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

Marie L.J. Gilbertson[1]*, Dave Onorato[2], Mark Cunningham[3], Sue VandeWoude[4], Meggan E. Craft[1,5]

*Corresponding author: jone1354@umn.edu

[1]Department of Veterinary Population Medicine, University of Minnesota, St Paul, MN 55108.

[2]Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, Naples, FL 34114.

[3]Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, Gainesville, FL 32601.

[4]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523

[5] Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN 55108.

Classification

Biological Sciences: Ecology

Keywords

disease model; simulation modeling; network modeling; feline leukemia virus; vaccination; test-and-removal; spatial restrictions; *Puma concolor coryi*

Abstract

Management of pathogen transmission is often hindered by uncertainties in the efficacy of and interactions between intervention strategies, sometimes resulting in unintended negative consequences. Yet 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 2002-2004, and continues to be affected by this deadly virus. Ongoing management efforts aim to mitigate the effects of FeLV on panthers with limited empirical information about which strategies are most effective and efficient. We used a simulation-based approach to determine optimal FeLV management strategies. We simulated use of proactive management interventions (i.e., proactive vaccination) as well as three reactive strategies, including vaccination in the face of an outbreak, test and removal protocols, and temporary spatial segregation of the panther population. Vaccination strategies included partial vaccine immunity, an understudied type of vaccine imperfection. We compared the effectiveness of different strategies in mitigating FeLV mortalities and duration of outbreaks. Results showed that inadequate proactive vaccination can paradoxically increase the number of disease-induced mortalities in FeLV outbreaks, at least in part due to 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 were unlikely to be effective under realistic conditions. Our results highlight the importance of integrating management and modeling approaches to identify unexpected consequences and synergies in pathogen management interventions and aid in conservation of at-risk species.

Significance Statement

Managing outbreaks of infectious disease is fraught with uncertainties such that seemingly helpful interventions can have unintended negative consequences. We used a simulation approach to determine optimal pathogen management strategies in an endangered carnivore, the Florida panther (*Puma concolor coryi*), which continues to be affected by outbreaks of feline leukemia virus. We tested interventions including proactive vaccination, reactive vaccination, test-and-removal, and temporary spatial restrictions. We found that inadequate proactive

vaccination can counterintuitively increase disease-related mortalities. In contrast, we identified synergies between proactive and reactive strategies that will be key for ongoing conservation of the iconic Florida panther. The results of this study demonstrate the importance of linking modeling and management to optimize pathogen control and avoid unexpected negative consequences.

Introduction

Outbreaks of infectious disease can have significant impacts on the population health of free-ranging wildlife, and are of heightened importance in species of conservation concern (1). 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 (2), spilling over from domestic cats, with subsequent direct transmission among panthers (3). In addition, there is recent evidence of ongoing FeLV spillover to and transmission among panthers (4), necessitating continued management of this deadly pathogen. FeLV inoculation with a domestic cat vaccine has been used previously, but with unknown efficacy in panthers (2). 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 offer a powerful tool for filling such disease management knowledge gaps (5). Models allow the ethical testing of a wide range of different management approaches and can reveal unexpected consequences of disease control interventions (6). Models have been used to optimize disease management protocols in a variety of free-ranging wildlife species of conservation concern, including Ethiopian wolves (7), chimpanzees (8), and Amur tigers (9). 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., 10, 11). Here, we use mathematical models of FeLV transmission in Florida panthers to determine optimal disease prevention and control strategies in this iconic carnivore.

85 Pathogen management strategies can be preventive (hereafter, *proactive*) or reactive.
86 Among proactive and reactive strategies, vaccination is a cornerstone in veterinary medicine (1,
87 12), and functions by reducing the availability of susceptible individuals. Vaccination can be
88 applied randomly, or targeted to high risk individuals (e.g., 13); to likely superspreaders (e.g., in
89 chimpanzees: 8); seasonally (e.g., 11, 14); or spatially, as with vaccine barriers or firewalls to
90 prevent spread of a pathogen to new areas or subsets of populations (e.g., 15–17). In panthers,
91 vaccination has been used for active management of FeLV (2, 4), but with uncertainties
92 regarding optimal distribution, individual efficacy, and level of population protection needed.
93 Among reactive pathogen management strategies, test-and-removal (or test-and-cull) is
94 commonly used in domestic species to functionally reduce the infectious period of infected
95 individuals (18). However, this approach is rarely used in wildlife due to the generally low
96 availability of field diagnostic tests and/or difficulty in recapturing individuals after a positive
97 diagnosis (18). Rather, pathogen control in wildlife often relies on non-selective culling in an
98 attempt to reduce density and—in theory—transmission (19). However, non-selective culling
99 can have a host of negative consequences for disease management including removal of
100 immune individuals (18, 20) or culling-induced perturbation (e.g., 21). Thanks to ready
101 availability of a field diagnostic test, FeLV management in domestic cats and Iberian lynx has
102 made use of test-and-removal or isolation of infected individuals (22, 23), and is part of future
103 FeLV mitigation plans in panthers. However, uncertainties remain regarding how test-and-
104 removal might interact with proactive vaccination efforts to more effectively reduce pathogen
105 impacts.
106 Larger-scale isolation or quarantine measures may also be used for disease
107 management in free-ranging wildlife, in the form of physical or behavioral barriers between
108 affected and unaffected subsets of a population. For example, fencing has been used to prevent
109 transmission of foot-and-mouth disease between wildlife and cattle in South Africa (24), and
110 winter feeding grounds may help prevent transmission of brucellosis between elk and cattle in

111 the Greater Yellowstone Ecosystem through behavioral separation (25). For panthers, while
112 physical barriers to prevent spillover from domestic cats are impractical, it may be possible to
113 use temporary spatial barriers to reduce the spatial spread of FeLV among panthers after a
114 spillover event. Specifically, the major I-75 freeway is fenced throughout Florida panther habitat
115 to reduce vehicle strikes, with regular wildlife underpasses the main means for wildlife to
116 traverse this barrier. While never before attempted, it may be possible to physically block these
117 underpasses under emergency conditions to prevent the spread of FeLV from the northern to
118 southern subsets of the panther population, or vice versa.

119 Given the ongoing risks of FeLV to panther conservation and the uncertainties regarding
120 application of proactive and reactive FeLV management strategies, the objectives of this study
121 were to test singly, and in combination, the effectiveness of: (1) proactive vaccination, (2)
122 reactive vaccination, (3) reactive test-and-removal, and (4) reactive temporary spatial
123 restrictions for reducing the population level impacts of FeLV in Florida panthers. We used a
124 mathematical modeling approach to address these objectives in order to efficiently and ethically
125 test a wide range of intervention protocols for the protection of this endangered carnivore.
126

127 **Methods**

128 *Simulation pipeline*

129 To examine the effect of different disease management regimes on FeLV control, we
130 used spatially-explicit, network simulations adapted from our research determining drivers of
131 retrovirus transmission in panthers (26). This approach involves two steps: (1) simulation of a
132 contact network among panthers, and (2) simulation of FeLV transmission on this network. In
133 brief, we simulated panther populations of 150 individuals (27) and used our previously
134 described exponential random graph model for retrovirus transmission in panthers (26) to
135 simulate contact networks constrained by network density among simulated populations (see
136 supplementary methods and Table S1 for further details).

