


Practical application of disease risk analysis for reintroducing gray wolves (*Canis lupus*) to Isle Royale National Park, USA

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Abstract

Evaluation of disease risks associated with wildlife translocations is important for minimizing unintended harm and achieving conservation goals. A framework for disease risk analysis (DRA) has been developed by the World Organization for Animal Health and International Union for Conservation of Nature, but applications for planning and implementation in wildlife conservation projects are limited. To fill this gap, we describe a DRA we conducted to identify, assess, and mitigate disease risks associated with reintroduction of gray wolves (*Canis lupus*) to Isle Royale National Park (IRNP). A total of 19 wolves were translocated from multiple locations within the Great Lakes Region to IRNP between September 2018 and September 2019. Integration of the DRA into project planning and use of diverse expertise among project personnel enabled a timely and cost-effective process that facilitated multidisciplinary and cross-cultural collaboration, transparent communication about risks and uncertainties, and practical management of disease risks for wildlife and personnel. Engaging disease experts and experienced field biologists in the assessment also helped to identify and account for potential sources of bias.

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We hope practical examples like this encourage wider adoption of DRA principles in translocations of wildlife for conservation purposes.

KEYWORDS

conservation, disease risk analysis, Isle Royale, ma'iingan, Minong, risk management, wildlife translocation, wolf

1 | INTRODUCTION

Translocation of wild animals is an effective tool for achieving wildlife management and conservation goals. Intentional movement of individuals or groups of animals from one location to another can help augment wildlife populations, facilitate adaptation to changing climates, increase genetic diversity or help restore populations of threatened or endangered species (IUCN/SSC, 2013; Seddon, 2010; Seddon et al., 2014). However, there are a variety of risks associated with translocation that can have unintended consequences for translocated animals, recipient populations, or associated communities, and ecosystem functions, as well as risks to domestic animals and humans (Berger-Tal et al., 2020; Cunningham, 1996; Karasov-Olson et al., 2021). Such risks may be ecological, social, economic, or health related, and must be weighed against projected benefits of translocation.

Historically, pathogens and disease were not considered in animal translocation projects (Griffith et al., 1993), but the importance of considering potential disease risks and managing them appropriately has become increasingly apparent (Kock et al., 2010). Pathogens present in recipient populations or communities can cause morbidity or mortality of translocated animals that may be naïve or highly susceptible to these pathogens, and in turn hamper species recovery efforts. Such was the case with plague (*Yersinia pestis*) in reintroduced black-footed ferrets (*Mustela nigripes*; Biggins & Godbey, 2003) and toxoplasmosis (*Toxoplasma gondii*) in Hawaiian crows (*Corvus hawaiiensis*; Work et al., 2000). New pathogens can also be inadvertently introduced into nonendemic areas, which can be detrimental to resident species and potentially humans in the destination location. Translocation of raccoons (*Procyon lotor*) from raccoon rabies enzootic areas in the southeastern United States into the mid-Atlantic for hunting purposes in the 1970s presumably introduced raccoon rabies into the region. This variant subsequently spread and resulted in raccoon rabies becoming enzootic throughout Atlantic states (Jenkins & Winkler, 1987; Nettles et al., 1979). The full and ongoing impacts of this pathogen introduction are now realized in expensive rabies control programs to prevent further spread of raccoon rabies west of the Appalachians (Chipman et al., 2008).

Disease risks associated with wildlife translocations can be difficult to assess and mitigate due to incomplete information on presence, prevalence, and epidemiology of disease-causing agents, as well as pathogenicity and resulting impacts of diseases on individuals and populations (Sainsbury & Vaughan-Higgins, 2012). This uncertainty can be daunting and inhibit informed decision-making and communication among project partners and stakeholders in the absence of an objective, repeatable, and transparent process for documenting risks and developing mitigation strategies (MacDiarmid, 1993). Assessing and managing disease risks requires diverse expertise related to wildlife diseases, species biology, population management, and ecology, as well as working knowledge of management policies, capacities, and resource limitations. Inclusion of traditional ecological knowledge (TEK), cultural values and needs, and socioeconomic considerations may also be required. Thus, effective communication and collaboration across disciplines, cultures, and project partners are critical to ensure that risk assessment is based on best-available knowledge, is inclusive of all partner and stakeholder interests, and leads to risk management strategies that are broadly acceptable and feasible to implement (Hartley & Sainsbury, 2017).

The disease risk analysis (DRA) framework developed by the World Organization for Animal Health (WOAH, formerly OIE) and International Union for Conservation of Nature (IUCN) provides a structured and evidence-based process to assess and manage disease risks in conservation planning, while also transparently addressing uncertainties associated with those risks (OIE and IUCN, 2014). This DRA framework involves five basic steps: problem description, hazard identification, risk assessment, risk management, and implementation and review. Problem description involves outlining the question or issue under consideration, stakeholder analysis, and communication planning. Conceptually diagramming the system, such as the pathway a translocated species may take from source to destination, is an important part of this step. Hazard identification involves identifying and prioritizing individual hazards (e.g., diseases), and identifying places along the translocation pathway where hazard risk may vary (e.g., pathogen introduction, recrudescence from latency, etc.). Each hazard is then assessed qualitatively or quantitatively in a risk assessment, where the likelihood

that each hazard is introduced into the system and associated consequences of that event are characterized. Uncertainty in these estimates is also described at this stage. Risk management involves outlining mitigation measures to reduce the likelihood and consequences of prioritized risks. Efficacy of each measure may be tested ahead of implementation if quantitative modeling approaches are used in the assessment. These measures are then integrated into translocation protocols. Finally, implementation and review involve implementation of selected risk management strategies and development of a clear action plan and timeline for the evaluation and refinement of the risk analysis and established risk management plan. Risk communication is an important, though sometimes overlooked, component of overall partner and stakeholder engagement and planning, and should occur during all steps of the DRA process. Applications of this framework with examples are described in the Manual of Procedures for Wildlife Disease Risk Analysis (Jakob-Hoff et al., 2013).

