

Title

**Predation-driven spillover: Pathogen bioaccumulation in top predators.**

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## Abstract

Predator-prey interactions present heightened opportunities for pathogen spillover, as predators are exposed to novel parasites through consumption of prey harboring potentially infectious agents. Epizootics with high morbidity and mortality have been recorded following prey-to-predator spillover events with significant conservation implications, particularly for sensitive species. However, relatively few virulent infections following prey consumption are reported, given the very large number of exposures that presumably occur. Further, many transmitted agents are infectious but clinically silent and thus go unrecognized. Mechanisms that determine outcome of predator exposure to prey-based pathogens therefore represent an important, understudied component of disease dynamics that should be considered in modeling approaches and empirical research to better understand disease risk and emergence, particularly in vulnerable or threatened species.

## Main Text

Spillover (see Glossary) as a phenomenon has taken on urgent significance due to the emergence of important diseases such as highly pathogenic avian influenza in North America, African swine fever in Asia and Eastern Europe, Ebola in Western and Central Africa, Hendra in Australia, and most recently the COVID-19 pandemic. Depending on the mode of transmission, host-switching events are dependent on proximity and interaction between a reservoir host and a new susceptible host species. Predation is one mechanism by which intraspecific contact occurs naturally by exposing predators to potential pathogens of prey species as a consequence of normal feeding behavior. While bioaccumulation of toxicants has been recognized as a significant risk to species at the top of food chains, little research describes the potential for predators to acquire or evade infectious diseases following exposure during hunting, capture, and ingestion of prey. This opinion piece examines this omission and provides recommendations for consideration of prey-to-predator spillover risk as an important but overlooked aspect of conservation medicine and predator ecology.

## **Spillover risk during predator-prey interactions**

Pathogen spillover, i.e., transmission of a pathogen from a reservoir host to a novel host, is a widely understood concept, particularly as it relates to emerging zoonotic diseases. Cross-species transmission occurs in species besides humans, resulting in major threats to wildlife survival and biodiversity (e.g., distemper in African lions [*Panthera leo*] [1], plague and distemper in black-footed ferrets [*Mustela nigripes*] [2, 3]), and disruption of agricultural industries (e.g., African swine fever [4], avian influenza [5]). The ecological determinants of spillover have been described as a series of permeable barriers that prevent infection of one host species by another

[6, 7]. While there is much variability across host-pathogen systems, the initial mechanistic barrier for all systems is sufficient contact for pathogen transmission. Predator-prey interactions bring both closely and distantly related host species into close contact, creating opportunities to surmount the initial barrier to spillover infection.

The concept of predators bioaccumulating toxins has been considered extensively [8], so the observation that predators “bioaccumulate” pathogens should not be surprising. However, there is a paucity of literature that estimates the risk of predators as spillover recipients based upon their prey-consumption behavior, and a surprising lack of scholarly work that has directly evaluated this phenomenon. **Table 1** lists fifteen examples of prey to predator spillover that have been well characterized by experimental or observational studies, with a variety of outcomes for the predator. Because the outcome of a predator-prey interaction would nearly always result in survival of the predator versus the prey, spillover from these encounters would therefore be of consequence only to the predator.

### **Outcomes of spillover transmission**

The majority of well-publicized spillover events result in catastrophic consequences to the new host, as these infections result in significant economic or conservation impacts. Recent studies indicate, however, that cross-species transmission frequently occurs without substantial population-level impacts to the recipient host. In the case of predation, a number of disease outcomes may result following predator consumption of prey harboring a potential spillover agent (i.e. consumption of a reservoir host), illustrated in **Figure 1** and **Table 1**. These range

from no infection in the predator, to adaptation and replication of the pathogen in the predator host, and transmission within the predator population, with either virulent or avirulent outcomes.

New advances in molecular technologies, including next generation sequencing and sensitive serosurveillance, have afforded opportunities for detection of microparasites from free-ranging wildlife [9-12]. Protocols permitting detection of pathogens in excreta have been perfected, allowing noninvasive sampling techniques that augment sample collection protocols that would otherwise be invasive, expensive, and potentially harmful to either animals or field personnel [13-15]. Further, significant advances in bioinformatics approaches have accelerated pathogen genomic characterization that estimates time and place of transmission events based on phylodynamic and phylogeographic analyses [9, 16-21]. Because of these recent refinements in field and diagnostic methods, inferring source of infection and tracking of pathogen host switching have become substantially more feasible and accurate.