137 We used a transmission model developed in Gilbertson et al. (26). Briefly, this model
138 used a susceptible-infectious-recovered compartmental framework, where individuals could be
139 progressively, regressively, or abortively infected (2). Progressive infections always resulted in
140 death, while regressive infections eventually recovered with immunity, and abortive infections
141 were always considered immune. Importantly, based on our previous work, we allowed both
142 progressives and regressives to be infectious, though regressives were less likely to transmit
143 (Table S1). Duration of infectiousness is long for FeLV (estimated as a mean of 18 weeks; 2 but
144 see 26 and Table S1). As in Gilbertson et al. (26), we included only disease-induced mortality,
145 and, in order to preserve key network structure, allowed territories vacated by deaths to be
146 reoccupied by new susceptible individuals (hereafter, *respawning*). Outbreaks were initiated by
147 a single, randomly selected non-isolate individual in the population (see supplement
148 discussion), and proceeded in weekly time steps for up to five years.

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

158 For a *baseline scenario*, we recorded key epidemic outcomes in the absence of
159 interventions. For *management scenarios* we recorded key outcomes in the presence of
160 interventions (Figure 1). These key outcomes were (1) the number of mortalities, (2) the
161 duration of an epidemic, and (3) the proportion of epidemics that “failed” per 100 successful
162 epidemics. A failed epidemic (i.e. stochastic fadeout; 28) was one in which fewer than 5

individuals acquired progressive or regressive infections. The outcomes of mortalities and epidemic durations were summarized as median values per parameter set, as results were often skewed; all outcomes were compared between baseline and management scenarios. All simulations were performed in R version 3.6.3 (29).

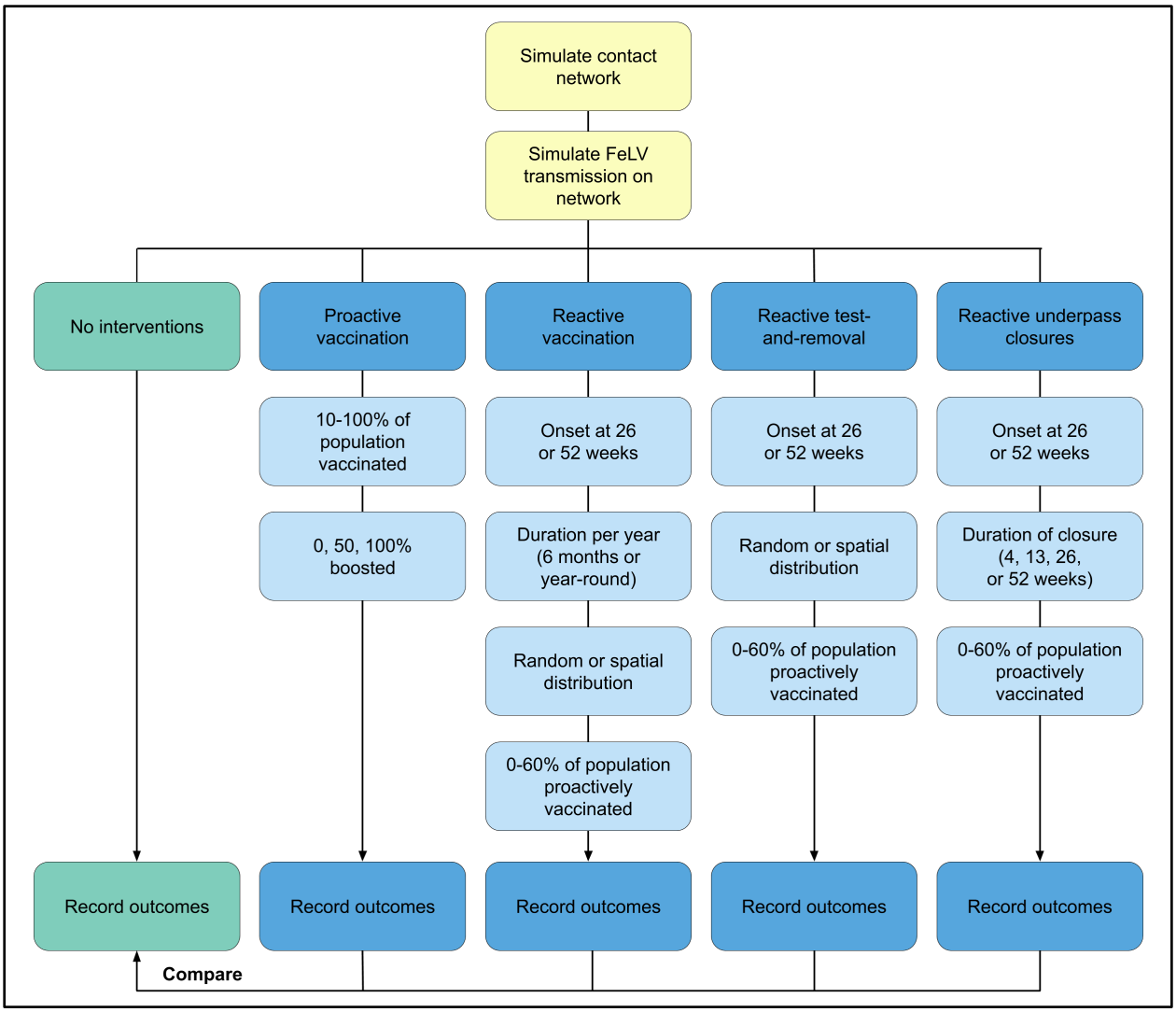

Figure 1: *Steps of the simulation process across baseline and management scenarios. The basic network and FeLV transmission simulation steps are shown in yellow, the baseline (no intervention) scenario in green, and the four different overarching management scenarios in*

172 *blue. Within each management scenario, we investigated several variations for the given*
173 *approach (shown in light blue) in a factorial design.*
174
175 *Proactive vaccination*
176 In the first management scenario, we examined the effect of different levels of population
177 proactive vaccination (proportion of the population vaccinated prior to an outbreak), and
178 different ratios of single versus boosted vaccination. We simulated from 10-80% (in 10%
179 increments) of the population having some degree of vaccine-induced immunity to FeLV at the
180 onset of an outbreak. These vaccinations were distributed randomly in the population. Among
181 the vaccinated individuals, 0, 50, or 100% received a second boosting inoculation. Actual
182 vaccine efficacies are unknown for panthers, but based on efficacy studies in domestic cats (30,
183 31), we conservatively assumed that boosted vaccination would prevent 80% of infections and
184 single vaccination would prevent 40% of infections. We modeled this efficacy as a binomial
185 probability to represent partial vaccine immunity (versus binary immunity; 32). We conducted
186 proactive vaccination scenarios in a full factorial design, for a total of 24 proactive vaccination
187 parameter sets, and 2,400 full simulations (100 full simulations per parameter set).
188
189 *Reactive vaccination*
190 During simulated periods of reactive vaccination administration, panthers were selected
191 at a rate of one panther per week for vaccination. We assumed that managers would not know
192 the disease status of an individual selected for vaccination and that vaccination would be
193 ineffective in infectious or recovered individuals. In the case of previously vaccinated individuals,
194 a re-vaccination changed the vaccine efficacy of singly vaccinated individuals (efficacy of 40%)
195 to the efficacy for boosted individuals (efficacy of 80%). We further assumed that managers
196 would know which individuals had received two vaccinations, so boosted individuals were not
197 selected for additional vaccination attempts.