Case examples describing DRAs for wildlife conservation projects are scarce in published literature despite the recognized importance of disease risks associated with wildlife translocations (Kock et al., 2010). In a review of over 700 translocation projects, 24% did not include any disease screening, and disease-related mortality could only be assessed in 22% of projects because animals were not monitored postrelease (Griffith et al., 1993). To help fill this gap and encourage wider adoption of DRAs, we present a DRA we conducted using the OIE/IUCN framework for reintroduction of gray wolves (*Canis lupus*, ma'iingan in Ojibwe) to Isle Royale National Park (IRNP) and Minong Traditional Cultural Property of the Grand Portage Band of Lake Superior Chippewa in Michigan. Here, we provide a case study for integration of a DRA into wildlife conservation project planning. We demonstrate how expertise of project personnel was used to complete a timely, cost-effective DRA that aligned with project objectives, facilitated transdisciplinary and cross-cultural collaboration and communication among partners and stakeholders, accounted for potential sources of bias, and managed disease risks for animals, humans, and the ecosystem.

2 | METHODS

2.1 | Study site

IRNP is an archipelago located in northwestern Lake Superior, about 24 km offshore of the Minnesota-Canadian border. It is designated an International Biosphere Reserve with 99% of the park managed as wilderness, which has helped to preserve this unique and dynamic ecosystem with limited human disturbance since the park was established in 1946. In 2019, this island,

known as “Minong” in Ojibwe, was listed on the National Register of Historic Places as the Minong Traditional Cultural Property, which recognizes its lasting relationship and cultural significance with the Grand Portage Band of Lake Superior Chippewa. About 16,000 visitors explore the island by boat or on foot each year from April to November; the park is closed to the public the remainder of the year and the only human activity is associated with research teams that have been studying wolves and moose on the island annually since 1958 (Allen, 1979; Mech, 1966; Nelson et al., 2011; Peterson, 2007).

Gray wolves and moose (*Alces alces*) are the prominent megafauna in IRNP. Other mammals include red fox (*Vulpes vulpes*), short-tailed weasel (*Mustela erminea*), river otter (*Lutra canadensis*), beaver (*Castor canadensis*), muskrat (*Ondatra zibenthiscus*), snowshoe hare (*Lepus americanus*), red squirrel (*Tamiasciurus hudsonicus*), little brown bat (*Myotis lucifugus*), and big brown bat (*Eptesicus fuscus*; Peterson, 1977). Wolves are apex predators on the island and have cultural significance for the Ojibwe. Wolves are primary predators of moose and also prey on beaver (Romanski, 2010), which are influential ecosystem engineers. Through effects on abundance and spatial distribution of prey species (Ditmer et al., 2018; Vucetich et al., 2011), wolves indirectly influence the terrestrial and aquatic plant communities, nutrient cycling, and other ecosystem processes.

Over the past 30 years, the numbers of wolves in IRNP declined from a high of 50 individuals in 1980 to a point that extirpation seemed likely (Peterson et al., 1998; Vucetich & Peterson, 2015). Thus, in 2018, the National Park Service (NPS) decided to restore the wolf population in IRNP via an immediate and limited reintroduction of wolves as described in IRNP's Final Environmental Impact Statement (EIS) to Address the Presence of Wolves (National Park Service, 2018). The goal was to introduce 20–30 wolves with sufficient genetic diversity to establish a viable population over the next 20 years. To accomplish this goal, the NPS, in collaboration with multiple federal, state, Tribal, provincial, academic, and nongovernmental organization partners, planned to capture and translocate free-ranging wolves from multiple locations near Lake Superior to IRNP over a 3- to 5-year period (Romanski et al., 2020). As part of the planning process for reintroduction, we conducted a DRA to identify, prioritize, and mitigate potential disease risks associated with capture and translocation of wolves to IRNP. We used the DRA framework developed by the OIE and IUCN (2014).

2.2 | Problem description

Starting in July 2018, we developed the problem description based on operational goals, objectives, and input

from project personnel during scheduled planning calls with partners (Table 1). We identified the overall goal and objectives and defined our scope and focus for the

TABLE 1 Problem description used to frame a disease risk analysis for capture and translocation of wolves to Isle Royale National Park (IRNP)

Question: What are infectious disease risks associated with translocation of wolves to IRNP and how can these risks be mitigated?

Goal	Objectives
Capture and translocate healthy wolves that would be effective predators, able to reproduce and not introduce new pathogens or new strains of endemic pathogens to IRNP	Minimize risks of: <ol style="list-style-type: none"> 1. Adverse health outcomes of translocated wolves 2. Introduction of novel pathogens or new strains of endemic pathogens 3. Zoonotic disease exposure for personnel involved in translocation operations, and staff and visitors in IRNP 4. Spill-back of disease to northeastern Minnesota or Ontario, Canada via natural movement of wolves across ice bridges from IRNP to the mainland after translocation

Scope and focus

- Populations of interest are wolves in IRNP, northeastern Minnesota, Upper Peninsula of Michigan, Michipicoten Island and Wawa, Ontario, Canada, and other native species in IRNP potentially susceptible to pathogens of concern
- Focus on infectious disease hazards; other hazard types are addressed in other aspects of project planning

Assumptions, limitations, and acceptable risks

- Multiple pathogens and vectors may cause clinical disease and mortality in wolves and other species; some have potential for population-level effects
- Introduction of novel or new strains of endemic pathogens may affect wolves or other native species in IRNP
- Data and knowledge gaps exist about pathogen prevalence and epidemiology in our populations of interest, resulting in uncertainties in the risk assessment
- Background pathogen pressures in source populations may vary resulting in likely but unknown differences in immunocompetence and disease risk
- Exact sources, timing, and numbers of wolves captured and translocated from each location cannot be guaranteed and will vary
- Infections may not be apparent on physical exam and diagnostic limitations may inhibit pathogen detection at the time of capture
- Inherent risks associated with capture, anesthesia, and translocation can be minimized but not eliminated
- Unpredictable risks will likely occur during this complex and dynamic operation

DRA. We also articulated assumptions, limitations, and acceptable risks.

