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

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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, 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 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.

Keywords

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

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.

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

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

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

121

122 **Methods**

123 *Simulation pipeline*

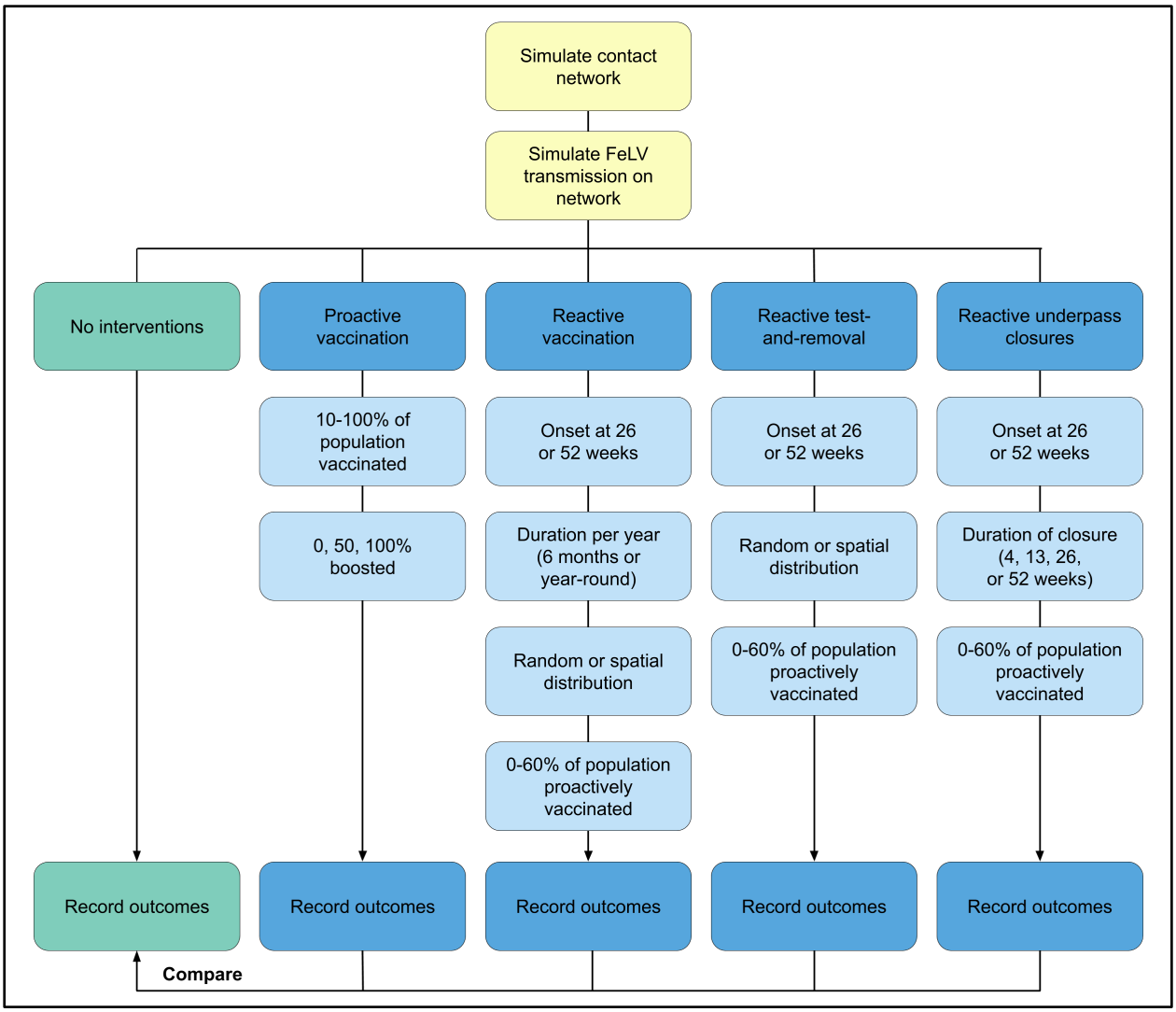
124 To examine the effect of different disease management regimes on FeLV control, we
125 used spatially-explicit, network simulations adapted from our research determining drivers of
126 retrovirus transmission in panthers (26). This approach involves two steps: (1) simulation of a
127 contact network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

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

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

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

158 individuals acquired progressive or regressive infections. The outcomes of mortalities and
159 epidemic durations were summarized as median values per parameter set, as results were
160 often skewed; all outcomes were compared between baseline and management scenarios. All
161 simulations were performed in R version 3.6.3 (29).
162



