DISEASE ECOLOGY OF FREE-RANGING DOGS IN CENTRAL INDIA: IMPLICATIONS FOR WILDLIFE CONSERVATION

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

DISEASE ECOLOGY OF FREE-RANGING DOGS IN CENTRAL INDIA: IMPLICATIONS FOR WILDLIFE CONSERVATION

presented by Aniruddha V. Belsare,

a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

I would like to dedicate this Doctoral Dissertation to Rupali. As cliché as it may sound, without her unequivocal support and encouragement I would not have had the courage to embark on this journey in the first place. Neither could I have completed this journey without Rupali by my side. I deeply appreciate her for tolerating my idiosyncrasies, my quirks and my migraine attacks. Rupali has shouldered far more than her fair share of family responsibilities while I pursued my PhD, and I promise to do the same for her in the coming years.

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DISSERTATION ABSTRACT

I planned my study around mass dog vaccination campaigns organized in six villages bordering the Great Indian Bustard Wildlife Sanctuary (GIB WLS) in central India. My first objective was to obtain baseline demographic data for free-ranging rural dog populations. Photographic surveys were used to create encounter histories, which were analyzed to obtain estimates of dog abundance in the six villages. I used two methods to analyze the encounter histories: Beck's method and Program CAPTURE. In the context of free-ranging dogs, Beck's method violates the assumption of equal detectability. Therefore the estimates of dog abundance obtained by the Beck's method were consistently lower than the estimates obtained using program CAPTURE, and even lower than the minimum number of dogs known to be present at each study site. The village dog populations in the study area consist of owned, quasi-owned, as well as ownerless dogs. The study villages had high dog densities (median density 719 dogs per km^2), the dog populations were male-biased (1.55 males per female) and comprised mostly of adult dogs (67-86%). Owned or ownerless, virtually all the dogs in such settings are free-ranging, and are not habituated to restraint of any sort (even by the putative owners). Capture, handling and restraint of such dogs pose a major logistical challenge. The study highlights the applicability of a relatively simple photographic mark-recapture method that does not require handling of dogs, to obtain vital demographic data for free-ranging dog populations.

My second objective was to document the baseline seroprevalence of viral pathogens in these dog populations. I collected blood samples during the mass vaccination campaigns, and used commercial enzyme-linked immunosorbent assay (ELISAs) kits to detect serologic evidence of exposure to three viral pathogens of dog: canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV). A high prevalence of exposure to these pathogens was documented (88% for CPV, 73% for CDV and 68% for CAV). As none of the sampled dogs had a history of prior vaccination, the high seroprevalence implies these pathogens are enzootic in the region. To further validate this finding, I collected longitudinal serologic data from the study area, and analyzed the seroprevalence data for patterns due to age-class and sex. Overall, 93% percent dogs were exposed to one or more of the three pathogens. Exposure rates in dogs were consistently high: >88% for CPV, >72% for CDV and 71% for CAV. A large proportion of unvaccinated adult dogs had antibodies against these pathogens indicating seroconversion following early natural infection. These results further support the finding that the pathogens are enzootic, and actively circulating in the dog populations. Serologic data for Indian foxes from the study area indicated susceptibility and exposure to the pathogens. The findings suggest that dogs could be playing a role in the maintenance and transmission of these pathogens in the fox population, and likely in other sympatric carnivore species as well.

Mass vaccination of dogs has been suggested as a tool for mitigating the disease threat to sympatric carnivores. Vaccination provides an antibody-

mediated mechanism against pathogens, thereby protecting individuals against infectious diseases and also contributing to herd immunity. But this study shows that most dogs in the population are immune to these pathogens by virtue of early natural infection, and therefore these individuals make little current or future contribution to viral maintenance. My third objective was to more directly assess this issue and determine the extent to which such mass vaccination programs are practicable and appropriate for large, free-ranging dog populations. A village-level vaccination experiment was undertaken to determine the efficacy and applicability of mass vaccination of dogs against CAV, CPV and CDV as a disease mitigation intervention strategy around GIB WLS. Vaccination failed to increase the proportion of adult dogs with IgG antibodies against CAV, CPV or CDV in the treatment group compared to the control group, because much of the effort was put into vaccinating dogs that were already antibody positive. In such situations, vaccination of adult dogs against these enzootic viral pathogens seems unnecessary, and would escalate the cost-benefit ratio of dog disease control programs.

I have modeled the interactions of dogs and foxes, and the resultant CDV transmission using agent-based stochastic simulations in NetLogo. My objective was to explore and contrast potential disease control strategies by comparing various scenarios under different assumptions. From the model output I generated the following predictions: (1) local dog vaccination programs will not reduce the number of CDV spillover events, even in scenarios where access to all dogs is attained; (2) reducing dog density or dog roaming can reduce the

number of spillover events, but will not fully eliminate such occurrences at the levels used in the models; (3) vaccination of foxes can eliminate CDV spillover events independent of CDV dynamics in dogs.

PREFACE

This research was conducted from February 2011 to July 2012. Serologic data from samples collected during the mass dog vaccination campaigns (February 2011 - July 2011) is reported in Chapter 1. Vaccinated and unvaccinated dogs in the study villages were subsequently sampled on several occasions between September 2011 and July 2012. Dog blood samples were also obtained from these villages during a pilot study undertaken in 2005-2007. Seroprevalence data reported in Chapter 2 is from unvaccinated dogs sampled during Session 1 (October 2005-February 2006), Session 2 (December 2006-March 2007), Session 3 (February 2011-July 2011) and Session 4 (September 2011-April 2012). Foxes were sampled during 2005-2007, and the seroprevalence data for fox samples is reported in Chapter 2. Chapter 3 reports the outcome of the vaccination experiment. Results are based on analysis of blood samples collected from treatment and control groups prior to the vaccination, and 6, 9 and 12 months post-vaccination.

Chapter 1 has been published, and other chapters of this dissertation are either in review or in preparation for submission to peer-reviewed journals. Below I detail the citation of Chapter 1:

Belsare, A.V., Gompper, M.E., 2013. Assessing demographic and epidemiologic parameters of rural dog populations in India during mass vaccination campaigns. Preventive Veterinary Medicine 111, 139-146.

Chapter 1: ASSESSING DEMOGRAPHIC AND EPIDEMIOLOGIC PARAMETERS OF RURAL DOG POPULATIONS IN INDIA DURING MASS VACCINATION CAMPAIGNS

ABSTRACT

Mass vaccination of dogs is a mainstay for efforts to control rabies and other viral pathogens. The success of such programs is a function of the ability to vaccinate sufficient proportions of animals to develop herd immunity. However, fully assessing success in reaching target vaccination-levels and in understanding the outcome of mass vaccination efforts is hindered if insufficient information is available on the demographics of dog populations and the prevalence of the targeted pathogens. While such information can sometimes be gained from questionnaire surveys, greater precision requires direct assessment of the dog populations. Here we show how such information can be gained from surveys of dogs conducted in association with mass-vaccination programs. We conducted surveys of dogs in six villages in rural Maharashtra, India, between February – July 2011 as part of an effort to reduce the risk of human rabies and virus transmission from dogs to wildlife. Mass vaccination efforts were conducted in each village, and paired with blood sample collection and photographic mark-recapture approaches to gain epidemiologic and demographic data. This data in turn facilitated estimates of dog abundance, population density and structure, vaccination coverage, and seroprevalence of antibodies against canine adenovirus (CAV), canine parvovirus (CPV), and canine distemper virus (CDV).

The median dog population size for the six villages was 134 (range 90-188), the median dog population density was 719 dogs per km^2 (range 526-969), and the median human:dog ratio for these six villages was 34 (range 30-47). The median household:dog ratio for the six villages was 6 (range 5-8). Following vaccination efforts, the median vaccination coverage achieved was 34% (range 24-42 %). The dog populations consisted mostly of adult dogs (67-86%) and the median sex ratio for the study area was male biased (1.55 males per female; range 0.9 – 2.5). The seroprevalence of antibodies against CAV, CPV and CDV was 68, 88 and 73% respectively. Mass vaccination campaigns provide an opportunity to obtain vital epidemiological and demographic data, and develop a clearer understanding of the threats and impacts of diseases and disease control measures.

INTRODUCTION

Dogs, as reservoirs and vectors of infectious pathogens, pose a serious threat to the health and well-being of humans and livestock (WHO, 1999; Cleaveland et al., 2002; Coleman et al., 2004; Knobel et al., 2005). In India, for instance, canine rabies is estimated to kill approximately 20000 people annually (Sudarshan et al., 2007). Pathogens transmitted from dogs also are a significant threat to the conservation of many wild carnivore species (Funk et al., 2001; Woodroffe et al., 2004). Epizootics putatively caused by viral transmission from free-ranging dogs are hypothesized to have caused several well-documented and precipitous declines in wildlife (Roelke-Parker et al., 1996; Laurenson et al., 1998; Kennedy et al., 2000). Vaccination is a mechanism for reducing the prevalence and incidence of viral loads in dogs and has been applied to African free-ranging dog populations to reduce risks to people and wildlife (Randall et al., 2006; Kaare et al., 2009; Fitzpatrick et al., 2012). Such approaches protect the vaccinated individual against infectious diseases and also contribute to 'herd immunity' within the broader population by reducing the population density of susceptible individuals to a point for which the basic reproduction number (R_o) for a pathogen is <1.

Mass vaccination of dogs has been suggested as the main tool for disease control to prevent disease transmission to humans, livestock and wildlife populations (Dodet and Meslin, 2001; Cleaveland et al., 2006). Epidemiological

models and field experiences from various countries indicate that outbreaks of rabies and canine distemper virus (CDV) can be prevented by mass vaccination (Cleaveland, 1996; Coleman and Dye, 1996). The World Health Organization (WHO, 1987) has proposed a target level of 70% for mass vaccination to eliminate or prevent outbreaks of rabies. Yet identifying the vaccination coverage of a mass vaccination program is dependent on precise assessment of the dog population size or density. Furthermore, addressing the population trajectories of dogs requires detailed information on dog demographics. While some of this information is sometimes gained from surveys of owners independent of vaccination programs (e.g. Acosta-Jamett et al., 2010), or during the mass vaccination program (e.g. Fiorello et al., 2006), such examples are the exception, as many vaccination programs collect limited information on the demographics of the dogs and few studies provide detailed information on the demographics of dog populations, and in particular, rural free-ranging dog populations.

In India, for example, field data on dog demographics and measures of the prevalence of important pathogens are virtually lacking. The actual incidence of rabies in dog populations is not known, and data are also lacking for the prevalence of other canine pathogens, such as CDV, canine parvovirus (CPV), and canine adenovirus virus (CAV; the causative agent of canine hepatitis), each of which may play a role in limiting dog populations in the region and may represent risks to wild canids. This lack of epidemiological data as well as demographic data on the free-ranging dog populations in India and elsewhere is

a major impediment in understanding the real threat of such pathogens, in achieving effective disease control, and in managing dog population growth.

Mass vaccination campaigns, if paired with surveys and sampling sessions, provide an opportunity to obtain high quality epidemiologic and demographic data for dog populations. Here we provide epidemiologic and demographic data collected from dog populations surveyed in six villages in rural Maharashtra, India. This work was spurred in part by an effort to reduce the risk of pathogen transmission from dogs to wild canids (Chapter 2) around the Great Indian Bustard Wildlife Sanctuary (GIB WLS), Nannaj, Maharashtra as well as to reduce the risk to humans in the focal villages where cases of human rabies occasionally occur. This region is of interest because CDV-related mortalities were documented in Indian foxes (*Vulpes bengalensis*) in 2006, and this mortality was putatively linked to spill-over of CDV from dogs (Vanak et al. 2007). Subsequently, mass dog vaccination campaigns were initiated by the Maharashtra Forest Department in villages around the GIB WLS, providing an opportunity to collect detailed information about dog pathogen exposure and demographics. Thus, this study was planned around the mass vaccination campaigns, using the opportunity to: 1) estimate dog abundance and density at each site, and then to estimate the vaccination coverage achieved during the mass vaccination campaigns; 2) document the dog population demographic characteristics at each site; 3) investigate the baseline seroprevalence of three viruses (CDV, CPV, and CAV). While our findings are specific to one region of

India, we believe they also provide insights for those attempting to formulate disease control strategies in rural regions elsewhere across the globe.

MATERIALS AND METHODS

Study location

The study was conducted in six villages (Wadala, Mardi, Nannaj, Akolekati, Karamba and Gawdi Darfal) bordering the GIB WLS at Nannaj, Maharashtra in central India between February and July 2011. The GIB WLS is a series of six protected grassland patches (6 km^2), which are remnants of the grassland ecosystem in a human-dominated landscape. The population finder feature on the Census of India website was used to obtain the human population of the study villages in 2001 (http://www.censusindia.gov.in/PopulationFinder/Population_Finder.aspx), and the decadal growth rate for rural Solapur district (2001-2011) (http://www.censusindia.gov.in/2011-prov-results/paper2/data_files/mah/8-POP-11-26.pdf). The later was used to calculate the projected population of the study villages in 2011. The projected human population sizes of these villages in 2011 ranged between 2973 and 7448, and the number of households ranged between 490 and 1300.

Dogs are ubiquitous in this region and have the phenotype typical of the village dogs of India. The local economy is based on agro-pastoralism, and thus the landscapes used by dogs consist of a matrix of sugarcane fields, vineyards, seasonal crops, communal grazing lands, protected grasslands and forestry plantations. Other carnivores that are found in the study area include Indian fox, gray wolf (*Canis lupus*), golden jackal (*C. aureus*), jungle cat (*Felis chaus*) and gray mongoose (*Herpestes edwardsi*). The movements and interactions of dogs and wildlife in this region have been the subject of detailed study (Vanak et al., 2007; Vanak and Gompper, 2009; Vanak et al., 2009; Vanak and Gompper, 2010).

Mass vaccination campaigns

Mass vaccination campaigns were organized in collaboration with the Maharashtra Forest Department in Wadala, Mardi, Nannaj, Akolekati and Karamba (February and March 2011), and in Gawdi Darfal (July 2011). Prior to every campaign, a meeting was convened in every village and the purpose of the campaign explained. The vaccination campaigns were scheduled on dates agreed during the meetings. Posters were put up to publicize the date and venue of the vaccination campaign. Dogs were vaccinated free of cost at a vaccination station (central point vaccination). We also did house-to-house vaccination for dogs that could not be brought to the vaccination station. Dogs were restrained by the putative owner or reference person; we provided leash and muzzle

whenever necessary. Free-roaming dogs that were either unowned or were difficult for the reference person to restrain were captured using box traps, vaccinated and released immediately.

All dogs were vaccinated with Rabigen mono (Virbac Animal Health) rabies vaccine, and dogs in three villages (Akolekati, Mardi and Wadala) were also vaccinated with Canigen DHPPi/L (Virbac Animal Health). Canigen DHPPi/L is a combination vaccine containing live CDV, CAV type 2, CPV and canine parainfluenza virus, along with inactivated whole organisms of *Leptospira canicola* and *L. icterohaemorrhagiae*. Every vaccinated dog was photographed, and by general visual examination the age class and sex of the dog was recorded. Dogs were classified as pups (0-4 mo), juveniles (5-12 mo) and adults (> 12 mo) on the basis of body size, allometry (visual estimate of head size and leg length compared to the body size), and behaviour (Daniels, 1983). Distinction between pups and juveniles was made on the basis of eruption pattern of their dentition (Kirk, 1977). Distinction between adults and juveniles was made on the basis of developed teats (adult females) or descended testes (adult males). For females, current reproductive state was recorded. The dog owner's name was recorded and coloured plastic cable ties were used as collars to temporarily mark vaccinated dogs. These cables could be easily removed by people, but unlike dog collars, the cable ties have to be cut and therefore have no value after removal. The cable ties were generally left on the dog, and therefore acted as supplemental identifying features when examining photographs of dogs during follow-up population surveys.

Estimation of dog abundance, dog population density and population characteristics

Pilot surveys undertaken in the study region, as well as in other regions, revealed that the highest number of dogs was encountered during early morning and early evening surveys. Therefore all surveys were undertaken between 7-9 am and 5-7 pm; this time period also ensured sufficient light for photography. Encounter histories were created from the pilot survey data and program CAPTURE was used to estimate detectability (encounter probability). Detectability is the same as capture probability and recapture probability in our study, as dogs were photographed from a distance ("captured" or "recaptured") without physically trapping them. The pilot survey data yielded a detectability of 0.41 ± 0.04 for dogs in the study area, and program CAPTURE indicated model M_h and the jackknife estimator as the most appropriate approach for dog abundance estimation (see below for further discussion of program CAPTURE and associated models). A minimum of 5 sampling sessions were recommended by Otis et al (1978) for average capture probabilities of at least 0.1 per session. For a detectability value of 0.4, five sampling sessions per village were deemed sufficient as per the sample size charts for mark-recapture experiments (Lee, 1972). Surveys were undertaken on motorcycle using pre-determined routes. Each route consisted of main and by-roads, ensuring that no part of the village was left out. Every dog encountered during the survey was photographed using a digital camera, and details (sex, age, color, markings) were noted, thus creating 'encounter histories' for each dog.

The survey data were used to estimate dog abundance at each study site using three approaches. First, the minimum number of dogs present at each site was calculated by adding the total number of individually recognizable dogs encountered during the 5 surveys/village plus the number of dogs from each village that were vaccinated but not encountered during any of the 5 surveys. Thus this represents the number of known individuals observed during the study. Second, we used Beck's method (Beck, 1973; WHO/WSPA, 1990), a simple capture-recapture approach commonly used for dog population size estimation (Fei et al., 2012), to extrapolate an estimate of dog population size. Beck's estimator is:

$$\check{N}_B = \Sigma^{t}_{j=1} (M_j x_j) / \Sigma^{t}_{j=1} m_j$$

where $\check{N}_B$ is the dog population estimator, t = the total number of photographic capture surveys, M_j is the number of dogs 'captured' in the j^{th} survey, m_j is the number of dogs 'recaptured' in the j^{th} survey, and x_j is the total number of distinct dogs captured in the j -1 surveys.

