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# The macroecology of viral coinfection

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Open Research Statement: Data are not yet provided. We downloaded PREDICT data from the USAID Development Data Library prior to the takedown of the USAID website in 2025. We affirm that a copy of these data will be permanently archived in Zenodo if the paper is accepted for publication. All steps of the analysis pipeline (data cleaning, statistical modeling, figure generation) can be reproduced using the R code available at https://github.com/viralemergence/macroecology-coinfection.

Keywords: coinfection, disease ecology, wildlife surveillance, macroecology, viral dynamics

**Abstract**

Coinfection is common in wild animals, and can profoundly influence disease outcomes and transmission. However, most coinfection research is based on laboratory experiments or a few well-studied wildlife systems. Here, we use data from the PREDICT project – the largest standardized wildlife disease surveillance project ever conducted – to describe patterns of viral coinfection and evaluate their association with host and virus factors. Within the viruses prioritized for testing, we find that coinfection is rare (detected in just 223 of 65,662 animals), but still more common than expected by chance – especially coinfections of coronaviruses, paramyxoviruses, and influenza A virus. We further find that coinfection is associated with host age, but bats and rodents exhibit opposing relationships. We find that captive wild animals (specifically rats and mallards) exhibit higher coinfection than free-ranging wild animals, highlighting potential risks to wildlife and human health from the wildlife trade. Finally, we find that biases in sampling and testing can shape observed patterns of coinfection. Our results characterize associations among viruses in the largest relevant dataset currently available. Factors associated with coinfection in this dataset such as host-level variation, virus-virus interactions, and human interference should be further tested via surveillance and laboratory approaches to better resolve the role of coinfection as an important and likely overlooked driver of viral dynamics in nature.

## Introduction

Parasites (including microparasites and macroparasites) exist in complex communities, where coinfection with multiple parasites at any time is the norm for wild animals (Cox 2001; Petney and Andrews 1998). Coinfections provide an opportunity for direct interactions between parasites, such as competition for resources (Budischak et al. 2017) or space (Knowles et al. 2013), or indirect interactions, such as those mediated by host immune responses (Ezenwa et al. 2021). These interactions can have a dramatic effect on outcomes at the host level, and transmission dynamics at the population level (Telfer et al. 2010; Gorsich et al. 2018; Pedersen and Fenton 2007; Graham 2008).

Several theoretical frameworks in disease ecology have incorporated the dynamics and consequences of coinfection (Pedersen and Fenton 2007; Fenton and Pedersen 2005; Graham 2008; Sweeny et al. 2021), but it can be difficult to test their predictions against empirical evidence. Within-host processes are easiest to study in laboratory settings (Fenton et al. 2014), but the controlled settings that make experimental approaches useful (e.g., laboratory organisms from a single host species, with known ancestry and no unknown parasites) also limit their similarity to real-world dynamics in natural communities. A few well-developed (and often long-term) field systems have also led to a number of insights about interactions within diverse host and parasite communities (Ezenwa et al. 2021), and some systems allow for natural experiments such as parasite removal or nutritional supplementation (Sweeny et al. 2020; Knowles et al. 2013; Ezenwa and Jolles 2015). However, neither field nor experimental case studies can provide insight into the full network of interactions occurring within parasite communities (or their consequences) at the ecosystem scale or above.

Whereas large-scale biodiversity data have reached volumes that allow for unprecedented research and monitoring, wildlife disease data collection has historically been fragmented between emerging infectious disease surveillance, wildlife management efforts, and ecological research. Across all three fields, there are significant global gaps in surveillance (Carlson et al. 2025; Cohen et al. 2023), and – despite broader progress towards open science in ecology and epidemiology – the sharing of spatially-explicit, host-level infection data remains rare (Schwantes et al. 2025; Albery et al. 2022). Some studies have used data synthesis to develop global infection datasets (Cohen et al. 2023, 2020; Heckley and Becker 2024; Warmuth et al. 2023), but in most cases, they have focused on single-host, single-parasite relationships, rather than single-host, multi-parasite dynamics.

Here, we explore coinfection dynamics at the global scale for the first time, using the single largest standardized study of wildlife disease ever conducted. The PREDICT project was a decade-long, multi-country initiative funded by the U.S. Agency for International Development (USAID) that conducted viral surveillance primarily in wild animals (PREDICT Consortium 2020). Focused on virus families of public health concern but capturing a sizable fraction of wildlife virus diversity, the project produced one of the largest standardized multi-virus datasets ever assembled and resulted in the discovery of new viruses (Anthony et al. 2017b; Goldstein et al. 2018), an improved understanding of drivers of virus infection and diversity (Anthony et al. 2017a; Eskew et al. 2025), and insight into human and virus-related risk factors for zoonotic spillover (Saylors et al. 2021). However, minimal work has been done using the PREDICT data to test macroecological hypotheses. Using these data, we set out to explore three questions: (1) How common is viral coinfection? (2) Is coinfection more common in certain host groups, life stages, and settings? And finally: (3) Are there non-random patterns of coinfection driven by

specific virus taxa, or by associations between specific pairs of virus taxa? Through this work, we also aimed to identify promising avenues for future research on viral coinfection.

## Methods

*Data cleaning*

Analyses were performed using the R statistical environment v4.5.1 (R Core Team 2025). All steps of the analysis pipeline (data cleaning, statistical modeling, figure generation) can be reproduced using the code available at https://github.com/viralemergence/macroecology-coinfection. Data from the PREDICT-2 project (2015-2019; the second half of the decade-long initiative) were openly shared on the USAID Development Data Library prior to the takedown of the USAID website. Our analyses relied on two PREDICT datasets: an animal sampling dataset containing details such as species, sex, and age class and a second dataset containing virus test results for individual specimens (which also included some basic host information). We cleaned the test result dataset by excluding human results and fixing minor inconsistencies in animal host taxonomy (e.g. host scientific name was mostly provided at the species level, but sometimes only at the genus or family level). We modified the animal taxonomic groups originally provided by joining “birds” and “poultry/other fowl” into one “birds” group, and separating a “rodents/shrews” group into “rodents” and “shrews” groups. Two detections of the hantavirus Thottapalayam virus were originally classified as belonging to the Bunyaviridae family; we reclassified them to the Hantaviridae family to align with current virus taxonomy(Kuhn et al. 2024). Finally, we joined the two datasets so that each test result was paired with the relevant animal data.

108 *Data overview*

109 Specimen collection (*n* = 126,952 unique specimens from 65,662 unique animals) was
110 concentrated in countries in West and Central Africa as well as South and Southeast Asia, with >
111 10,000 specimens collected in Sierra Leone, Guinea, and Bangladesh each (Fig. S1). Most
112 specimens were collected from bats (*n* = 77,334, 60.9%) and rodents (*n* = 25,683, 20.2%). The
113 dataset included some farm and companion animals, including poultry, dogs, and swine. Virus
114 screening was conducted using consensus polymerase chain reaction (PCR) assays (714,954 total
115 tests), mostly targeting virus families of significance to human health (e.g., coronaviruses,
116 filoviruses, and paramyxoviruses), but in some cases testing for specific viruses of interest (e.g.,
117 influenza viruses and ebolaviruses). Further details on PREDICT can be found in the PREDICT
118 Legacy Book (https://ohi.vetmed.ucdavis.edu/programs-projects/predict-project/reports).

119

120 *Analyses*

121 Across all animals and within each animal taxonomic group (16 total groups; Table S1), we
122 calculated infection prevalence (number infected divided by number tested) and coinfection
123 prevalence (number coinfected divided by number tested). We considered an animal to be
124 infected when one or more test results for specimens collected from that animal were positive for
125 at least one virus, and to be coinfected when test results were positive for two or more unique
126 viruses. For bats, rodents, and birds (the three largest taxonomic groups), we compared the
127 observed coinfection prevalence to the expected pairwise prevalence based on the prevalence of
128 each virus, using a permutation testing approach. Specifically, we randomized infection across
129 individual animals while maintaining the observed prevalence of each virus within the taxonomic
130 group, then calculated the number of simulated coinfected animals. We repeated this procedure

131 1000 times for each taxonomic group, then plotted the simulated number of coinfected animals
132 compared to the number observed in the PREDICT data.

133 To explore patterns of coinfection among virus taxa, we created a coinfection matrix at
134 the virus family level, where each count within the matrix represents a coinfection of two virus
135 families occurring in the same animal. We included virus family coinfections that represented
136 detections of two viruses in the same family (e.g. two coronaviruses) or in different families (e.g.
137 one coronavirus and one paramyxovirus). For animals infected with three viruses, we counted
138 each coinfection separately (e.g., an animal infected with coronavirus X, coronavirus Y, and
139 paramyxovirus Z would count for two coronavirus-paramyxovirus coinfections and one
140 coronavirus-coronavirus coinfection). We used the *ggraph* package (Pedersen 2024) to visualize
141 the frequency of virus family coinfection. To test if certain virus families were co-detected more
142 often than expected by chance, we first calculated the expected coinfection prevalence of two
143 virus families as the product of each virus family's overall prevalence in the dataset. We then
144 performed a binomial test for each virus family–virus family combination to determine if the
145 observed coinfection prevalence was significantly greater than expected. To account for multiple
146 comparisons, we adjusted *P* values using a Bonferroni correction. Finally, we repeated the above
147 analyses for two subsets of the full data: bats and rodents. We also created a coinfection matrix at
148 the individual virus level and visually explored coinfection patterns; however, due to high
149 sparsity in this matrix, we did not analyze these data further.