163

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

167 *blue. Within each management scenario, we investigated several variations for the given*
168 *approach (shown in light blue) in a factorial design.*
169
170 *Proactive vaccination*
171 In the first management scenario, we examined the effect of different levels of population
172 proactive vaccination (proportion of the population vaccinated prior to an outbreak), and
173 different ratios of single versus boosted vaccination. We simulated from 10-80% (in 10%
174 increments) of the population having some degree of vaccine-induced immunity to FeLV at the
175 onset of an outbreak. These vaccinations were distributed randomly in the population. Among
176 the vaccinated individuals, 0, 50, or 100% received a second boosting inoculation. Actual
177 vaccine efficacies are unknown for panthers, but based on efficacy studies in domestic cats (30,
178 31), we conservatively assumed that boosted vaccination would prevent 80% of infections and
179 single vaccination would prevent 40% of infections. We modeled this efficacy as a binomial
180 probability to represent partial vaccine immunity (versus binary immunity; 32). We conducted
181 proactive vaccination scenarios in a full factorial design, for a total of 24 proactive vaccination
182 parameter sets, and 2,400 full simulations (100 full simulations per parameter set).
183
184 *Reactive vaccination*
185 During simulated periods of reactive vaccination administration, panthers were selected
186 at a rate of one panther per week for vaccination. We assumed that managers would not know
187 the disease status of an individual selected for vaccination and that vaccination would be
188 ineffective in infectious or recovered individuals. In the case of previously vaccinated individuals,
189 a re-vaccination changed the vaccine efficacy of singly vaccinated individuals (efficacy of 40%)
190 to the efficacy for boosted individuals (efficacy of 80%). We further assumed that managers
191 would know which individuals had received two vaccinations, so boosted individuals were not
192 selected for additional vaccination attempts.

We varied the timing of the onset of reactive vaccination after the initiation of an FeLV outbreak to reflect the difficulty of epidemic detection in this elusive carnivore. We therefore began reactive vaccination at an optimistic, but difficult-to-attain time point of 26 weeks, and a more realistic time point of 52 weeks. In addition, we varied the distribution of reactive vaccination. While proactive vaccines were always distributed randomly, reactive vaccination was either randomly distributed or spatially distributed in an attempted vaccine barrier based on proximity to the I-75 freeway (see supplement for further details).

Because vaccination is resource and time intensive, we evaluated the effect of reactive vaccination for 6 months per year versus year-round. Among reactive vaccination scenarios, we also included scenarios with proactive vaccination, specifically where 0-60% of the population was proactively vaccinated (in 20% increments) at the initiation of an outbreak. Because it is highly unlikely that 100% of the proactively vaccinated population would have received boosted vaccination, we used a more conservative—yet still challenging to attain—ratio of 50% of the proactively vaccinated population being boosted (i.e. efficacy of 80%). We held this ratio of efficacy constant for proactive vaccination in all reactive scenarios. We again used a factorial design across all variations, resulting in 32 parameter sets and a total of 3,200 full simulations under reactive vaccination scenarios.

Reactive test-and-removal

Test-and-removal scenarios were built around a protocol in which panthers infectious at capture are removed from the population through humane euthanasia or temporary removal until recovery. For simplicity, we assumed that all progressively infected individuals would show clinical signs and be humanely euthanized at capture, while infectious regressive individuals were temporarily removed from the population until their recovery and re-released into any open territory. A maximum of five individuals were allowed to be temporarily removed in this way at 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

