The macroecology of viral coinfection

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Abstract

Coinfection is common in wild animals, and can have profound consequences for disease outcomes and transmission. However, most research on coinfection is based on laboratory experiments or well-studied wildlife systems. Here, we use the testing 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. We find that coinfection is rare because viral infections are rare, but coinfection is more common than expected by random chance – especially among coronaviruses, paramyxoviruses, and influenza A virus. We further find that coinfection risk changes over the host lifespan, but bats and rodents exhibit divergent responses to aging. Finally, we find that cave-roosting bats do not have a significantly higher rate of coinfection, but wild animals in captivity do – highlighting potential risks to wildlife and human health from the wildlife trade. Our results suggest that macroecological patterns of coinfection are driven by a mix of macroevolutionary and host-level variation in immunity, virus-virus interactions, and human influence, and point to coinfection as a potentially important (and frequently, undetected) driver of viral dynamics in nature.

Introduction

Parasites exist in complex communities, where coinfection with multiple parasites at any time is the norm for wild animals [1,2]. These coinfections provide an opportunity for direct interactions, such as competition for resources [3] or space [4], or indirect interactions, such as those mediated by host immune responses [5]. These parasite interactions can have a dramatic effect on outcomes at the host level, and transmission dynamics at the population level [6–9].

Several theoretical frameworks in disease ecology have incorporated the dynamics and consequences of coinfection [8–11], but it can be difficult to test their predictions against empirical evidence. Within-host processes are easiest to study in laboratory settings [12], 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. Well-developed (and often long-term) field systems have also led to a number of insights about interactions within diverse host and parasite communities [5], and some systems allow for natural experiments such as parasite removal or nutritional supplementation [4,13,14]. 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 [15,16], and – despite broader progress towards open science in ecology and epidemiology – the sharing of spatially-explicit, host-level infection data remains rare [17,18]. Some studies have used data synthesis to develop global datasets [16,19–21], but in most cases, they have focused on relationships between hosts and their parasites, rather than among the parasites themselves.

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 [22]. The project produced one of the largest standardized multi-virus datasets ever assembled and resulted in the discovery of new viruses [23,24], an improved understanding of drivers of virus infection and

diversity [25,26], and insight into human and virus-related risk factors for zoonotic spillover [27]. However, minimal work has been done using the PREDICT data to test macroecological hypotheses. Using these data, we set out to answer three questions: (1) Is viral coinfection common in nature? (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 interactions between specific pairs of virus taxa?

Methods

Analyses were performed using the R statistical environment v4.4.2 [28]. Prior to analysis, we cleaned the PREDICT data by excluding human test results and fixing minor errors in animal host taxonomy. We combined an animal sampling dataset that contained details such as species, sex, age class with another dataset of virus test results for individual specimens, so that each test result was paired with the relevant animal data.

Data overview

Data from the PREDICT-2 project (the second half of the decade-long initiative) was openly shared on the USAID Development Data Library prior to the takedown of the USAID website. Specimen collection (n = 126,952 unique specimens from 65,662 unique animals) was concentrated in West and Central Africa as well as South and Southeast Asia, with > 10,000 specimens collected in Sierra Leone, Guinea, and Bangladesh each (Fig. S1). Most specimens were collected from bats (n = 77,334, 60.9%) and rodents (n = 25,683, 20.2%). The dataset includes some farm and companion animals, including poultry, dogs, and swine. Virus screening was conducted using consensus polymerase chain reaction (PCR) assays (714,954 total tests), mostly targeting viral families of significance to human health (e.g., coronaviruses, filoviruses, and paramyxoviruses), but in some cases testing for specific viruses of interest (e.g., influenza viruses and ebolaviruses).

Analyses

For each animal associated with one or more virus detections, we quantified the number of viruses and virus families detected in that animal's specimens, and created binary classifications noting whether virus or virus family coinfection was detected. For each taxonomic group, we calculated the overall infection and coinfection prevalence. For bats, rodents, and birds (the three largest taxonomic groups), we compared the observed coinfection prevalence to the expected coinfection prevalence using a permutation testing approach. Specifically, we

randomized infection across individual animals while maintaining the observed prevalence of each virus within the taxonomic group, then calculated the number of simulated coinfected animals. We repeated this procedure 1000 times for each taxonomic group, then plotted the simulated number of coinfected animals compared to the true number.

We created a coinfection matrix at the virus family level for use in network analyses, where each count within the matrix represents a coinfection of two virus families occurring in the same animal. For the small number of animals that were infected with three viruses, we counted each coinfection separately (e.g., an animal infected with coronavirus X, coronavirus Y, and paramyxovirus Z would count for two coronavirus-paramyxovirus coinfections and one coronavirus-coronavirus coinfection). We used the *ggraph* package [29] to visualize the frequency of virus family coinfection. To test if certain virus families co-occurred more often than expected by chance, we first calculated the expected coinfection prevalence of two virus families as the product of each virus family's overall prevalence in the dataset. We then performed a binomial test for each virus family–virus family combination to determine if the observed coinfection prevalence was significantly greater than expected. To account for multiple comparisons, we adjusted *P* values using a Bonferroni correction. Finally, we repeated the above analyses for two subsets of the full data: bats and rodents.

