Paradoxes and synergies: optimizing management of a deadly virus in an endangered carnivore

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Keywords

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Abstract

1. Outbreaks of infectious disease can have serious consequences for wildlife population health, especially species of conservation concern. The endangered Florida panther, for example, experienced an outbreak of feline leukemia virus (FeLV) in 2002-04, and continues to be affected by this deadly virus. Ongoing management efforts aim to mitigate the effects of FeLV on panthers, but with limited information about which strategies may be most effective and efficient. Such uncertainties can benefit from

linking management with simulation models in order to optimize conservation efforts for populations of concern.

2. We used a simulation-based approach to determine optimal FeLV management strategies. We simulated use of proactive FeLV management strategies (i.e., proactive vaccination) and several reactive strategies, including reactive vaccination and test-and-removal. Vaccination strategies accounted for partial vaccine immunity, including when using single or boosted inoculations. We compared the effectiveness of these different strategies in mitigating the number of FeLV mortalities and the duration of outbreaks.
3. Results showed that inadequate proactive vaccination can paradoxically increase the number of disease-induced mortalities in FeLV outbreaks, most likely due to effects of partial vaccine immunity. Combinations of proactive vaccination with reactive test-and-removal or vaccination had a synergistic effect in reducing impacts of FeLV outbreaks. Temporary spatial restrictions, however, were unlikely to be effective under realistic conditions.
4. *Synthesis and applications:* Management-informed disease simulations demonstrated both unexpected negative consequences and synergies with active management strategies for a deadly virus in Florida panthers. We recommend a combination of proactive and reactive management approaches, and suggest prioritizing boosted vaccination over broad distribution of unboosted inoculations. Our results highlight the importance of integrating management and modeling approaches to aid in conservation of at-risk species.

Introduction

Outbreaks of infectious diseases can have significant impacts on the population health of free-ranging wildlife and are of heightened importance in species of conservation concern (Breed et al., 2009). For example, the feline retrovirus, feline leukemia virus (FeLV), has been the source of significant outbreaks in two endangered felids: Iberian lynx (*Lynx pardinus*) and Florida panthers (*Puma concolor coryi*). In the case of panthers, FeLV caused a deadly outbreak in 2002-2004 (Cunningham et al., 2008), spilling over from domestic cats with subsequent direct transmission among panthers (Brown et al., 2008). In addition, recent evidence demonstrates ongoing FeLV spillover to and transmission among panthers (Chiu et al., 2019), necessitating continued management to prevent future epidemics of this deadly pathogen. FeLV inoculation with a domestic cat vaccine has been used previously in panthers but with unknown efficacy (Cunningham et al., 2008). Further, the proportion of the population that must be vaccinated to most efficiently prevent future FeLV outbreaks is unknown, as is how proactive vaccination might interact with other reactive interventions to interrupt an FeLV outbreak. Such uncertainties are common among free-ranging wildlife threatened by infectious disease and hamper efforts to effectively control pathogen transmission.

Mathematical models of infectious disease transmission have been used to optimize disease management protocols in a variety of free-ranging wildlife species of conservation concern, including Ethiopian wolves (Haydon et al., 2006), chimpanzees (Rushmore et al., 2014), and Amur tigers (Gilbert et al., 2020). Further, models can serve the important function of balancing the realities of fieldwork with ideal disease control protocols to provide practical, effective guidance for wildlife managers (e.g., Robinson et al., 2018). Here, we use mathematical models of FeLV transmission in Florida panthers to determine optimal disease prevention and control strategies in this iconic carnivore.

Pathogen management strategies can be preventive (hereafter, *proactive*) or reactive. Among proactive and reactive strategies, vaccination is a cornerstone in veterinary medicine

79 (Breed et al., 2009) and functions by reducing the availability of susceptible individuals.
80 Vaccination can be applied randomly, or targeted to: high risk individuals or populations (e.g.,
81 Beyer et al., 2012); likely superspreaders (e.g., in chimpanzees: Rushmore et al., 2014);
82 seasonal dynamics (e.g., Baker et al., 2019); or spatial risk, as with vaccine barriers to prevent
83 spread of a pathogen to new areas or populations (e.g., Sanchez & Hudgens, 2020; Slate et al.,
84 2005).

85 Among reactive pathogen management strategies, test-and-removal (or test-and-cull) is
86 commonly used in domestic species to functionally reduce the infectious period of infected
87 individuals (Miguel et al., 2020). However, this approach is rarely used in wildlife due to the
88 generally low availability of field diagnostic tests and difficulty in recapturing individuals after a
89 positive diagnosis (Miguel et al., 2020). Rather, pathogen control in wildlife often relies on non-
90 selective culling in an attempt to reduce density and—in theory—transmission (Lloyd-Smith et
91 al., 2005). However, non-selective culling can have myriad negative consequences for disease
92 management including removal of immune individuals (Miguel et al., 2020; Potapov et al., 2012)
93 or culling-induced perturbation (e.g., Woodroffe et al., 2006). Thanks to ready availability of a
94 field diagnostic test, FeLV management in domestic cats and Iberian lynx has made use of test-
95 and-removal or isolation of infected individuals (Little et al., 2020; López et al., 2009), and is part
96 of future FeLV mitigation plans in panthers. However, uncertainties remain regarding sufficiency
97 of test-and-removal alone to mitigate pathogen spread or how it might interact with proactive
98 vaccination efforts to more effectively reduce pathogen impacts.

99 Given the ongoing risks of FeLV to panther conservation and the uncertainties regarding
100 application of proactive and reactive FeLV management strategies, the objectives of this study
101 were to test singly, and in combination, the effectiveness of: (1) proactive vaccination, (2)
102 reactive vaccination, and (3) reactive test-and-removal for reducing the population level impacts
103 of FeLV in Florida panthers. In addition, we evaluated the effectiveness of temporary panther
104 spatial restrictions via closure of wildlife highway underpasses, which we describe and discuss

105 in the supplementary materials. We used a simulation modeling approach to address our
106 objectives in order to efficiently and ethically test a wide range of intervention protocols for the
107 protection of this endangered carnivore.
108
109 **Methods**
110 *Simulation pipeline*
111 To examine the effect of different disease management regimes on FeLV control, we
112 used spatially explicit, network simulations adapted from our research determining drivers of
113 retrovirus transmission in panthers (Gilbertson et al., unpublished preprint). This approach
114 involves two steps: (1) simulation of a contact network among panthers, and (2) simulation of
115 FeLV transmission through this network. In brief, we simulated panther populations of 150
116 individuals (McClintock et al., 2015) and used our previously described exponential random
117 graph model for retrovirus transmission in panthers (Gilbertson et al., unpublished preprint) to
118 simulate contact networks constrained by network density among simulated populations (see
119 supplementary methods for further details and Table S1 for parameters).
120 Briefly, we used a stochastic chain binomial transmission model with a modified
121 susceptible-infectious-recovered compartmental framework, where individuals could be
122 progressively, regressively, or abortively infected (Cunningham et al., 2008; model developed in
123 Gilbertson et al., unpublished preprint). Progressive infections always resulted in death, while
124 regressive infections eventually recovered with immunity, and abortive infections were always
125 considered immune. Importantly, based on our previous work, we allowed both progressives
126 and regressives to be infectious, though regressives were less likely to transmit (Table S1). The
127 infectious period is long for FeLV (estimated as a mean of 18 weeks, though based on limited
128 observations: Cunningham et al., 2008; see also Gilbertson et al., unpublished preprint, and
129 Table S1). We included only disease-induced mortality, and, in order to preserve key network
130 structure, allowed territories vacated by deaths to be reoccupied by new susceptible individuals

131 (hereafter, *respawning*). Outbreaks were initiated by a single, randomly selected non-isolate
132 individual in the population (see supplement discussion), and proceeded in weekly time steps
133 for up to five years.