Third, the encounter histories were also analyzed using program CAPTURE, a feature in Program MARK (White and Burnham, 1999; White, 2008). Program CAPTURE has a long history of use in studies of wildlife demography, and it tests for variation in detectability, assuming that detectability may 1) vary with time (Model M_t); 2) vary with behavior response (Model M_b); 3) vary with individual animal (Model M_h). Program CAPTURE also considers all

possible combinations of these three variations, i.e., Models M_{tb}, M_{th}, M_{bh}, M_{tbh} as well as Model M_o, the null model with constant detectability. Program CAPTURE has a model selection algorithm to test 7 models that differ in their assumed sources of variation in detectability. By selecting the 'Appropriate' check box in the program CAPTURE models window in MARK, the model which best fits the capture history data is identified by the program. Specifically, chi-square tests are used to test for heterogeneity of detectability in the population, test for behavioral response after initial capture, test for time-specific variation in detectability, goodness of fit tests of model M_h, M_b and M_t, and test for behavioral response in presence of heterogeneity. Potential models are scored between 0.0 – 1.0, higher score indicates a better relative fit of the model to the capture history data. Population abundance is estimated using the model type assigned the highest rank by the program algorithm. The software manual available on MARK website (http://www.phidot.org/software/mark/docs/book/) provides detailed information about using the program.

Data recorded on the sex and age of dogs vaccinated during the campaign, as well as for those encountered during the surveys, was combined to obtain the sex ratio and the age structure of the dog populations. A two-tailed binomial test was used to test if the male:female sex ratio differed significantly from 1:1. Vaccination coverage was calculated based on the estimate of dog abundance and the number of dogs vaccinated at each site. Dog population density at each site was calculated by dividing the estimated dog population by the area of the study site. The latter was calculated in Google Earth Pro by

identifying the most external households of each village and connecting them to create a polygon.

Estimation of antibody seroprevalence

Blood was collected during the campaign if the reference person consented, and the dog tolerated handling with minimal restraint. Vaccuette 4 ml serum tubes with clot activating factor (Greiner Labortechnic, Germany) were used to collect blood samples by venipuncture of the cephalic or saphenous vein. Blood in the serum separator tubes was allowed to clot at ambient temperature, and the serum was then decanted and kept 48 hr before transporting to a -20º C freezer at the Serum Institute of India, Pune for storage. Strict Indian export laws combined with a lack of facilities with the capacity to conduct canine serological assessments via traditional methodologies (i.e. serum neutralization or haemagglutination-inhibition) necessitated the use commercial enzyme-linked immunosorbent assays (ELISAs) kits. The Immunocomb Canine VacciCheck (Biogal Galed Laboratories, Kibbutz Galed, Israel) test kit is based on solid phase immunoassay technology. Each kit consists of a comb shaped plastic card and a multi compartment developing plate. This kit utilizes a semi quantitative procedure based on color comparison between a standard and a test sample measured by using the color-coded scale (“CombScale”) provided in the kit. The results are documented in “S” units on a scale of 0 to 6, where S3 corresponded to a 1:16 titer by virus neutralization test (VN) for CAV, 1:80 titer for CPV by the

haemagglutination inhibition test (HI) for CPV, and 1:32 VN for CDV, and titers ("S = 1" or "S = 2") indicate that these dogs possess antibodies to the infectious agents. The presence of antibodies regardless of the titers demonstrates an active immune response due to past infection or vaccination (Schultz et al., 2010). The objective of our study was to survey dogs for serologic evidence of exposure to CAV, CPV & CDV. We therefore include dogs with "S" values ≥ 1 as positives in the prevalence calculations. The observed prevalence of exposure was calculated as percentage of sampled dogs diagnosed as seropositive. We calculated 95 % confidence limits for prevalence (Stern's exact method) using the software 'Quantitative Parasitology 3.0' (Reiczigel and Rózsa, 2011). The prevalence values are subdivided by sex and age-class.

RESULTS

Dog abundance and vaccination coverage

Program CAPTURE indicated model M_h as the most appropriate model and jackknife estimator was the suggested estimator for abundance estimation for all study sites. Estimates obtained by the Beck's method were consistently lower than the estimates obtained using CAPTURE, and lower than the minimum number of dogs known to be present at each study site (Table 1). Beck's population estimates were 64-94% the known minimum size of the six dog populations, and for two of the six populations the minimum known population

was greater than the upper 95% confidence interval for the Beck's estimator. Because we assumed that the number of observed dogs was an underestimate of the actual number of dogs, the estimates of dog abundance obtained using program CAPTURE were used for all subsequent calculations related to size, density and vaccination coverage.

The median dog population size for the six villages was 134 (range 90-188). For the six villages, the median dog population density for the six villages was 719 dogs per km^2 (range 526-969), the median human:dog ratio was 34 (range 30-47), and the median vaccination coverage achieved was 34% (range 24-42%) (Table 2). The median household:dog ratio for the six villages was 6 (range 5-8).

Dog demographics

The dog populations were comprised principally of adult animals; adults made up 67.2 – 77.9% of the dog populations in five villages excluding Gawdi Darfal (Table 3). The dog population in Gawdi Darfal was comprised of 86.4% adults. We report the age structure of Gawdi Darfal separately as it was surveyed at a different time of year (July) than the other villages (February-March).

The median male:female sex ratio for the six villages combined was 1.55 (range 0.9 – 2.5) (Table 3). A two-tailed binomial test did not reject the null

hypothesis of 1:1 sex ratio for four villages ($p = 0.198$ for Akolekati; $p = 0.62$ for Gawdi Darfal; $p = 0.76$ for Nannaj; $p = 0.107$ for Wadala), but was rejected for Karamba ($p = 0.01$) and Mardi ($p < 0.01$), and for six villages combined ($p < 0.01$).

Seroprevalence of viral antibodies

Blood samples were collected from n = 77 dogs from the six villages. In this pooled population of dogs, seroprevalence of CAV, CPV, and CDV antibodies was high, indicating that a large proportion of dogs were exposed to these pathogens (Table 4). For all individuals combined, post-hoc analysis revealed no significant difference in seroprevalence of CAV, CPV and CDV antibodies as a function of sex ($p=0.361$ for CAV, $p=0.192$ for CPV, and $p=0.747$ for CDV; Fisher's exact tests). Most adult dogs (n = 65) had been exposed to each of the three pathogens (observed prevalence = 72, 94, and 82% for CAV, CPV, and CDV respectively). The observed prevalence of antibodies in juveniles was relatively low (42% for CAV, 58% for CPV and 25% for CDV), based on a small sample size (n = 12) of juveniles tested for seroexposure. For the three pathogens, there was no significant difference in exposure rates of adult males and adult females ($p=0.316$ for CAV, $p=0.569$ for CPV, and $p=1$ for CDV; Fisher's exact tests).

DISCUSSION

Mass dog vaccination campaigns provide an excellent opportunity, with minimal additional efforts and resources, to assess demographic and epidemiologic parameters of dog populations. While many studies report data pertaining to dog vaccination coverage or dog population ecology, these data are primarily collected utilizing a household cluster survey approach (Davlin and VonVille, 2012). However, human-dog relationships vary dramatically across the globe as well as within locales, and therefore the conclusions derived from such questionnaire surveys may be biased by household response rates, motivations of responding individuals, and by a necessary focus on owned, easily handled, and more restricted dogs over unowned dogs or those dogs that are more difficult to handle or more difficult to locate because of free-ranging tendencies. Furthermore, many studies of dogs emphasize estimation of a human:dog ratio. While such ratios facilitate coarsely understanding the contact rates of humans and dogs, without a reference density for one of the populations they provide relatively little insight into dog demographics. Pairing mass vaccination campaigns with examinations of dog population biology allows a keener understanding of the dynamics of dog populations as well as the dynamics of micro- and macroparasites of dogs.

In addition, when dog population estimation is conducted, the field and analytic approach often fail to make use of advances available in modern animal demographics. For instance, and as was done in this study, a less biased

estimate of population size can be obtained by using relatively simple capture-mark-recapture methods in combination with the more sophisticated population estimation techniques commonly used in wildlife biology. We used two closed population capture-mark-recapture methods in this study. Note that because 'capture' as well as 'recapture' consisted of taking digital photographs of free-roaming dogs encountered during the surveys, physical handling was neither necessary to 'mark' the dogs (although a subset of dogs had the temporary collars placed on animals during vaccination), nor were physical 'recaptures' necessary to subsequently read the marks. Beck's method is commonly used when attempting to estimate dog population size. This method, which is a modification of Schnabel's variant of the Petersen-Lincoln Index using multiple recaptures assumes: 1) the population is closed and no births, deaths, immigration or emigration occurs during the sampling period; 2) all individuals in the population have equal probability of being detected (photographed); and 3) marked individuals do not lose marks between the surveys. The assumption of closure is reasonable as the surveys were carried out in a short span of time (4-6 days). Further, the photographs of 'marked' dogs and the relevant information (gender, age class, reference person) documented for each dog encountered during the surveys minimize the possibility of errors like double counting or failure to identify 'marked' dogs (similar to loss of marks). But factors like age, sex, temperament and inherent differences between individuals influence the activity pattern and thus the observability of dogs. Therefore the assumption of homogeneous detectability for the village dog populations is unrealistic. Beck's

method tends to be biased and to underestimate population size in situations where the fundamental assumption of equal detectability is violated (Fei et al., 2012). Accordingly, the dog population estimates obtained for the study sites using Beck's method were consistently lower than even the minimum number of dogs known to be present at each site. While Beck's method can result in good estimates of true population size under some conditions (Fei et al., 2012), our results suggest that under realistic field conditions this approach may considerably underestimate population size.

A more accurate approach to estimating rural dog population sizes may be to apply the strength of Program Capture, which examines the encounter histories for variation in detectability and tests a set of models that are able to account for the variations in the detectability associated with time, behavior and individual effects. Program Capture was designed for use by practitioners of wildlife biology, for whom biases in the likelihood of capturing or recapturing particular subsets of individuals are the norm, and therefore the need to contrast alternative models that account for capture biases is commonplace. In rural areas where a mix of owned and unowned dogs, as well as wide ranging dogs and dogs that rarely travel far, are likely to be found, addressing capture biases is fundamental to an accurate estimate of population size. For example, in our case, Program CAPTURE indicated model M_h and the jackknife estimator as the most appropriate approach for dog abundance estimation at each study site. Model M_h allows for heterogeneity in capture probabilities due to the inherent differences between individuals. The dog population estimates for each study site

obtained using this approach were higher than the minimum number of dogs known to be present at these sites.

Village dog populations in the study area consist of owned, quasi-owned, as well as ownerless dogs that are unconfined irrespective of the ownership status (A.V.Belsare, unpublished data). Yet most of these animals rarely leave the immediate vicinity of the village (Vanak and Gompper 2010). We therefore focused on the area where human dwellings occur rather than on the surrounding agricultural and protected lands while estimating the density of dogs at the study sites. The agricultural farms surrounding the villages, and the farm dogs, have not been included in this calculation. The estimated human:dog ratio 36.3 ± 6.1 for our study area is consistent with the findings of Sudarshan et al. (2006), and indicate that human:dog ratios in rural India are amongst the highest reported for studies from rural regions across the globe (Gompper, 2013).

Despite considerable effort to maximize vaccination coverage in these villages by focusing on relatively small villages, working with local governing agencies, and following up the central point vaccination with door-to-door vaccinations, the vaccination coverage achieved in our study is considerably lower compared to the proposed target coverage of 70% (WHO, 1987), and the coverage reported from rural areas in other countries: Tanzania 67.8% and 80.3%, Philippines 76% and 73%, Mexico 78.4% and Sri Lanka 66% (Davlin and Vonville 2012). The difference in vaccination coverage could be due to the dog ownership pattern and the attitudes of people regarding dogs in the region, or an

error in dog population estimation. Dogs in our study area can be grouped in three categories, village dogs, herding dogs and farm dogs (Vanak and Gompper 2010). The farm dogs and the herding dogs have owners, who can handle and restrain the dogs for the purpose of administering the vaccine. But a majority of the village dogs are quasi-owned, limiting their accessibility for vaccine administration. Though reference individuals are usually traceable for most village dogs, it does not guarantee accessibility for vaccination. Often the reference individual is unable to handle the dog. Furthermore these putative owners of the dogs often have misconceptions regarding dog vaccination. Many people in the region believe that dogs die or have reduced vigor after vaccination. The inability to handle dogs for vaccination can be dealt with by using trap cages, but the misconceptions regarding vaccination have to be dealt with by undertaking a long-term public education program in the region. Nevertheless, certain issues will always contribute to the difficulties in achieving the recommended 70% vaccination coverage. Even in developed countries, only 30-50% of the pet dog population is vaccinated against infectious diseases as per the estimates of the Vaccination Guidelines Group of the World Small Animal Veterinary Association (Day et al., 2010).

We recorded a high prevalence of antibodies against CAV, CPV and CDV in the blood samples collected during the vaccination campaigns. It should be noted that these estimates of seroprevalence are based on convenience sampling, and the results are not necessarily representative of the entire dog population of the study area. Nonetheless, as none of the sampled dogs had a

history of prior vaccination, the high seroprevalence implies these pathogens are enzootic in the region. Similar patterns have been observed on other continents (e.g. Fiorello et al., 2006; Laurenson et al., 1998). Given that these pathogens are of concern for dog welfare and also represent conservation concerns as they have the potential to cause morbidity and mortality in wild carnivores, especially canids (Barker and Parrish, 2001; Williams, 2001; Woods, 2001), longitudinal studies in combination with dog demographic studies should be undertaken to provide insights about the dynamics and persistence of these pathogens in free-ranging rural dog populations.

CONCLUSION

Mass vaccination campaigns provide an opportunity to obtain epidemiologic and demographic data for dog populations, and in the process develop a clearer understanding of dog population biology, the threats and impacts of the diseases, and the outcome of disease control measures. Such data would enormously aid the planning, implementation, and monitoring of future disease control programs.

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Table 1. Estimates of dog population sizes for six villages in rural Maharashtra, India obtained by three different methods.

Village	Beck's method (95% CI)	Model M_h (95% CI)	Minimum number of known animals from surveys and vaccination campaign
Wadala	99 (78, 120)	142 (127, 168)	108
Mardi	95 (75, 115)	188 (166, 222)	148
Nannaj	98 (78, 119)	157 (141, 194)	132
Akolekati	94 (74, 114)	126 (110, 159)	104
Karamba	70 (52, 88)	98 (83, 127)	78
Gawdi Darfal	65 (47, 82)	90 (75, 120)	69

Table 2. Dog population density, human:dog ratio, number of dogs vaccinated and vaccination coverage (%) for six villages in rural Maharashtra, India.

Village	Dog density (dogs/km^2)	Human:dog ratio	Number of dogs vaccinated	Vaccination coverage (%)
Wadala	526	34	60	42
Mardi	606	31	69	37
Nannaj	785	47	62	40
Akolekati	969	36	34	27
Karamba	653	30	30	31
Gawdi Darfal	818	34	22	24

Table 3. Dog sex ratios (males per female) and age structures (% pup = 0-4 mo; % juvenile = 6-12 mo, % adult = >12 mo) for six villages in rural Maharashtra, India.

Village	Males:females	Pup (%)	Juvenile (%)	Adult (%)
Wadala	1.4:1	10.8	15.1	74.1
Mardi	2.5:1	6.7	26.1	67.2
Nannaj	0.9:1	9.8	12.3	77.9
Akolekati	1.3:1	9.3	17.8	72.9
Karamba	1.9:1	18.2	6.5	75.3
Gawdi Darfal	1.7:1	4.5	9.1	86.4

Table 4. Observed prevalence (OP) and 95% confidence intervals (CI) of exposure to canine adenovirus (CAV), canine parvovirus (CPV), and canine distemper (CDV) in 77 dogs sampled in six villages in rural Maharashtra. Prevalence is subdivided by age (juvenile=4-12 mo; adult = > 12 mo) and sex class.

	n	OP (95%CI)		
		CAV	CPV	CDV
All	77	68 (56-77)	88 (79-94)	73 (62-82)
Adults	65	72 (60-82)	94 (85-98)	82 (70-90)
Juveniles	12	42 (18-71)	58 (29-82)	25 (7-54)
Males	62	64 (52-76)	86 (74-92)	71 (58-81)
Females	15	80 (53-94)	100 (78-100)	80 (53-94)
Adult males	51	69 (55-80)	92 (82-97)	80 (67-90)
Adult females	14	86 (57-97)	100 (76-100)	86 (57-97)
Juvenile males	11	46 (20-74)	54 (26-80)	27 (8-60)
Juvenile females	1	0	100 (50-100)	0

Chapter 2: EPIDEMIOLOGY OF VIRAL PATHOGENS OF FREE-RANGING DOGS AND INDIAN FOXES IN A HUMAN-DOMINATED LANDSCAPE IN CENTRAL INDIA

ABSTRACT

There is an increasing concern that free-ranging domestic dog (*Canis familiaris*) populations may serve as reservoirs of pathogens which may be transmitted to wildlife. We documented the prevalence of antibodies to three viral pathogens, canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV), in free-ranging dog and sympatric Indian fox (*Vulpes bengalensis*) populations in and around the Great Indian Bustard Wildlife Sanctuary, in Maharashtra, central India. A total of 219 dogs and 33 foxes were sampled during the study period. Ninety-three percent dogs and 87% foxes were exposed to one or more of the three pathogens. Exposure rates in dogs were high: >88% for CPV, >72% for CDV and 71% for CAV. A large proportion of adult dogs had antibodies against these pathogens due to seroconversion following early natural infection. The high prevalence of exposure to these pathogens across the sampling sessions, significantly higher exposure rates of adults compared to juveniles, and seroconversion in some unvaccinated dogs documented during the study period suggests that these pathogens are enzootic. The prevalence of exposure to CPV, CDV and CAV in foxes was 48%, 18% and 52%, respectively.

Further, a high rate of mortality was documented in foxes with evidence of ongoing CDV infection. Dogs could be playing a role in the maintenance and transmission of these pathogens in the fox population, but our findings show that most dogs in the population are immune to these pathogens by virtue of early natural infection, and therefore these individuals make little current or future contribution to viral maintenance. Vaccination of this cohort will neither greatly improve their collective immune status nor contribute to herd immunity. Our findings could have important implications for dog disease control programs that propose using canine vaccination as a tool for conservation management of wild carnivore populations.