We used generalized linear models to explore host correlates of virus coinfection. **1)** For an all-animals model (n = 2,462 animals), we excluded taxonomic groups with ≤ 10 animals, animals that were missing sex or age class data, and a small number of neonates and fetuses. Using the 'glm' function in the *stats* package, we modeled animal coinfection status (binary: either single infection or coinfection) as a function of geographic region (Africa, Asia), taxonomic group (bats, birds, rodents, shrews), sex (female, male), age class (adult, subadult, juvenile), and captivity status (wild free-ranging, owned domesticated, wild in captivity). Given inconsistency in host identification (i.e., not all animals could be 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 roosts in caves using data from Willoughby *et al.* [30] and the IUCN Red List. We excluded any bats for which cave roosting behavior or taxonomic family was not available and included the same restrictions on sex and age class as above. Using the 'glmmTMB' function in the *glmmTMB* package [31], we modeled bat coinfection status as a function of four fixed effects variables (geographic region, sex, age class, and cave roosting status), and one random effects variable (family). **3)** For a rodents-only model (n = 488), we

included the same restrictions on sex and age class as above and modeled rodent coinfection status as a function of those two predictors (collapsing subadults and juveniles into one category due to sample size restrictions). Family and geographic region were not included as variables because nearly all rodents were murids and were collected in Asia.

Results

We found that both infection and coinfection were rare overall. Out of 65,662 unique animals, 3,271 (4.98%) were infected with one or more viruses (Fig. 1A); viral prevalence in birds (487/5,617; 8.67%) and bats (2,059/36,181; 5.69%) was somewhat higher than average, while prevalence in rodents (549/12,876; 4.26%) was slightly lower than average. Animals with at least one virus detection were most often captured in South and Southeast Asia (Fig. 1B).

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

Coinfection is non-random across geography, phylogeny, and life stage

Bats, rodents, and birds exhibited unique patterns of coinfection, even accounting for their over-representation in the dataset. Coinfections were more common than expected based on the observed prevalence of all viruses: for all three taxonomic groups, we applied a permutation testing approach, and found that true coinfection rates exceeded the maximum random frequency observed across 1,000 simulations (Fig. S2). These results suggest that coinfection is more common than expected, indicating that animals either share risk factors for exposure, or viral infections tend to increase susceptibility to secondary infections. However, after accounting for other host factors, we found that birds were significantly less likely to be coinfected than bats (odds ratio (OR) = 0.30, 95% confidence interval (CI): 0.11-0.67, *P* = 0.007; Fig. 2A, Table S2), and rodents and shrews were marginally less likely than bats to be coinfected (rodents: OR = 0.76, 95% CI: 0.47-0.1.19, *P* = 0.239; shrews: OR = 0.43, 95% CI: 0.02-2.03, *P* = 0.404). These

168 findings could support the widespread hypothesis (and growing evidence) that bats are able to
169 tolerate viral infection, and potentially coinfection, through a unique set of immune adaptations
170 [32–34]. However, we note that virus coinfection rates within the all-animals model dataset (i.e.,
171 animals with available sex and age data) differed compared to the coinfection rates in the entire
172 dataset: while coinfection was still high in rodents (46/488, 9.43%) and bats (112/1,628, 6.88%),
173 it was noticeably lower in birds (12/316, 3.80%).
174
175 Animals sampled in Asia were more likely to be coinfected than those sampled in Africa in both
176 the all-animals model (OR = 2.07, 95% CI: 1.40-3.08, $P < 0.001$; Fig. 2A, Table S2) and the
177 bats-only model (OR = 2.61, 95% CI: 1.65-4.13, $P < 0.001$; Fig. 2C, Table S3). Rather than
178 reflecting any characteristic of one region versus another, we believe that this result was instead
179 driven by the geographic mosaic of specific coevolutionary associations between host and virus
180 groups [35]. In particular, flying foxes (*Pteropus* spp.; n = 3,084 animals) and rats (*Rattus* spp.;
181 n = 5,109) were heavily sampled but infected animals were primarily found in Asia, and both of
182 these genera had high coinfection rates (*Pteropus*: 37/245; 15.1%; *Rattus*: 44/403; 10.9%).
183
184 Coinfection was associated with life history, but those relationships varied between groups.
185 Based on previous studies [25,36], we hypothesized that infection prevalence – and therefore
186 coinfection rates – would be higher in younger animals, due to waning of maternal antibodies
187 and less-developed immune systems [37]. We found that younger rodents (juveniles and
188 subadults) were more likely to be coinfected than adults (OR = 2.14, 95% CI: 1.12-4.00, $P =$
189 0.018; Fig. 2E, Table S4). However, juvenile bats were less likely to be coinfected than adults
190 (OR = 0.14, 95% CI: 0.03-0.57, $P = 0.006$), and this effect was strong enough that it dominated
191 the all-animals model (OR = 0.34, 95% CI: 0.10-0.84, $P = 0.040$). This pattern is aligned with the
192 widespread hypothesis that bats' unique immune adaptations allow them to tolerate persistent
193 viral infections [38,39], which might accumulate over the course of an individual's lifespan.
194 Alternatively, depending on the timing of sampling by the PREDICT study, juvenile bats may
195 have been captured at times when levels of maternal antibodies were still high. Though
196 logistically challenging, longitudinal sampling within populations can help elucidate patterns of
197 virus infection and coinfection in relation to host maturation [36,40,41].
198
199 Finally, we found that host social structure could drive coinfection patterns, but found divergent
200 effects between natural and un-natural settings. For bats, cave-roosting behavior was not a
201 significant predictor of coinfection status. Cave-roosting bats can exist in large groups in close