150 For each infected animal *(n* = 3,271), we quantified the number of unique viruses
151 detected in its test results and classified its coinfection status as either single infection (exactly
152 one virus detected) or coinfection (two or more viruses detected). We then used generalized
153 linear models to explore correlates of coinfection status. For all model datasets, we excluded

taxonomic groups with ≤ 10 animals (carnivores, cattle/buffalo, dogs, goats/sheep, other), animals that were missing sex or age class data, and neonates and fetuses (*n* = 809 total animals excluded). **1)** For an all-animals model (*n* = 2,462), we used the 'glm' function in the *stats* package (R Core Team 2025) to model animal coinfection status as a function of geographic region (Africa and West Asia; South, East, and Southeast Asia), taxonomic group (bats, birds, rodents, shrews), sex (female, male), age class (adult, subadult, juvenile), captivity status (wild free-ranging, owned domesticated, wild in captivity), and the number of virus families tested for across all specimens associated with that animal. Given inconsistency in host identification (i.e., not all animals were identified to species level), we did not include any species-level traits as model predictors. **2)** For a bats-only model *(n* = 1,519), we classified whether each bat species is known to roost in caves using data from Willoughby *et al.* (Willoughby et al. 2017) and the IUCN Red List. We excluded bats for which cave roosting behavior was not available and bats not identified to at least family level. Using the 'glmmTMB' function in the *glmmTMB* package (Brooks et al. 2017), we modeled coinfection status as a function of five fixed effects variables (geographic region, sex, age class, cave roosting status, number of virus families tested), and one random effects variable (host family). **3)** For a rodents-only model (n = 488), we modeled coinfection status as a function of sex, age class, and number of virus families tested. Subadults and juveniles were collapsed into one age class category due to sample size restrictions, while host family and geographic region were not included as variables because nearly all rodents were murids and were collected in South, East, and Southeast Asia.

## Results

176 We found that both infection and coinfection were rare overall for viruses prioritised in
177 sampling. Out of 65,662 unique animals, 3,271 (4.98%) were infected with one or more viruses
178 (Fig. 1A); virus prevalence in birds (487/5,617; 8.67%) and bats (2,059/36,181; 5.69%) was
179 somewhat higher than average, while prevalence in rodents (549/12,876; 4.26%) was slightly
180 lower than average (Table S1). Animals with at least one virus detection were most often
181 captured in South, East, and Southeast Asia (Fig. S2). Within infected animals, there was uneven
182 geographic distribution of animal taxonomic groups; infected bats, rodents, and shrews were
183 widely distributed, while infected birds and swine were found only in South, East, and Southeast
184 Asia (Fig. 1B).

185 A single virus was detected in most infected animals (*n* = 3,048, 93.18%); coinfections of
186 two (*n* = 211, 6.45%) or three (*n* = 12, 0.37%) viruses in the same animal were infrequent. Of the
187 223 coinfected animals, over half were bats (*n* = 128, 57.4%) with the remainder being 50
188 rodents, 39 birds, 5 swine, and 1 shrew (Table S1). Within infected animals, coinfection rates
189 were highest in rodents (50/549, 9.11%), birds (39/487, 8.01%), and bats (128/2,059, 6.22%).
190 Roughly half of the triple coinfections (7/12; Table S2) were domesticated ducks and geese,
191 which were all infected with duck coronavirus, influenza A virus, and either Newcastle virus or
192 avian paramyxovirus 6; the remainder were bats infected with a mix of coronaviruses,
193 paramyxoviruses, influenza A virus, and a novel rhabdovirus.
194

195 *Coinfection is non-random across phylogeny and life stage*

196 Bats, rodents, and birds exhibited unique patterns of coinfection, even accounting for their over-
197 representation in the dataset. Coinfections were more common than expected based on the
198 observed prevalence of all viruses: for all three taxonomic groups, we applied a permutation

testing approach, and found that observed coinfection rates exceeded the maximum simulated coinfection rates (across 1,000 simulations for each taxonomic group; Fig. S3). However, when accounting for geographic region, sex, age class, captivity status, and number of virus families tested in a generalized linear model, we found that both birds (odds ratio (OR) = 0.24, 95% confidence interval (CI): 0.09-0.54, $p = 0.001$) and rodents (OR = 0.55, 95% CI: 0.33-0.89, $p = 0.018$) were significantly less likely to be coinfected than bats (Fig. 2A, Table S3). These findings could support the widespread hypothesis (and growing evidence) that bats are able to tolerate viral infection, and potentially coinfection, through a unique set of immune adaptations (Letko et al. 2020; Irving et al. 2021; Becker et al. 2025). However, we note that coinfection rates within the all-animals model dataset (i.e., animals with available sex and age data) differed compared to the coinfection rates in the dataset of all 3,048 infected animals: while coinfection was still high in rodents (46/488, 9.43%) and bats (112/1,628, 6.88%), it was noticeably lower in birds (12/316, 3.80%).

Coinfection was associated with life history, but those relationships varied between groups. Based on previous studies (Anthony et al. 2017a; Peel et al. 2025), we hypothesized that infection prevalence – and therefore coinfection rates – would be higher in younger animals, due to waning of maternal antibodies and less-developed immune systems (Goenka and Kollmann 2015). We found that younger rodents (juveniles and subadults) were more likely to be coinfected than adults, though this result was not statistically significant (OR = 1.84, 95% CI: 0.95-3.51, $p = 0.066$; Fig. 2E, Table S5). However, juvenile bats were less likely to be coinfected than adults (OR = 0.13, 95% CI: 0.03-0.55, $p = 0.006$; Fig. 2C, Table S4), and this effect was strong enough that it dominated the all-animals model (OR = 0.32, 95% CI: 0.10-0.80, $p = 0.030$; Fig. 2A, Table S3). This pattern is aligned with the widespread hypothesis that bats' unique

222 immune adaptations allow them to tolerate persistent viral infections (Calisher et al. 2006;
223 Plowright et al. 2016), which might accumulate over the course of an individual's lifespan
224 (Aguillon et al. 2025). Alternatively, depending on the timing of sampling by the PREDICT
225 study, juvenile bats may have been captured at times when levels of maternal antibodies were
226 still high. Though logistically challenging, longitudinal sampling within populations can help
227 elucidate patterns of virus infection and coinfection in relation to host maturation
228 (Wacharapluesadee et al. 2018; Brook et al. 2019; Peel et al. 2025).

229 Finally, we found that host social structure could drive coinfection patterns, but found
230 divergent effects between natural and un-natural settings. For bats, cave-roosting behavior was
231 not a significant predictor of coinfection status (OR = 0.93, 95% CI: 0.53-1.63, $p$ = 0.796; Fig.
232 2C, Table S4). Cave-roosting bats can exist in large groups in close proximity, potentially
233 increasing infection rates, but other roosting strategies can also create large, close aggregations
234 of bats (e.g., tree-roosting *Pteropus* species that cluster together). Finer-scale contact patterns
235 that we could not examine here (e.g., segregation by age or sex, or centrality in social networks)
236 might lead to more correlated infection risk across viruses at the individual level. Sickness
237 behaviors (e.g., reduced social interactions) after an initial infection could also reduce an
238 individual's subsequent likelihood of infection with other viruses (Ripperger et al. 2020; Moreno
239 et al. 2021).

240 In contrast, across all animals, we found that wild animals in captivity were significantly
241 more likely to be coinfected than free-ranging wild animals (OR = 2.12, 95% CI: 1.16-3.82, $p$ =
242 0.013; Fig. 2A, Table S3). These results support the hypothesis that captivity can increase
243 parasite transmission through both crowding, stress, and unusual patterns of interspecific contact.
244 This hypothesis has been strongly supported by previous analyses of coronavirus testing

245 conducted by the PREDICT project (Anthony et al. 2017b; Huong et al. 2020), and is a particular
246 point of concern, given the potential for coinfection to lead to recombinant coronaviruses (Wells
247 et al. 2023), and the high risk of spillover in wildlife farms and markets (Sánchez et al. 2021).
248 However, we note that of the 163 animals classified as "wild animals in captivity" in the all-
249 animals model dataset, 131 (80.4%) were rats (*Rattus* spp.) and 28 (17.2%) were mallards (*Anas*
250 *platyrhynchos*). Further work quantifying coinfection rates in captive counterparts of a broader
251 range of species is needed to better understand if these results are generalisable, and to examine
252 the role of potential mechanisms in driving coinfection in captivity.
253
254 *Sampling and testing biases can shape observed coinfection patterns*
255 Animals sampled in South, East, and Southeast Asia were more likely to be coinfected than those
256 sampled in Africa and West Asia in both the all-animals model (OR = 2.42, 95% CI: 1.63-3.61, $p$
257 $< 0.001$; Fig. 2A, Table S3) and the bats-only model (OR = 2.79, 95% CI: 1.78-4.39, $p < 0.001$;
258 Fig. 2C, Table S4). Rather than reflecting any characteristic of one region versus another, this
259 result might be driven by sampling artifacts. For instance, flying foxes (*Pteropus* spp.) had a high
260 coinfection rate (all-animals model dataset: 37/245; 15.1%) and were sampled only in South,
261 East, and Southeast Asia. Rats (*Rattus* spp.) were sampled in both geographic regions, but
262 infected animals were almost entirely found in South, East, and Southeast Asia, and coinfection
263 was high within this group (44/403; 10.9%). Virus family testing effort was also positively
264 correlated with coinfection in the all-animals model (OR = 1.11, 95% CI: 1.00-1.24, $p = 0.042$;
265 Fig. 2A, Table S3) and the rodents model (OR = 1.57, 95% CI: 1.29-1.94, $p < 0.001$; Fig. 2E,
266 Table S5).
267

268 *Evidence of virus-virus associations*

269 Coinfection networks revealed that certain viruses–namely, coronaviruses (CoVs),
270 paramyxoviruses (PMVs), and orthomyxoviruses (specifically, influenza A virus)–were most
271 frequently detected together (Fig. 2B, 2D, 2F). Notably, there were 108 CoV-CoV coinfections
272 (57 in bats, 48 in rodents), 69 CoV-PMV coinfections (44 in bats, 2 in rodents), 45 CoV-
273 influenza coinfections (23 in bats), and 10 PMV-influenza coinfections (1 in bats). Binomial
274 tests indicated that CoV-PMV, CoV-influenza, and PMV-rhabdovirus coinfections occurred
275 significantly more than expected in all animals and in bats, while coinfections with multiple
276 coronaviruses occurred significantly more than expected in rodents (Tables S6-S8). All other
277 virus family coinfections occurred < 10 times, and no coinfections involving arenaviruses,
278 filoviruses, or hantaviruses were detected.