134 Our primary objective was to examine the effect of different FeLV management regimes
135 on epidemic outcomes, so in our primary simulations, we held network generation and
136 transmission parameters constant at previously supported values (see supplementary methods
137 and Table S1 for further details). We evaluated the consistency of our results to the choice of
138 parameter values with a sensitivity analysis (see below). Hereafter, a *parameter set* represents
139 the unique set of network and transmission parameters for any given set of simulations. A *full*
140 *simulation* includes simulation of a single contact network and FeLV transmission through that
141 network (with or without management interventions). For each parameter set, we performed 100
142 full simulations.

143 For *baseline* and *management scenarios*, we recorded key epidemic outcomes in the
144 absence and presence of interventions, respectively (Figure 1). These key outcomes were (1)
145 the number of mortalities, (2) the duration of an epidemic, and (3) the proportion of epidemics
146 that "failed" per 100 successful epidemics. A failed epidemic (i.e. stochastic fadeout; Craft et al.,
147 2013) was one in which fewer than 5 individuals acquired progressive or regressive infections.
148 As at least 5 individuals acquired progressive infections during the 2002-04 outbreak
149 (Cunningham et al., 2008), this fadeout cutoff allowed for realism in simulated outbreak sizes,
150 while also reducing noise among "successful" epidemics resulting from especially small
151 outbreaks. The outcomes of mortalities and epidemic durations were summarized as median
152 values per parameter set, as results were often skewed; all outcomes were compared between
153 baseline and management scenarios, including quantification with Mann-Whitney *U* tests
154 (supplementary results). All simulations were performed in R version 3.6.3 (R Core Team,
155 2018).
156

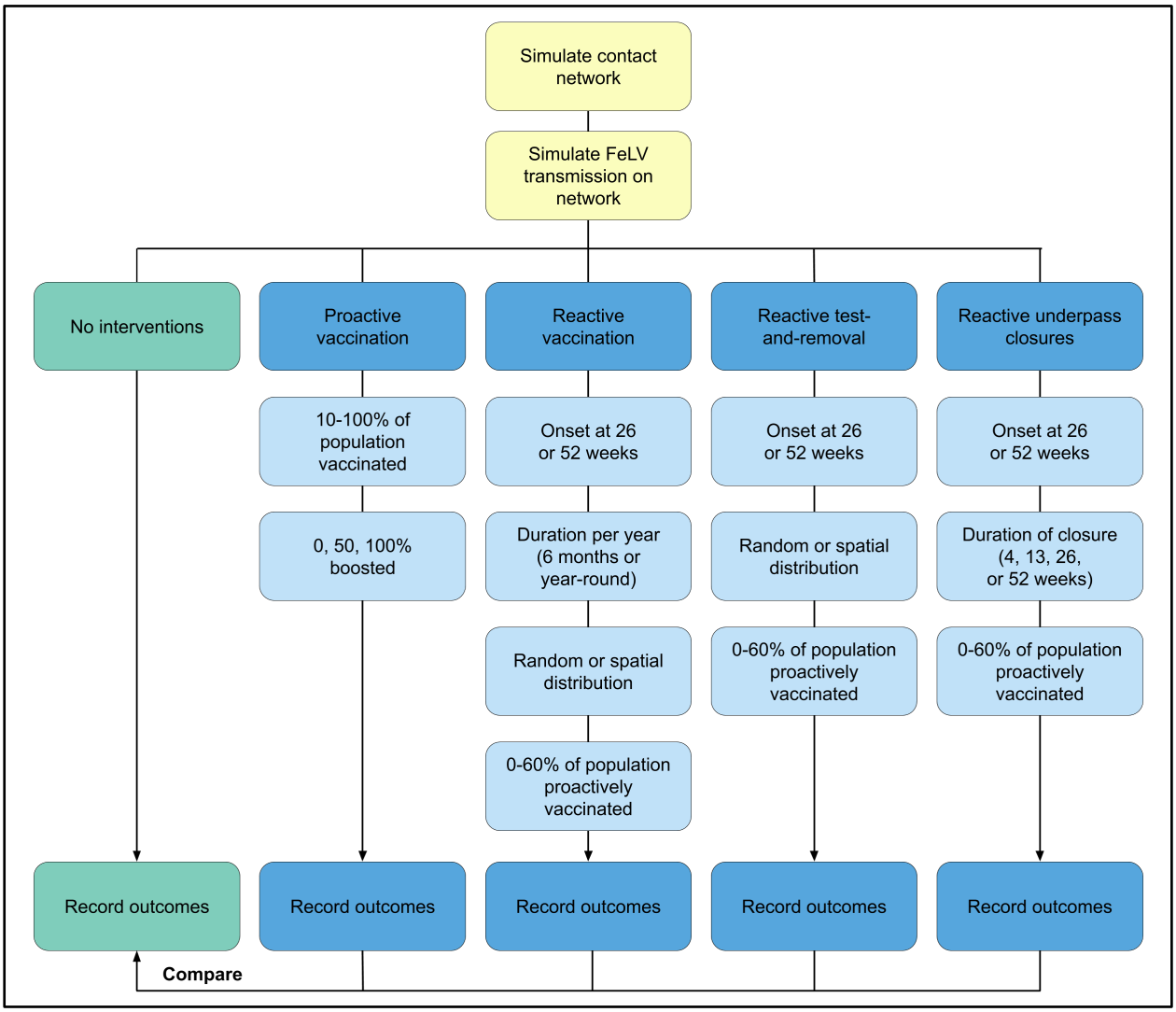

Figure 1: *Steps of the simulation process across baseline and management scenarios. The basic network and FeLV transmission simulation steps are shown in yellow, the baseline (no intervention) scenario in green, and the different overarching management scenarios in blue. Within each management scenario, we investigated several variations for the given approach (shown in light blue) in a factorial design. Note that methods and results for reactive underpass closures are described in the supplementary materials.*