198 We varied the timing of the onset of reactive vaccination after the initiation of an FeLV
199 outbreak to reflect the difficulty of epidemic detection in this elusive carnivore. We therefore
200 began reactive vaccination at an optimistic, but difficult-to-attain time point of 26 weeks, and a
201 more realistic time point of 52 weeks. In addition, we varied the distribution of reactive
202 vaccination. While proactive vaccines were always distributed randomly, reactive vaccination
203 was either randomly distributed or spatially distributed in an attempted vaccine barrier based on
204 proximity to the I-75 freeway (see supplementary methods for further details).
205 Because vaccination is resource and time intensive, we evaluated the effect of reactive
206 vaccination for 6 months per year versus year-round. Among reactive vaccination scenarios, we
207 also included scenarios with proactive vaccination, specifically where 0-60% of the population
208 was proactively vaccinated (in 20% increments) at the initiation of an outbreak. Because it is
209 highly unlikely that 100% of the proactively vaccinated population would have received boosted
210 vaccination, we used a more conservative—yet still challenging to attain—ratio of 50% of the
211 proactively vaccinated population being boosted (i.e. efficacy of 80%). We held this ratio of
212 efficacy constant for proactive vaccination in all reactive scenarios. We again used a factorial
213 design across all variations, resulting in 32 parameter sets and a total of 3,200 full simulations
214 under reactive vaccination scenarios.
215
216 *Reactive test-and-removal*
217 Test-and-removal scenarios were built around a protocol in which panthers infectious at
218 capture are removed from the population through humane euthanasia or temporary removal
219 until recovery. For simplicity, we assumed that all progressively infected individuals would show
220 clinical signs and be humanely euthanized at capture, while infectious regressive individuals
221 were temporarily removed from the population until their recovery and re-released into any open
222 territory. A maximum of five individuals were allowed to be temporarily removed in this way at
223 one time.

We expected that managers would be able to capture and test one panther per week at most, and that captures occurred during a 17 week (about 4 month) capture season, in accordance with current panther capture protocols. We assumed that managers would not know the disease status of a target individual until the capture occurred, so captures were not targeted by infection state. We varied the onset of test-and-removal, such that the intervention began 26 or 52 weeks after the initiation of an epidemic. Captures were also random or spatially targeted. If spatially targeted, captures (and consequent removals) only occurred on the same side of the I-75 freeway as the initial FeLV infection.

As in the reactive vaccination scenarios, we again also included varying degrees of proactive vaccination (0-60% in 20% increments). Reactive test-and-removal scenarios were simulated in a full factorial design across variations, for a total of 16 parameter sets and 1,600 full simulations.

Reactive underpass closures

To determine the potential utility of closing I-75 wildlife underpasses in a FeLV outbreak, we considered a “best case” scenario in which underpass closures were completely effective at preventing transmission across the freeway. Underpasses were closed instantaneously either 26 or 52 weeks after the initiation of an FeLV outbreak, and remained closed for 4, 13, 26, or 52 weeks. We again included variations in proactive vaccination (0-60% in 20% increments), as in other reactive management scenarios. The factorial design here resulted in 32 parameter sets for underpass closure scenarios, for a total of 3,200 full simulations.

Sensitivity analysis

We used a latin hypercube sampling (LHS) approach to generate 50 sensitivity analysis parameter sets across our 8 network and transmission parameters using the *lhs* package in R

249 (33). We repeated our baseline scenario simulations across these 50 parameter sets and
250 completed 50 simulations per parameter set for all sensitivity analyses.
251 Due to the high computational effort required to perform sensitivity simulations across all
252 management scenarios, we focused only on the proactive vaccination scenarios. Specifically,
253 we evaluated a subset of 12 LHS parameter sets across a subset of the proactive vaccination
254 conditions in a factorial design (see supplementary methods for further details), resulting in 108
255 parameter sets with 50 full simulations per parameter set (5,400 full simulations). This approach
256 allowed us to examine sensitivity of our proactive vaccination results across different outbreak
257 sizes and network and transmission parameters, while mitigating computational effort
258 associated with exploring such a wide range of parameters and scenario variations. Sensitivity
259 analysis simulation results were evaluated using scatterplots and Partial Rank Correlation
260 Coefficients (PRCC; 34, 35); proactive vaccination scenarios were evaluated for alignment with
261 our qualitative result that low proactive vaccination can increase disease-induced mortalities.
262
263 **Results**
264 Progressive infections are expected to result in death in panthers, and are therefore of
265 key concern for management efforts. For simplicity, hereafter, we refer to the number of
266 progressive infections in simulations as the number of mortalities. In the baseline, no-
267 intervention scenario, the median number of mortalities was 34 (range: 1-54); median duration
268 of epidemics was 119.5 weeks, and 34 epidemics failed (fewer than 5 progressive or regressive
269 infections) per 100 successful epidemics.
270
271 *Proactive vaccination alone*
272 Proactive vaccination paradoxically increased the number of mortalities across a range
273 of conditions, especially without vaccine boosting (Figures 2, S2). Even with 50% of vaccinates
274 receiving a booster, proactive vaccination only reduced mortalities from the baseline scenario at

high levels of population vaccination (i.e., 60-80%). With 100% boosting, proactive vaccination increased mortalities at low levels of population vaccination (10-20%), had marginal effects at 30-40% population vaccination, and was strongly effective at about 50% population vaccination levels and higher (at 80% population vaccination, median 17.5 mortalities versus 34 with no interventions). Proactive vaccination consistently lengthened the duration of epidemics, relative to the baseline scenario (up to a median duration of 143.5 weeks; Figures S3-5). Of those individuals which became infected in proactive vaccination scenarios, susceptibles were typically infected earliest, followed by vaccinated individuals, and susceptible respawned individuals (Figures S6). When all vaccinated individuals received a booster, vaccination reduced the probability of a successful outbreak even at 40% population vaccination (52 failures versus 34 failures per 100 successful epidemics; Figure S15). In contrast, when no vaccinated individuals received a booster, proactive vaccination was largely only effective at reducing the probability of epidemics at very high levels of population vaccination.