279 Examining coinfection patterns at the level of individual viruses, we found that viruses
280 that are transmitted by poultry and other livestock (influenza A virus, Newcastle disease virus,
281 and infectious bronchitis virus) had the highest degree centrality in the network (Fig. 3).
282 However, a handful of commonly occurring betacoronaviruses, including murine coronavirus,
283 PREDICT CoV-16 and PREDICT CoV-17, and Bat coronavirus HKU9, were also well
284 connected within the network.

285

286 **Discussion**

287 Here, we conducted the first analysis of viral coinfection patterns across multiple continents and
288 host taxonomic groups. We found that within the virus groups sampled, coinfection is rare –
289 detected in just 223 of 65,662 animals – but occurs more frequently than expected, particularly
290 among specific virus groups. Our findings suggest that widely-occurring viruses could be

291 important to account for when researching other pathogens. For example, influenza A virus was
292 often detected in combination with other viruses; one possible explanation for this finding is that
293 influenza A virus might alter host susceptibility to secondary infections. Future work could
294 explore how specific viral coinfections influence factors such as the magnitude and duration of
295 shedding, particularly as it could relate to zoonotic spillover risk (Schuh et al. 2025). We also
296 found coinfection dynamics that suggest further testing would be beneficial to resolve whether
297 these associations are modulated by broad differences in host immunology and/or fine-scale host
298 contact processes, including in anthropogenic settings like farms and the wildlife trade. These
299 findings are also of potential importance for public health, as they highlight the need for both
300 basic research on host-parasite interactions, and surveillance of environments where coinfection
301 can produce new reassortant and recombinant viruses with epidemic or pandemic potential
302 (Anthony et al. 2017a).

303 Although our study is the largest macroecological study of coinfection to date, our
304 findings should be interpreted in light of the limitations of our data. Most importantly, the
305 PREDICT project was not designed with ecological research as the primary objective, and so the
306 testing data have uneven coverage both across and within taxonomic groups. For instance, the
307 PREDICT project was almost single-handedly responsible for bats becoming the best-studied
308 wildlife hosts of viruses (Gibb et al. 2022). Given that over 60% of specimens were collected
309 from bats, these data probably drove many of our broad-scale findings. However, only half of all
310 bat families (10 of 21) were sampled, and an emerging body of work highlights that while bats
311 are unique viral reservoirs, there is still considerable important (and interesting) variation within
312 the group (Becker et al. 2025). We also note that data collection for PREDICT was cross-
313 sectional in nature, and therefore interpret our results as associations that warrant further

314 mechanistic testing, for example using longitudinal or experimental approaches to test causality
315 of interactions. Finally, the surveillance nature of the data should not be viewed as entirely
316 representative of underlying populations. More complete sampling agnostic to host and virus
317 taxonomy is therefore required for more confident understanding of coinfection dynamics across
318 and within host groups. However, we note that more complete, unbiased datasets do not yet
319 exist, and leveraging existing sampling efforts provide a foundation for future sampling
320 priorities.

321 Future work could build on initial steps here by designing surveillance to more explicitly
322 search for and report coinfection. A recent systematic review found that coinfections were
323 common in bats, but were incidentally reported within study findings (Jones et al. 2023). More
324 deliberate focus on sampling to capture coinfection dynamics may also require specific sampling
325 strategies: for example, reliably detecting coinfection in an individual host requires capture of
326 animals in-hand (versus, e.g., pooled or environmental sampling). Similarly, different parasites
327 are associated with different organs and sites in the body, and so prioritizing particular samples
328 or assays could be necessary, depending on study aims. Although broad sample and parasite
329 coverage presents substantial cost considerations, several recent developments offer possible
330 solutions. Multiplex, metagenomic, and metatranscriptomic methods all provide a fuller view of
331 the host virome, and are especially useful when sample sizes are small due to biological
332 limitations (e.g., collecting blood from small animals) (Boyd et al. 2015; Raghwani et al. 2023;
333 Chang et al. 2020). Longitudinal surveillance also improves the odds of viral detection, and
334 allows researchers to test hypotheses about asynchronous interactions between parasites, host
335 immunity, and external environmental factors (Plowright et al. 2019). Finally, host-level data on
336 wildlife disease are also shared infrequently (Schwantes et al. 2025), preventing the kinds of re-

analyses we conducted here, or approaches that synthesize findings through systematic reviews and meta-analyses (Jones et al. 2023). Developing a better global view of wildlife disease dynamics is an essential step to conducting more macroecological research on the consequences of coinfection.

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**Conflict of Interest Statement**

We declare no conflicts of interest.

## References


Aguillon, Samantha, Magali Turpin, Gildas Le Minter, et al. 2025. "Bat Things Come in Threes: Within-Host Dynamics of Herpesvirus Triple Infection in Bats." *Journal of Virology* 99 (11): e0160525.

Albery, Gregory F., Amy R. Sweeny, Daniel J. Becker, and Shweta Bansal. 2022. "Fine-scale Spatial Patterns of Wildlife Disease Are Common and Understudied." *Functional Ecology* 36 (1): 214–225.

Anthony, Simon J., Christine K. Johnson, Denise J. Greig, et al. 2017a. "Global Patterns in Coronavirus Diversity." *Virus Evolution* 3 (1): vex012.

Anthony, S. J., K. Gilardi, V. D. Menachery, et al. 2017b. "Further Evidence for Bats as the Evolutionary Source of Middle East Respiratory Syndrome Coronavirus." *mBio* 8 (2). https://doi.org/10.1128/mBio.00373-17.

Becker, Daniel J., Amanda Vicente-Santos, Ashley B. Reers, et al. 2025. "Diverse Hosts, Diverse Immune Systems: Evolutionary Variation in Bat Immunology." *Annals of the New York Academy of Sciences* 1550 (1): 151-172. https://doi.org/10.1111/nyas.15395.

Boyd, Victoria, Ina Smith, Gary Crameri, et al. 2015. "Development of Multiplexed Bead Arrays for the Simultaneous Detection of Nucleic Acid from Multiple Viruses in Bat Samples." *Journal of Virological Methods* 223 (October): 5–12.

Brook, Cara E., Hafaliana C. Ranaivoson, Christopher C. Broder, et al. 2019. "Disentangling Serology to Elucidate Henipa- and Filovirus Transmission in Madagascar Fruit Bats." *The*

*Journal of Animal Ecology* 88 (7): 1001–1016.

Brooks, Molliee, Kasper Kristensen, Koenj van Benthem, et al. 2017. "GlmmTMB Balances Speed and Flexibility among Packages for Zero-Inflated Generalized Linear Mixed Modeling." *The R Journal* 9 (2): 378.

Budischak, Sarah A., Christina B. Hansen, Quentin Caudron, et al. 2017. "Feeding Immunity: Physiological and Behavioral Responses to Infection and Resource Limitation." *Frontiers in Immunology* 8: 1914.

Calisher, Charles H., James E. Childs, Hume E. Field, Kathryn V. Holmes, and Tony Schountz. 2006. "Bats: Important Reservoir Hosts of Emerging Viruses." *Clinical Microbiology Reviews* 19 (3): 531–545.

Carlson, Colin J., Cole B. Brookson, Daniel J. Becker, et al. 2025. "Pathogens and Planetary Change." *Nature Reviews Biodiversity* 1 (1): 32–49.

Chang, Wei-Shan, John-Sebastian Eden, Jane Hall, Mang Shi, Karrie Rose, and Edward C. Holmes. 2020. "Metatranscriptomic Analysis of Virus Diversity in Urban Wild Birds with Paretic Disease." *Journal of Virology* 94 (18). https://doi.org/10.1128/JVI.00606-20.

Cohen, Jeremy M., Erin L. Sauer, Olivia Santiago, Samuel Spencer, and Jason R. Rohr. 2020. "Divergent Impacts of Warming Weather on Wildlife Disease Risk across Climates." *Science* 370 (6519). https://doi.org/10.1126/science.abb1702.

Cohen, Lily E., Anna C. Fagre, Binqi Chen, Colin J. Carlson, and Daniel J. Becker. 2023. "Coronavirus Sampling and Surveillance in Bats from 1996–2019: A Systematic Review

390 and Meta-Analysis." *Nature Microbiology* 8 (6): 1176–1186.