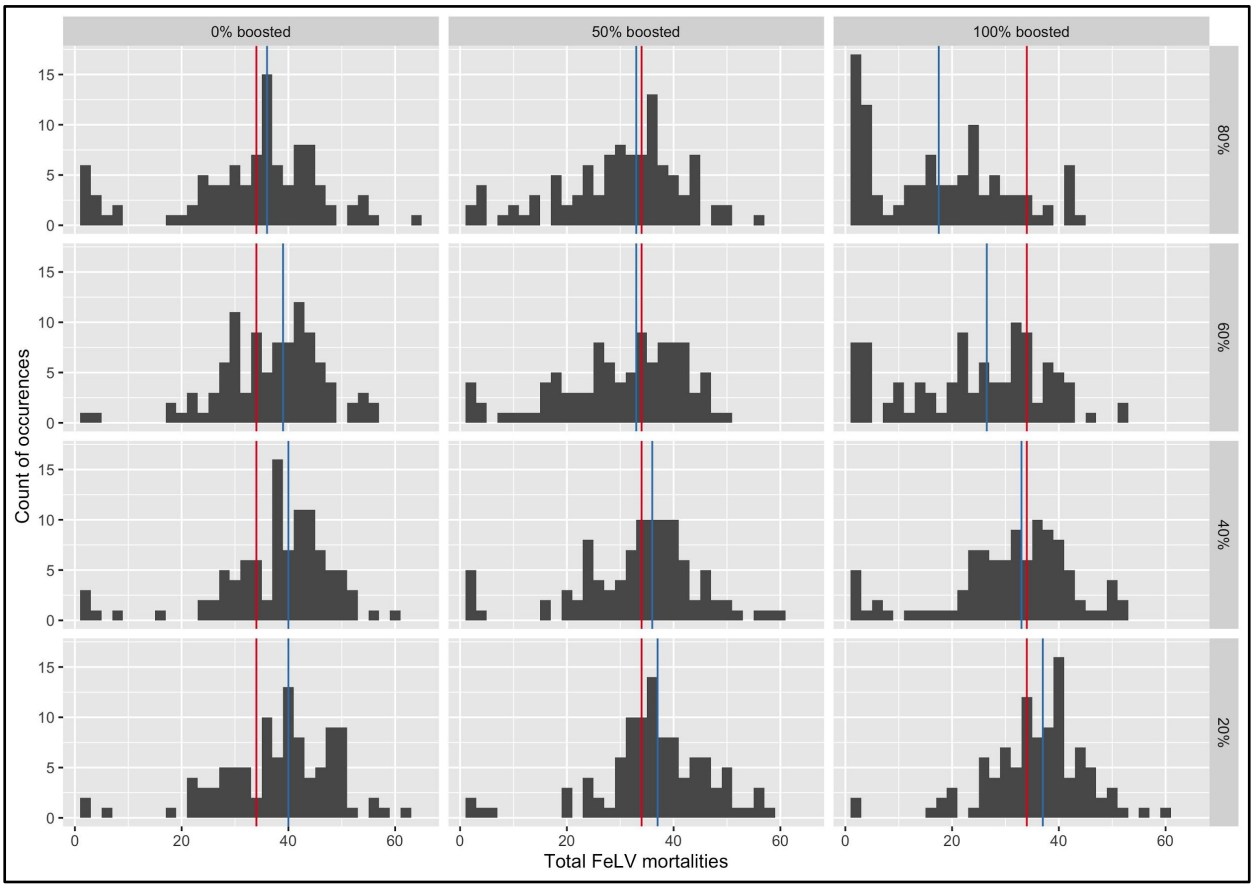
To determine the potential utility of closing I-75 wildlife underpasses in a FeLV outbreak, we considered a “best case” scenario in which underpass closures were completely effective at preventing transmission across the freeway. Underpasses were closed instantaneously either 26 or 52 weeks after the 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

244 (33). We repeated our baseline scenario simulations across these 50 parameter sets and
245 completed 50 simulations per parameter set for all sensitivity analyses.
246 Due to the high computational effort required to perform sensitivity simulations across all
247 management scenarios, we focused only on the proactive vaccination scenarios. Specifically,
248 we evaluated a subset of 12 LHS parameter sets across a subset of the proactive vaccination
249 conditions in a factorial design (see supplementary methods for further details), resulting in 108
250 parameter sets with 50 full simulations per parameter set (5,400 full simulations). This approach
251 allowed us to examine sensitivity of our proactive vaccination results across different outbreak
252 sizes and network and transmission parameters, while mitigating computational effort
253 associated with exploring such a wide range of parameters and scenario variations. Sensitivity
254 analysis simulation results were evaluated using scatterplots and Partial Rank Correlation
255 Coefficients (PRCC; 34, 35); proactive vaccination scenarios were evaluated for alignment with
256 our qualitative result that low proactive vaccination can increase disease-induced mortalities.
257
258 **Results**
259 Progressive infections are expected to result in death in panthers, and are therefore of
260 key concern for management efforts. For simplicity, hereafter, we refer to the number of
261 progressive infections in simulations as the number of mortalities. In the baseline, no-
262 intervention scenario, the median number of mortalities was 34 (range: 1-54); median duration
263 of epidemics was 119.5 weeks, and 34 epidemics failed (fewer than 5 progressive or regressive
264 infections) per 100 successful epidemics.
265
266 *Proactive vaccination alone*
267 Proactive vaccination paradoxically increased the number of mortalities across a range
268 of conditions, especially without vaccine boosting (Figures 2, S2). Even with 50% of vaccinates
269 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 S3-4). 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 S13). 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.