Management scenarios

166 We examined the effect of different levels of population proactive vaccination (proportion
167 of the population vaccinated prior to an outbreak) and different ratios of single versus boosted
168 vaccination. We simulated from 10-80% (in 10% increments) of the population having some
169 degree of vaccine-induced immunity to FeLV prior to the onset of an outbreak. These proactive
170 vaccinations were distributed randomly in the population. Among the vaccinated individuals, 0,
171 50, or 100% received a second boosting inoculation. Actual vaccine efficacies are unknown for
172 panthers, but based on efficacy studies in domestic cats (Sparkes, 1997; Torres et al., 2005),
173 we conservatively assumed that boosted vaccination would prevent 80% of infections and single
174 vaccination would prevent 40% of infections. We modeled this efficacy as a binomial probability
175 to represent partial vaccine immunity, wherein increased exposures to infectious individuals
176 increase the likelihood of vaccine failure (versus binary immunity wherein some vaccinated
177 individuals are 100% protected and others receive 0% protection; Barnett & Civitello, 2020).
178 In contrast, for reactive vaccination management scenarios, panthers were selected for
179 vaccination *during* outbreaks at a rate of one panther per week. We assumed that managers
180 would not know the disease status of an individual selected for vaccination and that vaccination
181 would be ineffective in infectious or recovered individuals. In the case of previously vaccinated
182 individuals, re-vaccination changed the vaccine efficacy of singly vaccinated individuals (efficacy
183 of 40%) to the efficacy for boosted individuals (efficacy of 80%). We varied the timing of the
184 onset of reactive vaccination after the initiation of an FeLV outbreak to reflect the difficulty of
185 epidemic detection in this elusive carnivore. We therefore began reactive vaccination at an
186 optimistic, but difficult-to-attain time point of 26 weeks, and a more realistic time point of 52
187 weeks. In addition, we varied the distribution of reactive vaccination. While proactive vaccines
188 were always distributed randomly, reactive vaccination was either randomly distributed or
189 spatially distributed in an attempted vaccine barrier based on proximity to the I-75 freeway,
190 which runs east-west through primary panther habitat (see supplement for further details).

191 Because vaccination is resource and time intensive, we evaluated the effect of reactive
192 vaccination for 6 months per year versus year-round.
193 Test-and-removal management scenarios were built around a protocol in which panthers
194 infectious at capture were removed from the population through humane euthanasia or
195 temporary removal until recovery. For simplicity, based on expected fatality of progressive
196 cases, we assumed that all progressively infected individuals would be humanely euthanized at
197 capture, while infectious regressive individuals were temporarily removed from the population
198 until their recovery and re-released into any open territory. A maximum of five individuals were
199 allowed to be temporarily removed in this way at one time based on expected capacity for
200 housing and care.
201 We expected that managers would be able to capture and test one panther per week at
202 most, and that captures occurred during a 17 week (about 4 month) capture season, in
203 accordance with current panther capture protocols. We assumed that managers would not know
204 the disease status of a target individual until the capture occurred, so captures were not
205 targeted by infection state, and removed individuals were able to transmit prior to removal. We
206 varied the onset of test-and-removal, such that the intervention began 26 or 52 weeks after the
207 initiation of an epidemic. Captures were also random or spatially targeted. If spatially targeted,
208 captures (and consequent removals) only occurred on the same side of the I-75 freeway as the
209 initial FeLV infection.
210 In addition, we simulated temporary spatial segregation of panthers via closure of wildlife
211 highway underpasses along the I-75 freeway, methods and results for which are reported in the
212 supplementary materials. To examine the effects of proactive and reactive management
213 interventions conducted in concert, we included proactive vaccination in all reactive
214 management scenarios: specifically, with 0-60% of the population proactively vaccinated (in
215 20% increments) prior to initiation of an outbreak. Because it is highly unlikely that 100% of the
216 proactively vaccinated population would have received boosted vaccination, in reactive

217 management scenarios we used a more conservative—yet still challenging to attain—ratio of
218 50% of the proactively vaccinated population being boosted (i.e. efficacy of 80%). We
219 conducted all management scenarios in a full factorial design resulting in 24, 32, and 16
220 parameter sets for proactive vaccination, reactive vaccination, and reactive test-and-removal
221 scenarios, respectively (100 full simulations per set for a total of 7,200 simulations).
222
223 *Sensitivity analysis*
224 We used a latin hypercube sampling (LHS) approach to generate 50 sensitivity analysis
225 parameter sets across our eight network and transmission parameters (Table S1) using the *lhs*
226 package in R (Carnell, 2012). We repeated our baseline scenario simulations across these 50
227 parameter sets and completed 50 simulations per parameter set for all sensitivity analyses.
228 Due to the high computational effort required to perform sensitivity simulations across all
229 management scenarios, we focused only on the proactive vaccination scenarios. Specifically,
230 we evaluated a subset of 12 LHS parameter sets across a subset of the proactive vaccination
231 conditions in a factorial design (see supplementary methods for further details), resulting in 108
232 parameter sets with 50 full simulations per parameter set (5,400 full simulations). This approach
233 allowed us to examine sensitivity of our proactive vaccination results across different outbreak
234 sizes and network and transmission parameters, while mitigating computational effort
235 associated with exploring such a wide range of parameters and scenario variations. Sensitivity
236 analysis simulation results were evaluated using scatterplots and Partial Rank Correlation
237 Coefficients (PRCC; Marino et al., 2008; Wu et al., 2013); proactive vaccination scenarios were
238 evaluated for alignment with our qualitative result that low proactive vaccination can increase
239 disease-induced mortalities.
240
241 **Results**

242 Progressive infections are expected to result in death in panthers, and are therefore of
243 key concern for management efforts. For simplicity, hereafter, we refer to the number of
244 progressive infections in simulations as the number of mortalities. In the baseline, no-
245 intervention scenario, the median number of mortalities was 34 (range: 1-54); median duration
246 of epidemics was 119.5 weeks, and 34 epidemics failed (fewer than 5 progressive or regressive
247 infections) per 100 successful epidemics.
248
249 *Proactive vaccination alone*
250 Proactive vaccination paradoxically increased the number of mortalities across a range
251 of conditions, especially without vaccine boosting (Figures 2, S2; Table S2). Even with 50% of
252 vaccinated individuals receiving a booster, proactive vaccination only reduced mortalities from
253 the baseline scenario at high levels of population vaccination (i.e., 60-80%). With 100%
254 boosting, proactive vaccination increased mortalities at low levels of population vaccination (10-
255 20%), had marginal effects at 30-40% population vaccination, and was strongly effective at
256 about 50% population vaccination levels and higher (at 80% population vaccination, median
257 17.5 mortalities versus 34 with no interventions). Proactive vaccination consistently lengthened
258 the duration of epidemics, relative to the baseline scenario (up to a median duration of 143.5
259 weeks; Figures S3-5, Table S3). Of those individuals which became infected in proactive
260 vaccination scenarios, susceptibles were typically infected earliest, followed by vaccinated
261 individuals, and susceptible respawned individuals (Figures S6). When all vaccinated individuals
262 received a booster, vaccination reduced the probability of a successful outbreak even at 40%
263 population vaccination (52 failures versus 34 failures per 100 successful epidemics; Figure
264 S16). In contrast, when no vaccinated individuals received a booster, proactive vaccination was
265 largely only effective at reducing the probability of epidemics at very high levels of population
266 vaccination.