391 Cox, F. E. G. 2001. "Concomitant Infections, Parasites and Immune Responses." *Parasitology*
392 122 (S1): S23–S38.

393 Eskew, Evan A., Kevin J. Olival, Jonna A. K. Mazet, and Peter Daszak. 2025. "A Global-Scale
394 Dataset of Bat Viral Detection Suggests That Pregnancy Reduces Viral Shedding."
395 *Proceedings of the Royal Society B: Biological Sciences* 292 (2045): 20242381.

396 Ezenwa, Vanessa O., Sarah A. Budischak, Peter Buss, et al. 2021. "Natural Resistance to Worms
397 Exacerbates Bovine Tuberculosis Severity Independently of Worm Coinfection."
398 *Proceedings of the National Academy of Sciences* 118 (3).
399 https://doi.org/10.1073/pnas.2015080118.

400 Ezenwa, Vanessa O., and Anna E. Jolles. 2015. "Opposite Effects of Anthelmintic Treatment on
401 Microbial Infection at Individual versus Population Scales." *Science* 347 (6218): 175–177.

402 Fenton, Andy, Sarah C. L. Knowles, Owen L. Petchey, and Amy B. Pedersen. 2014. "The
403 Reliability of Observational Approaches for Detecting Interspecific Parasite Interactions:
404 Comparison with Experimental Results." *International Journal for Parasitology* 44 (7):
405 437–445.

406 Fenton, Andy, and Amy B. Pedersen. 2005. "Community Epidemiology Framework for
407 Classifying Disease Threats." *Emerging Infectious Diseases* 11 (12): 1815–1821.

408 Gibb, Rory, Gregory F. Albery, Nardus Mollentze, et al. 2022. "Mammal Virus Diversity
409 Estimates Are Unstable due to Accelerating Discovery Effort." *Biology Letters* 18 (1):

410 20210427.

411 Goenka, Anu, and Tobias R. Kollmann. 2015. "Development of Immunity in Early Life." *The*
412 *Journal of Infection* 71 Suppl 1 (June): S112–20.

413 Goldstein, Tracey, Simon J. Anthony, Aiah Gbakima, et al. 2018. "The Discovery of Bombali
414 Virus Adds Further Support for Bats as Hosts of Ebolaviruses." *Nature Microbiology* 3
415 (10): 1084–1089.

416 Gorsich, Erin E., Rampal S. Etienne, Jan Medlock, et al. 2018. "Opposite Outcomes of
417 Coinfection at Individual and Population Scales." *Proceedings of the National Academy of*
418 *Sciences* 115 (29): 7545–7550.

419 Graham, Andrea L. 2008. "Ecological Rules Governing Helminth–microparasite Coinfection."
420 *Proceedings of the National Academy of Sciences* 105 (2): 566–570.

421 Heckley, Alexis, and Daniel Becker. 2024. "Parasite Traits Shape the Association between
422 Forest Loss and Infection: A Global Meta-Analysis." In *EcoEvoRxiv*. December 19.
423 https://doi.org/10.32942/x2pd2q.

424 Huong, Nguyen Quynh, Nguyen Thi Thanh Nga, Nguyen Van Long, et al. 2020. "Coronavirus
425 Testing Indicates Transmission Risk Increases along Wildlife Supply Chains for Human
426 Consumption in Viet Nam, 2013-2014." *PloS One* 15 (8): e0237129.

427 Irving, Aaron T., Matae Ahn, Geraldine Goh, Danielle E. Anderson, and Lin-Fa Wang. 2021.
428 "Lessons from the Host Defences of Bats, a Unique Viral Reservoir." *Nature* 589 (7842):
429 363–370.

Jones, Brent D., Eli J. Kaufman, and Alison J. Peel. 2023. “Viral Co-Infection in Bats: A Systematic Review.” *Viruses* 15 (9): 1860.

Knowles, Sarah C. L., Andy Fenton, Owen L. Petchey, Trevor R. Jones, Rebecca Barber, and Amy B. Pedersen. 2013. “Stability of within-Host - Parasite Communities in a Wild Mammal System.” *Proceedings of the Royal Society of London B: Biological Sciences* 280 (1762). http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2013.0598.

Kuhn, Jens H., Katherine Brown, Scott Adkins, et al. 2024. “Promotion of Order Bunyavirales to Class Bunyaviricetes to Accommodate a Rapidly Increasing Number of Related Polyploviricotine Viruses.” *Journal of Virology* 98 (10): e0106924.

Letko, Michael, Stephanie N. Seifert, Kevin J. Olival, Raina K. Plowright, and Vincent J. Munster. 2020. “Bat-Borne Virus Diversity, Spillover and Emergence.” *Nature Reviews Microbiology* 18 (8): 461–471.

Moreno, Kelsey R., Maya Weinberg, Lee Harten, et al. 2021. “Sick Bats Stay Home Alone: Fruit Bats Practice Social Distancing When Faced with an Immunological Challenge.” *Annals of the New York Academy of Sciences* 1505 (1): 178–190.

Pedersen, Amy B., and Andy Fenton. 2007. “Emphasizing the Ecology in Parasite Community Ecology.” *Trends in Ecology & Evolution* 22 (3): 133–139.

Pedersen, Thomas Lin. 2024. “Ggraph: An Implementation of Grammar of Graphics for Graphs and Networks.” R Package Version 2.2.1. https://github.com/thomasp85/ggraph, https://ggraph.data-imaginist.com.

450 Peel, Alison J., Manuel Ruiz-Aravena, Karan Kim, et al. 2025. "Synchronized Seasonal
451 Excretion of Multiple Coronaviruses Coincides with High Rates of Coinfection in Immature
452 Bats." *Nature Communications* 16 (1): 6579.

453 Petney, Trevor N., and Ross H. Andrews. 1998. "Multiparasite Communities in Animals and
454 Humans: Frequency, Structure and Pathogenic Significance." *International Journal for*
455 *Parasitology* 28 (3): 377–393.

456 Plowright, Raina K., Daniel J. Becker, Hamish McCallum, and Kezia R. Manlove. 2019.
457 "Sampling to Elucidate the Dynamics of Infections in Reservoir Hosts." *Philosophical*
458 *Transactions of the Royal Society of London. Series B, Biological Sciences* 374 (1782):
459 20180336.

460 Plowright, Raina K., Alison J. Peel, Daniel G. Streicker, et al. 2016. "Transmission or within-
461 Host Dynamics Driving Pulses of Zoonotic Viruses in Reservoir-Host Populations." *PLoS*
462 *Neglected Tropical Diseases* 10 (8): e0004796.

463 PREDICT Consortium. 2020. *Advancing Global Health Security at the Frontiers of Disease*
464 *Emergence*. One Health Institute.

465 Raghwani, Jayna, Christina L. Faust, Sarah François, et al. 2023. "Seasonal Dynamics of the
466 Wild Rodent Faecal Virome." *Molecular Ecology* 32 (17): 4763–4776.

467 R Core Team. 2025. "R: A Language and Environment for Statistical Computing." R Foundation
468 for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

469 Ripperger, Simon P., Sebastian Stockmaier, and Gerald G. Carter. 2020. "Tracking Sickness

470 Effects on Social Encounters via Continuous Proximity Sensing in Wild Vampire Bats."
471 *Behavioral Ecology* 31 (6): 1296–1302.

472 Sánchez, Cecilia A., Hongying Li, Kendra L. Phelps, et al. 2021. "A Strategy to Assess Spillover
473 Risk of Bat SARS-Related Coronaviruses in Southeast Asia." *Nature Communications* 13
474 (4380). https://doi.org/10.1038/s41467-022-31860-w.

475 Saylors, Karen, David J. Wolking, Emily Hagan, et al. 2021. "Socializing One Health: An
476 Innovative Strategy to Investigate Social and Behavioral Risks of Emerging Viral Threats."
477 *One Health Outlook* 3 (1): 11.

478 Schuh, Amy J., Brian R. Amman, Jonathan C. Guito, James C. Graziano, Tara K. Sealy, and
479 Jonathan S. Towner. 2025. "Modeling Natural Coinfection in a Bat Reservoir Shows
480 Modulation of Marburg Virus Shedding and Spillover Potential." *PLoS Pathogens* 21 (3):
481 e1012901.

482 Schwantes, Collin J., Cecilia A. Sánchez, Tess Stevens, et al. 2025. "A Minimum Data Standard
483 for Wildlife Disease Research and Surveillance." *Scientific Data* 12 (1): 1054.

484 Sweeny, Amy R., Gregory F. Albery, Daniel J. Becker, Evan A. Eskew, and Colin J. Carlson.
485 2021. "Synzootics." *The Journal of Animal Ecology* 90 (12): 2744–2754.

486 Sweeny, Amy R., Courtney A. Thomason, Edwin A. Carbajal, Christina B. Hansen, Andrea L.
487 Graham, and Amy B. Pedersen. 2020. "Experimental Parasite Community Perturbation
488 Reveals Associations between Sin Nombre Virus and Gastrointestinal Nematodes in a
489 Rodent Reservoir Host." *Biology Letters* 16 (12): 20200604.

490 Telfer, Sandra, Xavier Lambin, Richard Birtles, et al. 2010. "Species Interactions in a Parasite
491 Community Drive Infection Risk in a Wildlife Population." *Science (New York, N.Y.)* 330
492 (6001): 243–246.

493 Wacharapluesadee, Supaporn, Prateep Duengkae, Aingorn Chaiyes, et al. 2018. "Longitudinal
494 Study of Age-Specific Pattern of Coronavirus Infection in Lyle's Flying Fox (*Pteropus*
495 *Lylei*) in Thailand." *Virology Journal* 15 (1): 38.