Our analysis of disease transmission among domestic and nondomestic felids using highly sensitive molecular methods has caused us to observe repeatedly that predator hosts are at risk for pathogen spillover from prey species, and that both symptomatic and asymptomatic cross-species transmission events can be readily documented from subordinate to apex host (i.e., FFV, FIV, FeLV, *Mycoplasma* spp.) [16, 17, 22-24] (**Figure 2**). Both ecological and host:virus interactions result in potential for successful spillover (**Figure 1B, 2**); however, in most cases mechanisms driving predator-prey disease transmission outcomes have not been determined.

## Ecology of predator-prey interactions

Spillover research has focused on categorizing risk based on species relatedness and geographic distribution via phylogenetic approaches [21, 25-27]. Some studies have identified links between host phylogeny and shared pathogens, consistent with the assumption that closely related species share similar cellular receptors and immunological pathways, favoring spillover and efficient adaptation in related hosts [27-29]. Others have demonstrated that geographic overlap also explains disease links between host species, with numerous instances of spillover from a reservoir host to a distantly related recipient host (e.g., bats and rodents to humans, birds to mammals, etc.) [21, 26]. Beyond strictly trophically transmitted parasites, additional phylogenetic analysis should be conducted on the risk of transmission between unrelated species [30].

Patterns of predator resource selection are also an important consideration for spillover and concurrent conservation efforts. There is evidence that with increasing urbanization and other anthropogenic change, prey base is shifting in response to availability and sympatry [31, 32]. Studies have shown that urbanized habitat can provide sufficient food for predators; however, alterations in ecological and trophic relationships should be expected [32]. For example, Moss et al. 2015 [31] documented that puma (*Puma concolor*) inhabiting urban interfaces shifted from predominant specialization on native herbivores to increased predation on synanthropic mesocarnivores and small domestic species. Such changes in predator-prey ecology present new opportunities for cross-species transmission, thereby increasing the potential for spillover epidemics. Further, these changes also create opportunities for predator superinfection and emergence of novel pathogen strains.

## Conservation impact of prey-transmitted infections

Management of infectious disease in wildlife is a paramount modern conservation challenge. Although spillover infections pose a risk to all taxa, carnivores are especially susceptible to long term, population level impacts of epizootics due to their life history characteristics and long generation times [33, 34]. Thus, infectious diseases present an additional risk to both apex predators and mesocarnivores already threatened by habitat loss and fragmentation, persecution, and prey depletion [33, 35-38]. The irrefutable value of carnivores to ecosystem health is evidenced by their role as both keystone and indicator species [39, 40]. Yet the risk of predation-driven spillover in sensitive carnivore species has not received merited comprehensive interest. This is despite multiple examples of catastrophic population declines following introduction of a new pathogen originating from prey (**Figure 1B, Table 1**), such as the extirpation and near extinction of the black footed ferret due to mortalities arising from plague and distemper [2, 3]. Well-documented examples such as this have to date not led to inclusive studies that investigate *risk factors* for disease in predators, or research focused on determining *how* predators are often able to subvert clinical disease, despite common exposure to a wide array of prey-based parasites. Such questions are highly deserving of further study to assist species conservation plans.

Our studies on feline lentiviruses have documented both silent and fatal spillover infections in puma originating from close contact with, and putative predation on sympatric bobcats (*Lynx rufus*) and domestic cats (*Felis catus*) (**Figure 2**). We have documented frequent cross-species transmission of: (1) feline immunodeficiency virus (FIV) from bobcat to puma [17, 22], (2) *Mycoplasma haemominutum* from domestic cats to bobcats and puma, and (3) from bobcat to puma [24], and (4) feline foamy virus (FFV) from domestic cat to puma [16], with no

detectable clinical outcomes. Interestingly, FIV infection is highly restricted following host switching in geographically limited sites [9], while FFV infection has rapidly disseminated to very high prevalence in free-ranging puma populations [16]. While of no apparent impact to the host, such asymptomatic prey-transmitted infections result in population level changes in microflora, which may have consequences for host immunity or susceptibility to other pathogens.