INTRODUCTION

Three viral pathogens, canine parvovirus (CPV), canine distemper virus (CDV), and canine adenovirus (CAV), have a global distribution and cause severe, life-threatening diseases in dogs (*Canis familiaris*) and wild canid species (Laurenson et al., 1998; Day et al., 2010). Because these multi-host pathogens infect a wide range of mammalian carnivore species, they may constitute an important threat for many populations of conservation concern (Knobel et al., 2013). Most regions of the developing world have large, unvaccinated dog populations that potentially interact with wildlife as predators, prey, competitors, and as reservoirs of pathogens (Butler, 2004; Vanak and Gompper, 2009b; Gompper, 2013). This later factor is an increasing concern (Alexander et al., 1996; Roelke-Parker et al., 1996; Funk et al., 2001; Bronson et al., 2008). Epidemics of CDV in species such as African wild dogs (*Lycaon pictus*), Island foxes (*Urocyon littoralis*), African lions (*Panthera leo*), Caspian seals (*Phoca caspica*) and Lake Baikal seals (*P. sibirca*) have been attributed to transmission from dogs (Cleaveland et al., 2006), and a recent report suggests that unvaccinated dogs could be a source of CDV for Siberian tigers (*Panthera tigris altaica*) (Quigley et al., 2010). While less studied than CDV, dogs have also been implicated as a source of CPV contributing to mortality in the gray wolves (*C. lupus*) (Peterson et al., 1998) and as a source of CAV transmitted to sympatric maned wolves (*Chrysocyon brachyurus*) (Bronson et al., 2008). Effective mitigation of such viral-associated pathogen threats requires unequivocal

identification of reservoir populations, and an understanding of the structure and transmission processes that occur within the reservoir populations (Haydon et al., 2002). While dogs are typically assumed to be the reservoirs of pathogens influencing wildlife, rarely are such assumptions closely examined (Knobel et al., 2013).

There has been a general lack of research on infectious diseases of dogs and wildlife in Asia, and even measures of the prevalence of important pathogens in dog populations are virtually lacking. In India, for instance, free-ranging dogs are ubiquitous, with an estimated population of 59 million (Gompper, 2013). Further, many wild carnivore species are known to persist in the human-dominated landscapes that these dogs inhabit, such as wolves, lions, leopards (*Panthera pardus*), snow leopards (*P. unica*) and hyenas (*Hyaena hyaena*) (Singh et al., 2010; Athreya et al., 2013). These animals often attack dogs. For example, in India dogs are an important component of the diet of leopard (Mukherjee and Sharma, 2001; Edgaonkar and Chellam, 2002; Shah et al., 2009) and are also killed by wolves (Jhala, 1993; Jethva and Jhala, 2004). Dogs are also known to attack wild carnivores, and are an important source of mortality for many species of mesocarnivores like the Indian fox (*Vulpes bengalensis*) (Vanak, 2008; Vanak et al., 2013). It is therefore possible that populations of native carnivores, including species of conservation concern, are regularly exposed through such interactions to pathogens that are maintained in the large dog populations, and that these native species suffer population declines due to pathogen transmission from dogs without the problem being

identified. For example, the Indian fox has been known to undergo large population fluctuations, and although disease has been suspected it has never been properly investigated (Manakadan and Rahmani, 2000; Vanak and Gompper, 2009b).

In 2005-2007, a study of Indian fox ecology indicated the potential for fox-dog interactions in and around the Great Indian Bustard Wildlife Sanctuary (GIB WLS), Nannaj, in Maharashtra (Vanak, 2008; Vanak and Gompper, 2009b; Vanak and Gompper, 2010). A pilot study of dogs was undertaken around the GIB WLS to determine the prevalence of exposure to CPV and CDV (Vanak et al., 2007). We also sampled foxes during this study, with the objective of obtaining data on prevalence of exposure to CPV, CDV and CAV. In 2011-2012, we expanded on the initial study and undertook an in-depth epidemiological study of dog populations in and around the GIB WLS. The objective of this study was to collect baseline epidemiological data for dog population around the GIB WLS, focusing on CPV, CDV and CAV, with the recognition that such epidemiological data can be used to evaluate the risks dog populations present to wild canids, to design effective disease management programs, and to assess the impact of such programs. Here we report the levels of seroexposure in both dogs and foxes, and discuss the implications of these findings in the context of assessing pathogen ‘spillover’ risk represented by dogs to wildlife.

MATERIALS AND METHODS

Study area and species

The study was conducted in villages bordering the GIB WLS (17° 49’ 40” N and 75° 51’35” E). While the GIB WLS is comprised of 1222 km^2 protected area, the focal study portion of the sanctuary consists of six protected grassland patches totaling approximately 6 km^2, which is spread over a wide area in a human-dominated landscape that includes the villages. Combining the protected area and the village lands surrounding the sanctuary resulted in a focal study region of ~ 51 km^2. The local economy is based on agro-pastoralism, and the landscape consists of a matrix of agricultural fields, vineyards, communal grazing lands and a few government-owned forestry plantations. Dogs are ubiquitous in this region and have the phenotype typical of the village dogs of India. Village dog populations in the study area consist of owned, quasi-owned, as well as ownerless dogs, and these dogs are all unconfined irrespective of the ownership status (Vanak and Gompper, 2010). In 2011, the median dog population size in six villages bordering GIB WLS was 134 (range 90-188), the median dog density was 719 dogs per km^2 and the median human:dog ratio was 6 (range 5-8) (Belsare and Gompper, 2013). The activity, movements and interactions of dogs and wildlife in this region have been the subject of detailed study and involves

ranging that bring them in contact with wildlife within and outside the sanctuary (Vanak et al., 2007; Vanak and Gompper, 2009a; Vanak et al., 2009; Vanak and Gompper, 2010).

Indian foxes are the most common wild carnivore in the region. Other species of the order Carnivora that are found in the study area include gray wolf, golden jackal (*C. aureus*), jungle cat (*Felis chaus*) and gray mongoose (*Herpestes edwardsi*).

Capture and handling

Indian foxes were captured in and around the GIB WLS between April 2006 and May 2007. The foxes were captured using Victor #1 soft-catch traps, and blood samples were obtained after immobilizing the foxes with a combination of ketamine hydrochloride and xylazine hydrochloride (Belsare and Vanak, 2013). Dogs were sampled from the villages of Nannaj, Wadala, Mardi, Akolekati, Karamba and Gawdi Darfal, bordering the GIB WLS. During Session 1 (October 2005-February 2006) and Session 2 (December 2006-March 2007), dogs from the villages of Nannaj, Wadala, Mardi and Akolekati were sampled. During Session 3 (February 2011-July 2011) and Session 4 (September 2011-April 2012) dogs from all six villages were sampled. During these sessions, blood was collected only if the reference person (owner, putative owner, or the person handling the dog) consented, and was able to physically restrain the dog. Dogs

that were either unowned or were difficult for the reference person to restrain were captured using box traps, vaccinated and released after obtaining a blood sample. We classified dogs as pups (0-4 mo), juveniles (5-12 mo) and adults (> 12 mo) based on body size, allometry (visual estimate of head size and leg length compared to the body size), and behavior (Daniels, 1983). Eruption pattern of dentition was used to distinguish pups from juveniles (Kirk, 1977). Adults were distinguished from juveniles on the basis of developed teats (adult females) or descended testes (adult males). Capture and handling procedures were approved by the Animal Care and Use Committee of the University of Missouri-Columbia (Protocol #4262 and #7049).

Estimation of antibody seroprevalence

For both foxes and dogs, Vaccuette 4 ml serum tubes with clot activating factor (Greiner Labortechnic, Germany) were used to collect blood samples by venipuncture of the jugular, cephalic or saphenous vein. Blood in the serum tubes was allowed to clot at ambient temperature, and the serum was then decanted and kept 48 hours before transporting to a -20º C freezer at the Serum Institute of India, Pune for storage.

The lack of facilities with the capacity to conduct serological assessments via traditional methodologies (i.e. serum neutralization or haemagglutination-inhibition), combined with strict Indian export laws necessitated the use of

commercially available dot-ELISA assay kits. For samples collected between 2005 and 2007 (Session 1 and Session 2), Immunocomb® dot-ELISA assay kits (Biogal Laboratories, Kibbutz Galed, Israel) were used to determine IgG titers against CPV and CDV. Fox samples were also tested for IgM antibodies against CPV and CDV using the ImmunoComb CDV and CPV IgM kit. Samples collected between 2011 and 2012 (Session 3 and Session 4) were tested using Canine VacciCheck Antibody test kit (Biogal Galed Laboratories, Israel), which included tests for determining IgG antibodies against CAV in addition to CPV and CDV. We also used Canine VacciCheck Antibody test kits to test some of the stored fox sera samples for IgG antibody titers against CAV. These test kits are based on solid phase immunoassay technology. Each kit consists of a comb shaped plastic card and a multi compartment developing plate. The concentration of antibodies in serum samples is measured using the color-coded scale ("CombScale") provided in the kit. The test kit results are documented in "S" units (ImmunoComb score) on a scale of 0 to 6, where S3 corresponds to a 1:16 titer by virus neutralization test (VN) for CAV, 1:80 titer for CPV by the haemagglutination inhibition test (HI), and 1:32 VN for CDV. As per the information provided by the manufacturer, while an ImmunoComb score of S3 and above is to be considered as a protective level of antibodies to CPV, CDV and CAV, scores of S1 and S2 are also indicative that the individual possesses antibodies to the infectious agents. For the Immunocomb CPV and CDV IgM antibody test kits, an ImmunoComb score of S2 or more was indicative of detectable levels of IgM antibodies. In an unvaccinated dog, any detectable titer

of IgG antibodies is indicative of past exposure to the pathogen, while a detectable titer of IgM antibodies indicates ongoing or recent infection.

The prevalence of exposure to a pathogen was calculated as percentage of sampled dogs with detectable IgG antibodies against the pathogen (≥ S1). We calculated 95% confidence intervals for prevalence of exposure (Stern's exact method) using the software 'Quantitative Parasitology 3.0' (Reiczigel and Rózsa, 2011). We used χ^2 or Fisher's exact tests for statistical comparisons of prevalence by sampling sessions, age class, and sex. Prevalence data was stratified by age class, and Cochran-Mantel-Haenszel (CMH) chi-square tests were used to assess patterns in prevalence due to sex.

RESULTS

Seroprevalence of viral antibodies in dogs

A total of 219 dogs from the study region were sampled during the study period: 34 (♂ =25, ♀=9) during Session 1, 39 (♂ =35, ♀=4) during Session 2, 77 (♂ =62, ♀=15) during Session 3, and 69 (♂ =50, ♀=19) during Session 4. While only adult dogs were sampled during Session 1 and Session 2, juvenile dogs were included in the sampling undertaken during Session 3 (n=12; 16%) and Session 4 (n=26; 38%). There was no significant difference between the

exposure rates for the three pathogens in Session 3 and Session 4 (χ^2=0.062, p=0.804 for CPV; χ^2=0.053, p=0.818 for CDV, and χ^2=0.713, p=0.398 for CAV), we therefore combined the data from Session 3 and Session 4 for further analysis. For CPV and CDV, there was no significant difference in the exposure rates of adult dogs between Session 1, Session 2, Session 3 and Session 4 (p=0.626 for CPV, and p=0.758 for CDV; Fisher's exact tests).

Seventy-nine percent dogs sampled in sessions 3 and 4 were exposed to more than one of the tested pathogen species (Figure 1). There were no significant patterns in exposure to pathogen species (none, any one, any two, all three) when data were contrasted by sex (p=0.172; Fisher's exact test); but there were significant age class differences (p<0.0001; Fisher's exact test), with a higher proportion of adults (88%) exposed to more than one type of pathogen compared to juveniles (53%).

CPV: The observed prevalence of exposure to CPV based on IgG antibodies was 100% (95% CI 90-100%) in Session 1, and 97% (95% CI 86-100%) in Session 2. For Session 3 and Session 4 together, the prevalence of exposure to CPV was 88% (95%CI 81-92%). CPV exposure rate was significantly greater in adults (94%) compared to juveniles (68%) (p=0.0001, Fisher's exact test), but there was no significant pattern in prevalence when data was contrasted between males (85%) and females (97%) (p=0.074; Fisher's exact test). There was no significant pattern in prevalence of exposure to CPV

when sex-prevalence data was stratified by age class (CMH statistic=2.29, p=0.130). Two of the three resampled seronegative dogs acquired high titers of anti-CPV antibodies after the first sampling.

CDV: The observed prevalence of IgG antibodies to CDV was 85% (95% CI 69-94%) and 90% (95% CI 76-96%) for Session 1 and Session 2 respectively. For Session 3 and Session 4, the overall prevalence of exposure to CDV was 72% (95% CI 64-79), with exposure rate significantly greater in adults (83%) than in juveniles (40%) (χ^2=26.77, p<0.0001). CDV exposure rate was significantly greater in females (88%) than in males (67%) for Session 3 and Session 4 (χ^2=5.84, p=0.016). The difference in prevalence pattern when sex-prevalence data was stratified by age class was of marginal significance (CMH statistic=3.69, p=0.055). Between two samplings, one resampled seronegative dog acquired low anti-CDV IgG antibody titer, while two resampled dogs remained seronegative.

CAV: Combining data from sessions 3 and 4, the IgG antibody prevalence to CAV was 71% (95%CI 63-77%). CAV exposure rate was significantly greater (χ^2=7.936, p=0.005) in adults (77%) than in juveniles (53%), but did not differ significantly between males and females (71% for each sex) (χ^2=0, p=0.995). There was no significant pattern in prevalence of exposure to CAV when sex-prevalence data was stratified by age class (CMH statistic=0.171, p=0.679). Five dogs that were seronegative for anti-CAV IgG antibodies when first tested had

detectable titers subsequently; four dogs had seroconverted with high and one with low anti-CAV IgG antibody titers.

Seroprevalence of viral antibodies in foxes

Sera samples obtained from 33 adult Indian foxes (♂ =18, ♀=15) were tested for exposure to CPV and CDV. Combining the results from IgG and IgM antibody tests, Nine percent (n = 3) of the foxes tested had been exposed to both CPV and CDV, 49% (n = 16) foxes were exposed to one of these pathogens, and 42% (n = 14) had not been exposed to either of the pathogens. Of the 33 samples, 23 (♂ =13, ♀=10) were also tested for IgG antibodies to CAV. For these 23 foxes, IgG tests revealed that 52% were exposed to one pathogen, 35% were exposed to two pathogens, and none of the foxes were exposed to all three pathogens (Figure 1). There were no significant patterns in exposure to pathogen species (none, any one, any two) when data were contrasted by sex (p=0.122, Fisher's exact test).

CPV: Combining the observations for IgG and IgM antibodies, the prevalence of exposure to CPV in foxes was 48% (95% CI 32-65%). The exposure rate did not differ significantly (χ^2=0.793, p=0.373) in males (56%) and females (40%). Twelve percent (n = 4) of the foxes tested had detectable levels of anti-CPV IgM antibodies, indicating recent or ongoing CPV infection. One female fox had high titers of anti-CPV IgG (S5) with concurrent anti-CPV IgM antibody titers. Foxes were radiocollared and monitored for at least 2 months

post-sampling, and during this period we did not observe any mortality in foxes with detectable IgM antibodies to CPV.

CDV: The prevalence of exposure to CDV in foxes, based on IgG as well as IgM antibodies, was 18% (95% CI 8-35%). The exposure rate for CDV was 22% in males and 13% in females, a difference that was not statistically significant (p=0.665; Fisher's exact test). Detectable levels of anti-CDV IgM antibody titers were found in 15% (n = 5) foxes. Three of these foxes had concurrent high anti-CDV IgG titers, and were found dead within a month of sampling.

CAV: The prevalence of IgG antibodies to CAV in foxes was 52% (95% CI 32-72%). The exposure rate for CAV was 62% in males and 40% in females, a difference that was not statistically significant (p=0.414; Fisher's exact test).

DISCUSSION

The rural dog populations around GIB WLS had high exposure rates to CPV, CDV and CAV during each of the four sampling sessions, suggesting that these pathogens are enzootic, and actively circulating in the dog populations. The fact that seroconversion against these pathogens were documented in some unvaccinated dogs during the study further supports the conclusion. These results reflect observations in other systems; several studies have documented

serologic evidence of high rates of exposure to each of these pathogens in unvaccinated rural dog populations on other continents (e.g. Bronson et al., 2008; Millán et al., 2013; Bryan et al. 2011; Cleaveland et al. 2000).

The nature of human-dog interactions and the concept of dog ownership varies in cultures throughout the world (Jackman and Rowan, 2007). In our study area, as in rural areas across India, most dogs are affiliated with neighborhoods, and therefore considered ‘owned’ by the community. Owned or ownerless, virtually all the dogs in such settings are free-ranging, and are not habituated to restraint of any sort (even by the putative owners). This ownership pattern and the free-ranging nature of dogs pose logistical challenges for any interventions necessitating handling and restraint of dogs. Our sampling depended on the willingness and ability of the putative owner(s) or reference person(s) to restrain the dog, and therefore it should be noted that the results of this study are based on a convenience sample. We believe, however, that the epidemiological parameters reported here are representative of the entire dog population, because the concept of ‘ownership’ in the rural Indian setting does not include vaccination or any other preventive health care, any form of birth control, or the need for confinement. As a result, the owned, quasi-owned and ownerless dogs belong to a single panmictic village dog population.

In dog populations with endemic CPV and CDV, clinical infections are known to occur at an early age, after the maternal antibody-based immunity has declined (Mason et al., 1987; Williams, 2001). In our study, evidence of exposure

to all three pathogens was documented in juvenile dogs supporting such early exposure. Yet the exposure rates of adults were significantly higher than those of juveniles, possibly indicating the continued potential for initial or repeated exposure even in older age classes. This finding also suggests that most dogs in the population have survived natural exposure to these pathogens and seroconverted. Dogs that recover from natural infection due to CPV, CDV or CAV develop a lifelong immunity to these pathogens (Schultz et al., 2010). Thus most dogs in the population are immune to these pathogens, and have no current or future role in their maintenance.

These findings could have important implications for dog disease control programs, and for the selection of appropriate management approaches that might be considered in efforts to reduce the likelihood of cross-species transmission of viral pathogens that occur at relatively high prevalence in reservoir populations (Wright et al., 2013; Chapter 3). Canine vaccination has been recommended as a mechanism for reducing the prevalence and incidence of viral diseases in dogs, and has been used in conservation management of wild carnivore populations (Cleaveland et al., 2006; Knobel et al., 2013). Vaccination provides an antibody-mediated protection against viral pathogens, thereby protecting individuals against infectious diseases and also contributing to 'herd immunity' by reducing the density of susceptible individuals in the population. But in settings where large populations of free-ranging dogs occur, and pathogens such as CPV, CDV and CAV occur at high prevalence, the rationale for vaccinating adult dogs is questionable. If, as our study indicates, a large

proportion of adult dogs in a population have protective immunity due to seroconversion following early natural infection, additional vaccination of this cohort will neither greatly improve their collective immune status nor contribute to herd immunity. Rather, vaccination should target younger age classes in such situations. However, further research on such approaches is necessary.