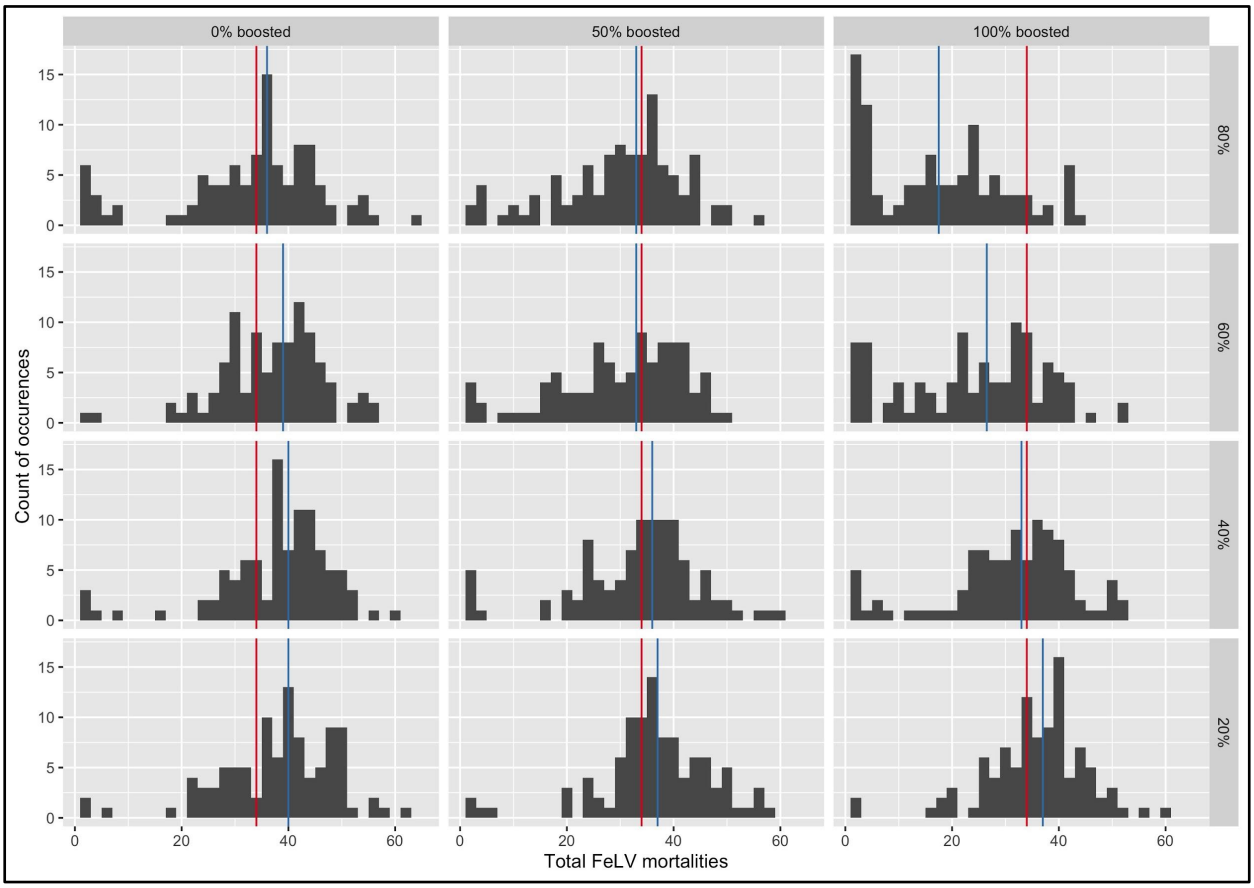

Figure 2: *Histograms of FeLV mortalities in simulated epidemics with proactive vaccination alone. Red vertical lines indicate the median number of mortalities from the baseline scenario without interventions; blue lines, scenarios with proactive vaccination. Panel rows represent the proportion of the population proactively vaccinated; columns represent ratios of vaccine efficacy among vaccinated individuals. Each histogram plot represents the results of 100 simulations. An expanded version of this figure is available in the supplementary materials (Figure S2).*

Proactive and reactive vaccination

Reactive vaccination alone did not reduce mortalities (Figure 3, Table S4). However, reactive vaccination appeared to work synergistically with proactive vaccination, particularly at moderate to high levels of proactive vaccination (i.e., at least 40-60% of the population proactively vaccinated). This largely held true regardless of the timing of intervention onset and

the strategy for reactive vaccination distribution (i.e., random versus spatial; Figures S7-8). The mortality-reducing effects of reactive vaccination were, however, slightly reduced if reactive vaccination occurred for only 6 months out of the year (Figure 3). A ratio of greater than 1.5 inoculations per vaccinated individual appeared to promote the largest reductions in mortalities (e.g., as few as a median of 25 mortalties with year-round reactive vaccination and 60% proactive vaccination versus 34 mortalities with no interventions; Figure 3). Adding reactive vaccination largely did not affect the durations of simulated epidemics (Figures S9-10, Table S5), and had little impact on the probability of epidemics failing, particularly in comparison to proactive vaccination alone (Figure S16).