282 ***Figure 2:*** *Histograms of FeLV mortalities in simulated epidemics with proactive vaccination*
283 *alone. Red vertical lines indicate the median number of mortalities from the baseline scenario*
284 *without interventions; blue lines, scenarios with proactive vaccination. Panel rows represent the*
285 *proportion of the population proactively vaccinated; columns represent ratios of vaccine efficacy*
286 *among vaccinates. Each histogram plot represents the results of 100 simulations. An expanded*
287 *version of this figure is available in the supplementary materials (Figure S2).*
288
289 *Proactive and reactive vaccination*
290 Reactive vaccination alone did not reduce mortalities (Figure 3). However, reactive
291 vaccination appeared to work synergistically with proactive vaccination, particularly at moderate
292 to high levels of proactive vaccination (i.e., at least 40-60% of the population proactively
293 vaccinated). This largely held true regardless of the timing of intervention onset and the strategy
294 for reactive vaccination distribution (i.e., random versus spatial; Figures S5-6). The mortality-
295 reducing effects of reactive vaccination were, however, slightly reduced if reactive vaccination
296 occurred for only 6 months out of the year (Figure 3). A ratio of greater than 1.5 inoculations per
297 vaccinated individual appeared to promote the largest reductions in mortalities (e.g., as few as a
298 median of 25 mortalties with year-round reactive vaccination and 60% proactive vaccination
299 versus 34 mortalities with no interventions; Figure 3). Adding reactive vaccination largely did not
300 affect the durations of simulated epidemics (Figures S7-8), and had little impact on the
301 probability of epidemics failing, particularly in comparison to proactive vaccination alone (Figure
302 S13).
303

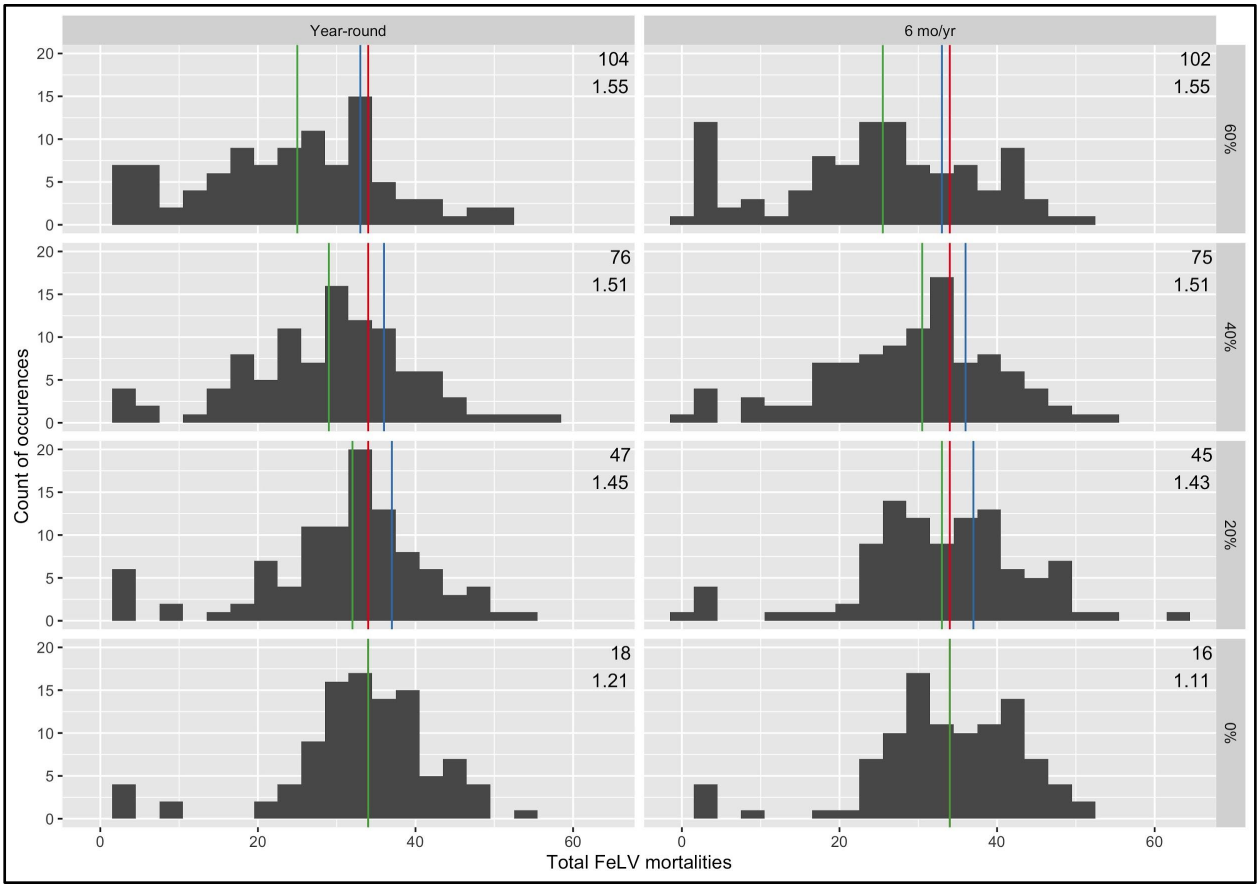


Figure 3: *Histograms of FeLV mortalities in simulated epidemics with both reactive and proactive vaccination. Results shown are for randomly distributed reactive vaccination starting 52 weeks after epidemic onset. Panel rows represent the proportion of the population proactively vaccinated (with 50% 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 S9). The addition of test-and-removal largely did not affect the durations of epidemics (Figure S10). 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 S13).