496 Warmuth, Vera M., Dirk Metzler, and Veronica Zamora-Gutierrez. 2023. "Human Disturbance
497 Increases Coronavirus Prevalence in Bats." *Science Advances* 9 (13): eadd0688.

498 Wells, Heather L., Cassandra M. Bonavita, Isamara Navarrete-Macias, Blake Vilchez, Angela L.
499 Rasmussen, and Simon J. Anthony. 2023. "The Coronavirus Recombination Pathway." *Cell*
500 *Host & Microbe* 31 (6): 874–889.

501 Willoughby, Anna, Kendra Phelps, Kevin Olival, and PREDICT Consortium. 2017. "A
502 Comparative Analysis of Viral Richness and Viral Sharing in Cave-Roosting Bats."
503 *Diversity* 9 (3): 35.

504

505 **Figure captions**

506

507 **Figure 1. A.** Number of unique animals infected with one or more viruses, separated by
508 taxonomic group. Groups in which only single infections were observed are not displayed (dogs:
509 $n$ = 10; goats/sheep: $n$ = 4; cattle/buffalo: $n$ = 2; carnivores: $n$ = 1; other: $n$ = 1). **B.** Distribution
510 of animals infected with one or more viruses, colored by taxonomic group. Point size
511 corresponds to the number of animals captured at a given location; coordinates were truncated so
512 that nearby locations could be grouped together. Points are partially transparent and jittered for
513 improved visibility.

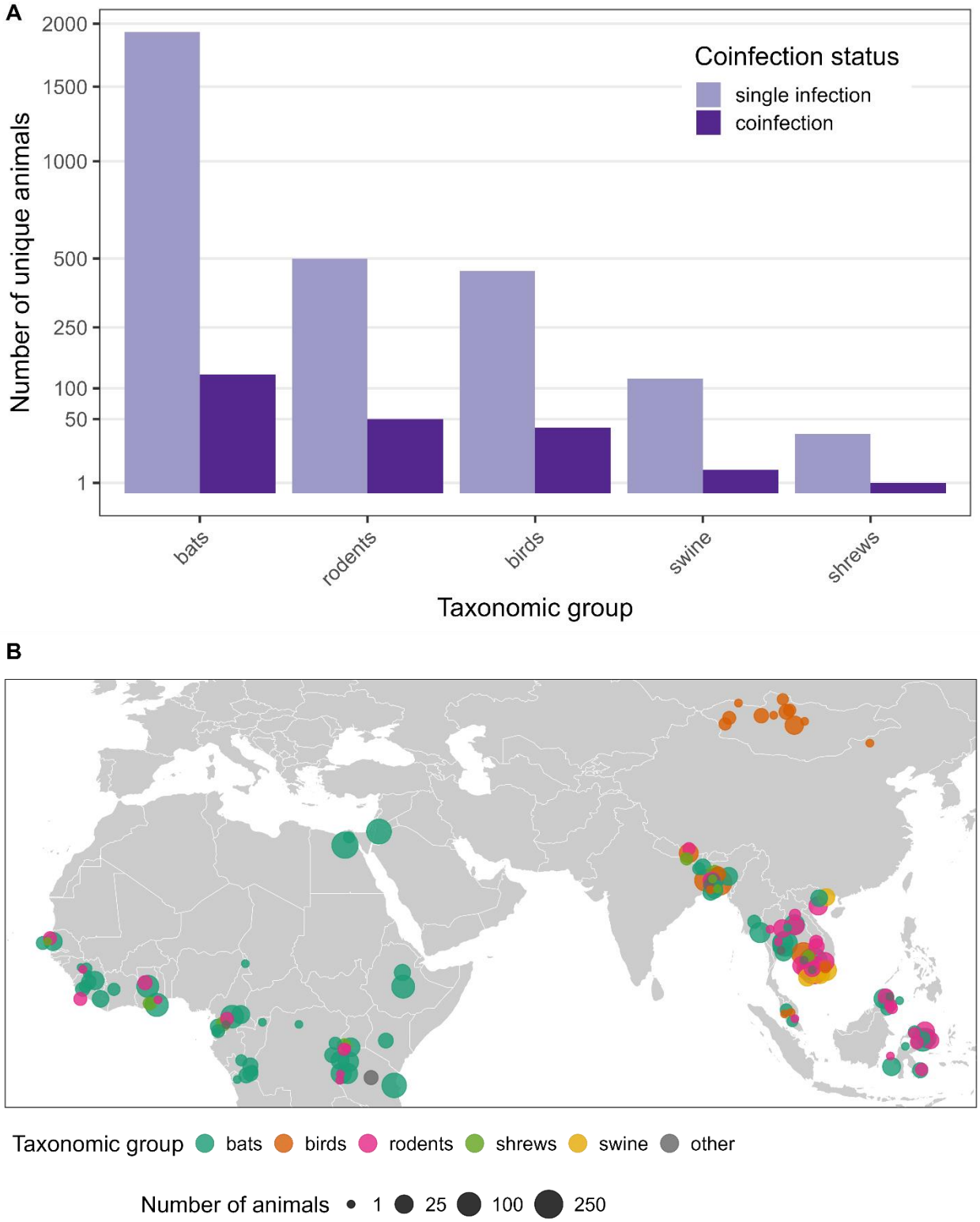
514

515 **Figure 2.** Predictors of virus coinfection (**A**, **C**, **E**) and virus family coinfection networks (**B**, **D**,
516 **E**). **Left column:** generalized linear model outputs (odds ratios and 95% confidence intervals
517 (CIs)) for predictors of coinfection are shown for all animals (**A**), bats only (**C**), and rodents only
518 (**E**). CIs that do not cross the vertical line at 1 (i.e., statistically significant) are purple. Reference
519 levels for categorical predictors are Africa and West Asia (geographic region), bats (taxonomic
520 group), female (sex), adult (age class), wild free-ranging (captivity status), and non-cave roosting
521 (roosting status). SES Asia = South, East, and Southeast Asia. **Right column:** networks of virus
522 family coinfection are shown for all animals (**B**), bats only (**D**), and rodents only (**F**). Nodes
523 represent virus families, while edges represent the frequency of virus family coinfection.
524 Asterisks indicate coinfections that occurred significantly more than expected.

525

526 **Figure 3.** Virus coinfection network. Nodes represent individual viruses, while edges represent
527 the frequency of virus coinfection. Only nodes with degree centrality ≥ 4 are labeled, and nodes
528 not involved in coinfections are not displayed.
529