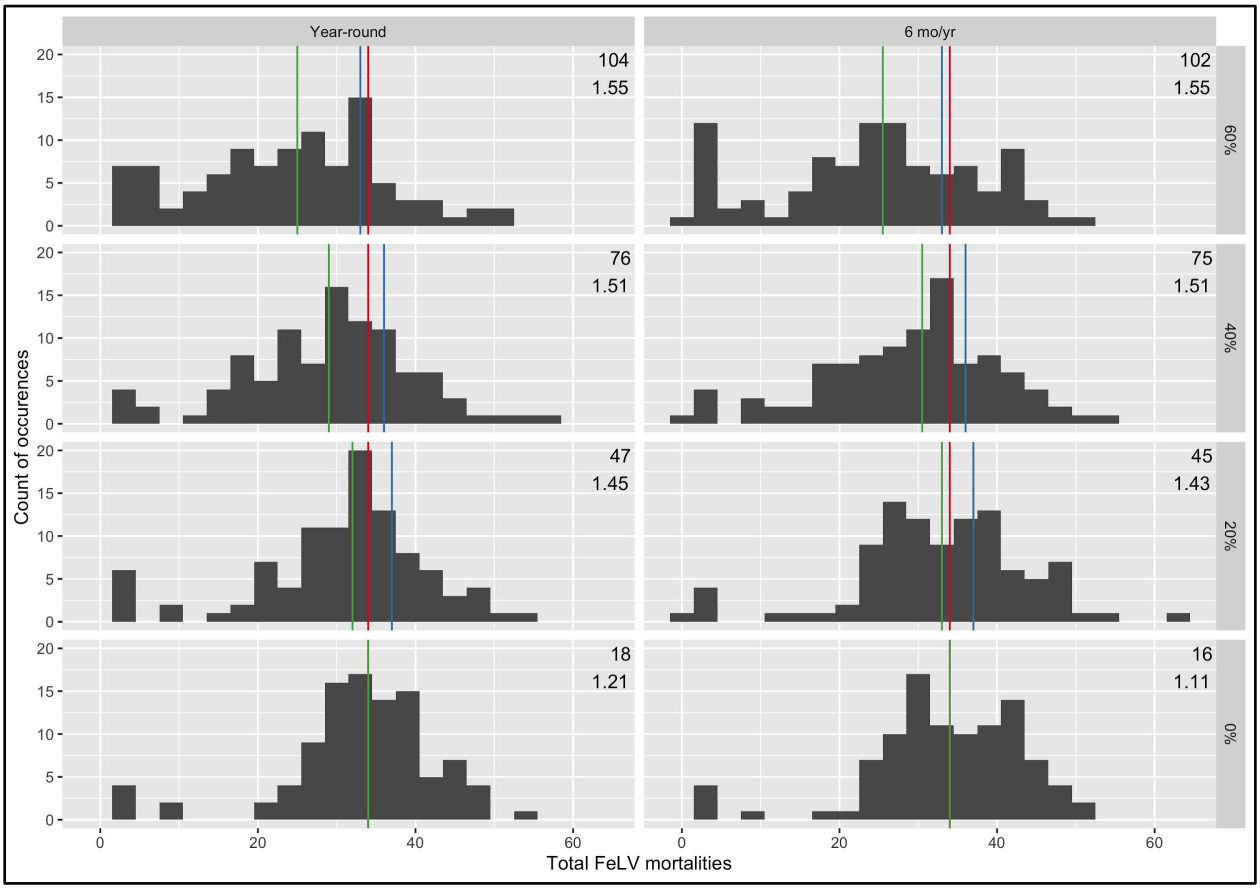

Figure 3: *Histograms of FeLV mortalities in simulated epidemics with both reactive and proactive vaccination. Results shown are for randomly distributed reactive vaccination starting*

52 weeks after epidemic onset. Panel rows represent the proportion of the population proactively vaccinated (with 50% boosted vaccination); note that the bottom row, with 0% proactive vaccination, demonstrates reactive vaccination alone. Columns represent duration of reactive vaccination per year. Red vertical lines indicate the median number of mortalities from simulations without interventions; blue lines for proactive vaccination alone; green lines for the given combination of reactive and proactive vaccination (note green lines overlap red lines in the 0% proactive vaccination scenarios). Numbers in the upper right of each histogram represent (above) median total number of vaccinated individuals (both proactive and reactive), and (below) median value for the total vaccinations used per total vaccinated individuals.

Proactive vaccination with test-and-removal

Test-and-removal alone did not reduce mortalities (Figure 4, Table S6). Like reactive vaccination, however, test-and-removal appeared to work synergistically with proactive vaccination, especially at moderate to high levels of proactive vaccination (i.e., at least 40-60% of the population proactively vaccinated; as few as a median of 26 mortalities). This largely held true regardless of the timing of the onset of the intervention or the targeting of captures (i.e., random versus spatial). Notably, simulated captures were only conducted for about 4 months per simulation year, in contrast to at least 6 months of reactive vaccination per year. Captures were marginally more likely to successfully identify actively infectious individuals when initiated earlier in an outbreak (Figure S11). The addition of test-and-removal largely did not affect the durations of epidemics (Figure S12, Table S7). When coupled with proactive vaccination, test-and-removal had a modest effect in reducing the probability of a successful epidemic (e.g., maximum of 50 failed epidemics per 100 successful versus 34 failed epidemics per 100 successes with no interventions; Figure S16).

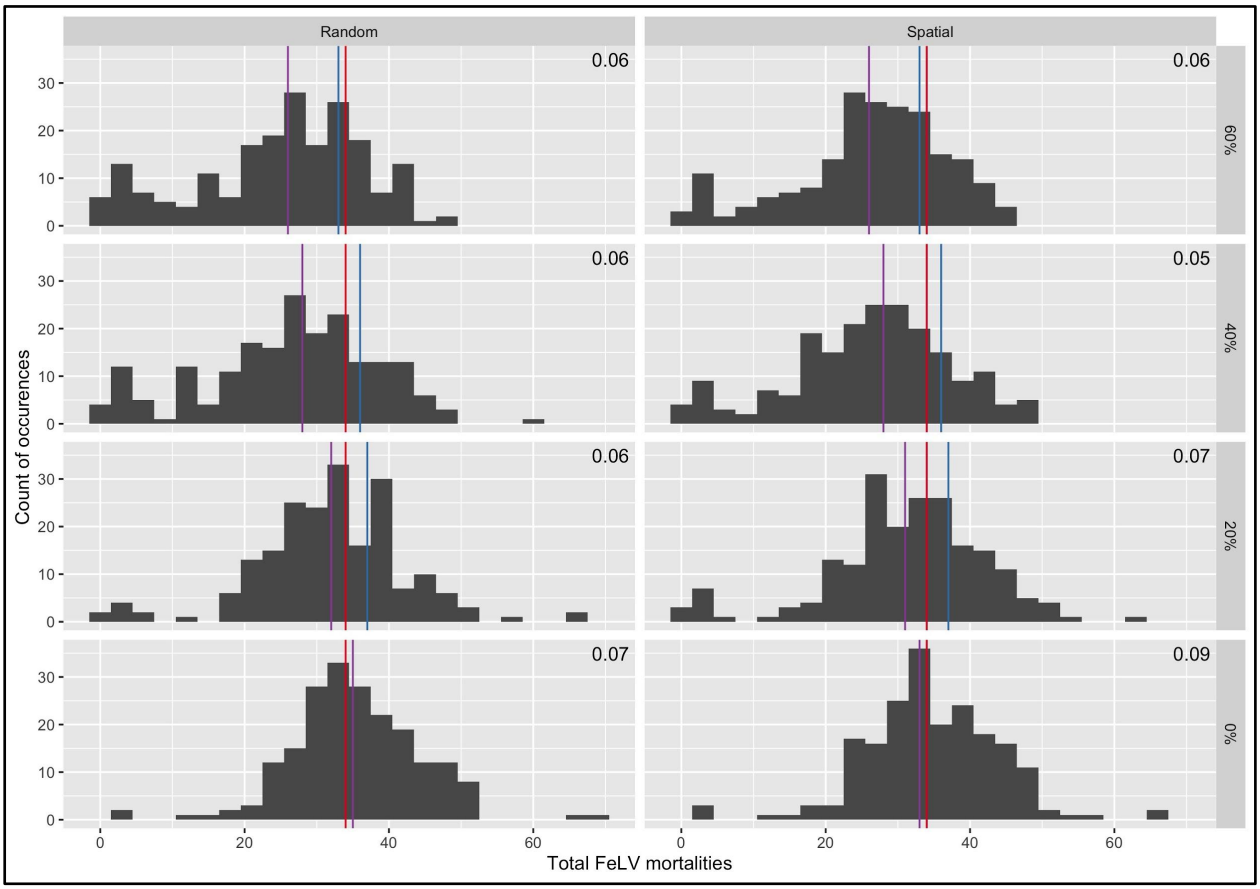

Figure 4: *Histograms of FeLV mortalities in simulated epidemics with both reactive test-and-removal and proactive vaccination. Panel rows represent the proportion of the population proactively vaccinated (with 50% boosted vaccination); note that the bottom row, with 0% proactive vaccination, demonstrates reactive vaccination alone. Columns represent the capture and testing targeting strategy (random versus spatially targeted). Vertical red lines indicate the median number of mortalities from simulations without interventions; blue lines for proactive vaccination alone; purple lines for the given combination of test and removal and proactive vaccination. Numbers in the upper right of each histogram represent the median value for the proportion of capture events that resulted in a removal or humane euthanasia.*