2.3 | Hazard identification

We created a list of hazards using published and unpublished data, including peer-reviewed scientific articles, agency databases and reports, and expert knowledge. We focused on infectious disease hazards since noninfectious hazards (e.g., trauma, anesthetic-related complications, stress, genetic fitness) were addressed in other aspects of project planning. We compiled available information on the presence or prevalence, morbidity, and mortality of each hazard within source and recipient populations (i.e., background hazard pressure). Where disease data were lacking for wolf populations in the Great Lakes Region, we used information from domestic dogs (e.g., cases of heartworm or blastomycosis) or humans (e.g., reported cases of tick-borne diseases) within the region. Where regional data were not available, we used published reports of disease in wolves in North America as indicators of potential consequences for each hazard. Finally, we developed a conceptual map to identify places where hazard risk might vary and be mitigated (i.e., critical control points [CCPs]) throughout the translocation process (Figure 1).

2.4 | Risk assessment

In August 2018, prior to the start of capture operations in September 2018, we conducted a half-day workshop, facilitated by project veterinarians (Drs. Michelle L. Verant and Tiffany M. Wolf), to share the project description, review the hazard list, and available information on background hazard pressures, and complete a risk assessment exercise to prioritize hazards for surveillance or mitigation. Participants included two tribal natural resources professionals from the Grand Portage Band of Lake Superior Chippewa and five federal and state wildlife biologists and a state wildlife veterinarian from the Great Lakes region involved in the project and with knowledge of the populations of interest. Additional input was collected from three regional wildlife or wolf biologists and one wildlife veterinarian after the workshop, using the same background materials and risk assessment tools. Following a review of the project description, hazard list, and conceptual map of the translocation pathway, each participant was asked to individually score each hazard in two categories: likelihood of risk and severity of consequence. Facilitators and participants used a qualitative scale of high, medium, low, and negligible for scoring. Assessments of likelihood and consequence were based on current knowledge and participants' experiences.

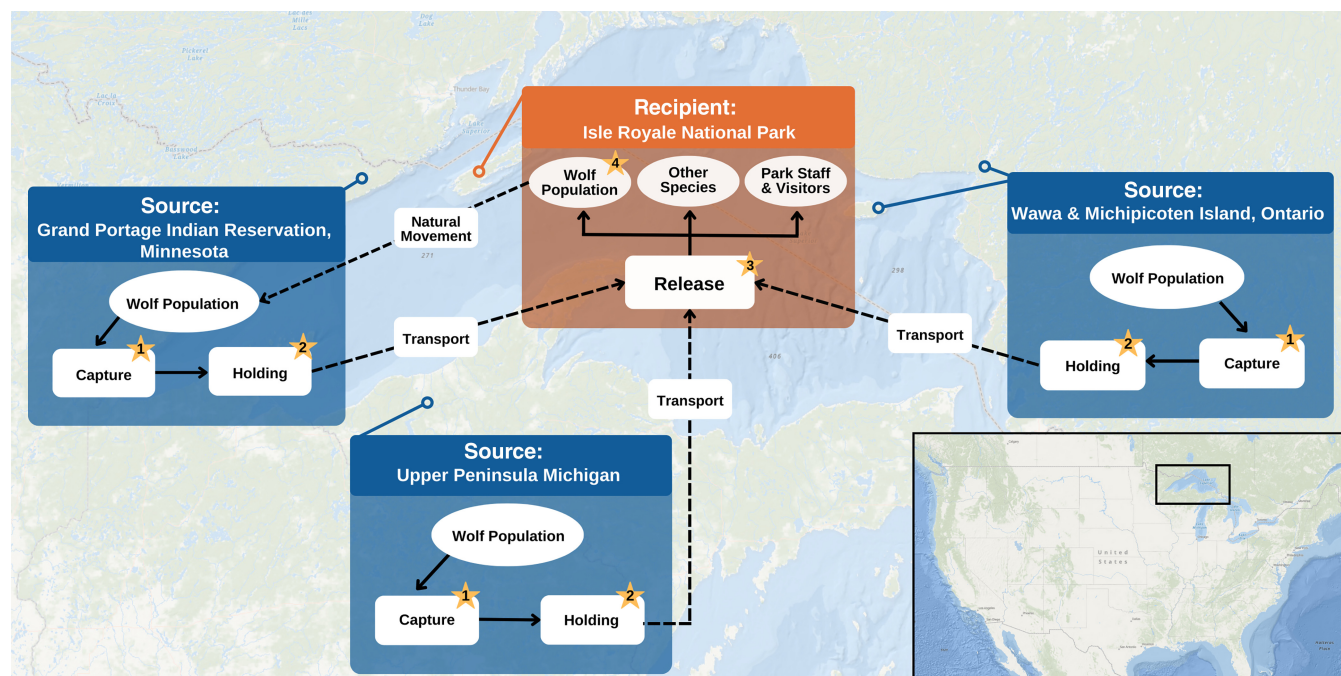


FIGURE 1 Conceptual map of the steps and pathways involved in capture and translocation of gray wolves to Isle Royale National Park from multiple source populations near Lake Superior. This diagram was used to facilitate hazard identification and critical control points (numbered stars) for risk mitigation by identifying potential sources and introductions of hazards throughout the translocation process

In assessing the likelihood of risk, participants were asked to consider the combined probability of capturing an infected animal and transmission of the pathogen to another individual during or post-translocation (in the absence of any mitigation). Assessment of consequence included direct effects on translocated individuals, as well as the wolf population in IRNP. Because risk to other species in IRNP was included in the scope of the DRA, but encompassed a lot of uncertainty, we included a separate scoring category for risk to other susceptible species. Each participant indicated their level of certainty of background hazard pressure in the populations of interest and in their assessment of risk for each hazard. Using these individual inputs, we performed a semiquantitative assessment by assigning numerical values for each qualitative score (high = 4, medium = 3, low = 2, and negligible = 1) and calculated the median score for each criterion (i.e., likelihood, consequence, uncertainty) with equal weighting for all individual responses. Results were shared with participants after the workshop and discussed as applicable to mitigation measures and protocols during subsequent project planning calls.