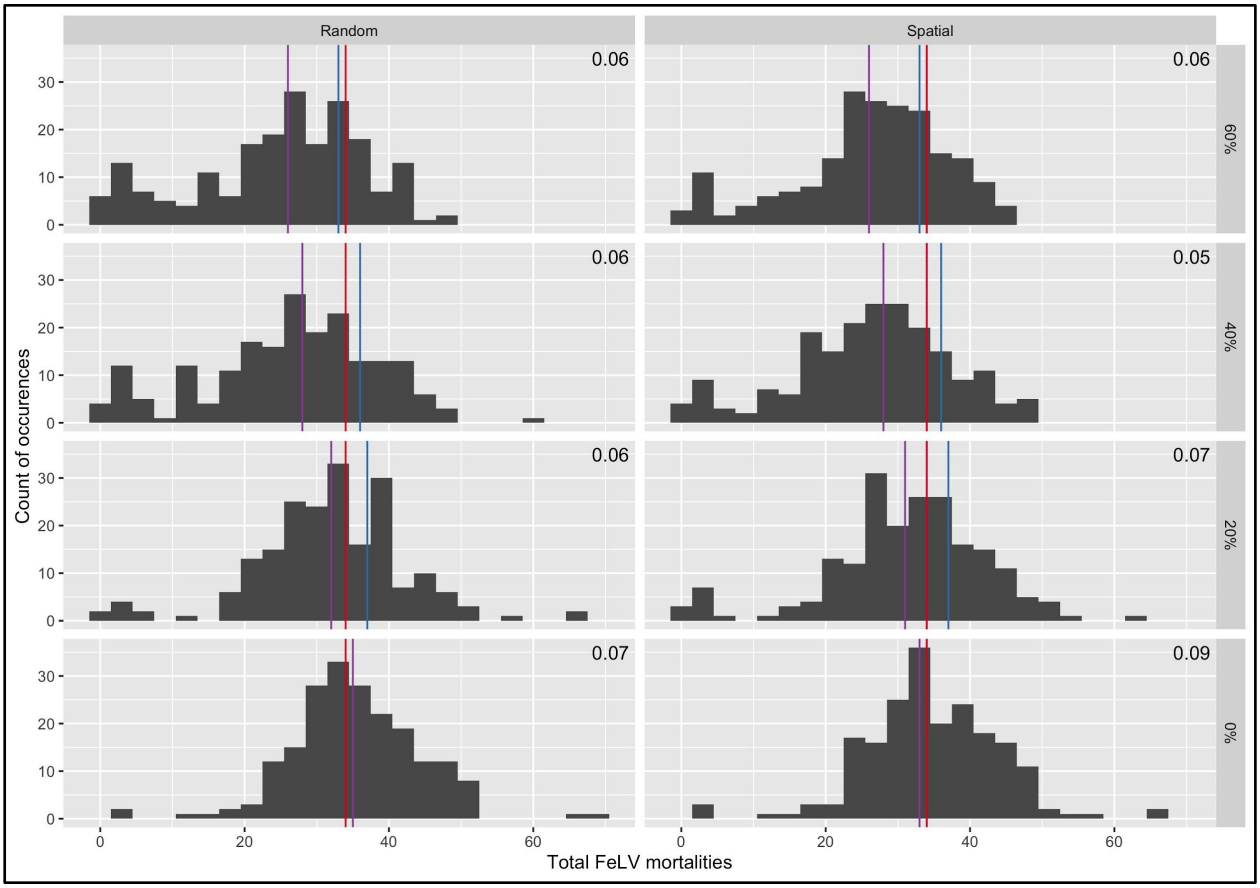


Figure 4: *Histograms of FeLV mortalities in simulated epidemics with both reactive test-and-removal and proactive vaccination. Panel rows represent the proportion of the population proactively vaccinated (with 50% boosted vaccination); columns represent the capture and testing targeting strategy (random 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 S11). 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 S13). 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 S12).