In contrast to these asymptomatic infections, spillover of feline leukemia virus (FeLV) from domestic cat to puma resulted in fatal outbreaks of disease in the endangered Florida panther (*Puma concolor coryi*) that significantly impacted the population and hindered the species recovery program [23, 41]. Recent evidence suggests FeLV may be able to replicate more competently in puma than the native domestic cat host [42] due to differences in host genomic content. FeLV originating in domestic cats has also been recorded to cause high mortality in Iberian lynx (*Lynx pardinus*) [43]. As additional examples of conservation impacts of prey-associated spillover in wild felids, reintroduction of the Canada lynx (*Lynx canadensis*) to native habitat has been impacted by mortalities caused by plague following exposure to infected prey [44], and Serengeti lions (*Panthera leo*) suffered an outbreak of distemper following putative spillover from domestic dogs (*Canis familiaris*) [45] (**Figure 1B**). These examples and others in **Table 1** highlight the conservation implications of this topic, and underscore a need for further assessment of the ecological and biological relationships between predators and prey-harbored pathogens.

#### **Mechanistic modeling of spillover risk to predators**



Disease modeling is one possible avenue to evaluate and explore the risk of spillover from prey to predators. However, mechanistic models of infectious diseases focusing on pathogen spillover and emergence are still uncommon [7, 46]. The majority of existing theory for predator-prey systems stems from community ecology, and explores the potential of predation to regulate disease in the prey population or disease as a mediator of competition. Models seeking to determine impact of predation on disease transmission have nearly uniformly considered how predation of prey results in changes in disease transmission in the *prey species*, either by removal of diseased animals or changes in prey population structure. For example, depending on the implicit modeling assumptions, various modeling studies have suggested that releasing prey from predation pressures can increase or decrease the risk of pathogen outbreaks in prey [47], aid in spillover to non-primary hosts [48], or in the case of selective predation on sick hosts, potentially increase the likelihood of parasite extinction [49]. These theoretical underpinnings are reflected in management philosophies where predation serves as a control of infectious disease in the host species, and therefore a potential ecosystem service [50-52].

While studies have certainly explored the interspecific dynamics of spillover in multi-host systems [53, 54], models have largely neglected to include predator behavior and prey consumption as important variables and possible feedbacks for pathogen spillover in the *predator*, especially in the context of human driven changes to the environment. This is a significant oversight given that urbanization and habitat fragmentation can drive significant changes in predators' prey composition and preferences [31]. For example, a recent model exploring pathogen spillover between core and matrix habitat during land conversion found key links between intermediate habitat lost and the risk of spillover in a multi-host system [55]. Another recent model suggests that generalist predators are better able to evade consequences

of infectious disease as compared to specialist predators, and that prey species may effectively use pathogens to deter susceptible predators [56]. Future extensions of these types of frameworks could explore feedbacks of predation-driven mortality and transmission, as well as the degree of specialization of the predator, as illustrated in **Figure 3**.

#### **Mechanisms underlying predator resistance**

There are few studies that provide evidence of mechanisms that have evolved to limit predator susceptibility to the large load of microparasites that are ingested every time a meal is taken. As in the case of modeling prey to predator spillover, there is more scholarly activity invested in examining how predation impacts the immune system, stress, and disease susceptibility in the *prey species*. Such studies have specifically focused on evaluation of likelihood of consumption of prey with active infection compared to consumption of uninfected prey, and subsequent outcomes to disease carriage within the prey community [57-59].

Feeding behavior, prey selection, host immunity, and host physiology are potential mechanisms underlying the ability of predators to withstand consequences of consumption of prey that carry commensal bacteria, viruses, and parasites, and potentially pathogenic disease agents. A wide range of morphologic, genetic, and functional variation underlies digestive physiologic adaptation suited for specific diet types [60]. Gastrointestinal physiology of predators has very likely adapted mechanistically to avoid infection following ingestion of prey harboring potentially infectious microparasites. For example, vultures resist disease despite consuming carcasses infected with anthrax, tuberculosis or brucellosis. It is thought this phenomenon is dependent upon very low gastric pH as well as a complex microbial community that constitutes the gut microbiome [61, 62].