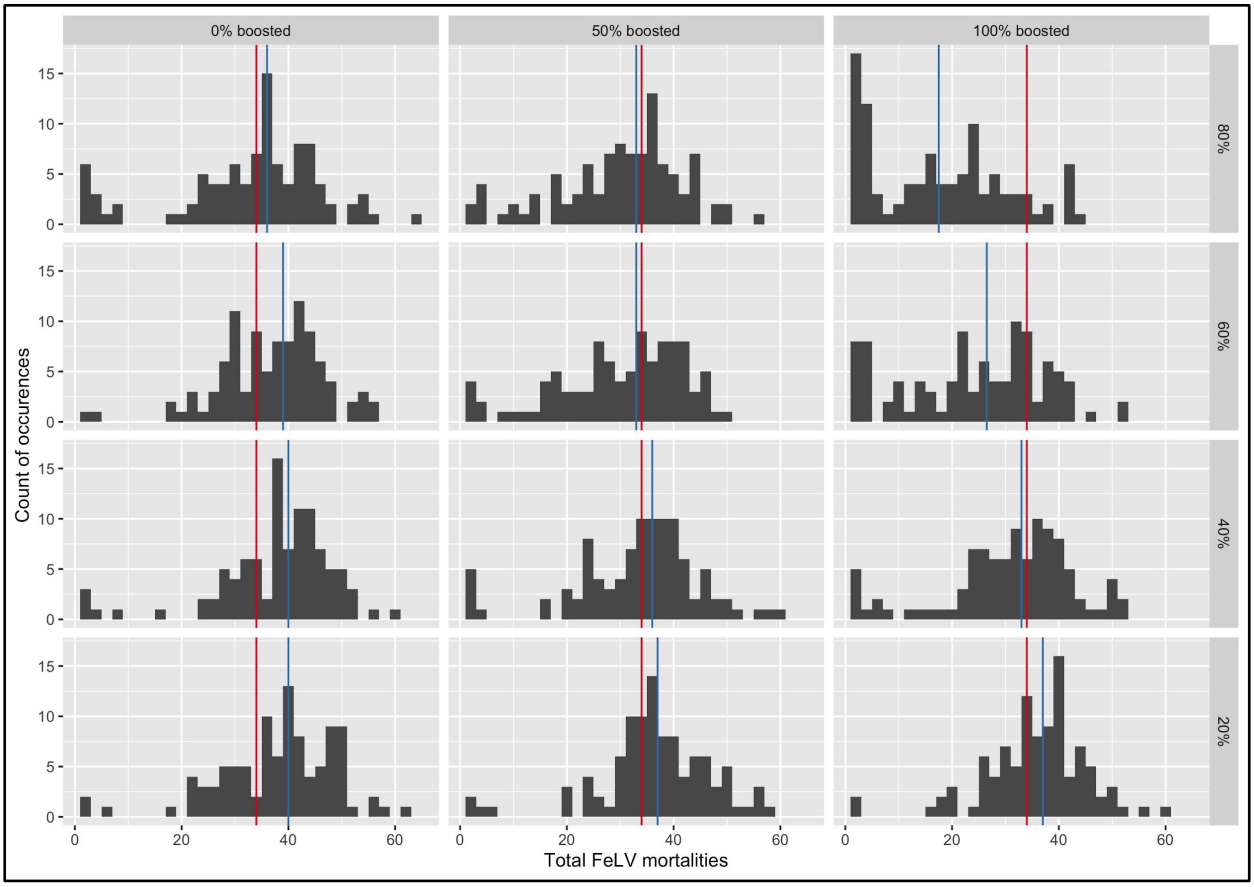

Figure 2: *Histograms of FeLV mortalities in simulated epidemics with proactive vaccination alone. Red vertical lines indicate the median number of mortalities from the baseline scenario without interventions; blue lines, scenarios with proactive vaccination. Panel rows represent the proportion of the population proactively vaccinated; columns represent ratios of vaccine efficacy among vaccinates. 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). 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), and had little impact on the probability of epidemics failing, particularly in comparison to proactive vaccination alone (Figure S15).

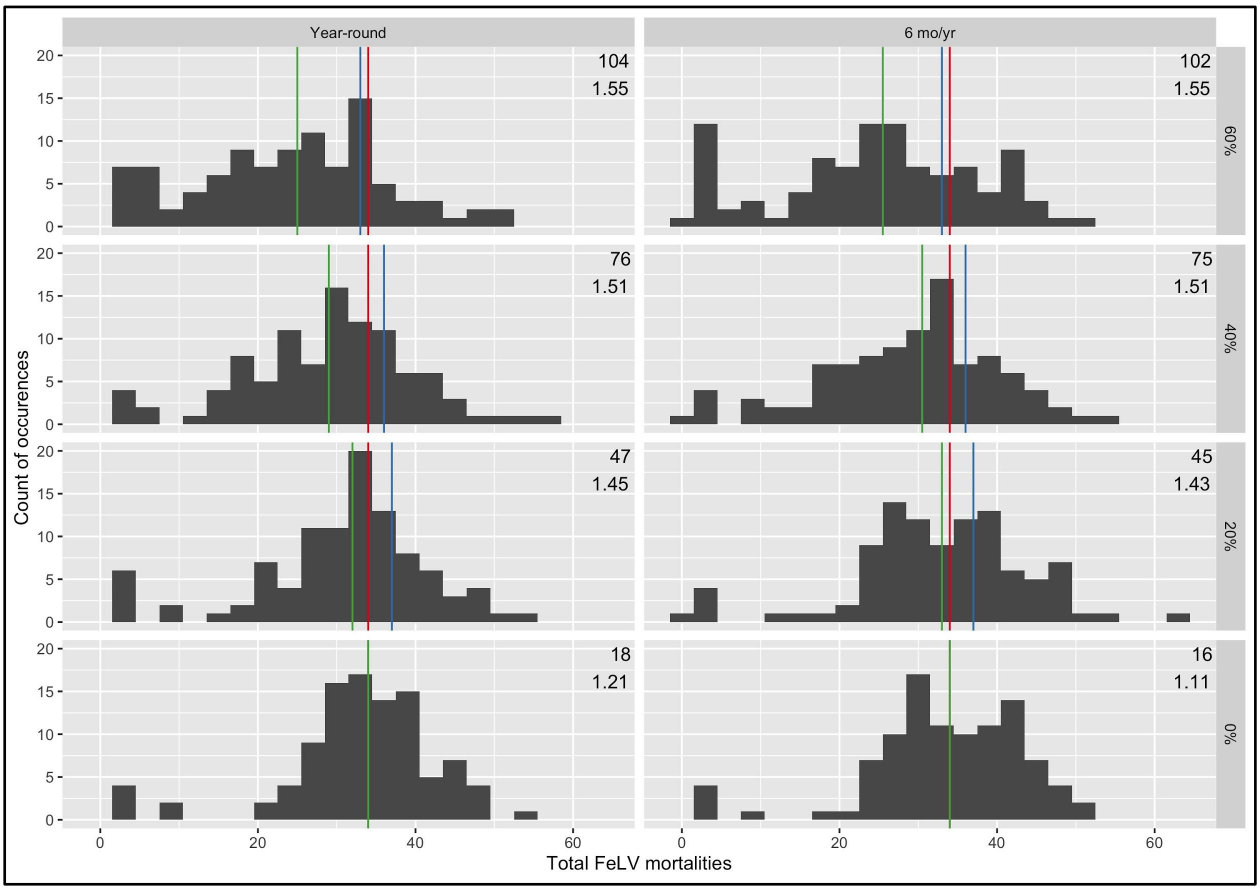

Figure 3: *Histograms of FeLV mortalities in simulated epidemics with both reactive and proactive vaccination. Results shown are for randomly distributed reactive vaccination starting*

52 weeks after epidemic onset. Panel rows represent the proportion of the population proactively vaccinated (with 50% boosted vaccination); 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). 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). 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 S15).