202 proximity, potentially increasing infection rates, but other roosting strategies can also create
203 large, close aggregations of bats (e.g., tree-roosting *Pteropus* species that cluster together).
204 Finer-scale contact patterns that we could not examine here (e.g., segregation by age or sex, or
205 centrality in social networks) might lead to more correlated infection risk across viruses at the
206 individual level. Sickness behaviors (e.g., reduced social interactions) after an initial infection
207 could also reduce an individual's subsequent likelihood of infection with other viruses [42,43].
208
209 In contrast, across all animals, we found that wild animals in captivity were significantly more
210 likely to be coinfected than free-ranging wild animals (OR = 2.16, 95% CI: 1.19-3.88, P = 0.010;
211 Fig. 2A, Table S2). These results support the hypothesis that captivity can increase parasite
212 transmission through both crowding, stress, and unusual patterns of interspecific contact. This
213 hypothesis has been strongly supported by previous analyses of coronavirus testing conducted
214 by the PREDICT project [23,44], and is a particular point of concern, given the potential for
215 coinfection to lead to recombinant coronaviruses [45], and the high risk of spillover in wildlife
216 farms and markets [46]. However, we note that our model was limited to an unrepresentative
217 sample of the data for which age and sex were available, and so of the 163 animals classified
218 as "wild animals in captivity," 159 (98%) were either rats (*Rattus* spp.) or mallards (*Anas*
219 *platyrhynchos*).
220
221 ***Evidence of virus-virus interactions***
222 Coinfection networks revealed that certain viruses–namely, coronaviruses (CoVs),
223 paramyxoviruses (PMVs), and orthomyxoviruses (specifically, influenza A virus)–were most
224 frequently detected together (Fig. 2B, 2D, 2F). Notably, there were 108 total coinfections with
225 multiple CoVs (57 in bats, 48 in rodents), 69 CoV-PMV coinfections (44 in bats, 2 in rodents), 45
226 CoV-influenza coinfections (23 in bats), and 10 PMV-influenza coinfections (1 in bats). Binomial
227 tests indicated that CoV-PMV, CoV-influenza, and PMV-rhabdovirus coinfections occurred
228 significantly more than expected in all animals and in bats, while coinfections with multiple
229 coronaviruses occurred significantly more than expected in rodents (Tables S5-S7). All other
230 virus family coinfections occurred < 10 times, and no coinfections involving arenaviruses,
231 filoviruses, or hantaviruses were detected.
232
233 Examining coinfection patterns at the level of individual viruses, we found that viruses that are
234 transmitted by poultry and other livestock (influenza A virus, Newcastle disease virus, and
235 infectious bronchitis virus) had the highest centrality in the network. However, a handful of

236 commonly occurring betacoronaviruses, including murine coronavirus, PREDICT CoV-16 and
237 PREDICT CoV-17, and Bat coronavirus HKU9, were also well connected within the network.
238