The seroprevalence of anti-CPV, anti-CDV and anti-CAV IgG antibodies in the foxes indicated prior exposure to these pathogens. However, the lower seroprevalence of anti-CDV IgG antibodies in foxes (12%), in combination with the high rates of mortality among the handful of foxes that were diagnosed as IgM antibody positive, is likely a function of higher CDV-related mortality in foxes. During this study, three foxes with detectable titers of IgM antibodies to CDV (indicating ongoing CDV infection) died within a month of their respective sampling occasions. Though the foxes were radio-collared and monitored as a part of an ecological study, we could not observe foxes for clinical signs prior to their deaths. We did however, observe clinical signs compatible with a neurologic disease like CDV in another fox (which was not a part of this study) in the study area. The ante-mortem blood sample from this animal indicated ongoing CDV infection (detectable anti-CDV IgM antibodies and high anti-CDV IgG antibodies). These findings support the assumption of a high CDV-related mortality in foxes.

The relatively high seroprevalence of anti-CAV IgG antibodies (52%) and anti-CPV IgG antibodies (39%) in adult foxes suggests that the pathogens are either endemic in foxes or that they commonly are transmitted from dog to foxes,

and that subclinical or mild disease with recovery is a relatively common outcome of exposure to these pathogens. Further, seroprevalence titers tend to vary inversely with the severity of disease (Greene and Appel, 1998). In our study, all foxes that were seropositive for CAV antibodies had high titers (≥ S4; A. Belsare, unpublished data); supporting the assumption of milder disease and recovery in foxes exposed to CAV. A caveat, however, is that because mortality from both CPV and CAV may be higher in juveniles whose maternally-derived antibodies have declined to low levels, and because serological tests are unable to distinguish between exposure to CPV and closely-related parvoviruses such as feline panleukopenia virus or between strains of virus such as type 1 and type 2 CAV (Knobel et al. 2013; Balboni et al. 2013), the assumption of a relatively mild disease in Indian foxes following infection by CPV or CAV should be made with caution.

One possible limitation of this study is that the methodology used to assess antibody levels has been designed for dogs, these test kits have not been fully validated for wild canid species. Validation of the kits with traditional methods would be critical to ensure the reliability of results in wild species. Nevertheless, the serology findings of high IgG titer and concurrent IgM titer, in particular, are characteristic of the immune response in clinically ill animals. Similar immune response was documented in the 4 foxes that died, and one of the foxes also exhibited symptoms typical of CDV infection. This provides some evidence of susceptibility of Indian foxes to CDV, and of the value of the test kits to assess CDV in foxes.

We have documented high exposure rates to CPV, CDV and CAV in the dog populations around GIB WLS. The large population size, the free-ranging nature of these dogs, and the enzootic status of the pathogens collectively suggest that these viruses potentially pose a threat to the wild canids in the region. Assuming that the pathogens diagnosed in dogs and foxes are truly the same, rather than misidentified due to serological cross-reactivity of different viruses or viral strains, dogs could be playing a role in the maintenance and transmission of these pathogens in the fox population, and likely in other sympatric carnivore species as well. Given that India has several species of globally threatened carnivore species occurring in close proximity to high densities of dog populations, further research to better understand the disease threats, and to identify potential disease management interventions is strongly recommended.

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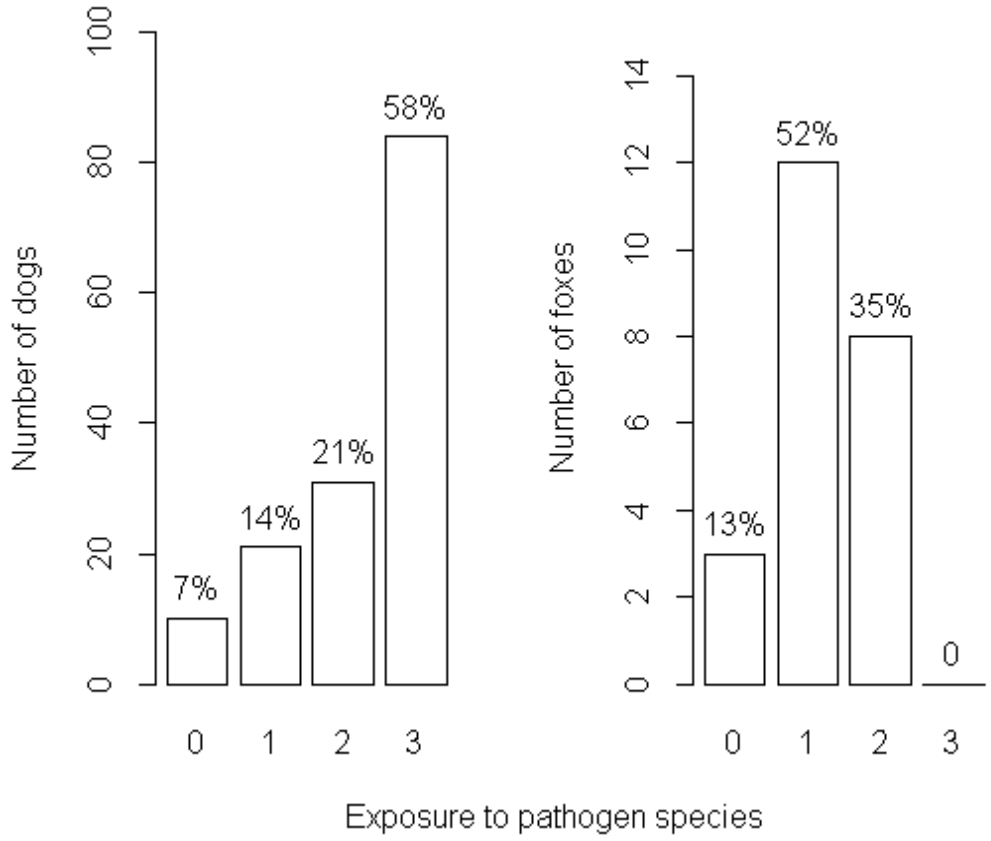
Figure 1. Distribution of seropositivity in dogs and foxes sampled around GIB WLS. Foxes (n = 23) and dogs (n = 146; sampled during Session 3 and Session 4) were assayed for exposure to canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV).



Chapter 3: TO VACCINATE OR NOT TO VACCINATE: LESSONS LEARNED FROM AN EXPERIMENTAL MASS VACCINATION OF FREE-RANGING DOG POPULATIONS

ABSTRACT

Domestic animal populations have the potential to act as reservoirs for multi-host pathogens, which may be transmitted to native species and cause population declines or extirpations. Domestic dogs are known reservoirs for several multi-host pathogens that may be transmitted to native carnivores. Mass vaccination of dogs has been suggested as a tool for mitigating the disease threat to sympatric carnivores. Following the observation of a putative canine distemper virus (CDV) epizootic in wild carnivores inhabiting the Great Indian Bustard Wildlife Sanctuary (GIB WLS) in central India, mass dog vaccination programs were initiated in six villages around the protected area. To determine the extent to which such mass vaccination programs are practicable and appropriate for large, free-ranging dog populations, a vaccination experiment was undertaken. Dogs from three villages were vaccinated against rabies virus, canine adenovirus (CAV), canine parvovirus (CPV) and CDV (treatment dogs), while those from three other villages were only vaccinated against rabies virus (control dogs). IgG antibody titers against CAV, CPV and CDV in control and treatment dogs were determined on four occasions during the study. When first examined, a large proportion of

the initially unvaccinated adult dogs in both the control and treatment group was IgG antibody positive, and thus protected against these pathogens. Background antibody seroprevalence rates were generally > 72% for each of the three viruses. Furthermore, several unvaccinated adult dogs acquired protection against these pathogens during the study. Vaccination failed to increase the proportion of adult dogs with IgG antibodies against CAV, CPV or CDV in the treatment group compared to the control group, as much of the effort was put into vaccinating dogs that were already antibody positive. In such situations, vaccination of adult dogs against these enzootic viral pathogens seems unnecessary, and would escalate the cost-benefit ratio of dog disease control programs.

INTRODUCTION

Large, unvaccinated populations of free-ranging domestic animals occur in many parts of the developing world, and there is an increasing concern that such populations may serve as sources and reservoirs of multi-host pathogens transmitted to wildlife. Domestic dogs in particular have been subject to focused attention because of multiple, well-documented cases of such transmission involving an array of microparasitic and macroparasitic species (Alexander et al., 1996; Roelke-Parker et al., 1996; Funk et al., 2001; Bronson et al., 2008; Knobel et al., 2013). For instance, serologic studies have documented evidence of exposure to a variety of canine multi-host viral pathogens in unvaccinated dog populations, and several of these pathogens have been identified as key threats to wild carnivore species (Woodroffe, 1999). Under an assumption that dogs are the principle reservoir of these pathogens, and thus the principal driver of the seemingly high incidence in wild carnivores, vaccination of dog populations has been suggested as one of the management options to protect wild carnivore species from such multi-host pathogens (Laurenson et al., 1998; Bronson et al., 2008; Bryan et al., 2011).

Vaccination has been the mainstay of successful canine rabies virus control and eradication programs across the globe (WHO, 2004; Belotto et al., 2005; Cleaveland et al., 2006). The success of canine rabies eradication programs as measured by declines in human rabies has underpinned efforts to

use mass vaccination as a tool for reducing the likelihood of viral pathogen transmission to wildlife in situations where the persistence of wildlife is perceived as at risk because of the high density (and high reservoir capacity) of dogs. For instance, mass vaccination trials of dogs undertaken around Serengeti demonstrated that disease threats to wild carnivores could be reduced by vaccination of dogs against canine distemper virus (CDV) and rabies virus (Cleaveland et al., 2007). Similarly, the absence of rabies or CDV cases in Ethiopian wolves in the Bale Mountains National Park between 1998 and 2003 was attributed in part to the mass dog vaccination campaigns conducted in the region (Laurenson et al., 2005).

Vaccination provides an antibody-mediated mechanism against pathogens, thereby protecting individuals against infectious diseases and also contributing to herd immunity by reducing the density of susceptible individuals in the population to a point for which the basic reproduction number (R_0) for a pathogen is less than one. Dogs are often assumed to function as reservoirs of pathogens of conservation concern. If the R_0 for a pathogen can be reduced to below one in this reservoir population by a mass vaccination campaign, then the persistence of the pathogen in this population as well as the likelihood of transmission to the target wildlife species could potentially be reduced. The implicit assumption here is that a large proportion of individuals in the reservoir population are susceptible to the pathogens, rather than infected or recovered. However, for viral pathogens such as CDV, the effectiveness of vaccination

programs in reducing the transmission risks into wild carnivore population has not yet been thoroughly evaluated (Prager et al., 2013).

In 2006-07, a study undertaken in the Great Indian Bustard Wildlife Sanctuary (GIB WLS), Nannaj in central India revealed high exposure rates to CPV and CDV in dogs, (Vanak et al., 2007). Indian foxes (*Vulpes bengalensis*) were also sampled during the study and a putative epizootic of CDV was documented, potentially attributed to transmission from local dogs. Despite the putative CDV-associated mortalities, foxes occurring in the region had lower antibody seroprevalence rates to these pathogens than did dogs, suggesting high mortality rates of foxes following exposure. Thus a tentative working hypothesis was put forth (Vanak et al., 2007) that high dog-fox contact rates facilitated the transmission of CDV from dogs to foxes, as the latter species would not have maintained CDV in isolation given its relatively low population density and the apparently high pathogenicity of CDV in foxes. These observations prompted the Maharashtra Forest Department (MFD), which oversees the GIB WLS, to undertake mass dog vaccinations in the villages surrounding the GIB WLS as an approach to protecting wild carnivores inhabiting the protected area. An in-depth epidemiological study of dogs around the GIB WLS was undertaken along with the mass vaccination campaigns, and the results indicated that CPV, CDV and CAV are enzootic in the dog populations around GIB WLS (Belsare and Gompper, 2013). These pathogens can infect a wide range of mammalian carnivore species, and may constitute an important conservation threat (Knobel et al., 2013).

As a part of the MFD-associated vaccination program, a small-scale (village-level) vaccination experiment was undertaken to determine the efficacy and applicability of mass vaccination of dogs against CAV, CPV and CDV as a disease mitigation intervention strategy around GIB WLS. Our initial interest was whether vaccination of dogs might result in altered dog demographics and therefore increased population size due to decreases in disease-related morbidity. However, as a part of the study we were also interested in rates of seroconversion and levels of herd immunity following vaccination, as such information is lacking for free-ranging dog populations. Here we report an outcome of the vaccination experiment with regards to natural and vaccine-related seroconversion, and the implications of the outcome for ongoing and future dog disease control programs and research.

MATERIALS AND METHODS

The GIB WLS is a 1,222 km^2 protected area spread across grassland regions of Maharashtra, in central India. The work described in this study took place at Nannaj, Maharashtra (17° 49’ 40” N and 75° 51’35” E) where the focal GIB WLS lands consist of six protected grassland patches totaling approximately six km^2, which are spread over a wide area in a human-dominated landscape. Six villages bordering the GIB WLS were included in this study. The protected area and the village lands surrounding the sanctuary together comprised the study region of ca 51 km^2. The number of households in these villages ranged

between 490 and 1300, and the projected human population sizes in 2011 ranged between 2973 and 7448; for the six villages the median dog population size was 134 (range 90-188) and the median dog population density was 719 dogs per km^2 (range 526-969) (Belsare and Gompper, 2013). Dogs are ubiquitous in this region, and are essentially unconfined irrespective of their ownership status. The activity pattern of dogs in the region involves ranging that may bring them in contact with wildlife (Vanak and Gompper, 2010).

Mass vaccination campaigns were organized in collaboration with the MFD, and dogs were vaccinated free of cost. The median vaccination coverage achieved in the six villages was 34% (range 24-42%) (Belsare and Gompper, 2013). Dogs in the villages of Gawdi Darfal, Karamba and Nannaj were included in the control group, and were vaccinated with Rabigen mono (Virbac Animal Health) rabies vaccine only. Dogs in the villages of Akolekati, Mardi and Wadala were included in the treatment group, and were vaccinated with Canigen DHPPi/L (Virbac Animal Health) and Rabigen mono rabies vaccine. Canigen DHPPi/L is a combination vaccine containing live CDV, CAV type 2, CPV and canine parainfluenza virus, along with inactivated whole organisms of *Leptospira canicola* and *L. icterohaemorrhagiae*. All dogs in the control and treatment villages were vaccinated against rabies because of the public health risks posed by dog-transmitted rabies in the region. For the purpose of this experiment, the 'treatment' consists of immunization against CAV, CPV and CDV.

The mass vaccination campaigns were organized in the villages of Akolekati, Karamba, Mardi, Wadala and Nannaj during February and March 2011, and in in the village of Gawdi Darfal during July 2011. Dogs were sampled just before the vaccine was administered during the mass vaccination campaigns (Session 1). We sampled a dog only if the reference person (owner, putative owner, or the person handling the dog) consented, and was able to physically restrain the dog. A leash and nylon muzzle was provided whenever necessary. If a dog struggled too much, or the reference person seemed incapable of properly restraining the dog, we did not sample that dog so as not to jeopardize human or dog health. Using the same approach, blood samples were collected from vaccinated and unvaccinated dogs in these villages during subsequent sessions following the mass vaccination campaigns: 6 months post-vaccination (Session 2; August-September 2011 for 5 villages and January 2012 for Gawdi Darfal), 9 months post-vaccination (Session 3; November-December 2011 for 5 villages and April 2012 for Gawdi Darfal), and 1 year post-vaccination (Session 4; February-March 2012 for 5 villages and July 2012 for Gawdi Darfal). No additional vaccinations took place after Session 1. Though both adult and juvenile dogs were vaccinated and sampled in the broader study, only adult dogs (>12 months) were included in the vaccination experiment so as to avoid confounding maternally and non-maternally derived antibodies.

Blood sample was collected in Vaccuette 4 ml serum tubes with clot activating factor (Greiner Labortechnic, Germany) by venipuncture of the cephalic or saphenous vein. Blood in the serum tubes was allowed to clot at

ambient temperature, and the serum was then decanted and kept 48 hr before transporting to a -20º C freezer at the Serum Institute of India, Pune for storage. We used a dot-ELISA kit (Canine VacciCheck Antibody test kit, Biogal Galed Laboratories, Israel) to determine the IgG antibody titers against CAV, CPV and CDV.

The observed prevalence (number of seropositive dogs / total number of dogs tested) of exposure for each session was calculated for the treatment and the control group by pooling data for three villages in each group. We calculated 95 % confidence limits for prevalence (Sterne's exact method) using the software Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2011). We used Fisher's exact test for comparing prevalence across sessions within and between the treatment and the control groups. Where no significant differences between sessions or between control and treatment dogs were observed, we also pooled the data for unvaccinated dogs sampled during the study to obtain a better estimate (that is, with the maximal number of individual dogs, facilitating a narrower confidence interval) of prevalence across the entire study period using the pooled sample.

Some dogs do not respond to vaccination and fail to develop sufficient antibody response (non-responders) (Larson and Schultz, 2007). To quantify the rates of non-response, dogs in the treatment group that were seronegative prior to vaccination, and were sampled during subsequent sampling sessions were

assessed for the response to vaccination. We assumed that seroconversion of these animals was due to vaccination rather than natural infection.

We evaluated the efficacy of mass vaccination against enzootic dog pathogens using a simple deterministic model. Pathogens like CAV, CPV and CDV are enzootic in many parts of the developing world, where dog populations comprise of free-ranging and unvaccinated dogs. Dogs exposed to CAV, CPV and CDV have detectable titers of antibodies against the pathogens, and develop lifelong immunity to these diseases (Schultz et al., 2010). The prevalence of pathogen exposure (P_{exp}) is the proportion of dogs in a population that are seropositive due to prior natural exposure. Such seropositive dogs will inadvertently be vaccinated during mass vaccination campaigns, but vaccination does not improve their immune status or contribute to the herd immunity. Therefore, we define the efficacy of mass vaccination (E_{mv}) as the proportion of seronegative dogs vaccinated during a vaccination campaign. We assume that the proportion of seronegative dogs vaccinated during mass vaccination campaigns will be a function of the prevalence of pathogen exposure and the percent vaccination coverage (V). We calculated the efficacy of mass vaccination as:

$$E_{mv} = V \times (1-P_{exp})$$

We assume that all vaccinated seronegative dogs will seroconvert after vaccination. Under these assumptions, we evaluated the efficacy of mass

vaccination for a range of vaccination coverage values (25-100%) in populations with different initial exposure rates (20-80%).