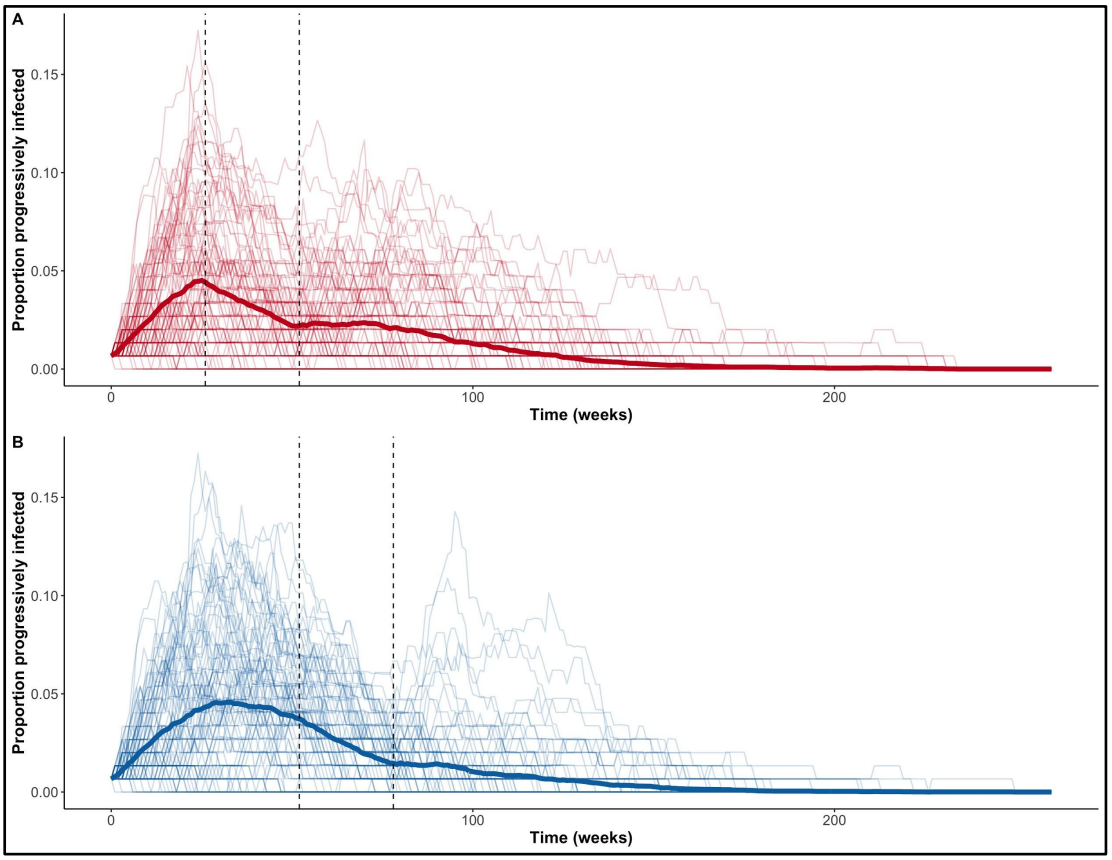


Figure 5: *Epidemic curves showing the proportion of the population progressively infected over time when 60% of the population was proactively vaccinated. Lighter lines show individual*

358 *simulation results; dark lines show mean values across all simulations. Vertical black dashed*
359 *lines show when underpasses were closed (left) and reopened (right). Panel A shows*
360 *underpasses closed for 26 weeks starting 26 weeks after an epidemic started; panel B shows*
361 *underpasses closed for 26 weeks starting at 52 weeks.*
362
363 *Sensitivity analyses*
364 Simulated epidemic sizes were variable across the full 50 sensitivity analysis parameter
365 sets in the absence of FeLV interventions (range: median 7.5 mortalities-median 47.5
366 mortalities; Figure S14). Based on PRCC, the parameters for network density, transmission
367 potential from regressives, weekly contact rates, and baseline transmission potential were
368 positively associated with median mortalities in the absence of interventions; the parameter for
369 the infection-induced mortality rate was negatively associated with median mortalities (see
370 supplementary results, Figure S15).
371 When focusing on a subset of parameter sets for proactive vaccination sensitivity
372 analysis, low levels of proactive vaccination (e.g., 20% population proactive vaccination) were
373 sometimes effective in reducing the number of mortalities, in contrast to our primary results.
374 PRCC results from proactive scenario sensitivity analysis suggested that the parameters for
375 network density, transmission potential from regressives, and weekly contact rates were
376 positively correlated with increased mortalities at low levels of vaccination (Figure S16).
377 However, network density did not have a clearly monotonic relationship with the difference
378 between mortalities with and without proactive vaccination (Figure S17). We therefore
379 performed additional *post-hoc* sensitivity analyses to further interrogate the relationship between
380 network density and our qualitative outcome of increased mortalities at low levels of proactive
381 vaccination (see supplementary results for detailed discussion of *post-hoc* analysis). These
382 additional analyses found some evidence that low levels of proactive vaccination were least
383 effective at reducing mortalities at intermediate values of parameters governing network