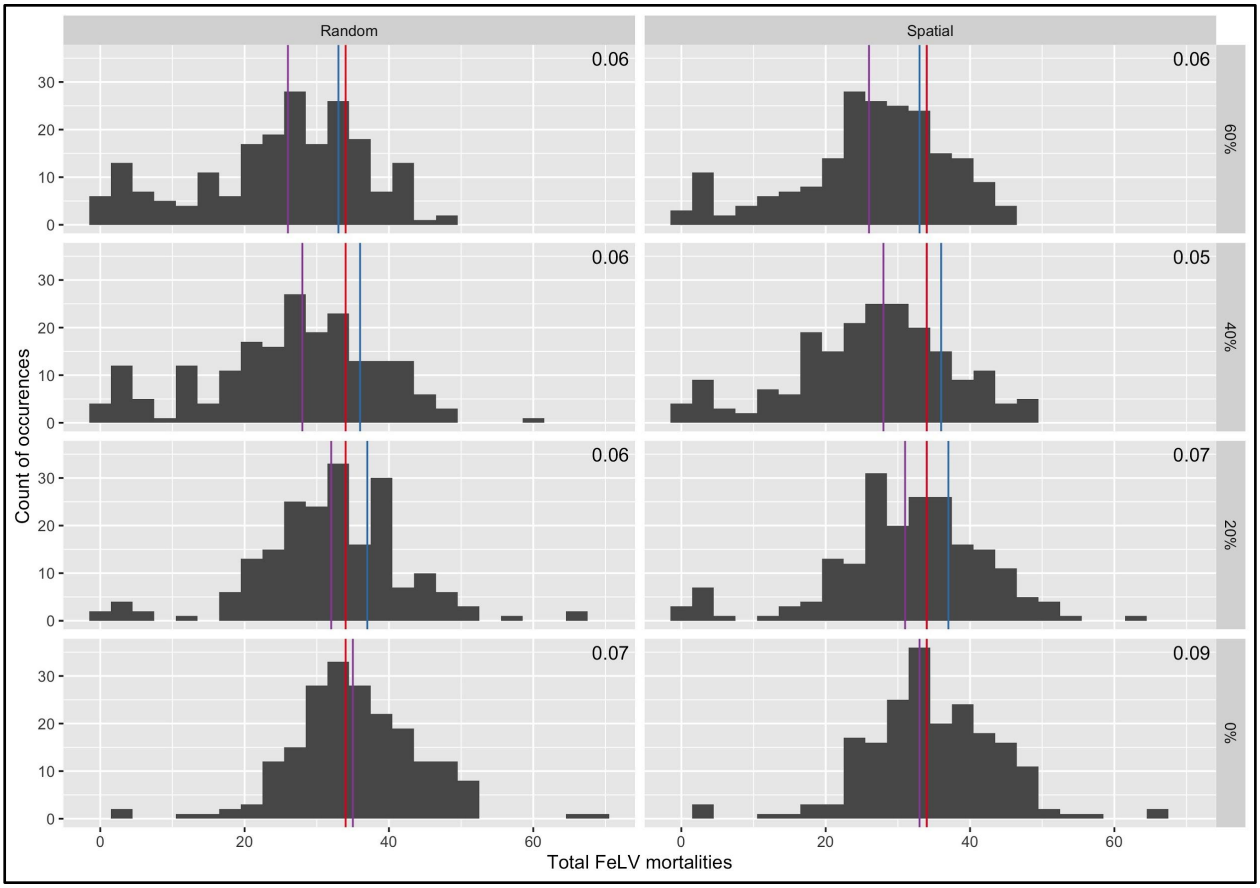

Figure 4: *Histograms of FeLV mortalities in simulated epidemics with both reactive test-and-removal and proactive vaccination. Panel rows represent the proportion of the population proactively vaccinated (with 50% boosted vaccination); columns represent the capture and testing targeting strategy (random 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.*

Reactive underpass closures

Reactive underpass closures were ineffective in the absence of proactive vaccination (Figure S13). The most effective impact of underpass closures on reducing FeLV mortalities

occurred when onset of closure was early (26 weeks after epidemic initiation), lasted for at least 13 weeks, and occurred in conjunction with at least 40-60% of the population being proactively vaccinated (though the clearest effects occurred when at least 60% were proactively vaccinated). Under these conditions, underpass closures synergistically reduced mortalities from baseline scenarios (as few as a median of 22 mortalities with underpass closures) and increased the probability that an epidemic would fail (maximum of 75 failed epidemics per 100 successes with underpass closures; Figure S15). In some cases, simulations showed a marked decrease in transmission during the period of underpass closures, but reopening often resulted in a subsequent resurgence of infections (Figure 5). Underpass closures had no clear impact on the duration of outbreaks (Figure S14).

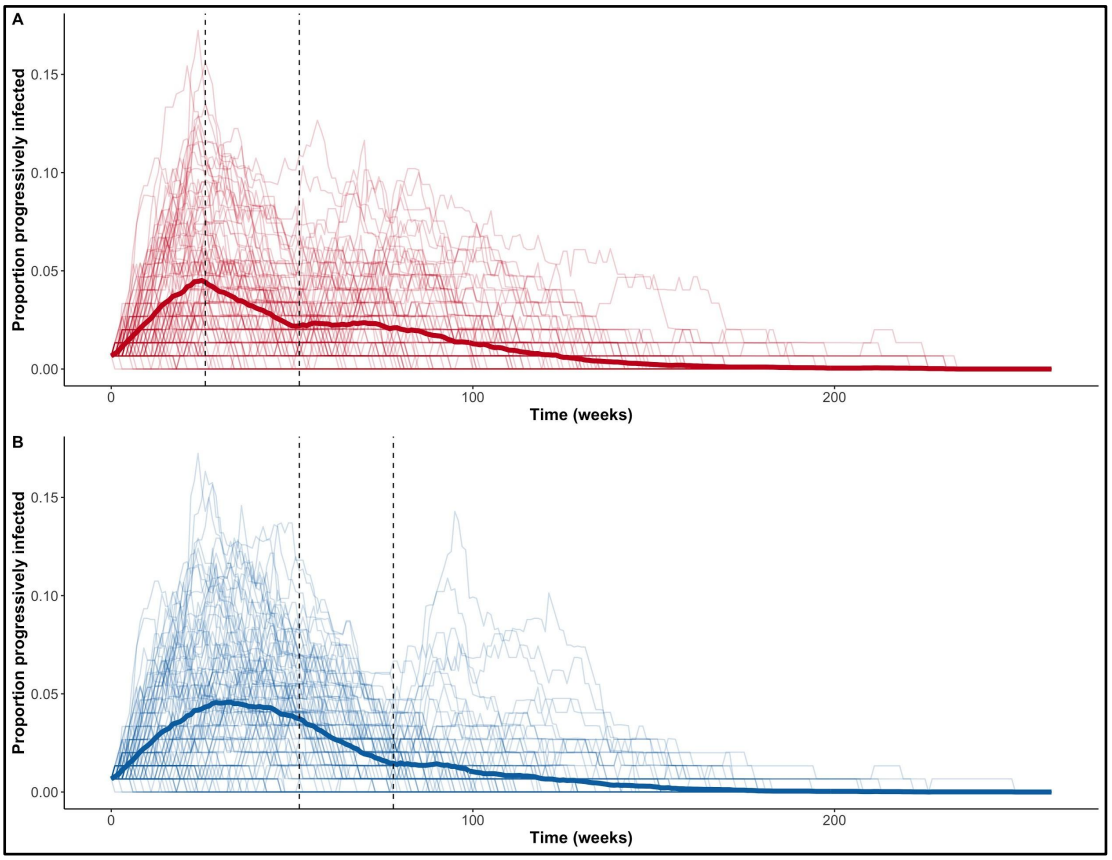

Figure 5: *Epidemic curves showing the proportion of the population progressively infected over time when 60% of the population was proactively vaccinated. Lighter lines show individual*