Predators may avoid eating species to which they are closely related, thereby avoiding exposure to pathogens that might be more easily transmitted from a phylogenetically and biologically similar species [63, 64] as described above. Additionally, diet selection might bestow specific antiparasitic compounds, or could alter internal physiology to enhance disease resistance [64]. Caching behavior, widespread in mammals and birds, results in degradation of food quality over time [65]. While the effect of caching on potential pathogen content of prey has not been well-studied, it is possible that such food hoarding behavior may result in degradation of potential pathogens prior to ingestion. There is substantial evidence that immune or resistance genes evolve to combat specific pathogens; coincidentally, pathogens are also under strong evolutionary pressure to adapt to hostile host environments—a circumstance referred to as the ‘red queen hypothesis’ [66]. A comprehensive analysis of predator genes under selection relative to related omnivore or herbivore species may reveal classes of gene families that maintain health in predator species. Additional studies to inform mechanisms of disease resistance would reveal important tenets of carnivore evolution, and could have practical value by revealing new host immunological or biological resistance factors against pathogen invasion.

### Concluding Remarks

Spillover from prey to predator has significantly impacted conservation efforts and may exacerbate loss of biodiversity stemming from anthropogenic change. While the frequency of predator exposure to pathogens of prey is high, only a subset of exposures result in clinically significant infection, and many asymptomatic infections go undetected. The mechanisms that determine clinical outcome in exposed predators are poorly understood, yet critically important considerations for conservation and management of vulnerable species. Additionally, elucidation

235 of underlying susceptibility and resistance factors that explain prey to predator disease  
236 transmission would assist in predictive models of spillover more generally. Disease models have  
237 largely ignored transmissions from prey to predators that may have substantial impacts on fragile  
238 populations; predation should thus be incorporated into disease modeling approaches when  
239 appropriate to the system under study. Focused studies on the interesting dynamic of passage of  
240 parasites between prey and predator are recommended to better understand risk of pathogen  
241 evolution and disease emergence as an outcome of naturally occurring predation in unstable or  
242 sensitive ecosystems (see Outstanding Questions).

243 Tables

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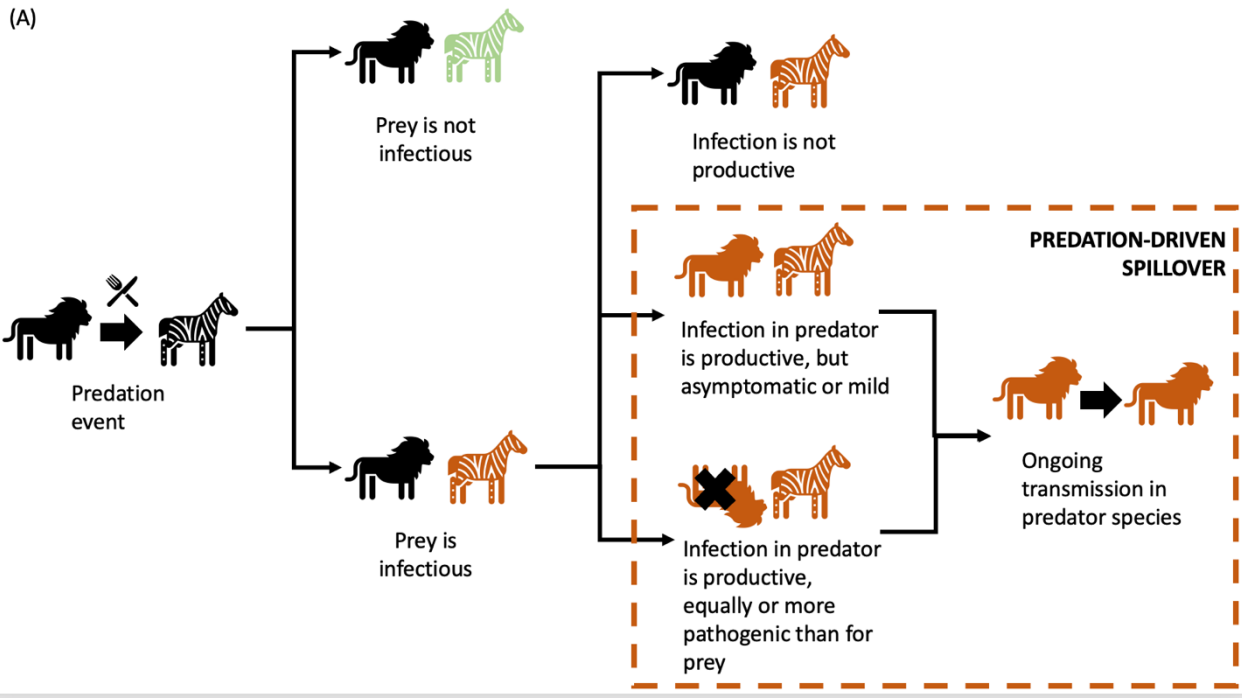
Agent	Predator	Prey	Outcome of spillover in the predator	Evidence for transmission route
Prion (PrP <sup>CWD</sup> /chronic wasting disease)	Coyote ( <i>Canis latrans</i> )	Deer ( <i>Odocoileus virginianus</i> and <i>O. hemionus</i> ), elk ( <i>cervus elaphus</i> ), and moose ( <i>Alces alces</i> )	Nonproductive infection, but with fecal shedding of infectious prions	Experimental [67]
Feline immunodeficiency virus (FIV)	Puma ( <i>Puma concolor</i> )	Bobcat ( <i>Lynx rufus</i> )	Productive subclinical infection	Phylogenetic [17, 22]
Feline foamy virus	Puma ( <i>Puma concolor</i> )	Domestic cat ( <i>Felis catus</i> )	Productive subclinical infection	Phylogenetic [16]
Salmonellosis	Red fox ( <i>Vulpes vulpes</i> ), other carnivores	Numerous mammalian prey species	Productive subclinical infection	Natural and experimental [68, 69]
Parvoviruses	Numerous wild carnivores	Numerous mammalian prey species	Productive infection, variable clinical outcome	Phylogenetic [70, 71]
Anthrax ( <i>Bacillus anthracis</i> )	Wolf ( <i>Canis lupus</i> ), African wildlife	Plains bison ( <i>Bison bison</i> ), various ruminants	Productive infection, variable clinical outcome	Natural observation [72]