2.5 | Risk management

We developed mitigation strategies that could be implemented during capture, handling, and transport that

focused on detection and/or elimination of hazards with high to medium risk. To do so, the project team evaluated where along the translocation pathway these hazards could be introduced or detected, CCPs where mitigation measures could be implemented, and the feasibility of selected strategies to mitigate the risk. Final decisions were made by consensus of project veterinarians and lead project personnel from participating agencies.

2.6 | Implementation and review

We incorporated selected risk management strategies into plans and protocols for capture, processing, holding, transport, and release of wolves. We also developed a plan for postrelease monitoring of translocated individuals that was integrated into ongoing monitoring and research of the wolf population on IRNP. Monitoring goals were to assess success of translocation and to evaluate our risk assessment and mitigation strategies to inform future translocations. Specific evaluation measures included cause-specific wolf mortality and disease outbreak and pathogen surveillance. At the time of capture, blood was collected for on-site diagnostics and retrospective analyses of pathogen exposure and infection. Each wolf was tested for heartworm (*Dirofilaria immitis*) infection at the time of capture using an IDEXX SNAP® 4Dx® Test Plus (IDEXX, Maine) or VetScan Heartworm

Rapid Test Kit (Abaxis, USA). The IDEXX kit also tested for antibodies against *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia* spp. Retrospective serologic analyses were completed at Cornell University Animal Health Diagnostic Center (Ithaca, New York) to detect antibodies against canine adenovirus (CAV), canine distemper virus (CDV), and West Nile virus (WNV; all via serum neutralization), canine parvovirus-2 (CPV, via hemagglutination inhibition), *B. burgdorferi*, *A. phagocytophilum*, and *Neospora caninum* (via indirect fluorescent antibody), and *Leptospira* serovars (via microscopic agglutination test). Analyses of whole blood including polymerase chain reaction (PCR), culture, and microscopic examination of blood smears to detect infections with tick-borne pathogens (Anaplasmataceae and *B. burgdorferi* sensu lato) were completed by the Munderloh Lab at the University of Minnesota, Department of Entomology (St. Paul, Minnesota). DNA was extracted from 100 to 200 µl blood using the Puregene Core Kit A (Qiagen Sciences, Valencia, California; Lynn et al., 2017). Primers Per1 and Per2 that amplify a 451 bp region of the 16S rDNA were used to detect presence of Anaplasmataceae (Goodman et al., 1996), and species identified by Sanger sequencing of the product in both directions (University of Minnesota Genomics Center) followed by BLASTN analysis. DNA from *A. phagocytophilum* strain HGE1 (Goodman et al., 1996) and water were amplified in parallel as positive and negative controls respectively. Primers that amplify *B. burgdorferi* sensu lato were used as described with DNA from the isolate B31 serving as positive and water as negative control (Marconi & Garon, 1992). Thin blood smears were air dried, fixed in absolute methanol for 10 min, stained with Giemsa's stain (KaryoMAX, Thermo Fisher Scientific, Waltham, MA), and examined under 100× magnification. Fecal samples collected at the time of capture were analyzed at Cornell University Animal Health Diagnostic center by modified Wisconsin double centrifugation floatation technique using sugar (1.33 specific gravity) and Zinc sulfate (1.18 specific gravity) as floatation solutions (Broussard, 2003). DNA extracted from fecal samples positive for Taeniid-type eggs (includes *Taenia* spp. or *Echinococcus* spp.) were subjected to PCR for the genetic marker, cytochrome c oxidase 1 using universal primers characterized previously (Bowles et al., 1994). Although results of serologic tests and fecal analyses were not available until after wolves were released in IRNP, this information was intended to address data gaps and facilitate refinement of the DRA for future translocations.

All translocated wolves were fitted with Global Positioning System (GPS) collars (VECTRONIC Aerospace GmbH, Berlin, Germany) with very high frequency transmitters and marked with unique ear tag numbers to

monitor movements postrelease. Location data were acquired every 4–5 h and transmitted every 20 h (depending on satellite connection) and mortality signals were set to occur if the location did not change within 6 h. Initial location of each wolf was obtained within 24 h of release or as soon as the collar was able to establish a satellite connection and transmit data. Clusters of points identified by two or more consecutive GPS locations within 50 m of each other indicated areas where wolves spent significant amounts of time, such as resting sites, rendezvous locations, or where predations occurred. Scat was collected for ongoing pathogen surveillance from GPS clusters and along hiking trail transects throughout the park from May to September following release (Romanski et al., 2020). Aerial surveys from fixed-wing aircraft were used to monitor wolves in winter as part of ongoing research of wolves and moose in IRNP (Hoy et al., 2020). Mortalities were investigated if feasible with a postmortem exam, including gross necropsy, histopathology, and diagnostic testing at the US Geological Service National Wildlife Health Center (Madison, Wisconsin) if the carcass was in suitable condition. A network of trail cameras was also deployed across IRNP for ongoing monitoring and research.

3 | RESULTS

3.1 | Hazard identification and risk assessment

We identified 27 infectious disease hazards potentially associated with translocation of wolves from the Great Lakes Region to IRNP. Hazards included eight viral pathogens, five bacterial pathogens, one fungus, eight internal parasites or parasite groups, and five external parasites or parasite groups. Ticks were included as potential vectors of wildlife and zoonotic pathogens. CCPs were identified throughout the translocation process (Figure 1).

