. If juveniles seroconvert each year, the pathogen is likely to be persistent and endemic in that population rather than infrequent and oscillatory. Serosurveys of juveniles are particularly useful in systems where long-lived circulating antibodies are the only measurable indication of exposure (e.g. African bat henipaviruses, in which RNA rarely is found, and virus has not yet been isolated [76,77]), as long as maternal immunity is not mistaken for juvenile exposure [78]. Sampling of isolated populations (e.g. [76]) similarly can help distinguish between pathogen persistence at the population level versus spatially and temporally patchy transmission.

Seroprevalence can be useful for monitoring spatial and temporal trends in prevalence if few individuals are infected at any point in time (e.g. with infectious periods short or transmission rates low), or if detection is difficult (e.g. lethal sampling is required to test whether lyssaviruses are present in most mammals [79]). However, if antibodies

Table 2. Sampling designs used in studies of pathogen dynamics in reservoir hosts.[a]

sampling design	description	advantages	disadvantages
opportunistic	nearby populations are sampled following a spillover event	isolating pathogen, identify reservoir hosts, pragmatic	overestimate prevalence, cannot capture spatial or temporal variation, skew inference of prevalence
single longitudinal	repeated sampling of single population over time	infer some temporal dynamics	cannot capture spatial variation, regular intervals could consistently miss shedding pulses
replicated longitudinal	repeated sampling of multiple populations over time	infer some spatial and temporal dynamics	logistically challenging, regular intervals could consistently miss shedding pulses
random sample	random distribution of sampling events over space and time	reduce bias from sampling at regular intervals	may not capture spatial or temporal variation when truly random, may not be feasible for many species
random stratified	random sampling from predetermined regions in space and time	more likely to obtain a representative spatial and temporal sample	may require greater effort than a simple random sample
adaptive sampling	random sampling augmented by intensive spatio-temporal sampling near outbreaks	reduce bias while capturing benefits of opportunistic sampling (e.g. isolating pathogen)	uncertainty in final sample size
rotating panel	each site is sampled *x* number of times and then the next site is sampled *x* times	infer fine-resolution temporal dynamics efficiently over space	few longitudinal samples from any one population, can modify system if sampling of given sites is too frequent
augmented serial panel	increases the between-site interval from a rotating panel design, adds a longitudinal study for one site	higher power for trend detection, longitudinal analysis possible, less likely to modify system	may require greater effort in terms of time and funding
partially augmented serial	replaces the longitudinal sampling of the augmented serial panel with repeated sampling of multiple sites	higher power for trend detection, longitudinal analysis possible, minimized bias	may require greater effort in terms of time and funding

[a]See box 1 for references.

persist for long periods relative to the lifespan of individual hosts, seroprevalence can remain relatively stable over time, even if pathogen prevalence oscillates or the pathogen is extinct locally [80].

Longitudinal sampling of even a small number of known animals over the course of their infections can place preliminary constraints on disease process parameters, which in turn may prove useful for identifying the duration of infection and immunity with stratified or adaptive sampling of populations. This strategy has been used to study wildlife diseases in diverse hosts, from bighorn sheep [43] to bats [81,82]), and is essential for elucidating the within-host dynamics of poorly understood bat viruses [23].

One may identify spatial extents and resolutions for investigation of spillover risk by focusing early sampling at invasion fronts, as suggested for non-native invasive species [9,83,84]. Informal adaptive sampling often is employed following spillover events, but it would be valuable to use formal adaptive sampling [54–58]. Focused sampling at invasion fronts facilitates explicit estimation of transmission, recovery and disease-induced mortality rates before herd immunity shapes dynamics. Moreover, higher public health burdens are often observed at the invasion front because epidemic curves in the reservoir hosts peak at those fronts, exerting high pathogen pressure. Moreover, human populations at invasion fronts rarely are well prepared to reduce spillover [11]. Sampling at the invasion front can be informed by an iterative process of data assessment, dynamic modelling, spatial and temporal forecasting and model validation [60]. Adaptive sampling, which complements random or random stratified sampling across space and time with focused sampling (e.g. in the region and months following a spillover event), also could be informative, but rarely has been implemented [56,85–87].

(c) Characterization of spatial and temporal dynamics

If there is no recent outbreak epicentre, various statistical approaches can be used to estimate the spatial and temporal structure of a pathogen to inform the sampling design. At most scales of observation, infection dynamics have some level of spatial and temporal dependence that decreases