384 connectivity (network density and proportion adults), especially when coupled with increased
385 transmission potential (e.g. higher infectiousness of regressive individuals and/or increased
386 weekly contact rates; Figures S19-20).
387
388 **Discussion**
389 In this study we found unexpected consequences and impacts of several epidemic
390 management strategies for disease control in small populations of conservation concern.
391 Although our simulation results provide guidelines for FeLV management in Florida panthers,
392 they also demonstrate the power of partnering modeling approaches and population
393 management questions to test and optimize disease control strategies in free-ranging wildlife
394 (36). Furthermore, the principles of transmission and available methods of disease control
395 underlying our findings provide insights for pathogen control in other host-pathogen systems,
396 including humans and livestock.
397
398 *Proactive vaccination alone may worsen epidemic outcomes under some conditions*
399 Our simulation results showed a paradoxical increase in FeLV mortalities with low levels
400 of proactive vaccination. This counterintuitive finding is likely due, at least in part, to partial
401 vaccine immunity, a type of vaccine imperfection often overlooked in studies of wildlife disease
402 (32). Under partial vaccine immunity, vaccinates can act as a semi-protected susceptible pool,
403 contracting infection later in the course of an epidemic, ultimately prolonging epidemics and
404 increasing the total number of mortalities. Only with adequate herd immunity can these effects
405 be avoided. This counterintuitive result is consistent with findings by Rees et al. (37), who found
406 reduced vaccine effectiveness with heterogeneity in the spatial distribution of hosts. Importantly,
407 our results suggest that studies assuming full protection from vaccination may significantly
408 underestimate the level of vaccination needed for population protection—underestimates which
409 may even make epidemics worse if only partial vaccine immunity can be achieved.

410 Our sensitivity analysis suggests that populations with intermediate levels of network
411 connectivity and/or high transmission potential may be most vulnerable to these paradoxical
412 effects. As in spatially structured populations (38), high or low connectivity causes infection to
413 fade out quickly; with intermediate connectivity, vaccine failures provide a steady supply of new
414 susceptibles. Alternatively, in cases of high transmission potential, vaccination may shift a rapid
415 fade-out epidemic to a sustained epidemic scenario, as in Rees et al. (37). While our sensitivity
416 analysis was limited by computational demands and use of some discrete parameters (which
417 may affect PRCC inference; 34), our findings are consistent with this broader body of literature.
418 FeLV vaccine efficacy may operate differently in reality from our simulation structure: for
419 example, some vaccinates may have zero vaccine-induced immunity, while others have 100%
420 protection (binary immunity; 32). Alternatively, vaccination may not protect from infection but
421 could reduce viral shedding or increase survival of infected individuals (32). In the case of binary
422 immunity, vaccine efficacy would be unlikely to prolong and worsen epidemics as we saw here.
423 In contrast, increased survival of infected individuals without changes to shedding potential
424 could extend or worsen outbreaks, and even favor the evolution of virulence (32). Future
425 research should assess vaccinated panthers' immune response to FeLV infection *in vitro* to help
426 refine understanding of vaccine imperfection in panthers and how imperfect immunity may
427 further alter vaccination guidelines.
428 Based on our results, we argue that wildlife managers should continue vaccinating
429 available panthers and prioritize boosting vaccinated individuals to develop a core population of
430 high-immunity individuals, rather than a broadly distributed low-immunity population. This
431 recommendation should be most effective at increasing the probability of epidemic failure, and
432 aligns with other wildlife studies which have emphasized vaccination of core populations (39) or
433 risk-based sub-populations (13).
434
435 *Temporary spatial restrictions are unlikely to be effective under realistic scenarios*

436 Here, we examined the effect of wildlife underpass closures as a novel method to restrict
437 connectivity of the panther population under emergency disease control conditions. Such
438 temporary spatial restrictions could increase other types of panther mortality (e.g. vehicle strikes
439 or intraspecific conflict), so such an intervention would need to reduce FeLV mortalities at a rate
440 greater than these other types of panther mortality in order to be a viable disease control
441 strategy. Unfortunately, our simulations found that temporary underpass closures were
442 generally no more effective at reducing FeLV mortalities than less risky reactive interventions.
443 Further, underpass closures were less effective when occurring after the peak of simulated
444 epidemics, such that spatial restrictions would need to occur early in an epidemic to be
445 effective. However, it should be noted that spatial restrictions may be more effective if used in
446 combination with other reactive FeLV management strategies (e.g., reactive vaccination in
447 concert with temporary spatial restrictions to prevent transmission resurgence after restrictions
448 are removed).
449 Extensive research has considered how landscape barriers and fragmentation may
450 affect pathogen transmission and control in wildlife (e.g., 37, 40–42). By closing underpasses,
451 we artificially fragmented panther habitat, assuming complete efficacy of closures in preventing
452 transmission across the I-75 freeway. In reality, some individuals would likely successfully
453 traverse this barrier. Because habitat fragmentation can promote pathogen persistence (42), a
454 "semipermeable" freeway barrier may, in fact, ultimately worsen epidemic dynamics.
455 Alternatively, landscape restrictions to animal movement can facilitate more efficient disease
456 control, as in Haydon *et al.* (7), where vaccinating to control pathogen spread along habitat
457 corridors reduced the level of vaccine coverage needed to protect endangered Ethiopian
458 wolves. While panthers are generally well-connected spatially, expansion of the population north
459 of its current range may favor similar metapopulation-oriented disease control strategies.
460 However, given uncertainties in the effect of underpass closures on other sources of mortalities