239 Discussion

240 Here, we conducted the first comprehensive analysis of viral coinfection patterns across multiple
241 continents and an entire host community. We found that coinfection is rare, because viral
242 infections are rare, but coinfection occurs more frequently than expected, particularly among
243 specific virus groups. Our findings suggest that widely-occurring viruses like influenza A virus
244 could have important effects on host susceptibility to secondary infections, and might be
245 important to account for in research on other pathogens. Future work could explore how specific
246 viral coinfections influence factors such as the magnitude and duration of shedding, particularly
247 as it could relate to zoonotic spillover risk [47]. We also found that coinfection dynamics may be
248 modulated by both broad differences in host immunology and fine-scale host contact processes,
249 including in anthropogenic settings like farms and the wildlife trade. These findings are also of
250 potential importance for public health, as they highlight the need for both basic research on
251 host-parasite interactions, and surveillance of environments where coinfection can produce new
252 reassortant and recombinant viruses with epidemic or pandemic potential [25].
253
254 Although our study is the largest macroecological study of coinfection to date, our findings
255 should be interpreted in light of the limitations of our data. Most importantly, the PREDICT
256 project was not designed with ecological research as the primary objective, and so the testing
257 data have uneven coverage both across and within taxonomic groups. For instance, the
258 PREDICT project was almost single-handedly responsible for bats becoming the best-studied
259 wildlife hosts of viruses [48]. Given that over 60% of specimens were collected from bats, these
260 data probably drove many of our broad-scale findings. However, only half of all bat families (10
261 of 21) were sampled, and an emerging body of work highlights that while bats are unique viral
262 reservoirs, there is still considerable important (and interesting) variation within the group [34].
263
264 Future work could build on initial steps here by designing surveillance to more explicitly search
265 for and report coinfection. A recent systematic review found that coinfections were common in
266 bats, but were incidentally reported within study findings [49]. More deliberate focus on sampling
267 to capture coinfection dynamics may also require specific sampling strategies: for example,
268 reliably detecting coinfection in an individual host requires capture of animals in-hand (versus,
269 e.g., pooled or environmental sampling). Similarly, different parasites are associated with

different organs and sites in the body, and so prioritizing particular samples or assays may be necessary, depending on study aims. Although broad sample and parasite coverage presents substantial cost considerations, several recent developments offer possible solutions. Multiplex, metagenomic, and metatranscriptomic methods all provide a fuller view of the host virome, and are especially useful when sample sizes are small due to biological limitations (e.g., collecting blood from small animals) [50–52]. Longitudinal surveillance also improves the odds of viral detection, and allows researchers to test hypotheses about asynchronous interactions between parasites, host immunity, and external environmental factors [53]. Finally, host-level data on wildlife disease are also shared infrequently [17], preventing the kinds of re-analyses we conducted here, or approaches that synthesize findings through systematic reviews and meta-analyses [49]. 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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Data Accessibility

PREDICT data on animal sampling and viral testing will be made available to the reviewers and editors at the time of submission as .csv files. A public link to the data will be provided upon manuscript publication. All code to reproduce analyses is available at https://github.com/viralemergence/macroecology-coinfection.

Figures

Figure 1. A. Number of unique animals infected by one or more viruses, separated by taxonomic group. Groups with only single infections are not displayed (dogs: *n* = 10; goats/sheep: *n* = 4; cattle/buffalo: *n* = 2; carnivores: *n* = 1; other: *n* = 1). **B.** Distribution of unique animals associated with one or more virus detections. Points are partially transparent and jittered for improved visibility.