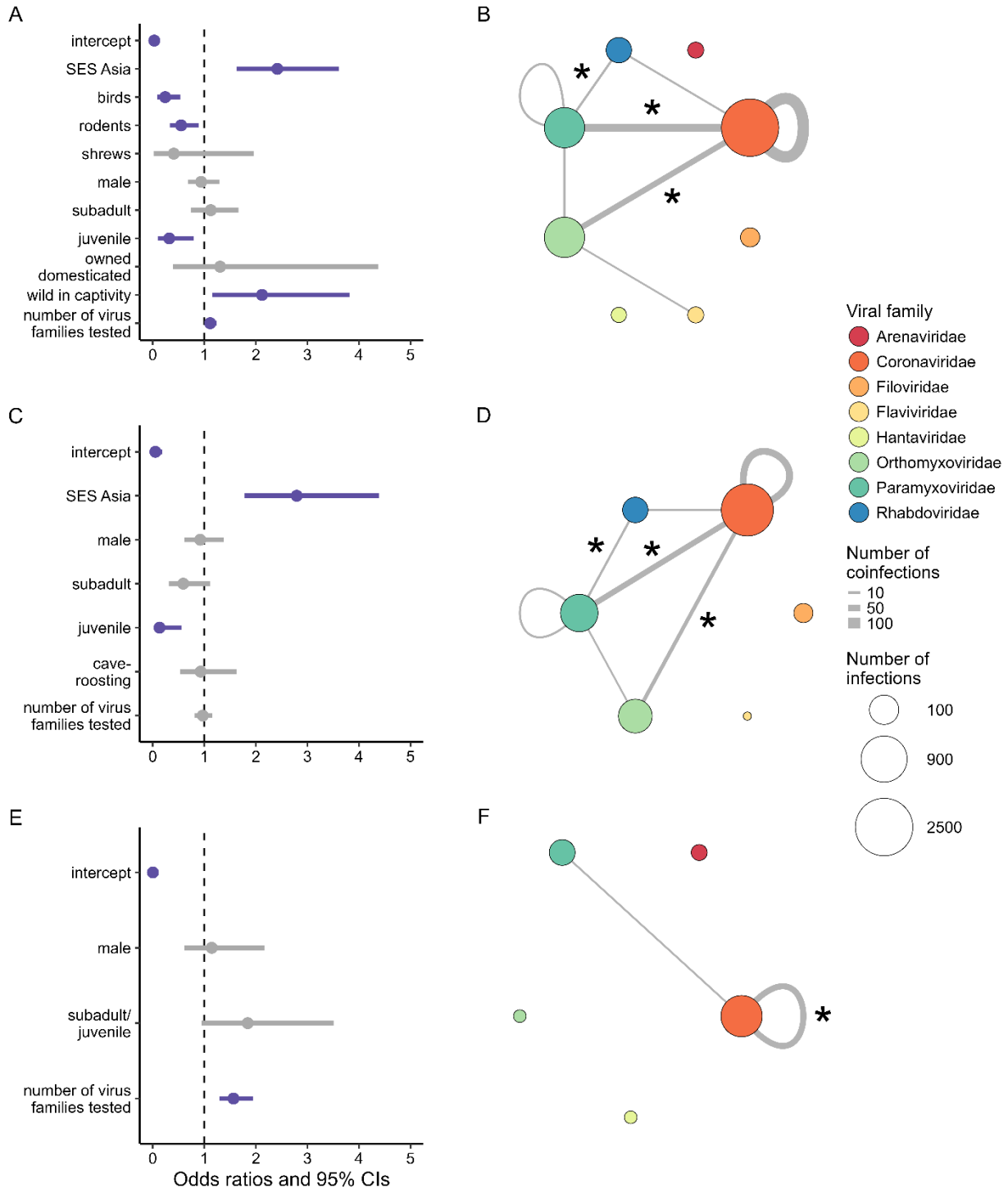
530 **Figure 1**




531

532

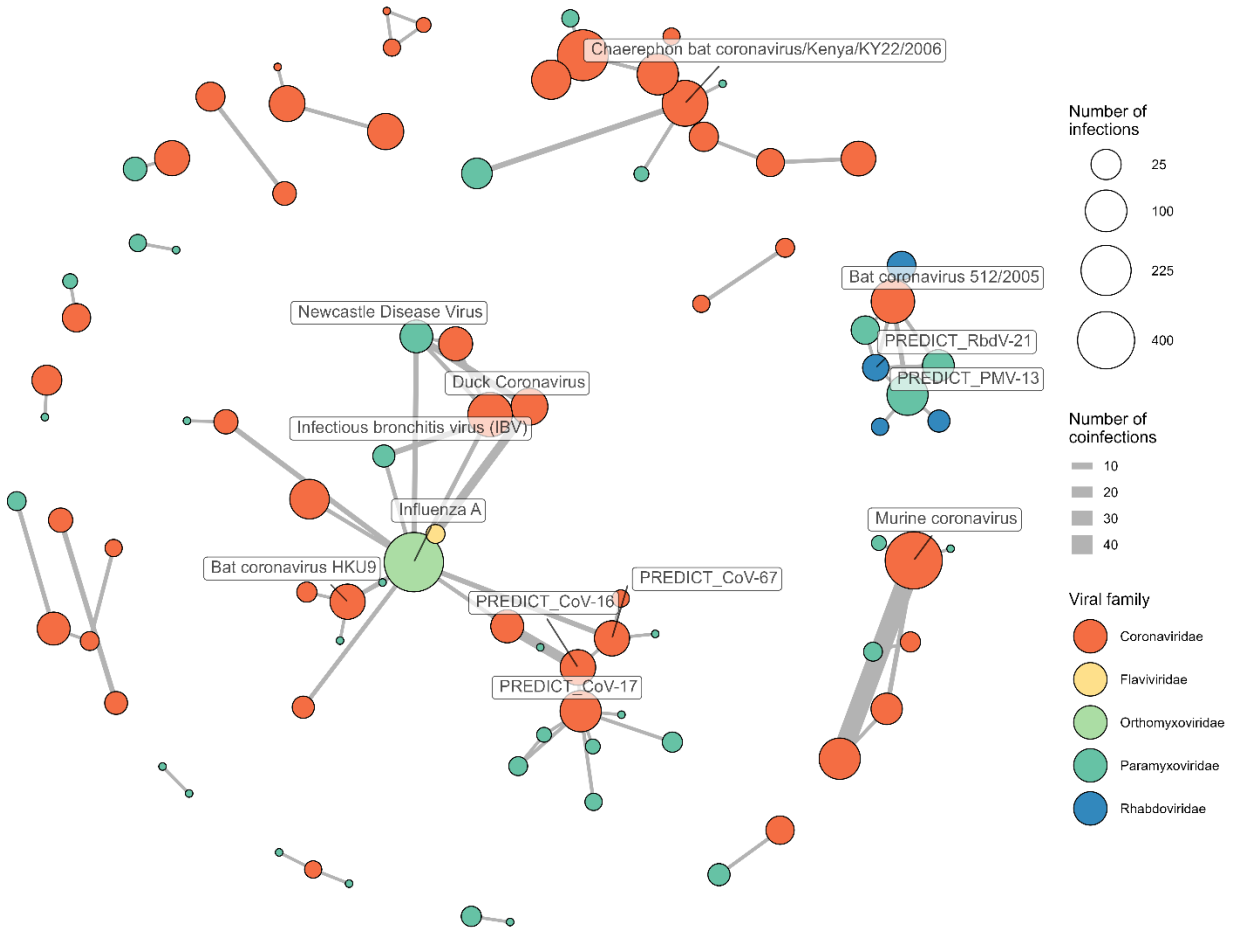
533 **Figure 2**




534

535

536 **Figure 3.**




537

538

**Supporting Information for:**

# The macroecology of viral coinfection

Cecilia A. Sánchez[1], Colin J. Carlson[1], Amy R. Sweeny[2*]

This Supporting Information contains Figures S1-3 and Tables S1-8.

**Figure S1.** Map of sampling effort in the PREDICT-2 dataset; color corresponds to the number of unique specimens collected in a country. Note that the color legend is on a log scale.

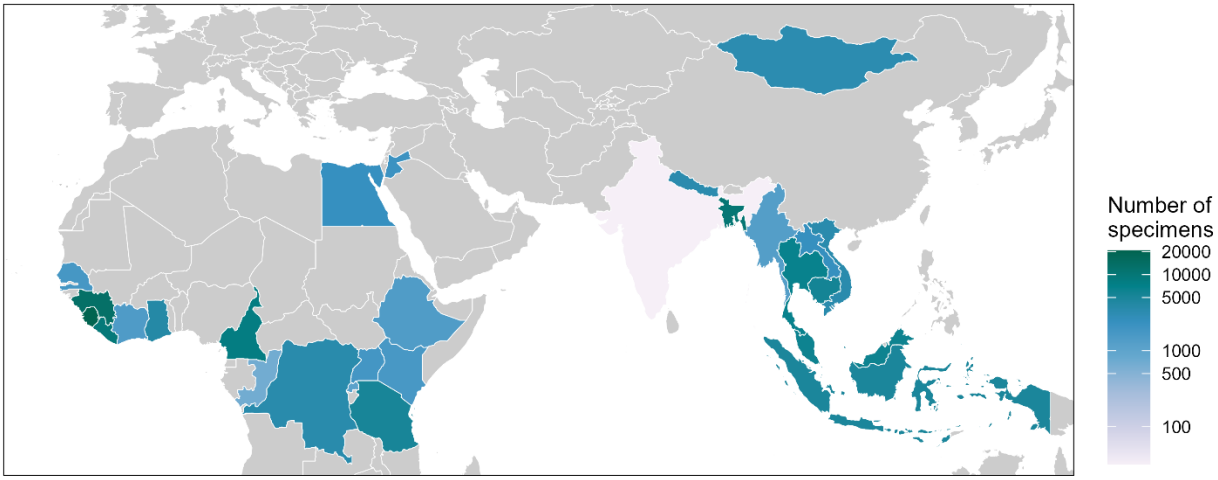
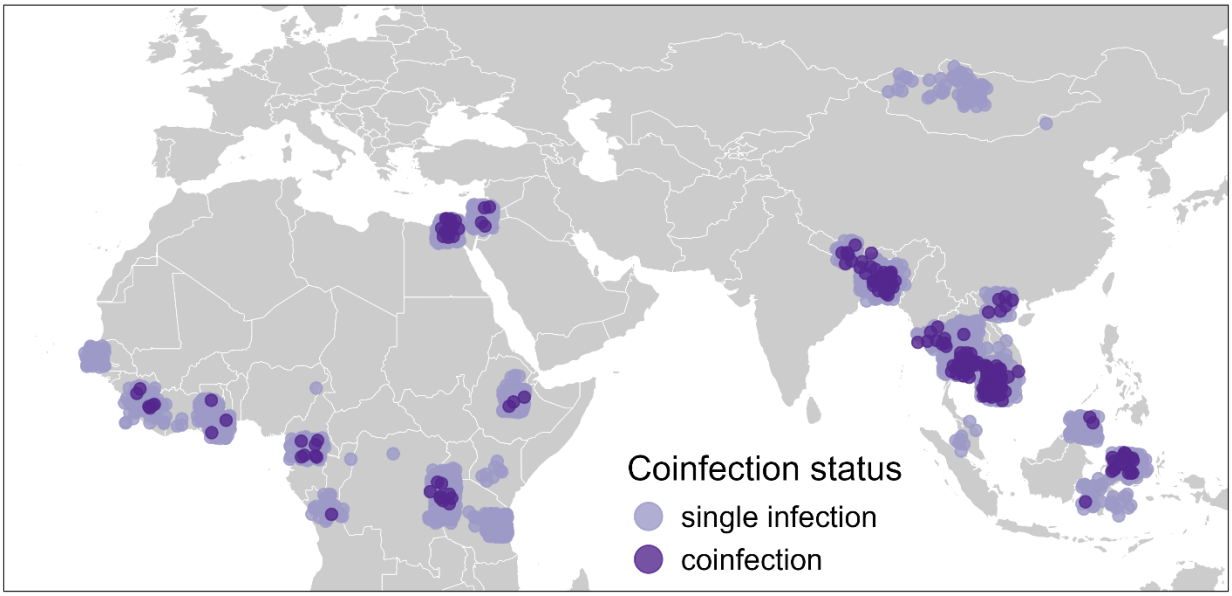


**Figure S2.** Geographic distribution of unique animals infected with one or more viruses. Points are partially transparent and jittered for improved visibility.