Haemoplasmosis ( <i>Candidatus Mycoplasma</i> spp.)	Bobcat ( <i>Lynx rufus</i> ) and puma ( <i>Puma concolor</i> )	Domestic cat ( <i>Felis catus</i> )	Productive infection, variable clinical outcome	Phylogenetic [24]
Feline leukemia virus	Puma ( <i>Puma concolor</i> )	Domestic cat ( <i>Felis catus</i> )	Productive virulent infection	Phylogenetic [23, 41]
<i>Toxoplasma gondii</i>	Southern sea otter ( <i>Enhydra lutris nereis</i> )	Snails, filter-feeding marine bivalves	Productive virulent infection	Natural and experimental [73, 74]
Rabies virus	African lion ( <i>Panthera leo</i> ), leopard ( <i>P. pardus</i> ), spotted hyena ( <i>Crocuta crocuta</i> )	Domestic dog ( <i>Canis familiaris</i> )	Productive virulent infection	Phylogenetic, natural observation [38]
Canine distemper virus	African lion ( <i>Panthera leo</i> ), Black-footed ferret ( <i>Mustela nigripes</i> ), other wild carnivores	Domestic dog ( <i>Canis familiaris</i> ), Prairie dog ( <i>Cynomys</i> spp.), other rodents	Productive virulent infection	Phylogenetic, natural observation [2, 45]
Plague ( <i>Yersinia pestis</i> )	Black-footed ferret ( <i>Mustela nigripes</i> ), Canadian lynx ( <i>Lynx Canadensis</i> ), puma ( <i>Puma concolor</i> )	Prairie dog ( <i>Cynomys</i> spp.), other rodents	Productive virulent infection	Natural observation [3, 44, 75]

Bovine tuberculosis ( <i>Mycobacterium bovis</i> )	Coyotes ( <i>Canis latrans</i> )	White-tailed deer ( <i>Odocoileus virginianus</i> )	Productive virulent infection	Natural observation [76]
Bluetongue virus	African carnivores	Ruminants, shrews, some rodents	Productive virulent infection	Natural observation [77]
African horse sickness virus	Domestic dog ( <i>Canis familiaris</i> ), African carnivores	Equid species	Productive virulent infection	Natural observation [78]

**Table 1. Spillover of pathogens from prey to predator is a common occurrence with numerous putative examples.** Evidence for predation as the putative route of transmission is often based on natural observation, experimental studies, and/or phylogenetic analysis. Gray shading in the table corresponds to outcome in the predator categorized as one of the following: nonproductive infection (lightest gray), productive subclinical infection, productive infection with variable clinical outcome, or productive virulent infection (darkest gray).