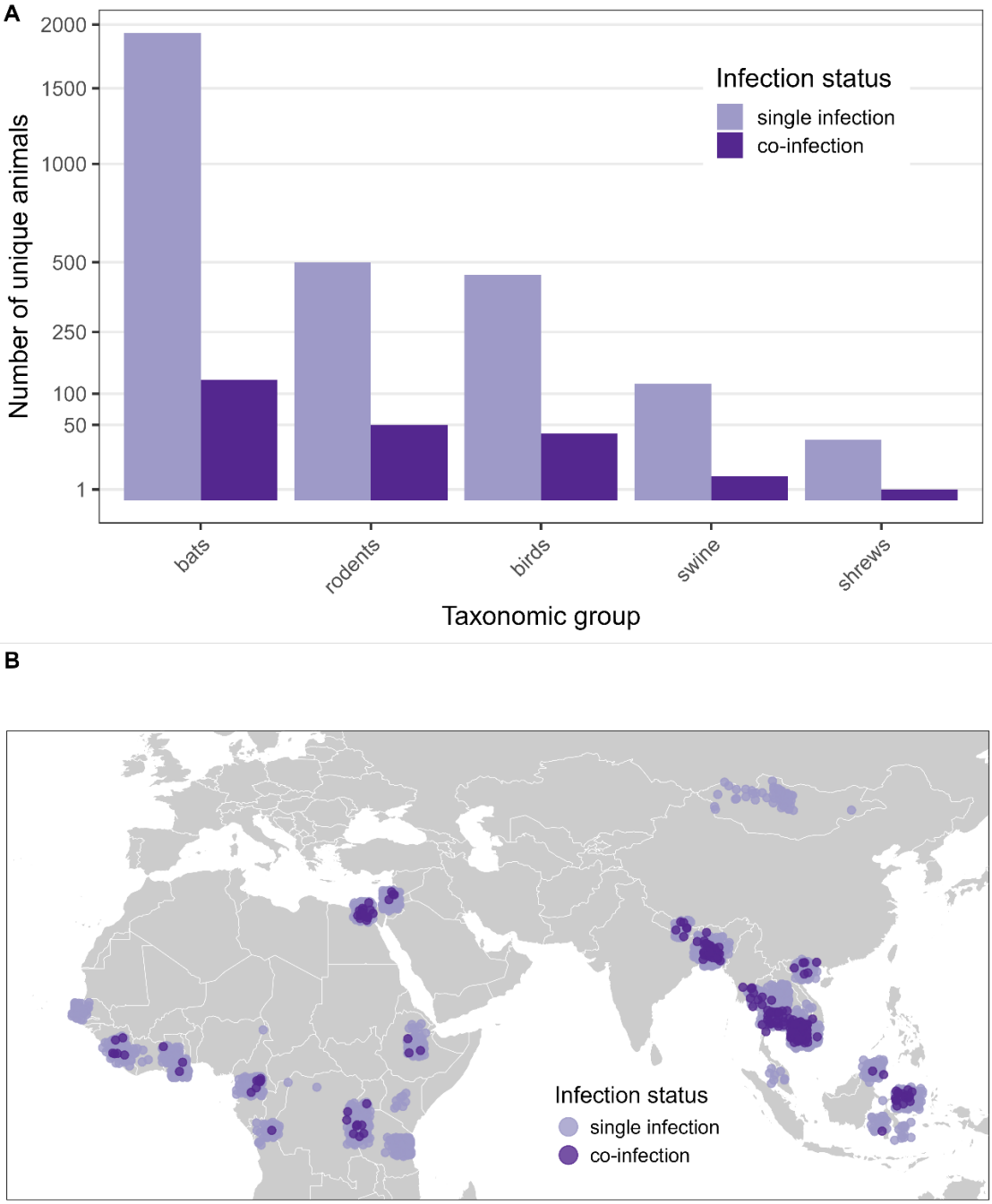


Figure 2. Predictors of virus coinfection (**A**, **C**, **E**) and virus family coinfection networks (**B**, **D**, **E**). In the left column, generalized linear model outputs (odds ratios and 95% confidence intervals (CIs)) for predictors of coinfection are shown for all animals (**A**), bats only (**C**), and rodents only (**E**). CIs that do not cross the vertical line at 1 (i.e., statistically significant) are purple, while CIs that do cross are gray. Reference levels are Africa (geographic region), bats (taxonomic group), female (sex), adult (age class), wild free-ranging (captivity status), and non-cave roosting (roosting status). In the right column, networks of virus family coinfection are shown for all animals (**B**), bats only (**D**), and rodents only (**F**). Nodes represent virus families, while edges represent the frequency of virus family coinfection. Asterisks indicate coinfections that occurred significantly more than expected.