Sensitivity analyses

330 Simulated epidemic sizes were variable across the full 50 sensitivity analysis parameter
331 sets in the absence of FeLV interventions (range: median 7.5 mortalities–median 47.5
332 mortalities; Figure S17). Based on PRCC, the parameters for network density, transmission
333 potential from regressives, weekly contact rates, and baseline transmission potential were
334 positively associated with median mortalities in the absence of interventions; the parameter for
335 the infection-induced mortality rate was negatively associated with median mortalities (see
336 supplementary results, Figure S18).
337 When focusing on a subset of parameter sets for proactive vaccination sensitivity
338 analysis, low levels of proactive vaccination (e.g., 20% population proactive vaccination) were
339 sometimes effective in reducing the number of mortalities, in contrast to our primary results.
340 PRCC results from proactive scenario sensitivity analysis suggested that the parameters for
341 network density, transmission potential from regressives, and weekly contact rates were
342 positively correlated with increased mortalities at low levels of vaccination (Figure S19).
343 However, network density did not have a clearly monotonic relationship with the difference
344 between mortalities with and without proactive vaccination (Figure S20). We therefore
345 performed additional *post-hoc* sensitivity analyses to further interrogate the relationship between
346 network density and our qualitative outcome of increased mortalities at low levels of proactive
347 vaccination (see supplementary results for detailed discussion of *post-hoc* analysis). These
348 additional analyses found some evidence that low levels of proactive vaccination were least
349 effective at reducing mortalities at intermediate values of parameters governing network
350 connectivity (network density and proportion adults), especially when coupled with increased
351 transmission potential (e.g. higher infectiousness of regressive individuals and/or increased
352 weekly contact rates; Figures S22-23).
353
354 **Discussion**

In this study we found unexpected consequences and impacts of several epidemic management strategies for disease control in small populations of conservation concern. Although our simulation results provide guidelines for FeLV management in Florida panthers, they also demonstrate the power of partnering modeling approaches and population management questions to test and optimize disease control strategies in free-ranging wildlife (Joseph et al., 2013). Furthermore, the principles of transmission and available methods of disease control underlying our findings provide insights for pathogen control in other host-pathogen systems, including humans and livestock.

Proactive vaccination alone may exacerbate epidemic outcomes under some conditions

Our simulation results showed a paradoxical increase in FeLV mortalities with low levels of proactive vaccination. This counterintuitive finding is likely due, at least in part, to partial vaccine immunity, a type of vaccine imperfection often overlooked in studies of wildlife disease (Barnett & Civitello, 2020). Under partial vaccine immunity, vaccinated individuals acted as a semi-protected susceptible pool, contracting infection later in the course of an epidemic, ultimately prolonging epidemics and increasing the total number of mortalities. Further, these effects likely interacted with the respawning process we used, which is similar to density-dependent birth processes which have been shown to increase pathogen prevalence and mortality under culling conditions (Choisy & Rohani, 2006). In the case of our simulations, proactive vaccination flattened the epidemic curve (Figure S5), leading to slow, steady introductions of both new susceptibles and vaccine “failures” which could themselves become infected and maintain epidemics. Only with adequate herd immunity could increased mortalities be avoided. This counterintuitive result is consistent with findings by Rees et al. (2013), who found reduced vaccine effectiveness in some conditions in which hosts had heterogeneous spatial distributions. Importantly, our results suggest that studies assuming full protection from vaccination may significantly underestimate the level of vaccination needed for population

381 protection—underestimates which may even make epidemics worse if only partial vaccine
382 immunity can be achieved.

383 Our sensitivity analysis suggests that populations with intermediate levels of network
384 connectivity and/or high transmission potential may be most vulnerable to these paradoxical
385 effects. As in spatially structured populations (McCallum, 2008), high or low connectivity causes
386 infection to fade out quickly; with intermediate connectivity, vaccine failures provide a steady
387 supply of new susceptibles. Alternatively, in cases of high transmission potential, vaccination
388 may shift a rapid fade-out epidemic to a sustained epidemic scenario, as in Rees et al. (2013).
389 While our sensitivity analysis was limited by computational demands and use of some discrete
390 parameters (which may affect PRCC inference; Marino et al., 2008), our findings are consistent
391 with this broader body of literature.

392 FeLV vaccine efficacy may operate differently in reality from our simulation structure: for
393 example, some vaccinated individuals may have zero vaccine-induced immunity, while others
394 have 100% protection (binary immunity; Barnett & Civitello, 2020). Alternatively, vaccination
395 may not protect from infection but could reduce viral shedding or increase survival of infected
396 individuals (Barnett & Civitello, 2020). In the case of binary immunity, vaccine efficacy would be
397 unlikely to prolong and worsen epidemics as we saw here. In contrast, increased survival of
398 infected individuals without changes to shedding potential could extend or worsen outbreaks,
399 and even favor the evolution of virulence (Barnett & Civitello, 2020). Future research should
400 assess vaccinated panthers' immune response to FeLV infection *in vitro* to help refine
401 understanding of vaccine imperfection in panthers and how imperfect immunity may further alter
402 vaccination guidelines.

403 Based on our results, we argue that wildlife managers should continue vaccinating
404 available panthers and prioritize boosting vaccinated individuals to develop a core population of
405 high-immunity individuals, rather than a broadly distributed low-immunity population. This
406 recommendation should be most effective at increasing the probability of epidemic failure, and

407 aligns with other wildlife studies which have emphasized vaccination of core populations (Vial et
408 al., 2006) or risk-based sub-populations (Beyer et al., 2012). Given ongoing uncertainties in
409 vaccine efficacy and the duration of immunity, in the event of an outbreak, we recommend
410 updating model parameterizations to reflect on-the-ground realities in an adaptive management
411 framework in order to provide the most useful predictions and guidance for managers. For
412 example, observed "breakthrough" infection rates (infections in vaccinated individuals) would be
413 a key observation for updating model predictions and adapting management responses.
414
415 *Reactive and proactive strategies can work synergistically to reduce epidemic impacts*
416 Our simulations showed that both reactive vaccination and test-and-removal strategies
417 reduced FeLV mortalities in panthers when used in combination with moderate levels of
418 proactive vaccination. Test-and-removal had more consistent effects with arguably less effort
419 than reactive vaccination (4 months of captures compared to year-round reactive vaccination).
420 We therefore suggest that test-and-removal be prioritized over reactive vaccination, especially if
421 identification of actively infectious individuals can be improved.
422 In our simulations, captures that were most aligned with the initial wave of infectious
423 individuals (i.e., with earlier onset) were more likely to identify actively infectious individuals for
424 removal. This finding highlights the importance of targeting captures to individuals likely to be
425 actively infectious. However, determining infection status in cryptic wildlife is difficult, and
426 consequently supports the increased use of remote tracking technologies that may be able to
427 (1) identify behavior changes associated with sickness, and (2) detect the onset of an epidemic
428 more quickly. This conclusion is consistent with similar findings in Channel Island foxes, where
429 increasing the number and frequency of tracking of sentinel individuals was important for early
430 identification of epidemics (Sanchez & Hudgens, 2020).
431 A key component of the success of test-and-removal here is the selective removal we
432 simulated, which avoids removing immune individuals that contribute to overall herd immunity

433 (Miguel et al., 2020; Potapov et al., 2012). However, we have simplified the field testing process
434 in our simulations. The common field FeLV diagnostic test identifies antigenemia, which is key
435 for identifying actively infectious individuals. However, the duration of antigenemia—and even
436 degree of infectiousness—in regressively infected individuals is unclear in panthers. We may
437 therefore overestimate the effect of removing regressive individuals, but given their reduced
438 infectiousness in our simulations, we still expect test-and-removal to be a key strategy for
439 mitigating FeLV impacts in panthers.