Figures



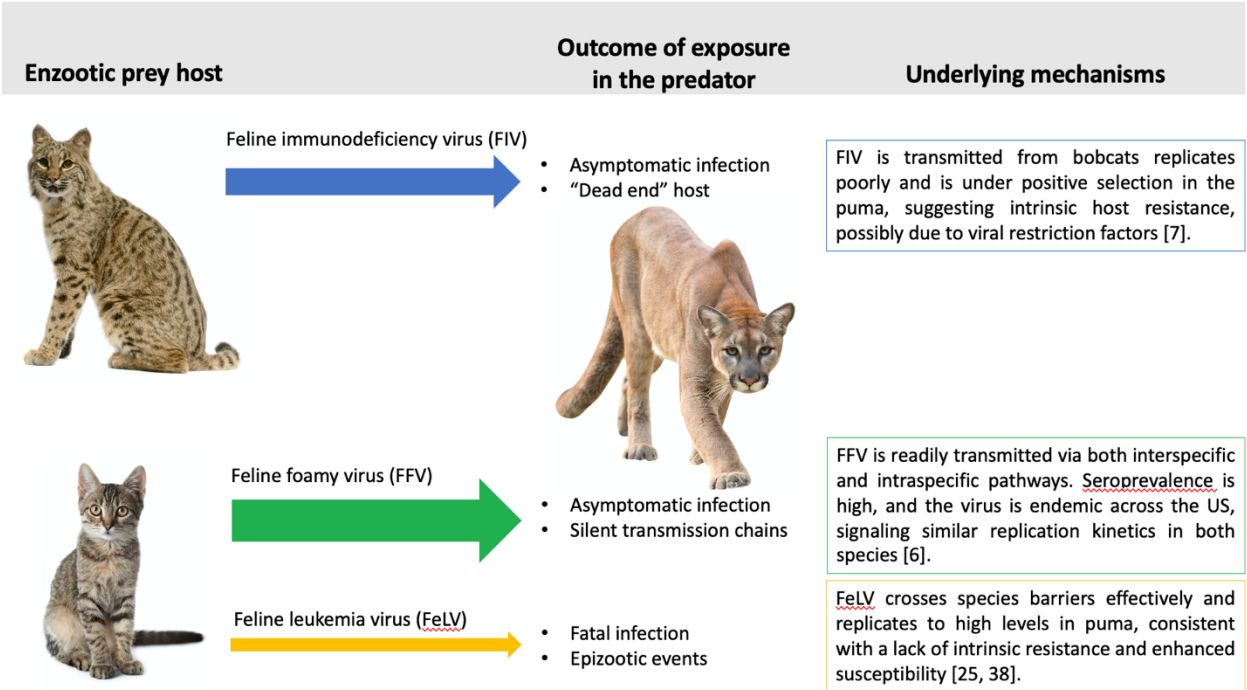
(B) PREDATION DRIVEN SPILLOVER OUTCOMES	 Infection is not productive	 Infection in predator is productive, but asymptomatic or mild	 Infection in predator is productive, equally or more pathogenic than for prey
HOST PATHOGEN SYSTEM EXAMPLES	Deer, elk, and moose are susceptible to chronic wasting disease (CWD)—a transmissible spongiform encephalopathy— that can be transmitted directly from animal to animal or indirectly through the environment. Coyotes consuming CWD-infected tissue do not exhibit viable infections, but do pass infectious CWD prions [62].  Deer with visible signs of CWD.	Salmonellosis is a zoonotic disease of concern worldwide. Beyond some endemic wildlife reservoirs, there is evidence that small, opportunistic predators like badgers and red foxes can become subclinically infected, while still actively shed <i>Salmonella</i> spp. in their feces [63, 64].  <i>Salmonella</i> Typhimurium (red) invading cultured human cells.	Canine distemper virus (CDV) remains a major disease of dogs, despite extensive vaccination. In 1994, an epidemic with significant mortality occurred in the lion population Serengeti-Mara ecosystem of East Africa. Isolates from infected lions were phylogenetically similar to isolates from infected domestic dogs [3].  Predators like lions, hyenas, bat-eared foxes, and leopards can be infected with CDV from domestic dogs.

Figure 1. Predation may result in pathogen spillover from prey to predator species.