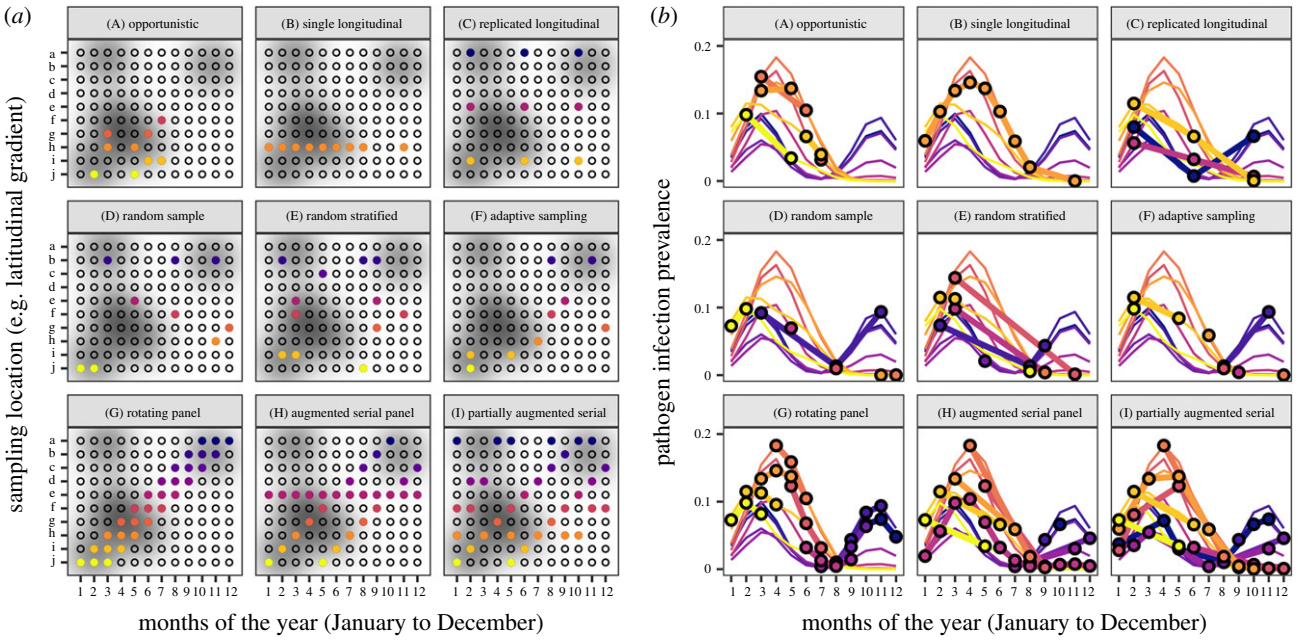

Figure 3. Allocation of sampling effort over time (months) and space (e.g. along a latitudinal gradient) and consequences for inference of pathogen prevalence. Grey shading in panel (*a*) denotes the underlying spatial and temporal pattern in infection prevalence (the kernel-smoothed intensity of a random realization of a binomial point process). Open circles represent sampling locations. Colours indicate sampling location. Panel (*b*) illustrates the observed and true temporal trends in infection prevalence across sampling sites. Thin lines indicate the known infection prevalence over the annual cycle, whereas filled circles are estimated prevalence values given each design and ignoring error in estimation of the prevalence. Thick lines indicate the observed time-series of infection prevalence and only connect points from a single location; locations that are only sampled once within a design have no corresponding thick line. Heuristic sampling designs are as follows: opportunistic (A), single longitudinal (B), replicated longitudinal (C), randomized (D), random stratified (E), adaptive (F), rotating (G), augmented, serially alternating (H) and partially augmented, serially alternating (I). Sampling effort is held relatively constant across A to F and G to I (for which designs can require higher sampling effort). Methods for the simulations are given in electronic supplementary material, Methods.

with distance or time. Understanding the extent of spatial and temporal autocorrelation can inform the allocation of sampling effort [59,73,74]; this can be accomplished formally through exploratory tools such as correlograms (e.g. plotting the between-site correlation as a function of between-site distance) or semivariograms, for which semivariance is modelled as a function of spatial and temporal lags [88]. Pairs or clusters of samples within regions can be compared with samples among regions to estimate spatial autocorrelation. For example, Tobin [84] demonstrated that within a given spatial extent, sampling clusters of points estimated spatial autocorrelation more accurately than sampling evenly distributed but distant points.

The larger the extent of spatial dependence, the further the sampling units (e.g. sampled populations) can be from each other to make general inferences about risk across a landscape. For example, systems with little spatial variability—e.g. highly connected systems and those in which the infectious period is long relative to the lifespan of the host (e.g. figure 2*c*,*d*)—require sampling of a small number of spatially disparate populations. In this case, more frequent sampling of one or two locations may be more informative than infrequent sampling of many locations. By contrast, spatially asynchronous infections may require infrequent sampling of many locations across the ranges of the pathogen and host.

In theory, the lower the temporal variance, the less frequently one needs to sample. Surveillance of the temporal dynamics of infection often is systematic, with sampling at regular intervals within single or multiple populations, and this is often dictated by logistical and funding constraints. For example, the Soay sheep (*Ovis aries*) population of St Kilda, Scotland, UK was sampled annually to reveal long-term fluctuations in parasite prevalence [64], and vampire bats (*Desmodus rotundus*) were sampled annually and biannually to reveal endemic viral and bacterial pathogens [65,66]. However, systematic sampling of a population in which prevalence is either extrinsically driven and seasonal or endogenous and epidemic may not detect temporal peaks in prevalence, particularly if the interval between samples is similar to or longer than the periodicity in the pathogen cycles or the peaks in epidemics. Random or rotating sampling designs can reduce the likelihood of this type of bias (box 1 and table 2). When temporal variance is high, as in cases with seasonal oscillations or multiple-year peaks, the sampling interval should reflect the periodicity of the disease. In such cases, generalized random stratified approaches may be used to avoid the pitfalls of systematic sampling and the clumping of simple random sampling [49].

The most challenging pathogens to sample, and therefore those that require the most-intense sampling, are those with highly localized infection dynamics [38]. If only one population is sampled, one erroneously might infer that all populations have similar dynamics. Characterization of infection structure in these systems is best captured with random, rotating or augmented sampling (box 1 and figure 3).

4. Conclusion

Uncertainty in predictions of spillover risk is reduced by knowledge of the spatial and temporal distribution of infection among populations of reservoir hosts. Financial and logistical constraints often force one to make inferences on the basis of small sample sizes and wide confidence intervals, and to

make trade-offs between spatial and temporal replication. Adopting wise design choices that are appropriate to the background dynamics of a particular system can extend the utility of even sparse data, and is essential to efficiently understand prevalence dynamics in reservoir host populations.

Data accessibility. This article has no additional data.

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