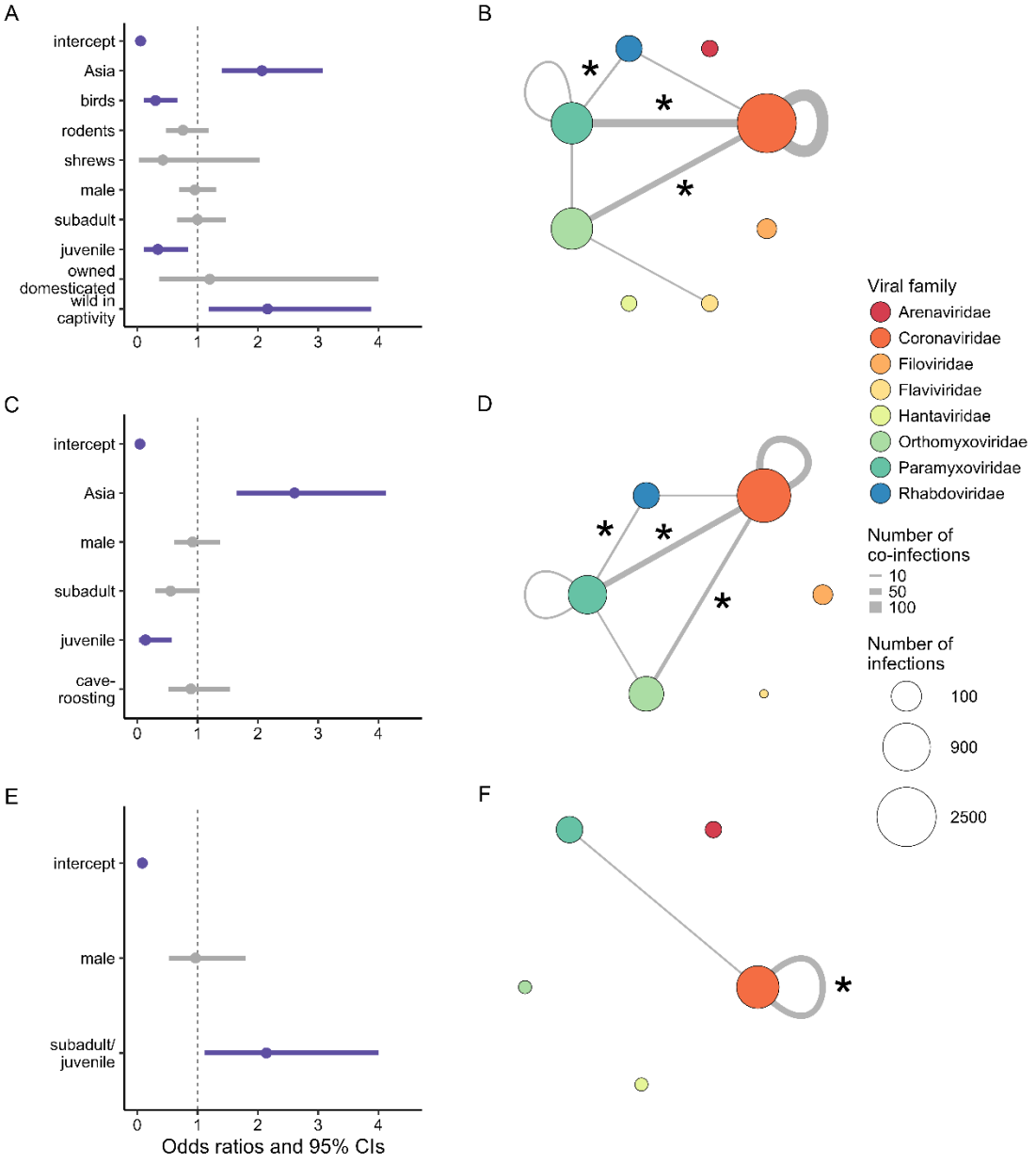
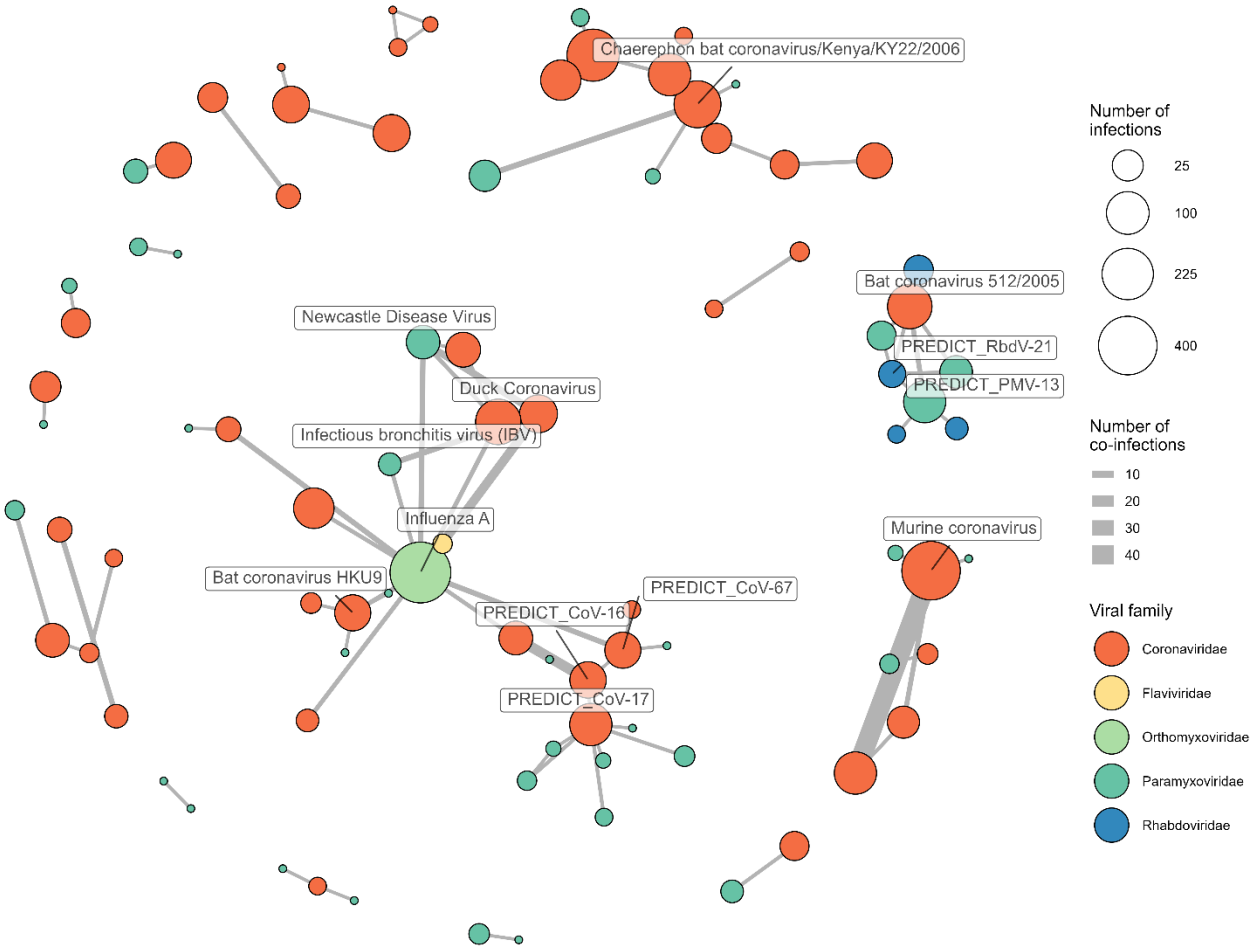