Of the 27 hazards identified, eight were of high to medium consequence, with four of the considered medium likelihood for infection and introduction to IRNP (sarcoptes mites, CPV, CAV, and heartworm), and one highly likely (ticks; Table 2). Likelihoods of the other three consequential hazards (rabies, CDV, and lice) were low. Hazards considered to have negligible likelihood or consequence were canine influenza, *Bordetella bronchiseptica*, and demodex mites. All other hazards were considered low consequence and of medium likelihood, except for *Echinococcus granulosus*, which was highly likely. Overall, participants indicated their certainty in these risk assessments as medium for 13 hazards and low certainty for 14 hazards (Table 2). Participants had high certainty in knowledge of background pathogen pressures for 10 hazards, medium

TABLE 2 Hazards are shown on a matrix according to median likelihood and consequence for each determined by subject matter experts based on semiquantitative scores

		Consequence		
		High	Medium	Low
Likelihood	High		Ticks ³	<i>Echinococcus granulosus</i> ³
	Medium	<i>Sarcoptes mites</i> ³ <i>Canine parvovirus</i> ³	<i>Canine adenovirus</i> ³ <i>Heartworm</i> ³	<i>Borrelia burgdorferi</i> ³ <i>Anaplasma phagocytophilum</i> ² <i>Neospora caninum</i> ³ <i>Sarcocystis</i> ¹ <i>Fleas</i> ² Roundworms ² Hookworms ³ Canine parainfluenza ¹
	Low	<i>Rabies virus</i> ²	<i>Canine distemper virus</i> ³ <i>Lice</i> ²	West Nile Virus ² Eastern Equine Encephalitis ² <i>Ehrlichia/Rickettsia</i> ¹ Trematodes ¹ <i>Toxoplasma gondii</i> ² <i>Leptospira serovars</i> ¹ <i>Blastomyces dermatitidis</i> ¹

Note: Colors indicate the median certainty of the risk assessment for each hazard as medium (orange) or low (blue). On average, experts did not express high certainty in the assessment for any of the hazards. Superscripts indicate the median certainty in knowledge of the background pathogen pressure for each hazard as high (3), medium (2), and low (1) as expressed by the experts. Hazards with negligible risks (*canine influenza*¹, *Bordetella bronchiseptica*¹ and *demodex mites*¹) are not shown.

certainty for 8 hazards, and low certainty for 9 hazards. Hazards considered to be high risk for species other than wolves were rabies and sarcoptes mites. With exception of *N. caninum*, which was considered a medium risk to moose, all other hazards were viewed as low or negligible risk to other species.

3.2 | Risk management

We developed mitigation measures to reduce risks associated with disease hazards, focusing primarily on hazards with high to medium consequences (Table 3). We did not address hazards with negligible risk. In general, risk management strategies fell into four categories: (1) selection of wolves for translocation according to established criteria (Table 4); (2) on-site diagnostic testing; (3) preventative treatments; and (4) biosecurity and public health measures to reduce risks of pathogen transmission between translocated wolves and project personnel. These measures were integrated into protocols and safety plans. For example, capture teams used the selection criteria at CCP1 (Figure 1) to determine if a wolf should be held for translocation or released on-site. If a wolf met the criteria, it was sampled and received preventative treatments at CCPs 1 and 2. Biosecurity and public health measures, such as disinfecting handling equipment and rabies pre-exposure prophylaxis for personnel, were

implemented at all CCPs. To minimize stress, wolves were transported and released in IRNP as soon as feasible following capture (typically within 24 h, one wolf was held for 72 h).

3.3 | Implementation and review

A total of 19 wolves were translocated to IRNP between September 2018 and September 2019 (Table S1). For additional details on capture and release of each wolf see Romanski et al. (2020). External parasites (mange mites, ticks, and lice) were not detected on any wolf and no signs of systemic disease were apparent during physical exams. Heartworm was also not detected using on-site diagnostic kits, although these assays are designed to detect antigen from adult female worms and could miss an early or male worm only infection. Concurrent antibodies to *B. burgdorferi* and *Anaplasma* spp. (*A. phagocytophilum*, *A. platys*) were detected in two wolves from Michigan using on-site diagnostic kits. One wolf from Ontario also had antibodies to *B. burgdorferi*. Translocated wolves received vaccines (Vanguard Plus 5 L4, Zoetis, New Jersey; IMRAB[®] 3, Merial Inc., Georgia) and injectable (Ivomec[®] 1%, Boehringer Ingelheim Vetmedica, Inc., Georgia; Droncit, Bayer Animal Health, Kansas) and topical (BRAVECTO, Merck Animal Health, New Jersey) antiparasitics. Wolves translocated

TABLE 3 Risk mitigation and evaluation measures for disease hazards associated with translocation of wolves to Isle Royale National Park