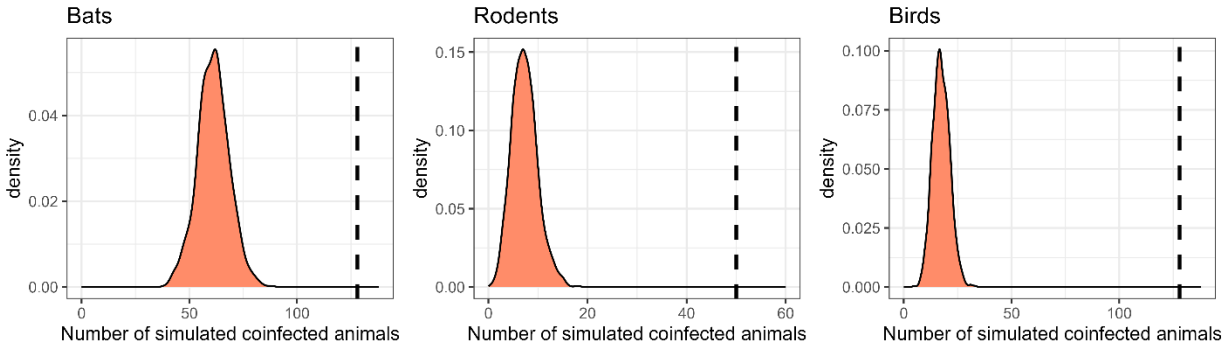


557 **Figure S3.** Density plots of 1000 coinfection simulations each for bats (*n* = 36,181), rodents (*n* =

558 12,876), and birds (*n* = 5,617). The observed number of coinfected animals (in the PREDICT-2

559 data) for each taxonomic group is plotted with a dashed vertical line.

560




561

562

563 **Table S1.** Number of infected animals and coinfected animals within the PREDICT-2 dataset,
564 separated by taxonomic group. NA = not applicable (if no animals were infected, then none
565 could be coinfected).
566

| **Animal taxonomic group** | **Total number tested** | **Number infected (≥ 1 viruses detected)** | **Number coinfected (≥ 2 viruses detected)** |
|---|---|---|---|
| Bats | 36181 | 2059 | 128 |
| Birds | 5617 | 487 | 39 |
| Camels | 356 | 0 | NA |
| Carnivores | 406 | 1 | 0 |
| Cats | 62 | 0 | NA |
| Cattle/Buffalo | 517 | 2 | 0 |
| Dogs | 961 | 10 | 0 |
| Goats/Sheep | 441 | 4 | 0 |
| Horses | 1 | 0 | NA |
| Non-human primates | 6196 | 0 | NA |
| Pangolins | 332 | 0 | NA |
| Rodents | 12876 | 549 | 50 |
| Shrews | 1050 | 33 | 1 |
| Swine | 611 | 125 | 5 |
| Ungulates | 32 | 0 | NA |
| Other | 23 | 1 | 0 |

567

568 **Table S2.** Characteristics of the 12 animals that were infected with three viruses. Note that host

569 and virus names are provided as originally described in the PREDICT dataset, and may not

570 conform to current taxonomies.

571

| PREDICT sample ID | Country | Taxonomic group | Species | Virus family | Virus |
|---|---|---|---|---|---|
| 56344 | Egypt | Bats | Rousettus aegyptiacus | Coronaviridae | Bat coronavirus HKU9 |
| | | | | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | PREDICT_PMV-116 |
| 108315 | Thailand | Bats | Pteropus lylei | Coronaviridae | PREDICT_CoV-17 |
| | | | | Paramyxoviridae | PREDICT_PMV-6 |
| | | | | Paramyxoviridae | PREDICT_PMV-128 |
| 108328 | Thailand | Bats | Pteropus giganteus | Coronaviridae | PREDICT_CoV-16 |
| | | | | Coronaviridae | PREDICT_CoV-68 |
| | | | | Paramyxoviridae | PREDICT_PMV-125 |
| 133237 | Cameroon | Bats | Rhinolophus cf. alcyone | Coronaviridae | Kenya bat coronavirus/BtKY83/59/58 |
| | | | | Coronaviridae | PREDICT_CoV-70 |
| | | | | Coronaviridae | PREDICT_CoV-109 |
| 145203 | Cambodia | Bats | Scotophilus kuhlii | Coronaviridae | Bat coronavirus 512/2005 |
| | | | | Paramyxoviridae | PREDICT_PMV-66 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | Rhabdoviridae | PREDICT_RbdV-21 |
| 54944 | Bangladesh | Birds | Anas platyrhynchos domesticus | Coronaviridae | Duck Coronavirus |
| | | | | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | Newcastle Disease Virus |
| 108865 | Bangladesh | Birds | Anas platyrhynchos domesticus | Coronaviridae | Duck Coronavirus |
| | | | | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | Newcastle Disease Virus |
| 126366 | Bangladesh | Birds | Cairina moschata | Coronaviridae | Infectious bronchitis virus (IBV) |
| | | | | Coronaviridae | Duck Coronavirus |
| | | | | Orthomyxoviridae | Influenza A |
| 126464 | Bangladesh | Birds | Anas platyrhynchos domesticus | Coronaviridae | Duck Coronavirus |
| | | | | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | Newcastle Disease Virus |
| 126519 | Bangladesh | Birds | Anas platyrhynchos domesticus | Coronaviridae | Duck Coronavirus |
| | | | | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | Avian Paramyxovirus 6 |
| 126536 | Bangladesh | Birds | Anser cygnoides domesticus | Coronaviridae | Duck Coronavirus |
| | | | | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | Avian Paramyxovirus 6 |
| 126561 | Bangladesh | Birds | Anas | Coronaviridae | Duck Coronavirus |

| | | | | | |
|---|---|---|---|---|---|
| | | | platyrhynchos domesticus | Orthomyxoviridae | Influenza A |
| | | | | Paramyxoviridae | Avian Paramyxovirus 6 |

572

573 **Table S3.** Model coefficients for a generalized linear model to examine effects of taxonomy,

574 sex, age, captivity status, and testing effort on virus coinfection status (binary: single infection or

575 coinfection) in bats, birds, rodents, and shrews. *p* values < 0.05 are in bold.

576

| **Variable** | | **Estimate** | **SE** | **z** | ***p*** | **Odds ratio (95% CI)** |
|---|---|---|---|---|---|---|
| Intercept | | -3.51 | 0.31 | -11.20 | **< 0.001** | 0.03 (0.02-0.05) |
| Geographic region | Africa and West Asia | – | – | – | – | – |
| | South, East, and Southeast Asia | 0.88 | 0.20 | 4.35 | **< 0.001** | 2.42 (1.63-3.61) |
| Taxonomic group | bats | – | – | – | – | – |
| | birds | -1.42 | 0.45 | -3.18 | **0.001** | 0.24 (0.09-0.55) |
| | rodents | -0.60 | 0.25 | -2.36 | 0.018 | 0.55 (0.33-0.89) |
| | shrews | -0.90 | 1.03 | -0.88 | 0.382 | 0.41 (0.02-1.96) |
| Sex | female | – | – | – | – | – |
| | male | -0.06 | 0.16 | -0.39 | 0.697 | 0.94 (0.68-1.29) |
| Age class | adult | – | – | – | – | – |
| | subadult | 0.12 | 0.20 | 0.58 | 0.563 | 1.13 (0.74-1.66) |
| | juvenile | -1.14 | 0.53 | -2.17 | **0.030** | 0.32 (0.10-0.80) |
| Captivity status | free-ranging wild animal | – | – | – | – | – |
| | owned domesticated | 0.27 | 0.60 | 0.44 | 0.658 | 1.31 (0.39-4.38) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | animal | | | | | |
| | wild animal in captivity | 0.75 | 0.30 | 2.48 | **0.013** | 2.12 (1.16-3.82) |
| Number of virus families tested | | 0.11 | 0.05 | 2.04 | **0.042** | 1.11 (1.00-1.24) |

577

578

579 **Table S4.** Model coefficients (fixed effects only) for a generalized linear mixed model to

580 examine effects of sex, age, cave roosting behavior, and testing effort on virus coinfection status

581 (binary: single infection or coinfection) in bats. *p* values < 0.05 are in bold.

582

| **Variable** | | **Estimate** | **SE** | **z** | ***p*** | **Odds ratio (95% CI)** |
|---|---|---|---|---|---|---|
| Intercept | | -2.97 | 0.64 | -4.61 | **< 0.001** | 0.05 (0.01-0.18) |
| Geographic region | Africa and West Asia | – | – | – | – | – |
| | South, East, and Southeast Asia | 1.03 | 0.23 | 4.44 | **< 0.001** | 2.79 (1.78-4.39) |
| Sex | female | – | – | – | – | – |
| | male | -0.08 | 0.21 | -0.40 | 0.686 | 0.92 (0.61-1.38) |
| Age class | adult | – | – | – | – | – |
| | subadult | -0.53 | 0.32 | -1.64 | 0.102 | 0.59 (0.32-1.11) |
| | juvenile | -2.03 | 0.73 | -2.77 | **0.006** | 0.13 (0.03-0.55) |
| Cave roosting | no | – | – | – | – | – |
| | yes | -0.07 | 0.29 | -0.26 | 0.796 | 0.93 (0.53-1.63) |
| Number of virus families tested | | -0.03 | 0.09 | -0.33 | 0.741 | 0.97 (0.81-1.16) |

583

584 **Table S5.** Model coefficients for a generalized linear model to examine effects of sex, age, and
585 testing effort on virus coinfection status (binary: single infection or coinfection) in rodents. *p*
586 values < 0.05 are in bold.
587

| **Variable** | | **Estimate** | **SE** | **z** | ***p*** | **Odds ratio (95% CI)** |
|---|---|---|---|---|---|---|
| Intercept | | -5.79 | 0.86 | -6.76 | **< 0.001** | 0.00 (0.00-0.01) |
| Sex | female | – | – | – | | – |
| | male | 0.13 | 0.32 | 0.42 | 0.676 | 1.14 (0.61-2.17) |
| Age class | adult | – | – | – | | – |
| | subadult/juvenile | 0.61 | 0.33 | 1.84 | 0.066 | 1.84 (0.95-3.51) |
| Number of virus families tested | | 0.45 | 0.10 | 4.36 | **< 0.001** | 1.57 (1.29-1.94) |

588

589 **Table S6.** Expected versus observed coinfection prevalence for virus families, for all animals. *P*

590 values were calculated from binomial tests and adjusted using a Bonferroni correction. $P_{adj}$

591 values < 0.05 are in bold.