However, clinical disease and follow on transmission in the predator is only one possible

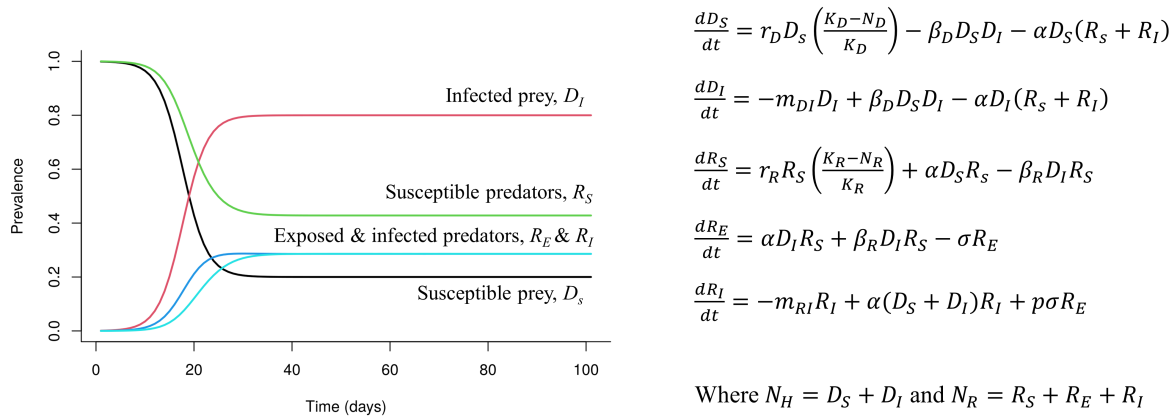


outcome. (A) When a predator consumes prey, the first determinant of spillover is whether or not the prey animal is infectious. Even if consuming an infectious prey animal, the infection may not be productive because of biological or physical defense mechanisms of the predator. If the infection is productive, the resulting infection may result in asymptomatic disease, or alternatively, the agent may be equally or more pathogenic than in the prey species. In some cases, ongoing transmission may occur in the predator species. (B) Three case studies of non-productive, asymptomatic or subclinical, and pathogenic infections in predators: chronic wasting disease in canids, salmonellosis in opportunistic carnivores, and canine distemper virus in African carnivores.



**Figure 2. Puma are susceptible to least three viruses for which bobcats and domestic cats serve as reservoir hosts.** Transmission of FIV, FFV, or FeLV requires close contact that would typically result in puma predation of subordinate feline species. Clinical outcomes in puma vary from asymptomatic to fatal. Further, intraspecific transmission in the puma following initial

spillover varies from absent (or below level of detection) to epizootic, and infection incidence in puma varies significantly. Both ecological and within host mechanisms have been identified relating to these variations. Arrow thickness is proportionate to frequency of spillover infection.



**Figure 3. Time course of prevalence for predator-prey system where transmission is driven by both predation and direct contact.** In this simple, modified Lotka-Volterra model, both the donor species (prey,  $D$ ) and recipient species (specialist predator,  $R$ ) are both susceptible to the same pathogen. The five sets of equations account for the respective susceptible ( $D_S$  and  $R_S$ ), exposed ( $R_E$ ) and infectious ( $D_I$  and  $R_I$ ) subclasses. Direct transmission occurs between susceptible and infectious donors at rate,  $\beta_D$ . Susceptible recipients are come into contact with infectious donors at rate,  $\beta_R$ . Recipients consume donors at a rate,  $\alpha$ , but if a susceptible recipient consumes an infectious donor, they also move into an exposed class ( $R_E$ ). Based on within-host processes, exposed recipients have a probability,  $p$ , of becoming infectious from direct contact or predation of the donor host. We assume that the latent period is comparatively short, such that susceptible and infectious recipients drive predation events. We further assume that the donors are not the only prey species for the recipient, and so we loosen the direct dependence of the recipient population on donor density by assuming logistic growth for donor and recipient

populations. This logistic growth is governed by species-specific growth rates (e.g.,  $r_D$  and  $r_R$ ) and carrying capacities (e.g.,  $K_D$  and  $K_R$ ). Infectious subclasses also have constant mortality rates that are species-specific (e.g.,  $m_{DI}$  vs.  $m_{RI}$ ). Initial conditions:  $D_S=999$ ,  $D_I=1$ ,  $R_S=10$ ,  $R_I=0$ . Parameters:  $r_D=1000$ ,  $K_D=1000$ ,  $m_{DI}=0.1$ ,  $\beta_D=0.0005$ ,  $r_R=0.0000001$ ,  $K_R=10$ ,  $m_{RI}=0.01$ ,  $\alpha=0.00001$ ,  $\beta_R=0.0005$ ,  $p=0.001$ .

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