**Supplementary Table 2.** Comparison of difference approaches to studying coronaviruses in bats. A total of 214 original studies on bat-associated coronaviruses were classified into study types. Study types were not exclusive, so a study may fit into multiple types depending on the sampling approach and analytical methods. All classified studies can be found in Supplementary Dataset 1.

| **Study type and description** | **Number of studies** | **Overview** | **What we can learn** | **Advantages** | **Caveats** |
| --- | --- | --- | --- | --- | --- |
| **Experimental** Experimental infection of individual bats or bat cell lines, or other viral manipulations in a controlled environment | Bat cell lines: 29  Live bats: 6 | Bat cell experiments   * Target cells: brain, embryo, intestine, kidney, lung * Tested viruses: multiple bat SARS-related CoVs, BatCoV HKU4, BatCoV HKU9, HCoV-229E, HCoV-NL63, MERS-CoV, PEDV, Ro-BatCoV GCCDC1, SADS-CoV, SARS-CoV, SARS-CoV-2, *Scotophilus* bat CoV 512, TGEV   Live bat experiments   * Tested hosts and viruses: *Artibeus jamaicensis* (MERS-CoV), *Eptesicus fuscus* (SARS-CoV-2), *Myotis lucifugus* (Myl-CoV), *Rousettus leschenaultii* (BatCoV HKU9), *Rousettus aegyptiacus* (bat SARSr-CoV WIV1, SARS-CoV-2) | * Characterization of newly detected viruses * Bat species susceptibility to infection and dose-response relationships * Magnitude, quality, and kinetics of immune responses to pathogens, and mechanisms of viral control or tolerance * Disease pathogenesis (or lack thereof) * Individual and within-host infection, disease, and immunological processes, especially those required for dynamic modeling (e.g., infectious periods, acute vs. latent infections, waning immunity, etc.) * Tissue tropism and routes of virus excretion and transmission * Receptor binding efficiency in bats and other potential hosts * Facilitative or antagonistic interactions between coinfecting viruses * Virus surface survival and sensitivity to heat or desiccation * Development of model systems, laboratory protocols, and screening tools for the field * Spillover potential to other/novel hosts | * Ability to test Koch's postulates using different strains and bat species * Causal inference * Controlled environment * Rapid technological advances make diagnostic tools affordable * Relatively rapid data acquisition | * Relies on existing viral isolates; cannot isolate new pathogens * No ecological context; impossible to accurately replicate environmental conditions * Lab conditions may not effectively mimic the environmental conditions that drive infections in reservoir hosts * Challenging and expensive to house and breed colonies of bats * Often requires biosafety level 3 or 4 facilities and specialized training * A bat is not a bat, and a virus is not a virus: species-specific responses to infection make it difficult to generalize across species or bat families * *In vitro* studies miss differences in cell recruitment and localization or cell-cell interaction * Immortalized cells behave differently from primary cells or cells in an *in vivo* context * Fundamental knowledge of bat immune systems and basic tools for probing bat immune responses are lacking * Experiments are usually time-limited (e.g., limited ability to study immune function senescence, viral recrudescence, etc.) |
| **Longitudinal**  Repeated sampling of individuals, single populations, or multiple populations over time; ideally, this occurs in closed populations with known individual life-histories | 14 | * Countries: Australia, China, Denmark, Germany, Malaysia, Singapore, South Korea, Thailand * Serially sampled species: *Eonycteris spelaea*, *Hipposideros cervinus*, *Myotis daubentonii*, *Myotis macropus*, *Myotis myotis*, *Pteropus lylei*, *Rhinolophus sinicus*, *Rousettus leschenaultii* | * Some spatial and temporal dynamics of pathogens in populations, and maybe in individuals * Spatiotemporal patterns of infection (e.g., travelling waves) * Transmission rates and dynamics, using carefully collected age-prevalence and age-seroprevalence data * Variation in prevalence/seroprevalence with host traits or environmental covariates * Parameters of the disease process in individuals and populations required for dynamic modeling (e.g., seasonality, maybe transmission rates, life-history traits) * Some dynamics of co-circulating viruses * Interventions that might reduce prevalence or magnitude of an epizootic or enzootic | * Ability to identify and isolate novel pathogens * May have ability to repeatedly collect covariate data or track life-histories of individuals * More power to exclude time-invariant differences between individuals, populations, or environments * Identification of temporal trends (e.g., seasonality) * Potential for forecasting and prediction * Intervention analysis * Relationship between time-series variables | * May not be truly longitudinal: without known recapture of individuals, repeated longitudinal monitoring at a geographic location may instead represent multiple cross-sectional surveys of the population * Expensive, time-consuming, and logistically challenging; slow data acquisition * Effective implementation requires a strong ecological understanding of the study system and collection of data to determine sampling frequency and duration * May be temporally biased; sampling at regular intervals may consistently detect or consistently miss viral shedding * May be spatially biased; difficult to sample spatially replicated populations * Determining disease dynamics is difficult: requires consistent recapture of individuals, longitudinal sampling that exceeds pathogen infectious period, nonlethal pathogen detection, and moderate prevalence * Large sample sizes, spatially replicated populations, and short sampling intervals are needed to understand environmental drivers, and individual and population-level variation in viral shedding * Relationships that exist for groups may not apply to individuals (ecological fallacy, e.g., virus x detected in all population subgroups sampled in Habitat A; therefore, all individuals or other population subgroups in Habitat A must also carry virus x. |