365 *simulation results; dark lines show mean values across all simulations. Vertical black dashed*
366 *lines show when underpasses were closed (left) and reopened (right). Panel A shows*
367 *underpasses closed for 26 weeks starting 26 weeks after an epidemic started; panel B shows*
368 *underpasses closed for 26 weeks starting at 52 weeks.*
369
370 *Sensitivity analyses*
371 Simulated epidemic sizes were variable across the full 50 sensitivity analysis parameter
372 sets in the absence of FeLV interventions (range: median 7.5 mortalities-median 47.5
373 mortalities; Figure S16). Based on PRCC, the parameters for network density, transmission
374 potential from regressives, weekly contact rates, and baseline transmission potential were
375 positively associated with median mortalities in the absence of interventions; the parameter for
376 the infection-induced mortality rate was negatively associated with median mortalities (see
377 supplementary results, Figure S17).
378 When focusing on a subset of parameter sets for proactive vaccination sensitivity
379 analysis, low levels of proactive vaccination (e.g., 20% population proactive vaccination) were
380 sometimes effective in reducing the number of mortalities, in contrast to our primary results.
381 PRCC results from proactive scenario sensitivity analysis suggested that the parameters for
382 network density, transmission potential from regressives, and weekly contact rates were
383 positively correlated with increased mortalities at low levels of vaccination (Figure S18).
384 However, network density did not have a clearly monotonic relationship with the difference
385 between mortalities with and without proactive vaccination (Figure S19). We therefore
386 performed additional *post-hoc* sensitivity analyses to further interrogate the relationship between
387 network density and our qualitative outcome of increased mortalities at low levels of proactive
388 vaccination (see supplementary results for detailed discussion of *post-hoc* analysis). These
389 additional analyses found some evidence that low levels of proactive vaccination were least
390 effective at reducing mortalities at intermediate values of parameters governing network

391 connectivity (network density and proportion adults), especially when coupled with increased
392 transmission potential (e.g. higher infectiousness of regressive individuals and/or increased
393 weekly contact rates; Figures S21-22).
394
395 **Discussion**
396 In this study we found unexpected consequences and impacts of several epidemic
397 management strategies for disease control in small populations of conservation concern.
398 Although our simulation results provide guidelines for FeLV management in Florida panthers,
399 they also demonstrate the power of partnering modeling approaches and population
400 management questions to test and optimize disease control strategies in free-ranging wildlife
401 (36). Furthermore, the principles of transmission and available methods of disease control
402 underlying our findings provide insights for pathogen control in other host-pathogen systems,
403 including humans and livestock.
404
405 *Proactive vaccination alone may worsen epidemic outcomes under some conditions*
406 Our simulation results showed a paradoxical increase in FeLV mortalities with low levels
407 of proactive vaccination. This counterintuitive finding is likely due, at least in part, to partial
408 vaccine immunity, a type of vaccine imperfection often overlooked in studies of wildlife disease
409 (32). Under partial vaccine immunity, vaccinates can act as a semi-protected susceptible pool,
410 contracting infection later in the course of an epidemic, ultimately prolonging epidemics and
411 increasing the total number of mortalities. Further, these effects likely interact with the
412 respawning process we used, which is similar to density-dependent birth processes which have
413 been shown to increase pathogen prevalence and mortality under culling conditions (37). In the
414 case of our simulations, proactive vaccination flattens the epidemic curve (Figure S5), leading to
415 a slow, steady introduction of new susceptibles which can themselves become infected and
416 maintain epidemics. Only with adequate herd immunity can increased mortalities be avoided.

417 This counterintuitive result is consistent with findings by Rees et al. (38), who found reduced
418 vaccine effectiveness with heterogeneity in the spatial distribution of hosts. Importantly, our
419 results suggest that studies assuming full protection from vaccination may significantly
420 underestimate the level of vaccination needed for population protection—underestimates which
421 may even make epidemics worse if only partial vaccine immunity can be achieved.

422 Our sensitivity analysis suggests that populations with intermediate levels of network
423 connectivity and/or high transmission potential may be most vulnerable to these paradoxical
424 effects. As in spatially structured populations (39), high or low connectivity causes infection to
425 fade out quickly; with intermediate connectivity, vaccine failures provide a steady supply of new
426 susceptibles. Alternatively, in cases of high transmission potential, vaccination may shift a rapid
427 fade-out epidemic to a sustained epidemic scenario, as in Rees et al. (38). While our sensitivity
428 analysis was limited by computational demands and use of some discrete parameters (which
429 may affect PRCC inference; 34), our findings are consistent with this broader body of literature.

430 FeLV vaccine efficacy may operate differently in reality from our simulation structure: for
431 example, some vaccinates may have zero vaccine-induced immunity, while others have 100%
432 protection (binary immunity; 32). Alternatively, vaccination may not protect from infection but
433 could reduce viral shedding or increase survival of infected individuals (32). In the case of binary
434 immunity, vaccine efficacy would be unlikely to prolong and worsen epidemics as we saw here.
435 In contrast, increased survival of infected individuals without changes to shedding potential
436 could extend or worsen outbreaks, and even favor the evolution of virulence (32). Future
437 research should assess vaccinated panthers' immune response to FeLV infection *in vitro* to help
438 refine understanding of vaccine imperfection in panthers and how imperfect immunity may
439 further alter vaccination guidelines.