440 Notably, reactive vaccination, in concert with proactive vaccination, is also a viable
441 alternative strategy to test-and-removal. Our simulations showed reactive vaccination to have
442 the strongest effects for reducing FeLV mortalities when at least 50% of the population was
443 vaccinated, and with a ratio of about 1.5 vaccines per vaccinated individual (i.e., 50% of
444 vaccinated individuals received a booster). We therefore suggest that managers should
445 prioritize boosting at least half of vaccinated individuals in a reactive vaccination response
446 scenario. We did not see a strong effect of attempting a vaccine barrier, in contrast to Sanchez
447 *et al* (2020), where a simulated vaccine barrier could effectively halt spread of a pathogen in
448 Channel Island foxes. This difference is likely due to the differences in home range size and
449 movement capacity between the two species, with foxes ranging less widely than panthers. In
450 reality—and as part of an adaptive management response—if an empirical outbreak of FeLV in
451 panthers exhibited a stronger spatial signal than was featured in our simulations, spatially
452 targeted reactive vaccination may yet be a worthwhile intervention strategy.

453 Importantly—particularly if levels of population protection from proactive vaccination are
454 unknown—both reactive vaccination and test-and-removal strategies mitigated the negative
455 effects of low levels of population protection seen with inadequate proactive vaccination. It is
456 therefore vital for managers to incorporate these reactive strategies in the event of future FeLV
457 outbreaks.

458

Limitations and future directions

In this study, we considered the effects of partial vaccine immunity, but we made the simplifying assumption of no waning vaccine or infection-induced immunity over time. However, our findings with regard to imperfect efficacy are representative of the likely consequences of waning immunity in that the loss of immunity supplies new susceptibles to the population. Should immunity not outlast the course of an FeLV outbreak, this process could prolong outbreaks and result in increased mortalities. Future research should therefore examine the effects of waning vaccine immunity, particularly considering the value of revaccinating individuals that may be experiencing loss of vaccine protection. Furthermore, an adaptive management framework should monitor for infection in individuals previously considered immune, which could represent vaccine failure or fading immunity. Model predictions could then be updated with these observations and revaccination rates adjusted accordingly.

Our simulation results found that relatively high levels of vaccination were required to reduce impacts of FeLV in panthers, in contrast to studies in other endangered species (Gilbert et al., 2020; Haydon et al., 2006). However, here we are investigating a pathogen with a long duration of infectiousness and lacking the advantages of distinct corridors between panther sub-populations for reducing required levels of vaccination. It is therefore unsurprising that panthers would require higher levels of FeLV population vaccination than was found, for example, for rabies vaccination in Ethiopian wolves (Haydon et al., 2006) or canine distemper virus vaccination in Amur tigers (Gilbert et al., 2020). However, we also did not assume the presence of preexisting population immunity prior to proactive vaccination, and some degree of population immunity likely already exists in panthers, given ongoing exposures (Chiu et al., 2019). This would reduce necessary vaccination levels in panthers, as would higher vaccine efficacy than we conservatively assumed here (Vial et al., 2006). Future research could prioritize better understanding the realized individual immunity after single and boosted FeLV vaccination in panthers to determine if inoculations are more protective than we conservatively assumed here.

485

486 *Conclusions*

487 Our simulation results highlight the risks of inadequate proactive vaccination, particularly
488 with partial vaccine immunity. We recommend prioritizing boosted vaccination in panthers, and
489 joint use of proactive vaccination and reactive strategies to mitigate the risks of imperfect
490 vaccination and most effectively reduce the impacts of FeLV in this iconic carnivore. This
491 research highlights the value of linking modeling and management priorities to identify
492 unexpected consequences of interventions and determine optimal pathogen management
493 strategies in free-ranging wildlife.

494

495 **Authors' Contributions**

496 All authors conceived the ideas and designed methodology; MLJG performed simulations,
497 analyzed data, and led the writing of the manuscript. All authors contributed critically to the
498 drafts and gave final approval for publication.

499

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510

Data Availability

Full R code for simulations is available on GitHub (https://github.com/mjones029/FeLV_Management_Simulations) and upon acceptance will be archived at Zenodo.

Works Cited

Baker, L., Matthiopoulos, J., Müller, T., Freuling, C., & Hampson, K. (2019). Optimizing spatial and seasonal deployment of vaccination campaigns to eliminate wildlife rabies. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *374*(1776), 20180280.

Barnett, K. M., & Civitello, D. J. (2020). Ecological and Evolutionary Challenges for Wildlife Vaccination. *Trends in Parasitology*, *36*(12), 970–978.

Beyer, H. L., Hampson, K., Lembo, T., Cleaveland, S., Kaare, M., & Haydon, D. T. (2012). The implications of metapopulation dynamics on the design of vaccination campaigns. *Vaccine*, *30*(6), 1014–1022.

Breed, A. C., Plowright, R. K., Hayman, D. T. S., Knobel, D. L., Molenaar, F. M., Gardner–Roberts, D., Cleaveland, S., Haydon, D. T., Kock, R. A., Cunningham, A. A., Sainsbury, A. W., & Delahay, R. J. (2009). Disease Management in Endangered Mammals. In R. J. Delahay, G. C. Smith, & M. R. Hutchings (Eds.), *Management of Disease in Wild Mammals* (pp. 215–239). Springer Japan.

Brown, M. A., Cunningham, M. W., Roca, A. L., Troyer, J. L., Johnson, W. E., & O'Brien, S. J. (2008). Genetic characterization of feline leukemia virus from Florida panthers. *Emerging Infectious Diseases*, *14*(2), 252–259.

Carnell, R. (2012). lhs: Latin hypercube samples. *R Package Version 0. 10, URL http://CRAN. R-Project.org/package= Lhs*.

Chiu, E. S., Kraberger, S., Cunningham, M., Cusack, L., Roelke, M., & VandeWoude, S. (2019). Multiple Introductions of Domestic Cat Feline Leukemia Virus in Endangered Florida Panthers. *Emerging Infectious Diseases*, *25*(1), 92–101.