592

| **Virus family combination** | **Expected coinfection prevalence** | **Observed coinfection prevalence** | **Observed > expected** | $P_{adj}$ |
|---|---|---|---|---|
| Arenaviridae-Arenaviridae | 5.80e-9 | 0 | No | 1 |
| Arenaviridae-Coronaviridae | 2.76e-6 | 0 | No | 1 |
| Arenaviridae-Filoviridae | 1.39e-8 | 0 | No | 1 |
| Arenaviridae-Flaviviridae | 5.80e-9 | 0 | No | 1 |
| Arenaviridae-Hantaviridae | 4.64e-9 | 0 | No | 1 |
| Arenaviridae-Orthomyxoviridae | 5.49e-7 | 0 | No | 1 |
| Arenaviridae-Paramyxoviridae | 5.42e-7 | 0 | No | 1 |
| Arenaviridae-Rhabdoviridae | 5.68e-8 | 0 | No | 1 |
| Coronaviridae-Coronaviridae | 1.31e-3 | 1.64-3 | Yes | 0.841 |
| Coronaviridae-Filoviridae | 6.62e-6 | 0 | No | 1 |
| Coronaviridae-Flaviviridae | 2.76e-6 | 0 | No | 1 |
| Coronaviridae-Hantaviridae | 2.21e-6 | 0 | No | 1 |
| Coronaviridae-Orthomyxoviridae | 2.61e-4 | 6.85e-4 | Yes | **< 0.001** |
| Coronaviridae-Paramyxoviridae | 2.58e-4 | 1.05e-3 | Yes | **< 0.001** |
| Coronaviridae-Rhabdoviridae | 2.70e-5 | 4.57e-5 | Yes | 1 |
| Filoviridae-Filoviridae | 3.34-8 | 0 | No | 1 |
| Filoviridae-Flaviviridae | 1.39e-8 | 0 | No | 1 |
| Filoviridae-Hantaviridae | 1.11e-8 | 0 | No | 1 |
| Filoviridae-Orthomyxoviridae | 1.32e-6 | 0 | No | 1 |

| | | | | |
|---|---|---|---|---|
| Filoviridae-Paramyxoviridae | 1.30e-6 | 0 | No | 1 |
| Filoviridae-Rhabdoviridae | 1.36e-7 | 0 | No | 1 |
| Flaviviridae-Flaviviridae | 5.80e-9 | 0 | No | 1 |
| Flaviviridae-Hantaviridae | 4.64e-9 | 0 | No | 1 |
| Flaviviridae-Orthomyxoviridae | 5.49e-7 | 1.52e-5 | Yes | 1 |
| Flaviviridae-Paramyxoviridae | 5.42e-7 | 0 | No | 1 |
| Flaviviridae-Rhabdoviridae | 5.68e-8 | 0 | No | 1 |
| Hantaviridae-Hantaviridae | 3.71e-9 | 0 | No | 1 |
| Hantaviridae-Orthomyxoviridae | 4.39e-7 | 0 | No | 1 |
| Hantaviridae-Paramyxoviridae | 4.33e-7 | 0 | No | 1 |
| Hantaviridae-Rhabdoviridae | 4.55e-8 | 0 | No | 1 |
| Orthomyxoviridae-Orthomyxoviridae | 5.19e-5 | 0 | No | 1 |
| Orthomyxoviridae-Paramyxoviridae | 5.12e-5 | 1.52e-4 | Yes | 0.090 |
| Orthomyxoviridae-Rhabdoviridae | 5.38e-6 | 0 | No | 1 |
| Paramyxoviridae-Paramyxoviridae | 5.06e-5 | 7.61e-5 | Yes | 1 |
| Paramyxoviridae-Rhabdoviridae | 5.31e-6 | 9.14e-5 | Yes | **< 0.001** |
| Rhabdoviridae-Rhabdoviridae | 5.57e-7 | 0 | No | 1 |

594 **Table S7.** Expected versus observed coinfection prevalence for virus families, for bats only. *P*

595 values were calculated from binomial tests and adjusted using a Bonferroni correction. $P_{adj}$

596 values < 0.05 are in bold.

597

| **Virus family combination** | **Expected coinfection prevalence** | **Observed coinfection prevalence** | **Observed > expected** | $P_{adj}$ |
|---|---|---|---|---|
| Coronaviridae-Coronaviridae | 1.80e-3 | 1.58e-3 | No | 1 |
| Coronaviridae-Filoviridae | 1.41e-5 | 0 | No | 1 |
| Coronaviridae-Flaviviridae | 1.17e-6 | 0 | No | 1 |
| Coronaviridae-Orthomyxoviridae | 2.37e-4 | 6.36e-4 | Yes | **< 0.001** |
| Coronaviridae-Paramyxoviridae | 3.89e-4 | 1.22e-3 | Yes | **< 0.001** |
| Coronaviridae-Rhabdoviridae | 5.75e-5 | 8.29e-5 | Yes | 1 |
| Filoviridae-Filoviridae | 1.10e-7 | 0 | No | 1 |
| Filoviridae-Flaviviridae | 9.17e-9 | 0 | No | 1 |
| Filoviridae-Orthomyxoviridae | 1.85e-6 | 0 | No | 1 |
| Filoviridae-Paramyxoviridae | 3.03e-6 | 0 | No | 1 |
| Filoviridae-Rhabdoviridae | 4.49e-7 | 0 | No | 1 |
| Flaviviridae-Flaviviridae | 7.64e-10 | 0 | No | 1 |
| Flaviviridae-Orthomyxoviridae | 1.54e-7 | 0 | No | 1 |
| Flaviviridae-Paramyxoviridae | 2.53e-7 | 0 | No | 1 |
| Flaviviridae-Rhabdoviridae | 3.74e-8 | 0 | No | 1 |
| Orthomyxoviridae-Orthomyxoviridae | 3.12e-5 | 0 | No | 1 |
| Orthomyxoviridae-Paramyxoviridae | 5.11e-5 | 2.76e-5 | No | 1 |
| Orthomyxoviridae-Rhabdoviridae | 7.56e-6 | 0 | No | 1 |
| Paramyxoviridae-Paramyxoviridae | 8.37e-5 | 1.11e-4 | Yes | 1 |

| | | | | |
|---|---|---|---|---|
| Paramyxoviridae-Rhabdoviridae | 1.24e-5 | 1.66e-4 | Yes | **< 0.001** |
| Rhabdoviridae-Rhabdoviridae | 1.83e-6 | 0 | No | 1 |

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599

600 **Table S8.** Expected versus observed coinfection prevalence for virus families, for rodents only.

601 *P* values were calculated from binomial tests and adjusted using a Bonferroni correction. $P_{adj}$

602 values < 0.05 are in bold.

603

| **Virus family combination** | **Expected coinfection prevalence** | **Observed coinfection prevalence** | **Observed > expected** | $P_{adj}$ |
|---|---|---|---|---|
| Arenaviridae-Arenaviridae | 1.51e-7 | 0 | No | 1 |
| Arenaviridae-Coronaviridae | 1.47e-5 | 0 | No | 1 |
| Arenaviridae-Hantaviridae | 6.03e-8 | 0 | No | 1 |
| Arenaviridae-Orthomyxoviridae | 6.03e-8 | 0 | No | 1 |
| Arenaviridae-Paramyxoviridae | 1.60e-6 | 0 | No | 1 |
| Coronaviridae-Coronaviridae | 1.44e-3 | 3.73e-3 | Yes | **< 0.001** |
| Coronaviridae-Hantaviridae | 5.90e-6 | 0 | No | 1 |
| Coronaviridae-Orthomyxoviridae | 5.90e-6 | 0 | No | 1 |
| Coronaviridae-Paramyxoviridae | 1.56e-4 | 1.55e-4 | No | 1 |
| Hantaviridae-Hantaviridae | 2.41e-8 | 0 | No | 1 |
| Hantaviridae-Orthomyxoviridae | 2.41e-8 | 0 | No | 1 |
| Hantaviridae-Paramyxoviridae | 6.39e-7 | 0 | No | 1 |
| Orthomyxoviridae-Orthomyxoviridae | 2.41e-8 | 0 | No | 1 |
| Orthomyxoviridae-Paramyxoviridae | 6.39e-7 | 0 | No | 1 |
| Paramyxoviridae-Paramyxoviridae | 1.69e-5 | 0 | No | 1 |

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