440 Based on our results, we argue that wildlife managers should continue vaccinating
441 available panthers and prioritize boosting vaccinated individuals to develop a core population of
442 high-immunity individuals, rather than a broadly distributed low-immunity population. This

443 recommendation should be most effective at increasing the probability of epidemic failure, and
444 aligns with other wildlife studies which have emphasized vaccination of core populations (40) or
445 risk-based sub-populations (13).
446
447 *Temporary spatial restrictions are unlikely to be effective under realistic scenarios*
448 Here, we examined the effect of wildlife underpass closures as a novel method to restrict
449 connectivity of the panther population under emergency disease control conditions. Such
450 temporary spatial restrictions could increase other types of panther mortality (e.g. vehicle strikes
451 or intraspecific conflict), so such an intervention would need to reduce FeLV mortalities at a rate
452 greater than these other types of panther mortality in order to be a viable disease control
453 strategy. Unfortunately, our simulations found that temporary underpass closures were
454 generally no more effective at reducing FeLV mortalities than less risky reactive interventions.
455 Further, underpass closures were less effective when occurring after the peak of simulated
456 epidemics, such that spatial restrictions would need to occur early in an epidemic to be
457 effective. However, it should be noted that spatial restrictions may be more effective if used in
458 combination with other reactive FeLV management strategies (e.g., reactive vaccination in
459 concert with temporary spatial restrictions to prevent transmission resurgence after restrictions
460 are removed).
461 Extensive research has considered how landscape barriers and fragmentation may
462 affect pathogen transmission and control in wildlife (e.g., 38, 41–43). By closing underpasses,
463 we artificially fragmented panther habitat, assuming complete efficacy of closures in preventing
464 transmission across the I-75 freeway. In reality, some individuals would likely successfully
465 traverse this barrier. Because habitat fragmentation can promote pathogen persistence (43), a
466 “semipermeable” freeway barrier may, in fact, ultimately worsen epidemic dynamics.
467 Alternatively, landscape restrictions to animal movement can facilitate more efficient disease
468 control, as in Haydon *et al.* (7), where vaccinating to control pathogen spread along habitat

corridors reduced the level of vaccine coverage needed to protect endangered Ethiopian wolves. While panthers are generally well-connected spatially, expansion of the population north of its current range may favor similar metapopulation-oriented disease control strategies. However, given uncertainties in the effect of underpass closures on other sources of mortalities and their limited effectiveness in our simulations, we suggest temporary underpass closures should currently be considered a low priority for FeLV management in panthers.

Reactive and proactive strategies can work synergistically to reduce epidemic impacts

Our simulations showed that both reactive vaccination and test-and-removal strategies reduced FeLV mortalities in panthers when used in combination with moderate levels of proactive vaccination. Test-and-removal had more consistent effects with arguably less effort than reactive vaccination (4 months of captures compared to year-round reactive vaccination). We therefore suggest that test-and-removal be prioritized over reactive vaccination, especially if identification of actively infectious individuals can be improved.

In our simulations, captures that were most aligned with the initial wave of infectious individuals (i.e., with earlier onset) were more likely to identify actively infectious individuals for removal. This finding highlights the importance of targeting captures to individuals likely to be actively infectious. However, determining infection status in cryptic wildlife is difficult, and consequently supports the increased use of remote tracking technologies that may be able to (1) identify behavior changes associated with sickness, and (2) detect the onset of an epidemic more quickly. This conclusion is consistent with similar findings in Channel Island foxes, where increasing the number and frequency of tracking of sentinel individuals was important for early identification of epidemics (16).

A key component of the success of test-and-removal here is the selective removal we simulated, which avoids removing immune individuals that contribute to overall herd immunity (18, 20). However, we have simplified the field testing process in our simulations. The common

field-available FeLV diagnostic test identifies antigenemia, which is key for identifying actively infectious individuals. However, the duration of antigenemia—and even degree of infectiousness—in regressively infected individuals is unclear in panthers. We may therefore overestimate the effect of removing regressive individuals, but given their reduced infectiousness in our simulations, we still expect test-and-removal to be a key strategy for mitigating FeLV impacts in panthers.

Notably, reactive vaccination, in concert with proactive vaccination, is also a viable alternative strategy to test-and-removal. Our simulations showed reactive vaccination to have the strongest effects for reducing FeLV mortalities when at least 50% of the population was vaccinated, and with a ratio of about 1.5 vaccines per vaccinated individual (i.e., 50% of vaccinated individuals received a booster). We therefore suggest that managers should prioritize boosting at least half of vaccinated individuals in a reactive vaccination response scenario. We did not see a strong effect of attempting a vaccine barrier, in contrast to Sanchez *et al* (16), where a simulated vaccine barrier could effectively halt spread of a pathogen in Channel Island foxes. This difference is likely due to the differences in home range size and movement capacity between the two species, with foxes ranging less widely than panthers. In reality, if an empirical outbreak of FeLV in panthers exhibited a stronger spatial signal than was featured in our simulations, spatially targeted reactive vaccination may yet be a worthwhile intervention strategy.

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

Limitations and future directions

521 In this study, we considered the effects of partial vaccine immunity, but we made the
522 simplifying assumption of no waning vaccine or infection-induced immunity over time. However,
523 our findings with regard to imperfect efficacy are at least partially representative of the likely
524 consequences of waning immunity, in that the loss of immunity supplies new susceptibles to the
525 population. Should immunity not outlast the course of an FeLV outbreak, this process could
526 prolong outbreaks and result in increased mortalities. Future research should therefore examine
527 the effects of waning vaccine immunity, particularly considering the value of revaccinating
528 individuals which may be experiencing loss of vaccine protection.

529 Our simulation results found that relatively high levels of vaccination were required to
530 reduce impacts of FeLV in panthers, in contrast to studies in other endangered species (7, 9).
531 However, here we are investigating a pathogen with a long duration of infectiousness and lack
532 the advantages of distinct corridors between panther sub-populations for reducing required
533 levels of vaccination. It is therefore unsurprising that panthers would require higher levels of
534 FeLV population vaccination than was found, for example, for rabies vaccination in Ethiopian
535 wolves (7) or canine distemper virus vaccination in Amur tigers (9). However, we also did not
536 assume the presence of preexisting population immunity prior to proactive vaccination, and
537 some degree of population immunity likely already exists in panthers, given ongoing exposures
538 (4). This would reduce necessary vaccination levels in panthers, as would higher vaccine
539 efficacy than we conservatively assumed here (40). Future research could prioritize better
540 understanding the realized individual immunity after single and boosted FeLV vaccination in
541 panthers to determine if inoculations are more protective than we conservatively assumed here.
542

543 *Conclusions*

544 Our simulation results highlight the risks of inadequate proactive vaccination, particularly
545 with partial vaccine immunity. We recommend prioritizing boosted vaccination in panthers, and
546 joint use of proactive vaccination and reactive strategies to mitigate the risks of imperfect

vaccination and most effectively reduce the impacts of FeLV in this iconic carnivore. This research highlights the value of linking modeling and management priorities to identify unexpected consequences of interventions and determine optimal pathogen management strategies in free-ranging wildlife.

Authors' Contributions

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

Acknowledgements

This research was supported by the National Science Foundation (DEB-1413925, 1654609, and 2030509). MLJG was supported by the Office of the Director, National Institutes of Health (NIH T32OD010993), the University of Minnesota Informatics Institute MnDRIVE program, and the Van Sloun Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Florida panther data collected by the Florida Fish and Wildlife Conservation Commission is fully supported by donations to the Florida Panther Research and Management Trust Fund via the registration of "Protect the Panther" license plates. We acknowledge the efforts of National Park Service staff in the collection of Florida panther data utilized in this study.

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.

573 **Works Cited**

574 1. A. C. Breed, *et al.*, “Disease Management in Endangered Mammals” in *Management of*
575 *Disease in Wild Mammals*, R. J. Delahay, G. C. Smith, M. R. Hutchings, Eds. (Springer
576 Japan, 2009), pp. 215–239.