Hazard	Detection method	Risk mitigation and evaluation measures
High to medium consequence		
Sarcoptes mites/mange	Physical exam and skin scrape if clinical signs	Do not translocate wolves with signs of mange (prevention) Topical antiparasitic (treatment and prevention) Dispose bedding, disinfect crates, and handling equipment (biosecurity)
Ticks (tick-borne pathogens)	Physical exam and on-site blood test	Topical antiparasitic (treatment and prevention) Remove any observed ticks (evaluation) Tick checks and tick repellent (public health prevention) Retrospective serology (evaluation) Retrospective whole blood analysis for active infections (evaluation)
Lice	Physical exam	Do not translocate wolves with signs of lice (prevention) Topical antiparasitic (treatment and prevention) Dispose bedding and disinfect crates/handling equipment (biosecurity)
Canine parvovirus	Clinical signs (active infection)	Vaccination (prevention) Retrospective serology (evaluation) Dispose bedding/waste and disinfect crates/handling equipment (biosecurity)
Canine adenovirus	Clinical signs (active infection)	Vaccination (prevention) Retrospective serology (evaluation) Dispose bedding/waste and disinfect crates/handling equipment (biosecurity)
Canine distemper virus	Clinical signs (active infection)	Vaccination (prevention) Retrospective serology (evaluation) Dispose bedding/waste and disinfect crates/handling equipment (biosecurity)
Rabies	Clinical signs (active infection)	Vaccination (prevention) Retrospective serology (evaluation) Rabies pre-exposure prophylaxis for all handling personnel (public health prevention)
Heartworm	On-site blood test	Do not translocate infected wolves (prevention) Ivermectin (treatment/prevention for microfilaria)
Low consequence		
Fleas	Physical exam	Do not translocate wolves with heavy infestations (prevention) Topical antiparasitic (treatment and prevention) Dispose bedding and disinfect crates/handling equipment (biosecurity)
<i>Echinococcus granulosus</i>	N/A	Praziquantel (treatment) Dispose bedding/waste and disinfect crates/handling equipment (biosecurity) Wear gloves and wash hands after handling (public health prevention) Retrospective fecal analysis (evaluation)
Intestinal parasites	N/A	Ivermectin (treatment for hookworms and roundworms) Retrospective fecal analysis (evaluation)
<i>Leptospira</i> serovars	N/A	Vaccination (prevention) Dispose bedding/waste and disinfect crates/handling equipment (biosecurity) Wear gloves and wash hands after handling (public health prevention) Retrospective serology (evaluation)
<i>Neospora caninum</i> , <i>Toxoplasma gondii</i> , and West Nile Virus	N/A	Retrospective serology (evaluation)

TABLE 4 Criteria for selecting wolves for translocation

Criteria checklist

1. Age 1 to about 5 years; not a pup
2. At least three intact, functional canines
3. Eyes both present and apparently functional (eyes clear, not cloudy, or opaque)
4. Decent body condition (for a wolf); not emaciated
5. No current fractures (healed/callused fractures that do not affect function are okay)
6. No missing limbs (foot to entire limb, missing toe okay)
7. No evidence of mange or lice infestation (e.g., moderate/severe hair loss, rough/scaly skin, visible lice)
8. Heartworm test negative

Note: Each wolf was assessed at the time of capture and if it did not meet the following criteria it was not translocated to Isle Royale National Park.

from northeastern Minnesota also received a topical treatment for chewing lice (Advantage II, Bayer Animal Health, Kansas), but given the lack of apparent lice infestations and perceived low risk from this specific hazard this treatment was discontinued for subsequent translocations. Use of BRAVECTO was continued given its effectiveness against ticks, fleas, and sucking lice. Antibiotics were administered parenterally to five wolves (26%) with apparent trauma associated with capture or pre-existing injuries.

Detailed summaries of wolf population monitoring including status, space use, social organization, reproduction, mortality, predatory activity, and genetic assessments of translocated individuals are reported elsewhere (Hervey et al., 2021; Orning et al., 2020; Romanski et al., 2020). As of April 2020, seven (36.8%) mortalities occurred among translocated wolves post-release. Mortalities occurred due to intraspecific aggression ($n = 3$), pneumonia ($n = 1$), cellulitis and septicemia secondary to trap injuries ($n = 1$), and undetermined causes ($n = 2$). The wolf that died of pneumonia about 35 days postrelease (008; Table S1) had evidence of lymphoid depletion, and CPV-2 and CDV were detected in lung tissue by PCR. CPV-2 was weakly positive (Ct 38.2) indicative of prior vaccination. Results for CDV (Ct 31.6) were less clear and it could not be determined whether presence of the virus was due to recent modified-live vaccination or active disease. There was no evidence of encephalitis on histology of brain sections, but decomposition and freeze-thaw artifacts precluded further evaluation of lung and other tissues.

Retrospective serologic testing revealed exposure to all pathogens of interest, except *Leptospira*, at each source location, with some differences in prevalence by location (Figure 2). All wolves from Minnesota and Michigan had exposure to CPV and CAV, with

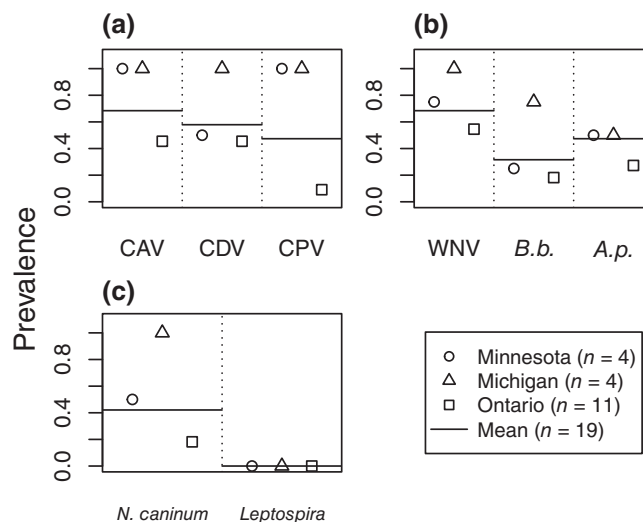


FIGURE 2 Seroprevalences of viral (A), vector-borne (B) and other (C) pathogens in wolves translocated to Isle Royale National Park from each source population. Horizontal lines show the mean seroprevalence for each pathogen among all translocated wolves. CAV is canine adenovirus, CDV is canine distemper virus, CPV is canine parvovirus, WNV is West Nile Virus, B.b. is *Borrelia burgdorferi* and A.p. is *Anaplasma phagocytophilum*. *Leptospira* serovars include *pomona*, *hardjo*, *icterohaemorrhagiae*, *grippityphosa*, and *canicola*.