| **Cross-sectional (intra-species)**  Sampling of a bat population or population subgroup(s) at a specific timepoint | 14 |  | * Genetic variation of strains within host population(s) * Spatial distribution of strains within host population(s) * Some differences between demographic stages (dependent on sampling time-point) * Possible to integrate with longitudinal studies of same species * Natural routes of excretion | * Relatively fast and inexpensive * Sampling of isolated populations can help distinguish between population-level pathogen persistence and spatiotemporally irregular transmission * Can sample populations adaptively in response to spillover * Ability to isolate pathogens * Some ability to detect spatial variation or statistically analyze differences. | * No ability to detect seasonality or other temporal trends * No causal inference * Large amounts of data are required to account for variation among individuals or populations * Effective implementation requires a strong ecological understanding of the study system * May be temporally biased: sampling during peaks or troughs in population prevalence will over- or underestimate geographic variation in prevalence or genetic diversity * May be spatially biased: at one timepoint, different population subgroups may have peaks or troughs in prevalence * Ecological fallacy (as in longitudinal studies) |
| **Cross-sectional (inter-species)**  Sampling of bat assemblages or a subset of a bat assemblage (>1 species) at a specific timepoint | 123 | * Sampled countries: 69 * Sampled bat families: 18 * Positive bat families: 14 * Sampled bat species: 543 * Positive bat species: 238 | * Identity of potential reservoir hosts * Potential exchange of strains between hosts * Host and geographic factors that impact viral diversity | * Rapid detection of viruses in multiple species * Ability to isolate pathogens * Some ability to detect species-level differences * Relatively fast and inexpensive | * Same caveats as intra-species cross-sectional studies * Often low sample sizes for opportunistically sampled species * Species bias: research effort may inadvertently skew importance of a particular species as a reservoir or spillover host * Ecological fallacy (as in longitudinal and intra-species cross-sectional studies) |
| **Multi-pathogen detection**  Detection of multiple pathogens (virus families, strains, or other parasite taxa) using metagenomic sequencing or other targeted methods on samples collected during cross-sectional or longitudinal sampling at the individual- or population-level | 36 |  | * Viral species diversity, abundance, and community dynamics * Some information about periods of potential spillover risk for newly detected viruses not yet known to be zoonotic * Coinfection and some insight into interactive effects of viruses on hosts | * Can be combined with next-generation sequencing to identify viral communities * May require little to no fieldwork if samples are already available * Can be relatively inexpensive with rapid data acquisition (design dependent) | * Same caveats as longitudinal or cross-sectional studies, depending on design * May be difficult to distinguish between facilitative or antagonistic interactions between coinfecting viruses or viruses synchronously shed from a bat population; requires large sample sizes combined with simulation or experimental studies * Drivers of multi-viral infection or shedding may be difficult to detect (e.g., may be driven by facilitative interaction between known or undetected coinfecting viruses, interactions with host physiology/immunity, and/or a response to optimal environmental conditions) * Biased detection: high titers of one virus in a sample may reduce assay sensitivity to other viruses * No causal inference * Co-detection of pathogens in pooled or population-level samples may reflect coinfection or contribution of multiple bats to the collected sample |
| **Sequencing only**  Viral sequencing on samples collected during longitudinal or cross-sectional sampling; little collection of data on other covariates | 29 |  | * Comparative genomics * Mutation and evolutionary rates * Virus discovery * Effective population size and genetic diversity of virus within or across subpopulations * Some information on viral dynamics may be possible (e.g., through phylodynamics) | * Requires little background knowledge of study system * Relatively inexpensive; rapid data acquisition * May require little to no fieldwork if samples are already available | * No ecological or physiological context * No causal inference |