461 and their limited effectiveness in our simulations, we suggest temporary underpass closures
462 should currently be considered a low priority for FeLV management in panthers.
463
464 *Reactive and proactive strategies can work synergistically to reduce epidemic impacts*
465 Our simulations showed that both reactive vaccination and test-and-removal strategies
466 reduced FeLV mortalities in panthers when used in combination with moderate levels of
467 proactive vaccination. Test-and-removal had more consistent effects with arguably less effort
468 than reactive vaccination (four months of captures compared to year-round reactive
469 vaccination). We therefore suggest that test-and-removal be prioritized over reactive
470 vaccination, especially if identification of actively infectious individuals can be improved.
471 In our simulations, captures that were most aligned with the initial wave of infectious
472 individuals (i.e., with earlier onset) were more likely to identify actively infectious individuals for
473 removal. This finding highlights the importance of targeting captures to individuals likely to be
474 actively infectious. However, determining infection status in cryptic wildlife is difficult, and
475 consequently supports the increased use of remote tracking technologies that may be able to
476 (1) identify behavior changes associated with sickness, and (2) detect the onset of an epidemic
477 more quickly. This conclusion is consistent with similar findings in Channel Island foxes, where
478 increasing the number and frequency of tracking of sentinel individuals was important for early
479 identification of epidemics (16).
480 A key component of the success of test-and-removal here is the selective removal we
481 simulated, which avoids removing immune individuals that contribute to overall herd immunity
482 (18, 20). However, we have simplified the field testing process in our simulations. The common
483 field-available FeLV diagnostic test identifies antigenemia, which is key for identifying actively
484 infectious individuals. However, the duration of antigenemia—and even degree of
485 infectiousness—in regressively infected individuals is unclear in panthers. We may therefore
486 overestimate the effect of removing regressive individuals, but given their reduced

487 infectiousness in our simulations, we still expect test-and-removal to be a key strategy for
488 mitigating FeLV impacts in panthers.

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

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

507

508 *Limitations and future directions*

509 In this study, we considered the effects of partial vaccine immunity, but we made the
510 simplifying assumption of no waning vaccine or infection-induced immunity over time. However,
511 our findings with regard to imperfect efficacy are at least partially representative of the likely
512 consequences of waning immunity, in that the loss of immunity supplies new susceptibles to the

513 population. Should immunity not outlast the course of an FeLV outbreak, this process could
514 prolong outbreaks and result in increased mortalities. Future research should therefore examine
515 the effects of waning vaccine immunity, particularly considering the value of revaccinating
516 individuals which may be experiencing loss of vaccine protection.

517 Our simulation results found that relatively high levels of vaccination were required to
518 reduce impacts of FeLV in panthers, in contrast to studies in other endangered species (7, 9).
519 However, here we are investigating a pathogen with a long duration of infectiousness and lack
520 the advantages of distinct corridors between panther sub-populations for reducing required
521 levels of vaccination. It is therefore unsurprising that panthers would require higher levels of
522 FeLV population vaccination than was found, for example, for rabies vaccination in Ethiopian
523 wolves (7) or canine distemper virus vaccination in Amur tigers (9). However, we also did not
524 assume the presence of preexisting population immunity prior to proactive vaccination, and
525 some degree of population immunity likely already exists in panthers, given ongoing exposures
526 (4). This would reduce necessary vaccination levels in panthers, as would higher vaccine
527 efficacy than we conservatively assumed here (39). Future research could prioritize better
528 understanding the realized individual immunity after single and boosted FeLV vaccination in
529 panthers to determine if inoculations are more protective than we conservatively assumed here.
530

531 *Conclusions*

532 Our simulation results highlight the risks of inadequate proactive vaccination, particularly
533 with partial vaccine immunity. We recommend prioritizing boosted vaccination in panthers, and
534 joint use of proactive vaccination and reactive strategies to mitigate the risks of imperfect
535 vaccination and most effectively reduce the impacts of FeLV in this iconic carnivore. This
536 research highlights the value of linking modeling and management priorities to identify
537 unexpected consequences of interventions and determine optimal pathogen management
538 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.

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