RESULTS

A total of 92 samples were obtained from 61 adult dogs (♂=41, ♀=20) from the control group and 119 samples were obtained from 69 adult dogs (♂=54, ♀=15) from the treatment group. Thirty seven dogs (61%) from the control group were sampled on one occasion, 18 dogs (30%) were sampled on two occasions, five dogs (8%) were sampled on three occasions and one dog (2%) was sampled on four occasions. Forty dogs (58%) from the treatment group were sampled on one occasion, 14 dogs (20%) were sampled on two occasions, nine dogs (13%) were sampled on three occasions and six dogs (9%) were sampled on four occasions (Table 1).

Seroprevalence among control and treatment animals

CAV: For Session 1, the overall proportion of dogs with anti-CAV IgG antibodies was 72% (95% CI = 60-82) among the combined population of control and treatment animals, and there was no significant difference (p = 0.790) between prevalence in the control (74%; 95% CI = 57-87%) and treatment groups (70%; 95% CI = 54-83%). In the treatment group, the proportion of dogs

with anti-CAV IgG antibodies increased significantly (p = 0.004) in the subsequent sessions compared to Session 1: 91% (95% CI = 75-97%) for Session 2, 100% (95% CI = 87-100%) for Session 3, and 92% (95% CI = 73-99%) for Session 4. In the control group, the proportion of dogs with anti-CAV IgG antibodies did not change significantly (p = 0.098) in the subsequent session compared to Session 1: 84% (95% CI = 67-94%) for Session 2, 100% (95% CI 80-100%) for Session 3, and 92% (95% CI = 63-100%) for Session 4. Contrasting control and treatment dogs within each sampling session, the proportion of dogs with anti-CAV IgG antibodies was similar in the treatment group and the control group during each post-vaccination session (p ≥ 0.708 for each of the subsequent sessions, Session 2, Session 3, and Session 4) (Figure 1). The overall prevalence of anti-CAV IgG antibodies in unvaccinated dogs (n = 98) sampled across the six villages during the entire study was 76% (95% CI = 66-83%).

CPV: During the initial sampling session, the overall proportion of dogs with anti-CPV IgG antibodies was 94% (95% CI = 86-98) among the combined population of control and treatment animals. There was no significant difference (p = 1) between prevalence in the control group (94%; 95% CI = 79-99%) and the treatment group (95%; 95% CI = 82-99%). Following vaccination of the treatment group, the proportion of dogs with anti-CPV IgG antibodies did not change significantly (p = 0.422) in the subsequent sessions compared to Session 1: 100% (95% CI = 90-100%) for Session 2, 96% (95% CI = 80-100%) for Session 3, and 92% (95% CI = 73-99%) for Session 4. A similar pattern occurred in the

control group, the proportion of dogs with anti-CPV IgG antibodies did not change significantly (p = 0.816) in the subsequent sessions: 94% (95% CI = 80-99%) for Session 2, 100% (95% CI = 80-100%) for Session 3, and 100% (95% CI = 76-100%) for Session 4. Contrasting control and treatment dogs within each sampling session, the proportion of dogs with anti-CPV IgG antibodies was similar in the treatment group and the control group during each post-vaccination session (p = 0.492 for Session 2, p = 1 for Session 3, p = 0.543 for Session 4) (Figure 1). Across the entire study, the overall prevalence of anti-CPV IgG antibodies in unvaccinated dogs (n = 98) sampled across the six villages was 95% (95% CI = 88-98%).

CDV: The initial overall proportion of dogs with anti-CDV IgG antibodies was 82% (95% CI = 71-90) among the combined population of control and treatment animals, and there was no significant difference (p = 0.2) between prevalence in the control group (90%; 95% CI = 74-97%) and the treatment group (76%; 95% CI = 60-87%). In the treatment group, the proportion of dogs with anti-CDV IgG antibodies did not change significantly (p = 0.918) in the subsequent sessions compared to Session 1: 81% (95% CI = 64-92%) for Session 2, 80% (95% CI = 60-92%) for Session 3, and 83% (95% CI = 63-94%) for Session 4. A similar pattern occurred in the control group, for which the proportion of dogs with anti-CDV IgG antibodies also did not change significantly (p = 0.414) in the subsequent sampling sessions: 78% (95% CI = 61-90%) for Session 2, 94% (95% CI = 71-100%) for Session 3, and 92% (95% CI = 63-100%) for Session 4. Contrasting control and treatment dogs within each

sampling session, the proportion of dogs with anti-CDV IgG antibodies was similar in the treatment group and the control group during each post-vaccination session ($p = 1$ for Session 2, $p = 0.374$ for Session 3, $p = 0.646$ for Session 4) (Figure 1). The overall prevalence of anti-CDV IgG antibodies in unvaccinated dogs (n = 98) sampled across the six villages during the entire study was 83% (95% CI = 74-89%).

Seroconversion and non-responders in control and treatment groups

Twenty nine dogs from the treatment group and 24 dogs from the control group were tested on two or more occasions. Eleven dogs from the control group that were initially seronegative for one of the three pathogens converted to seropositive. Five of five became positive for CAV, two of three for CPV, and one of three for CDV (Table 2). In both the control and treatment groups, no dogs that were initially seropositive became seronegative in subsequent samplings. Within the treatment group, a total of 11 dogs were initially seronegative for antibodies to one or more pathogens prior to vaccination. Of these, one of four vaccinated dogs remained negative for CAV and one of six for CDV (Table 2), indicating the potential for non-responders to exist within a population of vaccinated free-ranging dogs.

Efficacy of mass vaccination against enzootic pathogens

As per the model output, the efficacy of mass vaccination decreases with increasing prevalence of pathogen exposure (Figure 2). For a given prevalence of pathogen exposure, the efficacy of mass vaccination increases with the vaccination coverage. Where natural pathogen seroprevalence is low (20%), high levels of vaccination coverage (75-100%) results in 60-80% of vaccinated dogs benefiting (that is, seroconverting following vaccination). In contrast, where natural exposure rates are high (as is the case for CAV, CPV, and CDV in this study), even high levels of vaccination contributed relatively little additional benefit. For example, the CDV, CPV and CAV exposure rates in our study population were 83%, 95%, and 76%, respectively, and the median vaccination coverage was 34%. The model indicates that only 5-10% of the vaccinated dogs actually benefitted from this mass vaccination program for each of the pathogens.

DISCUSSION

A large proportion (>72%) of unvaccinated, adult dogs sampled during this study had IgG antibodies against each of the viral pathogens, indicating recovery from natural exposure. Further, most dogs in the small subset of control group who were seronegative when initially sampled acquired IgG antibody titers against these pathogens during the study, indicating continual natural exposure

and survival in these dogs. Dogs recovering from natural infection due to CAV, CPV and CDV develop a lifelong immunity to these pathogens (Schultz et al., 2010). Thus, a large proportion of unvaccinated adult dogs (76% for CAV, 95% for CPV, 83% for CDV) in our study area can be considered protected against these pathogens. Given this observation, it is unsurprising that vaccination of dogs in the treatment group failed to increase the proportion of dogs with IgG antibodies against CAV, CPV or CDV compared to the control group. Indeed, seroprevalence of each pathogen in the experimental and control groups were remarkably similar in each of the three post-vaccination sessions. Therefore, in settings like the GIB WLS, where exposure rates of diseases like CAV, CPV and CDV are high, the rationale of vaccinating adult dogs to enhance dog welfare or to protect wildlife populations is debatable. Vaccination of adult dogs against CAV, CPV and CDV in such populations would result in little additive benefit in terms of herd immunity, and would escalate the cost-benefit ratio of dog disease control programs.

Further, it has been recognized that some dogs do not develop sufficient antibody response after vaccination (non-responders) (Larson and Schultz, 2007). Some dogs are incapable of responding to immune stimulation following vaccination, while in other cases the non-response could be due to inadequate immune stimulation (improper or insufficient vaccination). The result is a lack of development of protective surface antibodies. Despite starting with a small number of dogs that entered the study as treatment (vaccinated) animals and

that were seronegative for one or more pathogens, we documented non-responders for CAV (one of four vaccinated dogs) and CDV (one of six vaccinated dogs). Non-responders to CPV were not documented in this study. Such observations suggest the need for further studies to estimate the proportion of non-responders in free-ranging dog population. If high rates of non-response occur, the likelihood of developing herd immunity is greatly reduced.

The high levels of background antibody seroprevalence rates of the three pathogens assessed in this study are not atypical of unvaccinated dog populations. Several studies have reported a high seroprevalence of antibodies in unvaccinated dogs. Rural dogs sampled around three national parks in Uganda revealed a high prevalence of CDV (100%) and CPV antibodies (65.2%) (Millán et al., 2013). Dogs sampled from around Madidi National Park in Bolivia had a high prevalence of CDV (92%), CPV (92%) and CAV (77%) antibodies (Fiorello et al., 2004). High prevalence of CDV (93%) and CPV (85%) antibodies (but a low prevalence CAV antibodies; 18%) was documented in dogs from Noel Kempff Mercado National Park in Bolivia (Bronson et al., 2008). Collectively these finding suggest that most adults in free-ranging dog populations have high antibody seropositive rates for several pathogens of conservation concern and therefore that vaccination efforts that target these animals provide no additional benefit.

Our working hypothesis for this pilot experiment was that mass vaccination against enzootic pathogens would reduce the number of

seronegative (susceptible) dogs in the population, thereby contributing to the herd immunity as well as reducing the occurrence of clinical and subclinical cases caused by these pathogens. Indirectly, such reductions would benefit wildlife by decreasing cross-species transmission opportunities. Yet the effect of decreased disease occurrence might also result in differences in population parameters such as adult survival, female reproduction, litter size and litter survival between the mass vaccinated populations and populations that were not vaccinated. But a large proportion of adult dogs in the population were already antibody positive by virtue of a natural exposure at an early age, and vaccination of such dogs would have no effect on demographics as no additional benefit is afforded to the individual or to the population as a whole. On the other hand, where prevalence is low (for instance, for pathogens such as rabies or in particular settings such as observed by Bronson et al. (2008) for CAV in Bolivia, vaccination of dogs may be a more appropriate strategy for enhancing levels of herd immunity.

In dog populations with enzootic CPV and CDV, clinical infections are known to occur at an early age, after the maternal antibody-based immunity has declined (Mason et al., 1987; Williams, 2001). We obtained blood samples from 12 juveniles (6-12 mo) and one pup during the mass vaccination campaigns. In juveniles, the prevalence of antibodies was lower, albeit with wide confidence intervals compared to adults: 42% (95% CI = 18-71%) for CAV, 58% (95% CI = 29-82) for CPV and 25% (95% CI = 7-54%) for CDV. The pup that was sampled did not have antibodies to any of the three pathogens. Pups and juveniles

therefore appear to play a critical role in the transmission epidemiology of these pathogens, and further research should be directed towards this cohort in an effort to delineate the mechanisms by which these pathogens persist in the dog populations.

Vaccination of dog reservoir populations has been recommended as a potential measure that can be used to protect wild carnivores from canid diseases. By vaccinating dogs against pathogens of conservation concern, it is expected that the number of susceptible dogs in the population will be reduced, thereby reducing the occurrence of clinical cases and the likelihood of transmission events between infected dogs and wild carnivores. Such a program will readily work for a pathogen such as rabies, for which only a small portion of the population is ever infectious (or immune) at any given moment. But for a pathogen like CDV, which has a relatively short incubation period and a longer infectious period, and induces long-lasting immunity in the survivors, the pool of susceptibles in a population depletes rapidly. The combination of such host-pathogen characteristics contributes to the herd immunity (Anderson and May, 1979). Mild disease with recovery is a common outcome in populations where CPV is enzootic (Barker and Parrish, 2001). Most new infections occur in juveniles exposed to CPV after the maternal antibodies have declined (Mason et al., 1987). CPV is known to be highly immunogenic, and recovered animals are known to develop a long-lasting immunity and the antibody titers are periodically boosted due to natural reexposure. Therefore, and as the results of this study demonstrate, if most of the dogs in a population are already IgG antibody positive

and therefore protected against the pathogen(s), vaccination will not provide any additional benefit unless the younger age class animals could be reliably targeted.

These findings indicate that before conducting mass vaccination programs of free-ranging dog populations for conservation programs (or dog welfare programs), an epidemiological study investigating the pattern of exposure to pathogens should be undertaken to assess the likely success of any such program. Further, it is likely that similar conclusion should be drawn for other domestic animal taxa. Vaccination-based management of multi-host parasites that persist in wildlife because of high densities of domestic hosts on the landscape may not always be cost-effective or appropriate if much of that effort indiscriminately targets domestic hosts that are already non-susceptible to the pathogen of concern.

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Table 1. Number of dogs tested for antibodies to canine adenovirus, canine parvovirus and canine distemper virus during four sampling sessions in the control and treatment group. The number of dogs that were resampled is given in parenthesis.

	Session 1	Session 2	Session 3	Session 4
Control group	31 (0)	32 (13)	17 (10)	12 (8)
Treatment group	37 (0)	32 (13)	25 (19)	25 (18)
Total	68 (0)	64 (26)	42 (29)	37 (26)

Table 2. Serologic status of dogs in the control and treatment groups, sampled on two or more occasions for antibodies against canine adenovirus (CAV), canine parvovirus (CPV) and canine distemper virus (CDV).

	CAV		CPV		CDV	
	Control	Treatment	Control	Treatment	Control	Treatment
positive-positive	21	27	23	30	23	25
negative-positive	5	3	2	1	1	5
negative-negative	0	1	1	0	2	1

Figure 1. Prevalence of exposure (± 95% CI) to canine adenovirus (CAV), canine parvovirus (CPV) and canine distemper virus (CDV) in dogs from the control (dark bars) and treatment (light bars) groups during 4 sampling sessions.

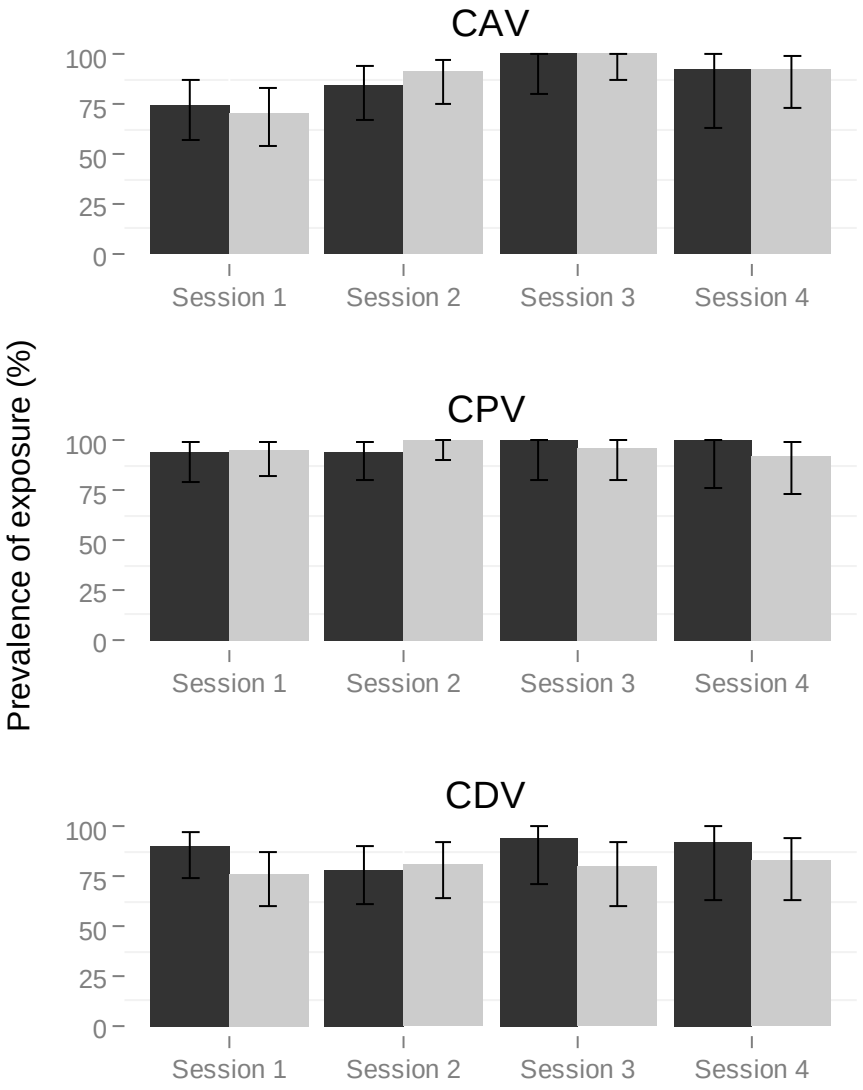
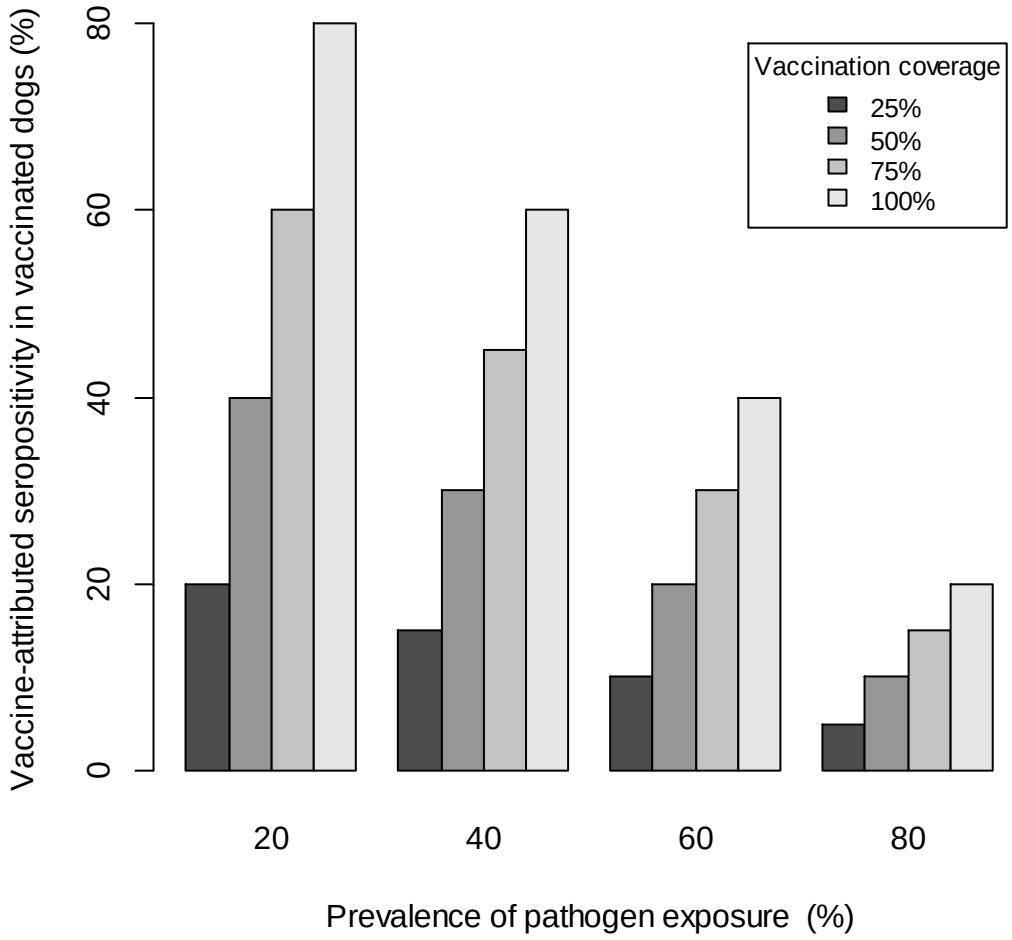


Figure 2. Efficacy of mass vaccination for a range of vaccination coverage values in populations with different population exposure rates.