Choisy, M., & Rohani, P. (2006). Harvesting can increase severity of wildlife disease epidemics. *Proceedings. Biological Sciences / The Royal Society*, *273*(1597), 2025–2034.

Craft, M. E., Beyer, H. L., & Haydon, D. T. (2013). Estimating the probability of a major outbreak from the timing of early cases: an indeterminate problem? *PloS One*, *8*(3), e57878.

Cunningham, M. W., Brown, M. A., Shindle, D. B., Terrell, S. P., Hayes, K. A., Ferree, B. C., McBride, R. T., Blankenship, E. L., Jansen, D., Citino, S. B., Roelke, M. E., Kiltie, R. A., Troyer, J. L., & O'Brien, S. J. (2008). Epizootiology and management of feline leukemia virus in the Florida puma. *Journal of Wildlife Diseases*, *44*(3), 537–552.

Gilbert, M., Sulikhan, N., Uphyrkina, O., Goncharuk, M., Kerley, L., Hernandez Castro, E., Reeve, R., Seimon, T., McAloose, D., Seryodkin, I. V., Naidenko, S. V., Davis, C. A., Wilkie, G. S., Vattipally, S. B., Adamson, W. E., Hinds, C., Thomson, E. C., Willett, B. J., Hosie, M. J., … Cleaveland, S. (2020). Distemper, extinction, and vaccination of the Amur tiger. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.2000153117

Gilbertson, M. L. J., Fountain-Jones, N. M., Malmberg, J. L., Gagne, R. B., Lee, J. S., Kraberger, S., Kechejian, S., Petch, R., Chiu, E., Onorato, D., Cunningham, M. W., Crooks, K. R., Funk, W. C., Carver, S., VandeWoude, S., VanderWaal, K., & Craft, M. E. (2021). Transmission of one predicts another: Apathogenic proxies for transmission dynamics of a fatal virus. *bioRxiv*. https://doi.org/10.1101/2021.01.09.426055

Haydon, D. T., Randall, D. A., Matthews, L., Knobel, D. L., Tallents, L. A., Gravenor, M. B., Williams, S. D., Pollinger, J. P., Cleaveland, S., Woolhouse, M. E. J., Sillero-Zubiri, C., Marino, J., Macdonald, D. W., & Laurenson, M. K. (2006). Low-coverage vaccination strategies for the conservation of endangered species. *Nature*, *443*(7112), 692–695.

Joseph, M. B., Mihaljevic, J. R., Arellano, A. L., Kueneman, J. G., Preston, D. L., Cross, P. C., & Johnson, P. T. J. (2013). Taming wildlife disease: bridging the gap between science and management. *The Journal of Applied Ecology*, *50*(3), 702–712.

Little, S., Levy, J., Hartmann, K., Hofmann-Lehmann, R., Hosie, M., Olah, G., & Denis, K. S. (2020). 2020 AAFP Feline Retrovirus Testing and Management Guidelines. *Journal of Feline Medicine and Surgery*, *22*(1), 5–30.

Lloyd-Smith, J. O., Cross, P. C., Briggs, C. J., Daugherty, M., Getz, W. M., Latto, J., Sanchez, M. S., Smith, A. B., & Swei, A. (2005). Should we expect population thresholds for wildlife disease? *Trends in Ecology & Evolution*, *20*(9), 511–519.

López, G., López-Parra, M., Fernández, L., Martínez-Granados, C., Martínez, F., Meli, M. L., Gil-Sánchez, J. M., Viqueira, N., Díaz-Portero, M. A., Cadenas, R., Lutz, H., Vargas, A., & Simón, M. A. (2009). Management measures to control a feline leukemia virus outbreak in the endangered Iberian lynx. *Animal Conservation*, *12*(3), 173–182.

Marino, S., Hogue, I. B., Ray, C. J., & Kirschner, D. E. (2008). A methodology for performing global uncertainty and sensitivity analysis in systems biology. *Journal of Theoretical Biology*, *254*(1), 178–196.

McCallum, H. (2008). Landscape structure, disturbance, and disease dynamics. *Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems*, 100–122.

McClintock, B. T., Onorato, D. P., & Martin, J. (2015). Endangered Florida panther population size determined from public reports of motor vehicle collision mortalities. *The Journal of Applied Ecology*, *52*(4), 893–901.

Miguel, E., Grosbois, V., Caron, A., Pople, D., Roche, B., & Donnelly, C. A. (2020). A systemic approach to assess the potential and risks of wildlife culling for infectious disease control. *Communications Biology*, *3*(1), 353.

Potapov, A., Merrill, E., & Lewis, M. A. (2012). Wildlife disease elimination and density

dependence. *Proceedings. Biological Sciences / The Royal Society*, *279*(1741), 3139–3145.

R Core Team. (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rees, E. E., Pond, B. A., Tinline, R. R., & Bélanger, D. (2013). Modelling the effect of landscape heterogeneity on the efficacy of vaccination for wildlife infectious disease control. *The Journal of Applied Ecology*, *50*(4), 881–891.

Robinson Stacie J., Barbieri Michelle M., Murphy Samantha, Baker Jason D., Harting Albert L., Craft Meggan E., & Littnan Charles L. (2018). Model recommendations meet management reality: implementation and evaluation of a network-informed vaccination effort for endangered Hawaiian monk seals. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1870), 20171899.

Rushmore, J., Caillaud, D., Hall, R. J., Stumpf, R. M., Meyers, L. A., & Altizer, S. (2014). Network-based vaccination improves prospects for disease control in wild chimpanzees. *Journal of the Royal Society, Interface / the Royal Society*, *11*(97), 20140349.

Sanchez, J. N., & Hudgens, B. R. (2020). Vaccination and monitoring strategies for epidemic prevention and detection in the Channel Island fox (*Urocyon littoralis*). *PloS One*, *15*(5), e0232705.

Slate, D., Rupprecht, C. E., Rooney, J. A., Donovan, D., Lein, D. H., & Chipman, R. B. (2005). Status of oral rabies vaccination in wild carnivores in the United States. *Virus Research*, *111*(1), 68–76.

Sparkes, A. H. (1997). Feline leukaemia virus: a review of immunity and vaccination. *The Journal of Small Animal Practice*, *38*(5), 187–194.

Torres, A. N., Mathiason, C. K., & Hoover, E. A. (2005). Re-examination of feline leukemia virus: host relationships using real-time PCR. *Virology*, *332*(1), 272–283.

Vial, F., Cleaveland, S., Rasmussen, G., & Haydon, D. T. (2006). Development of vaccination

strategies for the management of rabies in African wild dogs. *Biological Conservation*, *131*(2), 180–192.

Woodroffe, R., Donnelly, C. A., & Cox, D. R. (2006). Effects of culling on badger *Meles meles* spatial organization: implications for the control of bovine tuberculosis. *Journal of Applied Ecology,* 43(1), 1-10.

Wu, J., Dhingra, R., Gambhir, M., & Remais, J. V. (2013). Sensitivity analysis of infectious disease models: methods, advances and their application. *Journal of the Royal Society, Interface / the Royal Society*, *10*(86), 20121018.