Figure 3. Virus coinfection network. Nodes represent individual viruses, while edges represent the frequency of virus coinfection. Only nodes with degree centrality ≥ 4 are labeled.



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449

Supplementary Information

Figure S1. Map of sampling effort in the PREDICT dataset. Only one point is plotted for each country, with the size corresponding to the number of unique specimens collected in that country.

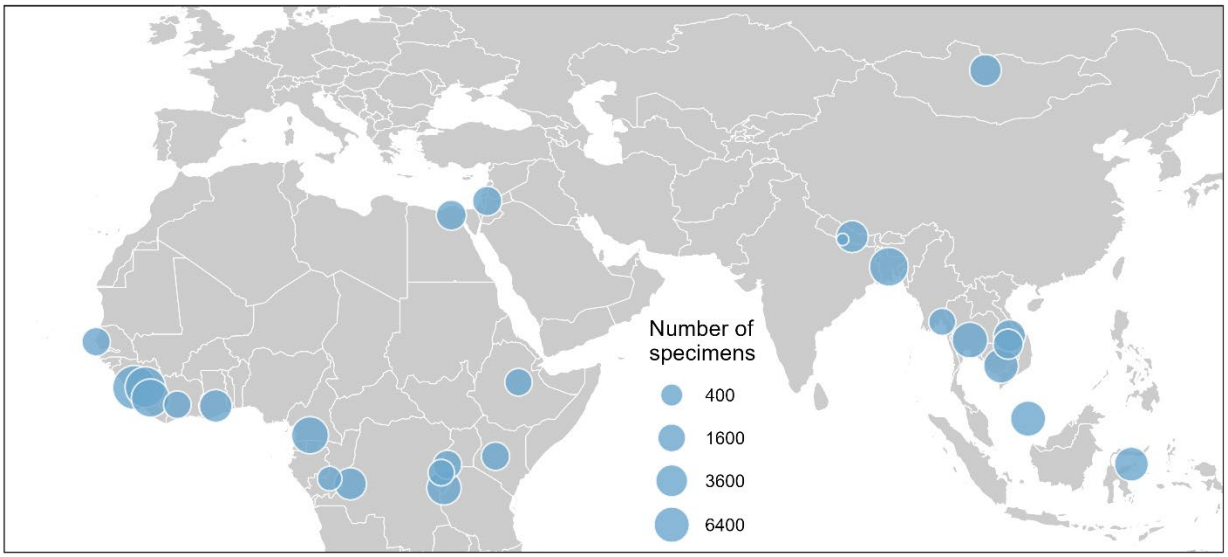
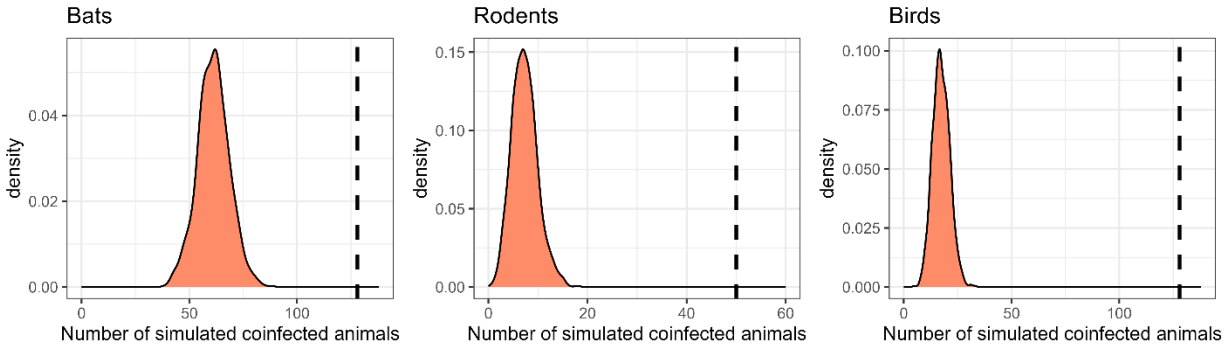


Figure S2. Density plots of 1000 coinfection simulations each for bats (*n* = 36,181), rodents (*n* = 12,876), and birds (*n* = 5,617). The true number of coinfected animals for each taxonomic group is plotted with a dashed vertical line.