Chapter 4: A MODEL-BASED APPROACH FOR INVESTIGATION AND MITIGATION OF DISEASE SPILLOVER RISKS TO WILDLIFE: DOGS, FOXES AND CANINE DISTEMPER IN CENTRAL INDIA

ABSTRACT

Multi-host pathogens can pose a serious conservation threat when free-ranging domestic animal populations occur alongside susceptible populations of wild species. An example is canine distemper virus (CDV), which can occur at high prevalence in domestic dog (*Canis familiaris*) populations from which it may be transmitted (spillover) into wild carnivore populations. Effective management of such disease threats is hindered by our limited understanding of the the dynamics of interspecific CDV transmission in natural settings. We used a modeling approach to better understand CDV spillover threats to wild Indian foxes (*Vulpes bengalensis*) occurring in a protected grassland habitat in central India. An agent-based stochastic simulation model was built, and parameterized with data from ecological and epidemiological studies. Based on the sensitivity analyses of the model, the CDV incidence rate in dogs was most influenced by the proportion of roamer dogs in the dog population. The CDV incidence rate in dogs was also sensitive to the CDV introduction frequency in the dog population. The proportion of roamer dogs in the dog population also influenced the number of CDV spillover events. The basic reproductive number (R_0) for CDV in the

model fox population was 0.85, indicating that CDV could not be independently sustained in the fox population. We used the model to explore potential management strategies to mitigate the risk of CDV spillover. Vaccination of local dog populations was an ineffective disease control strategy, while fox vaccination was highly effective. Interventions potentially resulting in lower contact rates between dogs and foxes, like reduction in village dog density and restricting dog movements in fox habitat, implemented in a sustained and integrated manner would be most effective in mitigating disease threats to foxes. Such modeling approaches can be used to better understand disease threats for other species of management concern, and to contrast potential management interventions.

INTRODUCTION

Large populations of free-ranging domestic animals, such as dogs (*Canis familiaris*) occur in most of the developing world, and these free-mixing, mostly unvaccinated populations provide opportunities for the persistence and transmission of multi-host pathogens. Transmission of viruses from dog reservoirs has resulted in population declines and local extirpations in many threatened carnivore species, including both wild canids and non-canids (reviewed by Funk et al. 2001; Woodroffe et al. 2004; Cleaveland et al. 2006; Knobel et al. 2013). While vaccination of the dog reservoir or the target wildlife population have been suggested as potential management options to protect wild carnivore species from such multi-host pathogens (Laurenson et al., 1997; Bronson et al., 2008; Bryan et al., 2011; Flacke et al., 2013), choosing appropriate management action is hindered by an incomplete understanding of the relative role of different host species in maintaining the pathogen within the broader multi-host species community.

Canine distemper virus (CDV) is one of the most important infectious microparasites of domestic and free-ranging carnivores worldwide. Dogs are often considered to be a primary reservoir of CDV and a source of infection for wild species, although CDV can persist in carnivore populations independent of dogs (Almberg et al., 2010). Epidemics in species such as African wild dogs (*Lycaon pictus*), Island foxes (*Urocyon littoralis*), African lions (*Panthera leo*),

Caspian seals (*Phoca caspica*) and Lake Baikal seals (*P. sibirca*) have been attributed to CDV derived from dogs (Cleaveland et al., 2006). Mass vaccination of dogs has been successful in controlling the incidence of viruses such as rabies in free-ranging dogs and preventing transmission of dog rabies to other host species (Hampson et al., 2009; Kaare et al., 2009). Although CDV vaccination of dogs has been incorporated in some conservation management programs, its effectiveness in controlling CDV in dog populations remains to be fully evaluated (Prager et al., 2013; Chapter 3).

An example of such difficulties in fully understanding the dynamics of interspecific CDV transmission in natural settings is observed in domestic canid-wild canid CDV transmission in rural Maharashtra, India. High prevalence of exposure (> 72%) to CDV has been documented in free-ranging dog populations around the Great Indian Bustard Wildlife Sanctuary (GIB WLS), Nannaj in central India (Chapter 2). Indian foxes (*Vulpes bengalensis*) sampled in the same locale were also exposed to CDV (18%), and CDV-related mortalities were documented for multiple radiocollared foxes (Vanak et al., 2007). To address this concern, the Maharashtra Forest Department (MFD), which oversees the GIB WLS, undertook mass dog vaccinations in the villages surrounding the GIB WLS as an approach to protecting wild carnivores inhabiting the protected area. Along with the mass vaccination programs, a study was undertaken to evaluate the applicability and efficacy of dog vaccination (against CDV, canine adenovirus and canine parvovirus) in free-ranging dog populations. The results indicated that vaccination failed to increase the proportion of dogs with antibodies against

enzootic pathogens because a large proportion of unvaccinated adult dogs (the primary cohort that received targeted vaccination) were already antibody positive for these pathogens and therefore protected against these pathogens (Chapter 3). Vaccination of adult dogs against CDV in such populations would be unnecessary, and would escalate the cost-benefit ratio of dog disease control programs. Further, given that most adult dogs are not infectious, it is not entirely clear how CDV could repeatedly move from the dog to the low density fox population.

An important outcome of this work was the realization that effective management of diseases in free-ranging populations requires a better understanding of the conditions favoring persistence of the pathogens in the system under study. Identifying a reservoir population and the mechanism of transmission between the reservoir hosts and the target hosts would help determine where the control efforts need to be directed. But undertaking experimental interventions to identify a reservoir host population, especially when wildlife species are involved, is a legally, ethically, and logistically difficult proposition. For instance, determining the mechanisms of disease transmission and estimating disease transmission rates in field situations pose serious challenges, as it depends upon repeated interventions requiring capture and handling of statistically large numbers of free-ranging animals. Thus, unequivocal identification of disease reservoirs and the underlying transmission mechanisms is often nearly impossible when the host populations are free-ranging.

Given the substantial uncertainty about the disease dynamics, a modeling approach can increase our understanding of the disease processes and define interventions that are epidemiologically and ecologically sound. Here we use an agent-based model of CDV dynamics in a two host system. We model CDV transmission between dogs and foxes in and around the GIB WLS based on our current best understanding of the system. The model provides opportunities to play out various scenarios under different assumptions, and explore potential disease control strategies. The modeling exercise also indicates the areas where further research could be directed.

BIOLOGICAL BACKGROUND

CDV is a multi-host pathogen with a global distribution, and causes severe, life-threatening diseases in dogs and wild canid species (Day et al., 2010). Canid species are the principal reservoir hosts for CDV (Greene and Appel, 2006). Several studies from across the globe have documented high seroprevalence of anti-CDV antibodies in free-ranging dogs, implying that CDV is endemic in such populations (Fiorello et al., 2004; Kelly et al., 2005; Bronson et al., 2008; Acosta-Jamett et al., 2011; Belsare and Gompper, 2013; Millán et al., 2013). In areas with large, free-ranging dog populations, most adult dogs have developed immunity to the pathogen (Chapter 3). A constant supply of puppies ensures availability of susceptible dogs in the population, and persistence of CDV in dog populations is attributed to contact among recently infected and

susceptible dogs (Greene and Appel, 2006). Between 25% and 75% of susceptible dogs get subclinical CDV infection, while clinical disease is mostly seen in pups following loss of maternal antibody at 3-6 months of age (Williams, 2001). The infectious phase starts 1 week post-infection when the infected dogs start shedding the virus and may last for up to 60 to 90 days, although shorter periods of virus shedding are more common. The virus is unstable in the environment and is rapidly destroyed outside the host (Greene and Appel, 2006).

The daily activity pattern of free-ranging dogs in areas bordering wildlife reserves or natural areas may involve ranging in wildlife habitat, and thus bring them into contact with wildlife. Free-ranging dogs interact with native wildlife at multiple levels (Butler et al., 2004; Vanak and Gompper 2009; Gompper, 2013) and a potential exists for transmission of pathogens from the abundant reservoir host (dogs) to sympatric wildlife. In the case of CDV, transmission of viral particulates between animals is via aerosol or contact with bodily excretions. The likelihood of such contact between infectious and susceptible animals can be mediated by resource distributions (e.g. food waste, crops, water sources) (Knobel et al., 2013). Furthermore, chasing, fights and simultaneous and sequential feeding events at carcasses have also been suggested as mechanisms facilitating contact and pathogen transmission (Kapil and Yeary, 2011).

Large fluctuations of Indian fox populations have been documented, and although disease has been suspected it has never been properly investigated

(Manakadan and Rahmani, 2000; Vanak and Gompper, 2009). A study undertaken in the GIB WLS in 2006-07 revealed a putative epizootic of CDV in Indian foxes, and the combination of low seroprevalence of anti-CDV antibodies in the population as a whole, but high mortality among recently exposed individuals, suggests high susceptibility and low probability of survival after exposure (Chapter 2).

THE MODEL

The model was developed in NetLogo version 5.0.2 (July 27, 2012) (Wilensky, 1999). Here we provide the overview, general concepts underlying the model's design and details as per the ODD (Overview, Design concepts, and Design) protocol for describing individual- and agent-based models (Grimm et al., 2006).

Purpose

The purpose of this model is to explore the transmission dynamics of CDV in a two-host system, comprised of an abundant dog population and a relatively low density Indian fox population in central India. Given the paucity of data, the modeling exercise was undertaken to elucidate the parameters that are most

important for disease transmission in this system, suggest potential management strategies and prioritize future research.

State variables and scales

Free-ranging domestic dogs (hereafter dogs), wild Indian foxes (hereafter foxes) and the landscape are the three entities of this model. The model landscape consists of square patches (0.04 km^2), each representing a habitat type (fallow land-grassland complex, farmlands and human habitations). Foxes occur on the fallow land-grassland complex, while dogs are associated with villages and farmlands. CDV is endemic in the dog population, and foxes are susceptible to CDV. The model dog population consists mostly of adult dogs; pups are added to the population once a year during the whelping season. Dogs and foxes are characterized by the identity of the home patch they occupy, and their disease status (susceptible, infected, and infectious). Additionally, dogs also can recover from CDV infection, and become immune for life. Foxes do not recover from CDV, and none of the foxes have immunity against CDV. Dogs are also characterized by their roaming tendencies; 'roamers' cover larger area during their daily excursions than 'non-roamers'. The time step for this model was 1 day, and a model run comprised of simulations for 20 years.

Process overview and scheduling

An overview of the processes and their daily run schedule is provided as a pseudo-code:

Set the day counter

Dogs-roam-infect

Dogs roam

If shedding virus, may transmit infection to susceptible dogs and foxes

If susceptible, may get infected

Return to own home-patch

Dog-disease-progression

Start shedding virus

Die or recover

Dogs-reproduce

Pups added to the population during whelping season

Disease-risk-dogs

Pups become susceptible

Dogs-die

Dogs die throughout the year due to other causes

Foxes-roam

If susceptible, may get infected

If shedding virus, may infect a susceptible fox

Return to own home-patch

Fox-disease-progression

Start shedding virus

Die

Foxes-die

Die from other causes

Update-fox-numbers

Dead foxes are replaced once per year

Reset the day counter after 365 days

All changes in state variables due to model processes are updated immediately.

Design concepts

Emergence: The movement of dogs and foxes, and therefore the contact pattern, as well as the transmissibility of CDV is represented by empirical rules and parameters. The patterns of CDV in the dog population and the fox population emerge from the model.

Stochasticity: The initial fox population is located with stochastic functions. The daily movements of foxes and dogs are also modeled by stochastic functions. Dogs are assigned ‘roamer’ status randomly during the setup.

Observation: The movement pattern of individuals and of the two populations (dogs and foxes) was observed to verify their respective home range sizes. During the model runs, we monitored and analyzed the proportion of susceptible, infected and recovered dogs and foxes.

Initialization

A fixed proportion of the model landscape is designated as human-modified landscape, and the remaining as fox habitat. The model is initialized by randomly assigning given number of dogs to village and farmland patches, and foxes to the fox habitat patches avoiding patches that are adjacent to patches with dogs. Initially, all dogs and foxes have their disease status as susceptible. Twenty five percent of the total dogs are randomly assigned ‘roamer’ status.

Input data

The only input data are the slider settings. This model does not use time-series inputs.

Submodels

Dogs-roam-infect: For each time step, dogs visit a random patch in and around the village, and return to their respective home-patch. In CDV endemic regions, transmission of CDV in a population is sustained via contact between recently infected and susceptible animals. Transmission of CDV can occur when an infectious dog encounters a susceptible dog or fox, either on the visited patch or on the home-patch. The probability of CDV transmission during such an encounter is set using the sliders 'dog-dog-transmissibility' and 'dog-fox-transmissibility'.

The daily movements of dogs in the model were based on the mean home-range size of 0.45 (± 0.11 SE) km^2 reported by Vanak and Gompper (2010) for dogs in the study area. We adhere to a simple definition of home range: the area used by an individual in its normal activities of food gathering, mating and caring for the young. The home range was calculated as the area of all the patches visited by an individual over a period of 1 year. The daily movements of dogs in the model landscape were modeled using a random-Poisson function and a mean daily distance travelled of 0.4 patches (0.08 km). One hundred iterations of the movement submodel simulated for 1 year yielded a mean (± SD) home-range of 0.55 (± 0.07) km^2.

Some dogs are known to travel larger distances during their daily excursions, and we term these dogs 'roamers'. Based on the data from

photographic surveys of village dogs undertaken in this region, the average daily excursions of roamers was approximately 1.2 km (Belsare, unpublished data). The daily movements of roamer dogs in the model landscape were modeled using a random-Poisson function and a mean daily distance travelled of 6 patches (1.2 km). One hundred iterations of the movement submodel simulated for 1 year yielded a mean (± SD) home-range of 4.42 (± 0.21) km^2.

Dog-disease-progression: As in most developing countries, large populations of free-ranging dogs in India are associated with human communities, and therefore ubiquitous throughout the landscape. These dog populations are interconnected due to movements of free-ranging dogs as well as human-mediated movements of dogs. A highly virulent pathogen like CDV is maintained in village dog populations by recurrent introductions of new infections from neighboring areas. Dogs start shedding the virus after a latent period of 7 days. The subsequent pathogenesis is influenced by the immune response of the individual. Dogs with strong immune responses clear the virus by 14 days post-infection, while those with poor immune response die in 3-4 weeks. Dogs with delayed immune response can shed the virus for 60-90 days post-infection, but shorter periods of virus shedding are more typical (Greene and Appel, 2006). Estimates suggest that between 25% and 75% of susceptible dogs become subclinically infected with CDV. An experimental mortality rate of around 43% has been reported for CDV in dogs (Krakowka and Koestner, 1976).

Based on field observations, we estimated the frequency of CDV introduction to be once in two months for the model dog population. Infected dogs in this model start shedding virus 7 days post-infection. We assume that 50% of the infections are subclinical, and these dogs shed the virus for an average of 30 days post-infection, then recover from the infection, stop shedding the virus and become immune. We assume 50% mortality in the clinically infected dogs. Fifty percent of the clinically infected dogs die on day 17 and the remaining recover after shedding the virus for an average of 30 days post-infection. Recovered dogs have lifelong immunity against CDV.

Dogs-reproduce: The mean whelping date (± SD) for a free-ranging dog population in Jaipur, India was distributed about 23rd November (± 58 days) (Reece et al., 2008). Similar findings have been reported for other free-ranging dog populations in India (Pal, 2001; Totton et al., 2010a). Further, free-ranging dog populations in the developing world have a high turnover rate. For example, the turnover rate of dog populations in Tunisia was found to be 30% (Matter, 1993). Based on the data from demographic surveys conducted in the study area, we estimate the dog population turnover rate at 40%. We therefore add 40% dogs to the model over a period of 4 months for every year of the model run.

Disease-risk-dogs: Pups lose the protection provided by maternally-derived antibodies between by 3 months of age, and a higher CDV prevalence rate is found in pups and juveniles between 3-6 months of age (Greene and

Appel, 2006). In the model, pups cannot be infected with CDV until 3 months of age, at which time they become susceptible.

Dogs-die: Dogs also die from other causes throughout the year. Other causes of dog mortality include motor vehicle accidents, other diseases, and killing by wolves (*C. lupus*) and humans (Belsare, unpublished data). As per the assumed turnover rate for the model dog population, 40% of dogs die throughout the year.

Foxes-roam: Each fox visits a random patch within its home-range every day of the model run, and returns to its home-patch. If a susceptible fox encounters an infectious dog or an infectious fox on the patch it has visited, or on its home-patch, it can get infected. The probability of CDV transmission during such an encounter is set using the slider 'fox-fox-transmissibility'.

Fox movements in the model landscape are based on the published home range size for Indian foxes. Vanak and Gompper (2010) reported a mean (± SD) home-range of 2.39 (± 0.31 SE) km^2 for foxes. One hundred iterations of the movement submodel simulated for 1 year, with an average daily distance travelled of 3 patches (0.6 km) modeled using a random-Poisson function, yielded a mean (± SD) home-range of 2.28 (± 0.14) km^2. Based on this calculation, foxes in the model were set to travel a mean distance of 3 patches per day.

Foxes-disease-progression: Although pathogenicity of CDV in dogs has been well-studied, little is known about CDV virulence in non-domestic canids. It is assumed that the clinical course of CDV in foxes is similar to that in dogs, except that foxes have low recovery rates from CDV. A serosurvey of foxes in the study area supports this assumption of no recovery and no immunity to CDV (Chapter 2). In the model, infected foxes begin to shed virus one week after infection and die of CDV after 21 days.