577 2. M. W. Cunningham, *et al.*, Epizootiology and management of feline leukemia virus in the
578 Florida puma. *J. Wildl. Dis.* **44**, 537–552 (2008).

579 3. M. A. Brown, *et al.*, Genetic characterization of feline leukemia virus from Florida panthers.
580 *Emerg. Infect. Dis.* **14**, 252–259 (2008).

581 4. E. S. Chiu, *et al.*, Multiple Introductions of Domestic Cat Feline Leukemia Virus in
582 Endangered Florida Panthers. *Emerg. Infect. Dis.* **25**, 92–101 (2019).

583 5. H. McCallum, Models for managing wildlife disease. *Parasitology* **143**, 805–820 (2016).

584 6. J. O. Lloyd-Smith, *et al.*, Epidemic dynamics at the human-animal interface. *Science* **326**,
585 1362–1367 (2009).

586 7. D. T. Haydon, *et al.*, Low-coverage vaccination strategies for the conservation of
587 endangered species. *Nature* **443**, 692–695 (2006).

588 8. J. Rushmore, *et al.*, Network-based vaccination improves prospects for disease control in
589 wild chimpanzees. *J. R. Soc. Interface* **11**, 20140349 (2014).

590 9. M. Gilbert, *et al.*, Distemper, extinction, and vaccination of the Amur tiger. *Proc. Natl. Acad.*
591 *Sci. U. S. A.* (2020) https:/doi.org/10.1073/pnas.2000153117.

592 10. Robinson Stacie J., *et al.*, Model recommendations meet management reality:
593 implementation and evaluation of a network-informed vaccination effort for endangered
594 Hawaiian monk seals. *Proceedings of the Royal Society B: Biological Sciences* **285**,
595 20171899 (2018).

596 11. L. Baker, J. Matthiopoulos, T. Müller, C. Freuling, K. Hampson, Optimizing spatial and
597 seasonal deployment of vaccination campaigns to eliminate wildlife rabies. *Philos. Trans.*
598 *R. Soc. Lond. B Biol. Sci.* **374**, 20180280 (2019).

599 12. S. Cleaveland, Viral threats and vaccination: disease management of endangered species.
600 *Anim. Conserv.* **12**, 187–189 (2009).

601 13. H. L. Beyer, *et al.*, The implications of metapopulation dynamics on the design of
602 vaccination campaigns. *Vaccine* **30**, 1014–1022 (2012).

603 14. K. M. McClure, A. T. Gilbert, R. B. Chipman, E. E. Rees, K. M. Pepin, Variation in host
604 home range size decreases rabies vaccination effectiveness by increasing the spatial
605 spread of rabies virus. *J. Anim. Ecol.* **89**, 1375–1386 (2020).

606 15. E. J. Newton, *et al.*, Differential impacts of vaccination on wildlife disease spread during
607 epizootic and enzootic phases. *J. Appl. Ecol.* **56**, 526–536 (2019).

608 16. J. N. Sanchez, B. R. Hudgens, Vaccination and monitoring strategies for epidemic

prevention and detection in the Channel Island fox (*Urocyon littoralis*). *PLoS One* **15**, e0232705 (2020).

17. D. Slate, *et al.*, Status of oral rabies vaccination in wild carnivores in the United States. *Virus Res.* **111**, 68–76 (2005).

18. E. Miguel, *et al.*, A systemic approach to assess the potential and risks of wildlife culling for infectious disease control. *Commun Biol* **3**, 353 (2020).

19. J. O. Lloyd-Smith, *et al.*, Should we expect population thresholds for wildlife disease? *Trends Ecol. Evol.* **20**, 511–519 (2005).

20. A. Potapov, E. Merrill, M. A. Lewis, Wildlife disease elimination and density dependence. *Proc. Biol. Sci.* **279**, 3139–3145 (2012).

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

22. S. Little, *et al.*, 2020 AAFP Feline Retrovirus Testing and Management Guidelines. *J. Feline Med. Surg.* **22**, 5–30 (2020).

23. G. López, *et al.*, Management measures to control a feline leukemia virus outbreak in the endangered Iberian lynx. *Anim. Conserv.* **12**, 173–182 (2009).

24. A. Mysterud, C. M. Rolandsen, Fencing for wildlife disease control. *J. Appl. Ecol.* **56**, 519–525 (2019).

25. G. G. Cotterill, *et al.*, Winter feeding of elk in the Greater Yellowstone Ecosystem and its effects on disease dynamics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **373** (2018).

26. M. L. J. Gilbertson, *et al.*, Transmission of one predicts another: Apathogenic proxies for transmission dynamics of a fatal virus. *bioRxiv* [Preprint] (2021) https:/doi.org/10.1101/2021.01.09.426055.

27. B. T. McClintock, D. P. Onorato, J. Martin, Endangered Florida panther population size determined from public reports of motor vehicle collision mortalities. *J. Appl. Ecol.* **52**, 893–901 (2015).

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

29. R Core Team, R: A Language and Environment for Statistical Computing (2018).

30. A. H. Sparkes, Feline leukaemia virus: a review of immunity and vaccination. *J. Small Anim. Pract.* **38**, 187–194 (1997).

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

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

33. R. Carnell, lhs: Latin hypercube samples. *R package version 0.10, URL http://CRAN.R-*

project.org/package=lhs (2012).

34. S. Marino, I. B. Hogue, C. J. Ray, D. E. Kirschner, A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J. Theor. Biol.* **254**, 178–196 (2008).

35. J. Wu, R. Dhingra, M. Gambhir, J. V. Remais, Sensitivity analysis of infectious disease models: methods, advances and their application. *J. R. Soc. Interface* **10**, 20121018 (2013).

36. M. B. Joseph, *et al.*, Taming wildlife disease: bridging the gap between science and management. *J. Appl. Ecol.* **50**, 702–712 (2013).

37. M. Choisy, P. Rohani, Harvesting can increase severity of wildlife disease epidemics. *Proc. Biol. Sci.* **273**, 2025–2034 (2006).

38. E. E. Rees, B. A. Pond, R. R. Tinline, D. Bélanger, Modelling the effect of landscape heterogeneity on the efficacy of vaccination for wildlife infectious disease control. *J. Appl. Ecol.* **50**, 881–891 (2013).

39. H. McCallum, Landscape structure, disturbance, and disease dynamics. *Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems*, 100–122 (2008).

40. F. Vial, S. Cleaveland, G. Rasmussen, D. T. Haydon, Development of vaccination strategies for the management of rabies in African wild dogs. *Biol. Conserv.* **131**, 180–192 (2006).

41. D. L. Smith, B. Lucey, L. A. Waller, J. E. Childs, L. A. Real, Predicting the spatial dynamics of rabies epidemics on heterogeneous landscapes. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 3668–3672 (2002).

42. J. A. Tracey, S. N. Bevins, S. VandeWoude, K. R. Crooks, An agent-based movement model to assess the impact of landscape fragmentation on disease transmission. *Ecosphere* **5**, 1–24 (2014).

43. L. A. White, J. D. Forester, M. E. Craft, Disease outbreak thresholds emerge from interactions between movement behavior, landscape structure, and epidemiology. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 7374–7379 (2018).