exposure to CDV in half from Minnesota and all from Michigan. Exposure to *N. caninum* and WNV was ubiquitous in Michigan wolves, with lower prevalence in Minnesota and Ontario. Exposure to *B. burgdorferi* was also higher in Michigan wolves, and half of the wolves in Minnesota and Michigan had antibodies against *A. phagocytophilum*, as indicated by indirect fluorescent antibody assays. Overall, pathogen prevalence was lower in wolves from Ontario. One wolf from Minnesota had *A. phagocytophilum* detected in whole blood by PCR, indicating bacteremia at the time of capture. This may have been a recent infection because antibodies were not detected. Morulae were not observed on blood smears and the bacterium was not recovered by culture; however granulocytic *A. phagocytophilum* inclusions are rarely seen in peripheral blood and the sample was contaminated. *B. burgdorferi* was not detected in whole blood samples from any of the wolves. Analyses of fecal samples collected from individual wolves at the time of translocation ($n = 18$) detected parasite stages of *Trichuris* spp. (7%), *Capillaria* spp. (13%), *Sarcocystis* spp. (27%), *Taeniidae* (7%), *Alaria* spp. (7%), *Eucoleus boehmi* (13%), and spurious *Eimeria* spp. (13%). *Sarcocystis* was the only parasite observed in all source populations. *Taenia hydatigena* was confirmed via PCR on *Taeniidae* positive samples in two wolves (13%). Notably, *E. granulosus* was not detected in the samples.

4 | DISCUSSION

Disease is a critical yet often overlooked element of wildlife conservation projects. Here, we demonstrate how DRA can facilitate identification, prioritization, and mitigation of disease risks associated with intentional movements of wildlife. The success of this DRA was associated with its relatively rapid completion and ease of integration into the translocation planning process, and its guidance in developing and implementing protocols for animal and human health and postrelease monitoring. In addition, it provided a structured, multidisciplinary process for proactively and transparently identifying and communicating risks and uncertainties to diverse stakeholders throughout the project.

The flexibility of this DRA framework (Jakob-Hoff et al., 2013) allowed our team to effectively and efficiently meet project objectives within existing constraints and timelines while facilitating collaboration across disciplines. The majority of operational planning and protocol development for reintroducing wolves to IRNP occurred over 3 months with the first wolves captured on September 25, 2018 following approval of the Record of Decision for the Final EIS to Address the Presence of Wolves at Isle Royale National Park on June 7, 2018 (National Park Service, 2018). This short timeframe necessitated efficient means of assessing risks and determining mitigation measures that could be easily incorporated into planning meetings with park staff and collaborators. While the DRA process is often completed during a multi-day organized and facilitated workshop with experts, stakeholders, and decision makers (Jakob-Hoff et al., 2013), we used a modified approach to accomplish this process with limited time and resources. Project veterinarians designed and led the DRA, engaged project personnel and partners as subject matter experts to assist with identifying and qualitatively prioritizing risks, and facilitated inclusion of disease risks and mitigations when developing protocols for capture, handling, translocation, and postrelease monitoring of wolves. This work was accomplished via email correspondence, inclusion within weekly operational planning calls with partners, and a half-day workshop during a 2-day in-person meeting a few weeks prior to the start of captures. Engaging project personnel and partners in this process created a shared understanding of potential risks and consequences and helped to ensure mitigation measures were realistic and supported by everyone responsible for implementing them.

The DRA process also facilitated discussion of recognized and uncertain risks among project personnel. This information was used to create key messages for proactive risk communication about this high-profile project with agency leadership, external partners, stakeholders,

and the public. For example, key messages highlighted ways in which the health of translocated wolves and other native species on IRNP was prioritized, and explained inherent, irreducible risks associated with capture and translocation of free-ranging wild animals. These messages were especially pertinent when communicating about mortalities that occurred among wolves. As an example, the wolf that died of pneumonia postrelease had a small amount of CDV detected in lung tissue postmortem. Although we cannot definitively conclude whether presence of CDV was due to recent vaccination or active disease, this wolf had pre-existing antibodies to CDV at the time of capture and ongoing CDV transmission was not observed within the source population or the group of wolves translocated to IRNP, indicating this finding was likely due to vaccination with a live-attenuated virus. This wolf also had evidence of an active *Anaplasma* infection at the time of capture and translocation. Although canine anaplasmosis is thought to be an acute illness, there is some evidence that it may cause chronic infections in dogs (Khatat et al., 2021). Thus, immunosuppressive effects of anaplasmosis and/or complications related to stress of capture, anesthesia, and translocation (Teixeira et al., 2007) may have contributed to this wolf's increased susceptibility to disease. However, the ultimate cause of death remains unknown. This example highlights that conducting a DRA will not eliminate risk or uncertainty, but can provide a structured means for transparently and proactively identifying and addressing risks to reduce disease-related harm associated with wildlife translocations.

A key challenge in risk assessment is often related to lack of data in the species or populations of interest. The DRA framework is a structured approach that can be used to integrate multiple sources and types of information to facilitate risk-based decision-making while transparently addressing uncertainty. In this project, participation of Grand Portage Band of Lake Superior Chippewa tribal natural resources professionals in the DRA, project planning, and implementation was inherent due to the long-standing relationship between the Band and IRNP, and the Band's ancestral and enduring connection to the island. As such, tribal perspectives and knowledge were included, but more intentional inclusion of different forms of TEK could have provided a greater breadth of perspectives on disease risks. Data on pathogen prevalence and disease in wolves historically on IRNP and within the source populations were primarily limited to disease outbreak reports and opportunistic mortality investigations, or observations and samples collected from individual animals when captured for other purposes. To try to fill these data gaps, we conducted a more comprehensive search of disease data in wolves

across the Great Lakes region and relied upon data in other susceptible species in the area as indicators of pathogen presence where data from wolves were lacking. Much of the data came from serosurveys, which although useful for assessing historical exposure and prevalence, are not as useful for assessing consequence since we cannot infer manifestations or impacts of acute or chronic disease in individuals or populations. Additionally, the variation in background pathogen pressures among the source populations further complicated the assessment of risk for translocated individuals, as well as for recipient populations. Finally, there is a general lack of diagnostic test validation specific to wolves and there can be differences in sensitivity between testing methods, which can result in inconsistent findings, such as the incongruence in the 4Dx Snap and IFA tests for exposure to tick-borne diseases in five of the translocated wolves. Taken together, these variables and limitations created uncertainty among experts in the overall risk assessment.