Foxes die: Apart from CDV-related mortality, foxes also die from other causes throughout the year. The fox population is assumed to have a turnover rate of 20%. Other causes of fox mortality include poaching by humans, other diseases, and predation by larger carnivores, including dogs (Vanak 2008).

Update fox numbers: Once per year (on day 358), the patches replace the foxes that have died.

PARAMETERIZATION AND SIMULATION EXPERIMENTS

Calibration

Vanak (2008) conducted a study to investigate the spatial ecology of Indian foxes in and around the GIB WLS. The study area (~51 km^2) comprised of a focal grassland-fallow land complex bordered by several villages with

farmlands radiating outward from the villages and gradually merging with the fallow land-grassland complex. The model landscape consisted of 1089 patches, representing 49 km^2 (each patch measuring 0.04 km^2), approximating the dimensions and the landcover/landuse pattern of this study area. Of the total area, 40% was assigned to human-modified landscape (villages and farmlands) and ~60% was assigned to fox habitat (grasslands, forestry plantations and fallow land).

Indian foxes occurred in the grasslands, fallow lands and forestry plantations, while dogs were associated with the villages and farmlands (Vanak and Gompper, 2010). The fox density in the model landscape was set at ~ 1.5 per km^2 (~1 fox per 17 grassland patches), and was based on reported densities of up to 1.6 per km^2 for Indian foxes (Manakadan and Rahamani, 2000). The median village dog density in this region is 719 dogs per km^2 (range 526-969) (Belsare and Gompper, 2013), while the farmland dogs occur at a density of 28 (± 3.2 SE) dogs per km^2 (Vanak et al., 2009). In the model landscape, five patches in each of the four corners represent a village, and each village patch has 28 dogs with a resultant village dog density of 700 dogs per km^2. Farmland patches in the model landscape have a dog density of 25 dogs per km^2. Based on the data from photographic surveys of village dogs undertaken in this region, around 20-25% of the dogs were ‘roamers’ with average daily excursions of around 1.2 km (Belsare, unpublished data).

There are no empirical estimates for the transmissibility parameter for CDV in dogs and foxes. An epidemiological network model built to infer CDV dynamics in the Serengeti lion population was analyzed using transmissibility values between 0 and 0.3, with values > 0.2 indicating high rates of transmissibility (Craft et al., 2009). As CDV is a highly contagious disease of domestic and wild carnivores, we use a value of 0.3 for dog-dog-transmissibility, 0.2 for dog-fox-transmissibility, and a slightly higher value of 0.25 for fox-fox-transmissibility. We assume that the disease causing contacts between foxes and dogs would include more indirect interactions like foxes investigating dog scat and urine, or sequential feeding events, as dogs are an important cause of Indian fox mortality and are avoided by foxes (Vanak, et al., 2009). Disease causing contacts between foxes would additionally include close proximity, chases, fights, mating, and sequential and simultaneous feeding events. We have not considered fox to dog transmission in this model. The ecological parameter values used in this model are listed in Table 1 and the epidemiological parameter values are listed in Table 2.

Simulation experiments

Experiments were set up using the BehaviorSpace feature in NetLogo. BehaviorSpace creates scenarios by changing the specified parameter values, and generates replicates for each scenario. For each scenario, 50 model runs were undertaken and the mean output parameter values were recorded. CDV incidence rate in the model dog population was one of the output parameter-of-

interest. Incidence rate is defined as the number of new cases of disease occurring in a population over a period divided by the sum of animal-years at risk for each individual in the population for that period, and indicates how fast a disease spreads in a population. We calculated the CDV incidence rate in dogs over 10 years (year 11 to 20 of the model run). The other output parameter-of-interest was the number of CDV spillover events between dogs (reservoir host) and foxes in a period of 10 years. We calculated the total number of CDV cases occurring in the fox population due to direct transmission from dogs between years 11 to 20 of each model run.

We first obtained output parameters for the baseline scenario, using the best estimates (as detailed above) for all input parameters. Sensitivity analysis was performed using an index that incorporates output variance in the local sensitivity analysis (Bar Massada and Carmel, 2008). The following parameters were selected for sensitivity analysis: village dog density, proportion of roamer dogs, CDV introduction frequency in dogs and dog-dog-transmissibility. For the output parameter ‘number of CDV spillover events’, we additionally included the parameter dog-fox-transmissibility for sensitivity analysis. Model parameters were increased one at a time by 20%, and mean output and standard deviation were calculated for each parameterization. The local sensitivity index around a

reference parameter value, S_t, was calculated using the following equation:

$$S_t = \frac{\left| \bar{Y}_{alt} - \bar{Y}_{ref} \right|}{(p_{alt} - p_{ref})\left((s^2_{alt} + s^2_{ref}) / n\right)^{1/2}} * p_{ref}$$

where Y_{ref} is the mean of the model output distribution generated from the reference parameter, Y_{alt} is the mean of the model output distribution generated from an altered parameter, s^2_{ref} and s^2_{alt} are the variances of these distributions, p_{ref} is the reference parameter value, p_{alt} is the altered parameter value and n is the sample size. Model parameters were ranked according to their sensitivity index value.

We estimated the basic reproductive number (R_0) for CDV in the fox population. Basic reproductive number (R_0) is defined as the expected number of secondary cases produced by a typical infectious individual in an entirely susceptible population (Heesterbeek, 2002). We ran the model with reference parameter values, but without CDV transmission from dogs (dog-fox-transmissibility set to 0). One fox was infected with CDV on the first tick, and we recorded the number of foxes infected by this infected fox (secondary cases). We averaged R_0 over 50 model runs.

We then investigated the applicability of potential disease control interventions to mitigate the disease spillover threat in the model fox population. Three potential approaches have been described for controlling diseases caused by multi-host pathogens in threatened hosts: a) Managing disease in the reservoir hosts, b) minimizing contacts between the source and the target population, and c) disease control in the target population (Woodroffe, 1999; Haydon et al., 2002).

Experiment 1: Dog vaccination: As dogs are assumed to be the reservoirs for CDV in this model, we evaluated dog vaccination as an intervention to manage CDV in the dog population. Vaccination is one mechanism of reducing the prevalence and incidence of infectious individuals in a population. Two scenarios, 50% and 100% vaccination coverage, were evaluated. We selected a scenario with 50% vaccination coverage as our work in the region indicates that it is an achievable target with reasonable effort (Belsare and Gompper, 2013). The other experimental scenario of 100% vaccination coverage is an extreme scenario, but was considered to evaluate the applicability of dog vaccination as a disease control intervention under ideal settings. Starting from the 11th year of each model run, dogs were vaccinated on the first day of every year to achieve the necessary vaccination coverage.

Experiment 2: Dog density reduction: Disease control in the target population can also be achieved by limiting the size of the reservoir host population. A smaller reservoir population would have a lower probability of

pathogen persistence, and would also result in lower contact rates with the target populations (Barlow, 1996). Scenarios with reduced village dog densities (compared to the baseline scenario) were evaluated. One approach for reducing dog densities is Animal Birth Control (ABC) programs, wherein dogs are surgically sterilized with the aim of controlling dog population size. A dog demographic model developed to understand the long term impact of ABC on dog population in Jodhpur (India), predicted a 69% decrease in dog population after 13-18 years of continuous implementation of ABC program (Totton et al., 2010b). Another approach for reducing dog densities is improved waste management (Manor and Saltz, 2004; Sobrino et al., 2009) which removes the food resources available to support dogs. Based on our interactions with the villagers in the region, a program combining these approaches could be implemented in collaboration with the local authorities. We consider two scenarios as a result of such a program: 50% and 75% reduction in village dog density. We also consider an extreme scenario with 90% reduction in village dog density.

Experiment 3: Restricting dog movements in fox habitat: Contacts between dogs and foxes can be minimized by removal of roamers and/or restricting dog movements in the fox habitat. This could be achieved by selectively trapping and removing dogs venturing into protected areas, and implementing an outreach program in villages to encourage responsible dog ownership. About half of the roamer dogs in the study villages did not have any owners or reference persons, and could be trapped and removed under such a

program (personal observations, Belsare). We therefore consider a scenario with a 50% reduction in the proportion of roaming dogs as well as an extreme situation with no roaming dogs in the model landscape.

Experiment 4: Fox vaccination: Vaccination of the target species can be employed to reduce the threat of disease spillover. Oral CDV vaccines that could be used in foxes are not available at present. The vaccination coverage achieved using appropriate parenteral CDV vaccines would be a function of the intensity of the efforts put into the disease control program. We evaluated four scenarios requiring an increasing intensity of efforts, achieving fox vaccination coverage of 25%, 50%, 75% and 100% respectively. Starting from the 11^{th} year of the model run, a given proportion of foxes were vaccinated on the first day of every year.

We compared the mean model outputs from experimental and baseline scenarios using one-way ANOVA. In cases where the null hypothesis was rejected ($p<0.05$), we used Tukey's HSD post-hoc test to determine the scenarios which resulted in significantly different outputs. For all experiments, the model output 'number of CDV spillover events' was used for comparisons between scenarios. For the first experiment, we also compared the model output 'mean CDV incidence rate in dogs' between scenarios.

RESULTS

Model testing

For model testing, we compared the epidemiological trends emergent from the model with observations from field studies and empirical knowledge. Initially the model dog population was comprised entirely of susceptible individuals. Based on estimate derived from field observations, the frequency of CDV introduction in the model dog population was set to once every two months. Thereafter the proportion of susceptible dogs in the population reduced rapidly, and after the first year > 50% of dogs in the population were in the CDV recovered category. The prevalence of CDV exposure was > 60% and remained above this level throughout the model run (Fig. 1). This trend is in agreement with the high CDV exposure rates (> 72%) documented on several occasions in the free-ranging dog populations around the GIB WLS (Belsare and Gompper, 2013; Chapter 2). This trend is also in agreement with the observation that a pathogen with a long infectious period and high transmissibility rapidly disseminates in a susceptible population (Swinton et al., 2002). The monthly prevalence rates of CDV in the model dog population were lowest during the whelping season as dogs were added to the population, and increased 3-4 months after the whelping period. Such fluctuations in CDV prevalence rates are in agreement with the observation that clinical disease is mostly seen in pups following the loss of maternal antibodies at 3-6 months in endemic areas with large dog populations (Williams, 2001).

We obtained a R_0 value of 0.85 for CDV in the model fox population, implying that CDV cannot be maintained in the fox population at a density of 1.5 foxes per km^2. This is in agreement with epidemiological theory and empirical data suggesting that wild carnivore populations, by virtue of small population size and lower population densities, are often not suitable to maintain highly pathogenic infections like CDV (Lyles and Dobson, 1993; Dye et al., 1995; Cleaveland et al., 2002) unless they form part of a broader multi-host community.

Sensitivity

The model parameters were ranked according to their local sensitivity index value (S_t) (Table 3). The CDV incidence rate in dogs was most sensitive to the proportion of roamer dogs in the dog population and to the CDV introduction frequency in dogs. The other output parameter, number of CDV spillover events, was also most strongly influenced by the proportion of roamer dogs in the dog population. Two other parameters, the dog-fox-transmissibility and the village dog density, also had some influence on the number of CDV spillover events. The frequency of CDV introduction in dogs, though important for CDV incidence rate in dogs, was relatively unimportant for the number of CDV spillover events.

Simulation experiments

Baseline scenario: The mean CDV incidence rate in dogs obtained for the baseline scenario was 0.36 per dog-month at risk, or 36 new cases of CDV per

100 susceptible dogs per month. An average of 14 CDV spillover events occurred every 10 years with the baseline scenario.

Experiment 1: Dog vaccination: The mean CDV incidence rate in dogs did not differ significantly between the baseline and experimental scenarios (F=0.47; p=0.626). With annual dog vaccination coverage of 50%, the mean CDV incidence rate in dogs was 0.35 per dog-month at risk. When all dogs in the population were vaccinated at the beginning of every year (100% annual vaccination coverage), the mean CDV incidence rate obtained was 0.35 per dog-month at risk. Thus, dog vaccination was not effective in controlling CDV in the dog population.

The difference in the mean number of CDV spillover events per 10 years between the baseline and experimental scenarios (Fig. 2) was of borderline statistical significance (F=3.133, p=0.047). With 50% annual vaccination coverage of the dog population, an average of 13 CDV spillover events per 10 years were recorded. With 100% annual vaccination coverage of the dog population, an average of 12 CDV spillover events were recorded. The difference between baseline scenario (14 spillover events) and 100% vaccination coverage was statistically significant (p=0.036). However, given the small effect size, dog vaccination appears to be an ineffective disease control intervention for mitigating the CDV spillover risks in foxes.

Experiment 2: Dog density reduction: The mean number of CDV spillover events per 10 years differed significantly between the baseline scenario of 14 spillover events and experimental scenarios with village dog density reduction ($F=17.07$, $p<0.0001$). With a 50% reduction in the village dog population, there were an average of 11 CDV spillover events per 10 years; with a 75% reduction there were an average of 10 CDV spillover events per 10 years, and this number declined to 8 per 10 year period with a 90% reduction in the village dog density (Fig. 3). The mean output differed significantly between the baseline scenario and experimental scenario with ≥50% reductions in village dog density (post-hoc tests: $p<0.005$ or lower for all pairwise comparisons).

Experiment 3: Restricting dog movements: The mean number of CDV spillover events per 10 years differed significantly between the baseline scenario and experimental scenarios with dog movement restriction ($F=56.6$, $p<0.0001$) (Fig. 4). With a 50% reduction in the proportion of roamer dogs, the average number of CDV spillover events per 10 year period declined to 11. Where no roamer dogs occurred in the model dog population the average number of CDV spillover events per 10 year period was 6, a 57% decline relative to baseline scenarios. The mean output differed significantly between the baseline scenario and each experimental scenario at levels of $p < 0.0001$ or less.

Experiment 4: Fox vaccination: The mean number of CDV spillover events per 10 years differed significantly between the baseline scenario and experimental scenarios with fox vaccination ($F=202$, $p<0.0001$) (Fig. 5). The

average number of CDV spillover events per 10 year period declined to 8 when 25% of the fox population was vaccinated annually and to 4 and 2 when 50% and 75%, respectively, of the fox population was vaccinated annually. Vaccination of 100% of the fox population every year eliminated CDV spillover. In any pairwise post-hoc comparison the mean output differed significantly between the baseline scenario and the experimental scenario ($p<0.0001$ in all cases).

DISCUSSION

Multi-host pathogens like CDV can pose a serious conservation threat when large, free-ranging domestic animal populations and populations of wild species occur together in a landscape. Management of disease threats in free-ranging populations such as dogs is challenging due to the complex ecological and epidemiological interactions of hosts and pathogen, and our limited understanding thereof. One of the strategies suggested for investigation and mitigation of disease risks is triangulation, a process of gathering scientific evidence about a system by using a combination of field studies, laboratory studies and model explorations (Plowright et al., 2008). Field studies indicate factors that can be explored in the laboratory, and also provide data to parameterize models. In turn, models generate predictions that can be tested in the field or laboratory settings. We have used a similar approach to explore the dynamics of CDV in a two-host system. Interactions of two species, dogs and foxes, both susceptible to CDV were modelled using agent-based stochastic

simulations. We defined a schematical environment representing our study area in central India, a protected grassland habitat surrounded by human-modified landscape. We used data from ecological and epidemiological studies conducted in the region to parameterize the model. From the model output we generate the following predictions: (1) local dog vaccination programs will not reduce the number of CDV spillover events, even in scenarios where access to all dogs is attained; (2) reducing dog density or dog roaming can reduce the number of spillover events, but will not fully eliminate such occurrences at the levels used in the models; (3) vaccination of foxes can eliminate CDV spillover events independent of CDV dynamics in dogs.

We have considered only two species in this CDV transmission model: foxes as the target population and dogs as the source population. As per the epidemiological theory, infectious pathogens can persist indefinitely in populations exceeding a critical community size (Bartlett, 1960). Dogs are the most common carnivore found in the study area, associated with human habitations and farmlands. Due to their free-ranging tendencies and human-mediated movements (herding dogs, villagers acquiring or abandoning dogs), dog populations in such human-dominated landscapes are epidemiologically interconnected and it is likely that the size of this metapopulation is well above the suggested critical community size necessary to maintain a morbillivirus such as CDV (Swinton et al., 2002). Wild carnivore populations generally cannot maintain pathogens like CDV that are highly pathogenic (Cleaveland et al., 2002) unless they occur at high densities or as part of a broader multi-host community.

If elimination of an infection like CDV is the objective, disease control strategies should prioritize maintenance hosts (Haydon et al., 2002). But vaccination of even 100% of dogs in the model landscape was ineffective in controlling CDV in the dog population, and therefore in mitigating CDV spillover risk in foxes. The failure of CDV vaccination in dogs as a disease control strategy could be attributed to two model assumptions. The village dog populations were assumed to be epidemiologically connected to other dog populations in the region, and thus part of a metapopulation, resulting in recurrent introductions of CDV. We also assumed a high turnover rate of the village dog populations, resulting in an annual influx of large number of susceptible dogs in the population. Both these assumptions are based on field observations, and corroborate with observations from studies conducted elsewhere (Kitala et al., 2001; Acosta-Jamett et al., 2010). Further, the CDV exposure pattern in the model dog population and the outcome of simulated dog vaccination is in agreement with the pilot vaccination experiment conducted in the study area (Chapter 3). Most of the adult dogs in the study area were immune to CDV by virtue of prior infection, and vaccination did not provide any additional benefit to such individuals and therefore to the herd immunity. Vaccination alone is never a sufficient intervention in circumstances where disease and exposure risks are high in open populations (Newbury et al., 2009).

We have considered dogs as the only maintenance population in our model. Apart from foxes, several wild carnivore species occur in the study area, including gray wolves, golden jackals (*C. aureus*), jungle cats (*Felis chaus*) and

gray mongooses (*Herpestes edwardsi*). One or more of these species could be functioning as part of a maintenance community, or as a part of the transmission link from the maintenance community to the target population (Haydon et al., 2002). In such a scenario, dog vaccination alone cannot be an effective disease control strategy if the critical community size in the absence of dogs is sufficient to maintain CDV.

When CDV was introduced into the model fox population, the virus could not be sustained independently, and the CDV pattern in foxes was a result of repeated introductions from the dogs. With $R_0 < 1$ for CDV, the model fox population was a non-percolating, non-maintenance population. Disease control measures in a non-percolating population should focus on preventing new introductions of disease (Craft et al., 2009). In the model landscape, we evaluated two disease control measures that focused on reducing the disease transmission from dogs to foxes. Experimental scenarios with smaller dog populations resulted in significant reduction in the number of CDV spillover events. Similarly, experimental scenarios with reduction in the proportion of roamers in the dog population also resulted in lesser number of CDV spillover events compared to the baseline scenario. These interventions could be combined in a disease control program to achieve better results.