464 **Table S1.** Characteristics of the 12 animals that were infected with three viruses. Note that host
465 and virus names are provided as originally described in the PREDICT dataset, and may not
466 conform to the latest taxonomies.
467

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	Coronaviridae	Duck Coronavirus

			platyrhynchos domesticus	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 platyrhynchos domesticus	Coronaviridae	Duck Coronavirus
				Orthomyxoviridae	Influenza A
				Paramyxoviridae	Avian Paramyxovirus 6

Table S2. Model coefficients for a generalized linear model to examine effects of taxonomy, sex, age, and captivity status on virus coinfection status in bats, birds, rodents, and shrews. *P* values < 0.05 are in bold.

Variable		**Estimate**	**SE**	**z**	***P***	**Odds ratio (95% CI)**
Intercept		-2.92	0.18	-16.52	**< 0.001**	0.05 (0.04-0.08)
Geographic region	Africa	–	–	–	–	–
	Asia	0.73	0.20	3.63	**< 0.001**	2.07 (1.40-3.08)
Taxonomic group	bats	–	–	–	–	–
	birds	-1.20	0.45	-2.69	**0.007**	0.30 (0.11-0.67)
	rodents	-0.28	0.24	-1.18	0.239	0.76 (0.47-1.19)
	shrews	-0.86	1.02	-0.84	0.404	0.43 (0.02-2.03)
Sex	female	–	–	–	–	–
	male	-0.05	0.16	-0.28	0.776	0.96 (0.70-1.31)
Age class	adult	–	–	–	–	–
	subadult	-0.004	0.20	-0.02	0.986	1.00 (0.66-1.47)
	juvenile	-1.07	0.52	-2.05	**0.040**	0.34 (0.10-0.84)
Captivity status	free-ranging wild animal	–	–	–	–	–
	owned domesticated animal	0.18	0.60	0.31	0.760	1.20 (0.36-4.01)
	wild animal in captivity	0.77	0.30	2.57	**0.010**	2.16 (1.19-3.88)

475 **Table S3.** Model coefficients (fixed effects only) for a generalized linear mixed model to
476 examine effects of sex, age, and cave roosting behavior on virus coinfection status in bats. *P*
477 values < 0.05 are in bold.
478

Variable		**Estimate**	**SE**	**z**	***P***	**Odds ratio (95% CI)**
Intercept		-3.13	0.47	-6.72	**< 0.001**	0.04 (0.02-0.11)
Geographic region	Africa	–	–	–	–	–
	Asia	0.96	0.23	4.10	**< 0.001**	2.61 (1.65-4.13)
Sex	female	–	–	–	–	–
	male	-0.09	0.21	-0.42	0.675	0.92 (0.61-1.37)
Age class	adult	–	–	–	–	–
	subadult	-0.59	0.32	-1.86	0.063	0.55 (0.30-1.03)
	juvenile	-2.00	0.73	-2.73	**0.006**	0.14 (0.03-0.57)
Cave roosting	no	–	–	–	–	–
	yes	-0.12	0.28	-0.42	0.673	0.89 (0.51-1.54)

479

480 **Table S4.** Model coefficients for a generalized linear model to examine effects of sex and age
481 on virus coinfection status in rodents. *P* values < 0.05 are in bold.
482

Variable		**Estimate**	**SE**	**z**	***P***	**Odds ratio (95% CI)**
Intercept		-2.48	0.26	-9.44	**< 0.001**	0.08 (0.05-0.14)
Sex	female	–	–	–		–
	male	-0.03	0.31	-0.11	0.913	0.97 (0.52-1.80)
Age class	adult	–	–	–		–
	subadult/juvenile	0.76	0.32	2.36	**0.018**	2.14 (1.12-4.00)

483

484 **Table S5.** Expected versus observed coinfection prevalence for virus families, for all animals. *P*
485 values were calculated from binomial tests and adjusted using a Bonferroni correction. P_{adj}
486 values < 0.05 are in bold.
487

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

488

489 **Table S6.** Expected versus observed coinfection prevalence for virus families, for bats only. *P*
490 values were calculated from binomial tests and adjusted using a Bonferroni correction. P_{adj}
491 values < 0.05 are in bold.
492

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

493

494 **Table S7.** Expected versus observed coinfection prevalence for virus families, for rodents only.
495 *P* values were calculated from binomial tests and adjusted using a Bonferroni correction. P_{adj}
496 values < 0.05 are in bold.
497
498

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

499