Interestingly, subject matter experts were more certain in their assessment of hazards that ranked as high to medium consequence compared with hazards that were considered to have a low consequence. One possible explanation is that pathogens of high consequence are more extensively studied, as they are more readily observed or actively monitored in wildlife populations. In contrast, there is less information on hazards of low consequence because infections are rare and/or typically have negligible health impacts. If this is the case, this difference in reporting can be a correlative measure of consequence for these pathogens. However, it is also possible that this data disparity creates a biased sense of confidence in more well-known pathogens and more uncertainty in less-known pathogens, particularly when considering their introduction into naïve or stressed populations.

An interesting observation from this DRA was that veterinarians expressed higher certainties overall in their risk assessments than field biologists. Overconfidence bias, which is excessive confidence in knowing the truth, is a common cognitive bias and is difficult to overcome (Bazerman & Moore, 2013; Moore & Healy, 2008). Multiple techniques have been described and tested to reduce bias during expert elicitation and decision analysis (Ferretti et al., 2016), and some advocate for completing a DRA with an independent group not influenced by decision-makers to avoid bias (Leighton, 2002). Others have recognized the value and necessity of including project personnel and decision-makers in the process to ensure relevant and feasible outcomes (Jakob-Hoff et al., 2013; Krueger et al., 2012). We specifically included project veterinarians and agency biologists in this DRA to combine specific disease expertise with years of experience in handling and studying free-ranging wolves.

Having a diversity of backgrounds and knowledge among experts was intended to help account for potential sources of bias and add rigor to our results. Additionally, inclusion of indigenous communities, cultural perspectives, and socioeconomic factors in hazard identification and assessment could add value to all steps of the DRA process and should be considered for projects involving culturally significant wildlife species and/or ecosystems.

Postrelease monitoring and disease surveillance data can be helpful in reducing uncertainty and refining a DRA for subsequent translocations (Hartley & Sainsbury, 2017). Serologic and fecal analyses of wolves at the time of translocation to IRNP confirmed presence of all pathogens assessed in the DRA (except *Leptospira* and *E. granulosus*) within each of the source populations. Although sample sizes were small, there were differences in background pathogen pressure by location with generally higher pathogen prevalence in wolves from the Upper Peninsula of Michigan compared with northeastern Minnesota or Ontario. Depending on the pathogen, prior exposure may be an advantage if it generates a protective immune response or it could be a reason to not select individuals from a more pathogen-rich population. Of course, disease risks must be considered in concert with other factors that contribute to overall fitness (e.g., genetics, body size, and experience with different types of prey) and operational feasibility (e.g., time of year, funding, partner and stakeholder support) when determining sources for future translocations. To expand upon this DRA, we intend to conduct a retrospective serologic study of wolves in source populations (as samples are available) to enhance our knowledge of background pathogen pressure in the region. Populations of particular interest are wolves in northeastern Minnesota and nearby regions of Ontario, due to potential for natural movements of wolves to or from IRNP via ice bridges. In fact, one female wolf translocated to IRNP from the Grand Portage Indian Reservation in September 2018, returned to the mainland over a temporary ice bridge that formed in January 2019. She returned briefly to her capture location where she may have reunited with a former pack mate (a previous season pup) before traveling throughout northeastern Minnesota and Ontario through September 2019 (Orning et al., 2020).

Potential disease risks to other native species on IRNP is also of interest and is being investigated as part of ongoing integrated ecological research in collaboration with the Grand Portage Band of Lake Superior Chippewa and other state, federal, provincial, and academic partners. In the DRA, risk to other species was added as a separate category, but we did not explicitly include this data in the hazard prioritization process; it did not meaningfully alter final outcomes and was already accounted

for within the scope of consequences. Participants also expressed considerable uncertainty and differing perspectives on potential risks to other species. In the future, consequences of certain hazards could be more fully assessed by considering potential impacts to subsistence practices dependent upon the health of subsistence species. As an example, *N. caninum* was a concern for some participants given known presence of this parasite in wolves in the region and effects on reproductive health in cattle (Brandell et al., 2021; Carstensen et al., 2017; Dubey et al., 2007; Dubey et al., 2011). Although there are no cattle in IRNP, this parasite has been detected in moose in the region, but consequences of infection for moose are unknown (Gondim et al., 2004). Additionally, *N. caninum* is known to infect other wild carnivore species that inhabit IRNP, including a case of clinical neosporosis in a red fox (Dubey et al., 2014). Given the recognized importance of disease spillover to other species, options for integrating this information more explicitly into the risk assessment warrants future consideration.

Wildlife translocations can be complex and dynamic given multiple sources of risk and uncertainty. Prevention and early detection of diseases are critical for avoiding or reducing harmful impacts to translocated individuals and recipient ecosystems. Once an introduced pathogen becomes established in free-ranging wildlife, eradication is costly and nearly impossible to achieve. Although disease risks and other hazards associated with wildlife translocation are never zero, the DRA process can be an effective tool for transparently identifying, managing, and communicating risks to maximize the likelihood of success in achieving conservation goals. We hope this report encourages wider adoption of risk analysis principles that can be practically and efficiently integrated into planning and implementation of intentional movements of wildlife for conservation.

AUTHOR CONTRIBUTIONS

Michelle L. Verant conceived and lead the risk analysis with Tiffany M. Wolf, Mark C. Romanski, and Seth Moore. Mark C. Romanski, Seth Moore, Brent R. Patterson, and Dean E. Beyer led wolf capture operations. Ulrike G. Munderloh, Lisa D. Price, and Mandigandan Lejeune analyzed samples. Michelle L. Verant, Tiffany M. Wolf, and Treana Mayer analyzed and summarized the data. Michelle L. Verant and Tiffany M. Wolf wrote the article with contributions from all coauthors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

Data underlying this article will be shared on reasonable request to the corresponding author with permission from contributing agencies.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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