Vaccination of foxes was the most effective intervention resulting in significant decrease in the number of CDV spillover events. Unfortunately, such an intervention has to be considered in the light of several practical constraints.

The availability of suitable vaccines is an important issue. Modified Live Virus (MLV) vaccines are the most commonly available CDV vaccines, but these are attenuated for use in dogs and should be used in other species only with the recognition that MLV vaccines can induce the disease in species in which they have not been tested (Montali et al., 1983; Day et al., 2010). In contrast, Vectored Recombinant (rCDV) vaccines are safe, and are often used and recommended for susceptible wild species, but are not available everywhere.

Another practical constraint would be the capturing and handling of wild species for the purpose of vaccination, as oral CDV vaccines are not available at present. Further, such interventions are expensive in the long run as spillover of CDV from dogs would be a perpetual threat and vaccination would have to be carried out every year. For the Indian fox, which is widely distributed and considered a relatively common carnivore in India, such an intervention need not be considered at present. However, such interventions have been conducted for other spillover scenarios (see e.g. Knobel et al., 2008 for rabies; López et al., 2009 for feline leukemia virus; Pusey et al. 2008 for polio). If CDV threatens an endangered species, vaccination of the threatened host should be considered. In India, threatened and near threatened wild carnivore species like lions (*Panthera leo*), tiger (*Panthera tigris*), and leopards (*Panthera pardus*) are not restricted to protected areas and often occur in human-dominated landscapes, coming in contact with dogs. Therefore a substantial risk exists for CDV spillover in these species. An important point to note here is that disease control measures like vaccination directed at dogs are unlikely to be effective, given the size and

turnover of dog populations, and the pattern of CDV in the dog populations. We reemphasize this point in the light of a recent advisory issued by the National Tiger Conservation Authority to vaccinate dogs around protected areas to prevent CDV transmission in tigers (http://projecttiger.nic.in/whtsnew/CVD.pdf).

Like for all models, the results are dependent on the simplifying assumptions. First, we have assumed that the dog population is stable. We have based this model on our study area around GIB WLS in central India. It is known that the size of dog population depends on the size of human population (Gompper, 2013). Based on the information provided by the village heads, the human population in our study area has not changed appreciably between 2001 and 2011; we therefore assume a stable dog population. We have also assumed a stable fox population in the model landscape. But habitat fragmentation and anthropogenic activities in the region could be negatively influencing the fox population, and therefore invalidate the assumption of a stable fox population. Further, the pathogen spreading interactions in this model depend primarily upon the movement of dogs and foxes. While modeling the movements of dogs and foxes in this model, we have not considered possible variations in the movement patterns of these animals which could result from behavioral changes after infection, juvenile dispersal, or temporal variations in movement during the breeding season.

Sensitivity analyses of this model indicate that the proportion of the dog population comprised of roamers has a strong influence on the CDV incidence

rate in dogs and on the number of CDV spillover events. We have defined roamer dogs as dogs that travel larger distances during their daily excursions, and therefore can potentially play an important role in transmission of the virus to other susceptible dogs or foxes. The estimated proportion of roamer dogs was based on preliminary observations from a demographic study undertaken in the study area. A better estimate of the proportion of roamer dogs or dogs regularly venturing in fox habitat can be obtained by using camera-based, telemetry-based, or observer-derived surveys. Similarly, cohort studies undertaken in village dog populations can contribute to a better understanding of CDV epidemiology, specifically the age at which dogs get infected and the duration for which infected dogs shed the virus. Dogs can be genetically assayed for viral shedding by assessment of nasal and conjunctival swabs. Such a study could also be supplemented by a pup vaccination experiment to better understand the population effects of vaccination intervention.

In conclusion, vaccination of local dog populations seems ineffective as a disease control strategy for CDV. In contrast, smaller village dog populations potentially result in lower contact rates between dogs and foxes, and therefore fewer spillover events, while wildlife vaccination virtually eliminates the spillover risk to the target population. Disease control programs should therefore have a strong component of public outreach, emphasizing responsible dog ownership. For example, animal birth control (ABC) programs implemented in and around areas of conservation concern, in combination with restrictions to the movements of dogs in habitats occupied by species of conservation concern would result in a

decrease in spillover events. From a research perspective, further epidemiological study should be undertaken to better understand the dynamics of CDV in natural systems to inform such models. As most adult dogs in such settings are immune to enzootic pathogens like CDV due to early natural exposure, pup vaccination rather than adult dog vaccination should be evaluated as a potential disease control intervention. While our model is specific to evaluation of potential disease control interventions to mitigate CDV spillover threat in Indian foxes in a grassland habitat in central India, the modeling approach we have used in this paper could be applied to other pathogens as well as other species of conservation concern to better understand the disease threats, and to identify potential disease management interventions.

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Table 1. Ecological parameter values used to model CDV dynamics in and around the GIB WLS involving two hosts, dogs and foxes.

Parameter	Value	Reference
Landscape		
Human-modified landscape	40% of the simulated landscape	Vanak and Gompper, 2010
(Villages and farmlands)	60% of the simulated landscape	
Fox habitat		
Dog demographics		
Village dog density	700 per km^2	Belsare and Gompper, 2013
Farmland dog density	25 per km^2	Vanak et al., 2009
Dog population turnover	40%	Belsare, unpublished data
Fox demographics		
Fox density	~ 1.5 per km^2	Manakadan and Rahamani, 2002
Fox population turnover	20%	Vanak, personal communication
Daily movements		
Dogs	0.08 km	Calibrated from mean home-range sizes reported by Vanak and Gompper, 2010 and Belsare, unpublished data
Roamer dogs	1.2 km	
Foxes	0.6 km	

Table 2. Epidemiological parameter values used to model CDV dynamics in and around the GIB WLS involving two hosts, dogs and foxes.

Parameter	Value	Reference
Frequency of CDV introduction in the dog population	Once every 60 days	Belsare, unpublished data
Latent period in dogs and foxes	7 days	Appel and Greene, 2006
Viral shedding in dogs	Average of 20 days	Appel and Greene, 2006
Viral shedding in foxes	14 days	Williams, 2001
dog-dog-transmissibility	0.30	Based on the range of transmissibility values used by Craft et al., 2009
dog-fox-transmissibility	0.20	
fox-fox-transmissibility	0.25	

Table 3. Local Sensitivity Index values (S_t) of the two output parameters, CDV incidence rate in dogs and number of CDV spillover events.

Output parameter	Parameter	S_t
CDV incidence rate in dogs	proportion of roamer dogs	9.37
	CDV introduction frequency in dogs	8.39
	dog-dog-transmissibility	3.49
	village dog density	1.49
Number of CDV spillover events	proportion of roamer dogs	9.11
	dog-fox-transmissibility	6.01
	village dog density	4.42
	dog-dog-transmissibility	3.66
	CDV introduction frequency in dogs	0.95

Figure 1. Proportion of CDV exposed dogs in the population during the model run. CDV exposure rates are high in the model dog population (>50%) after year one of the model run, and oscillate between a relatively lower exposure rate during the whelping season and a high exposure rate 3-4 months after the whelping period.

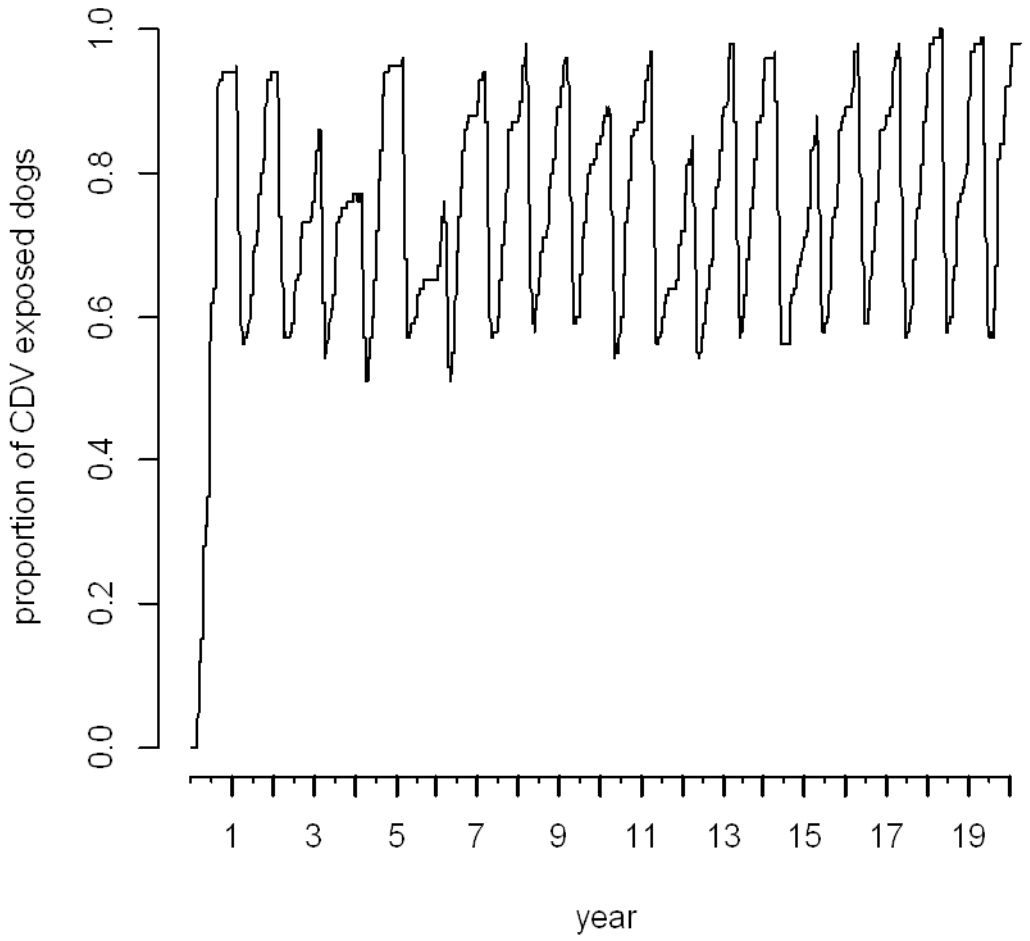
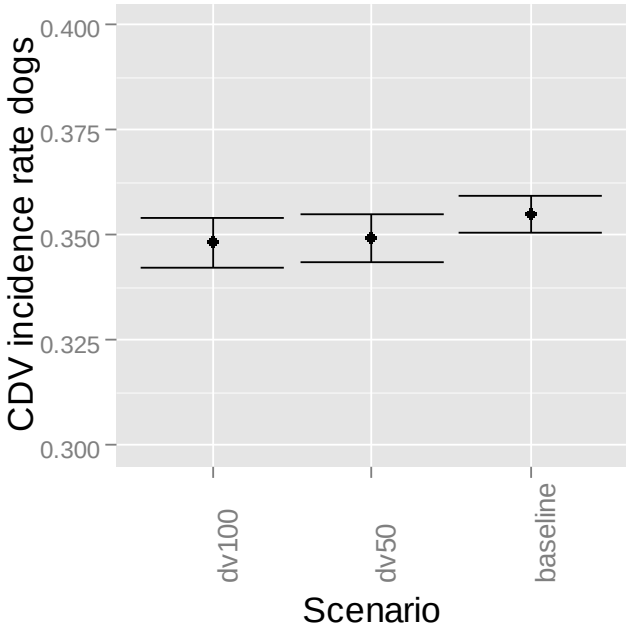


Figure 2. Mean output parameters (CDV incidence rate in dogs and number of spillover events) with error bars for two scenarios of dog vaccination coverage [50% vaccination coverage (dv50), 100% vaccination coverage (dv100)] and the baseline scenario.



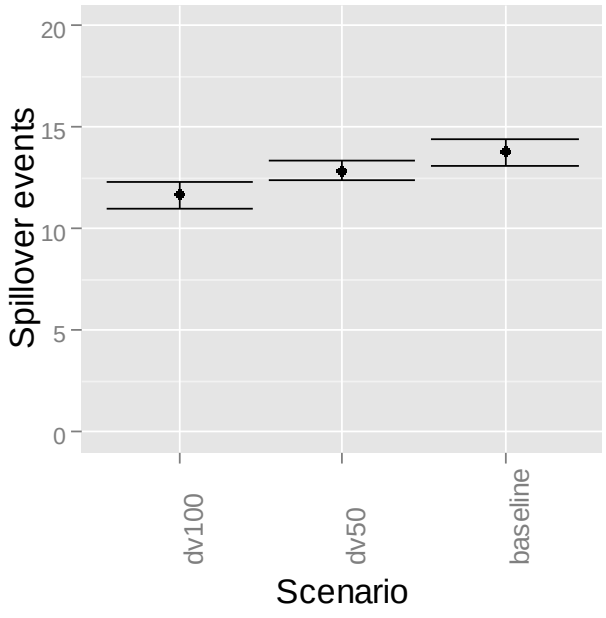


Figure 3. Mean output parameter (number of spillover events) with error bars for three scenarios of village dog density reduction [50% reduction (vdd350), 75% reduction (vdd175), 90% reduction (vdd70)] and the baseline scenario.

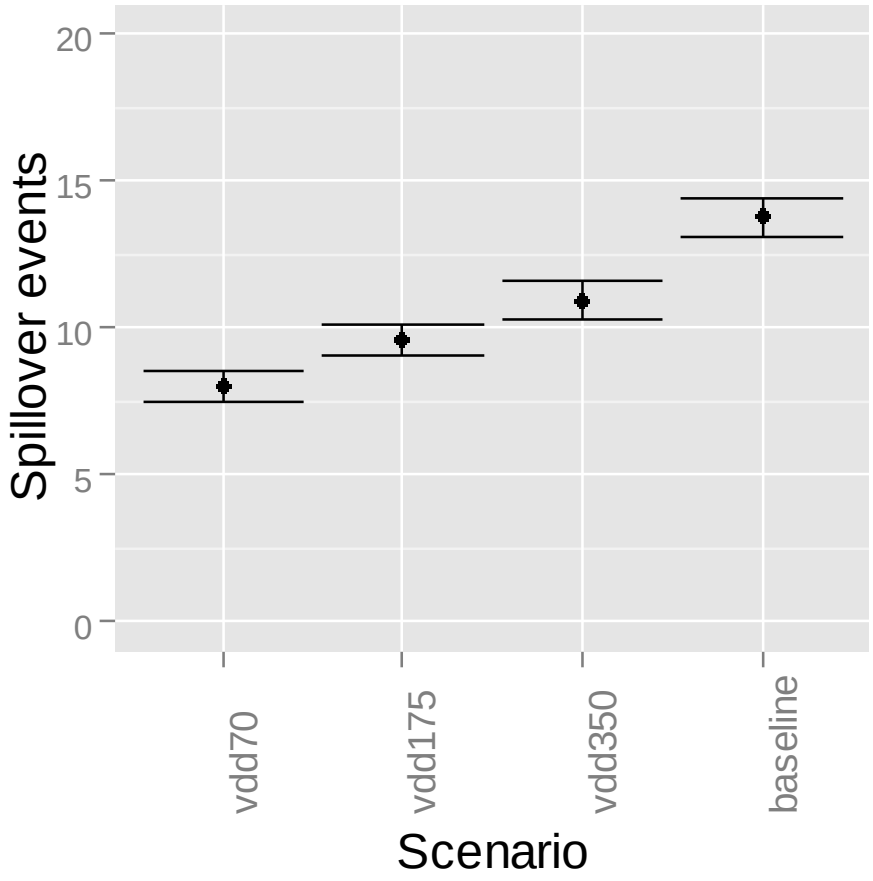


Figure 4. Mean output parameter (number of spillover events) with error bars for two scenarios of reduced proportion of roamer dogs [50% reduction (50%rd), no roamer dogs (0rd)] and the baseline scenario.

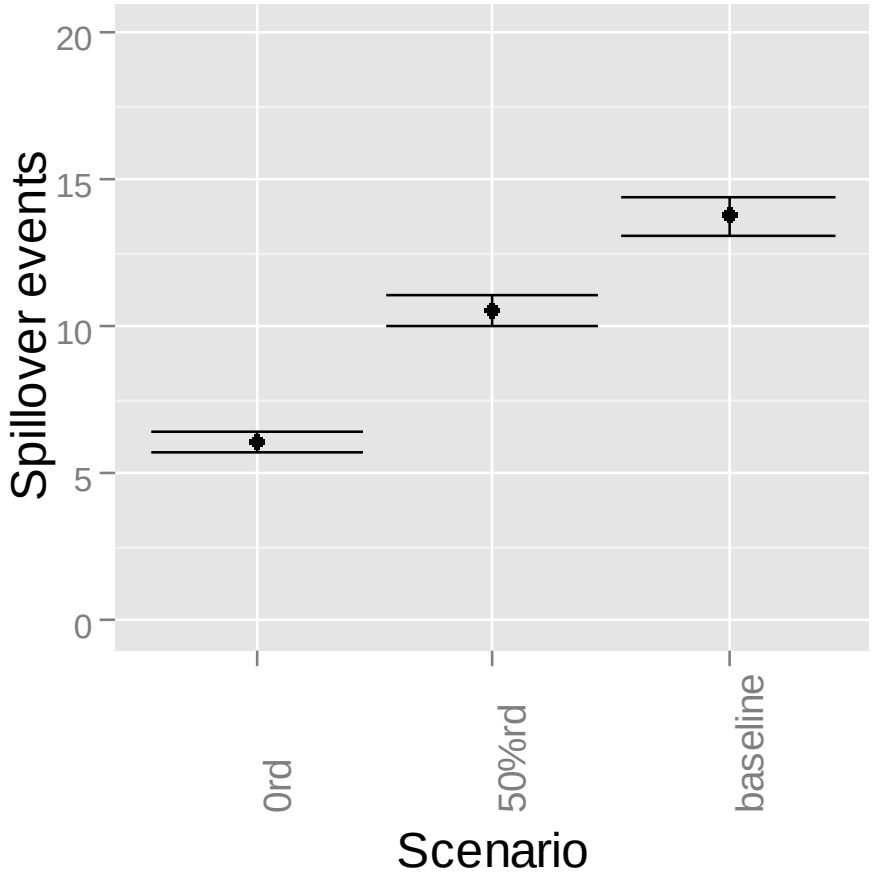
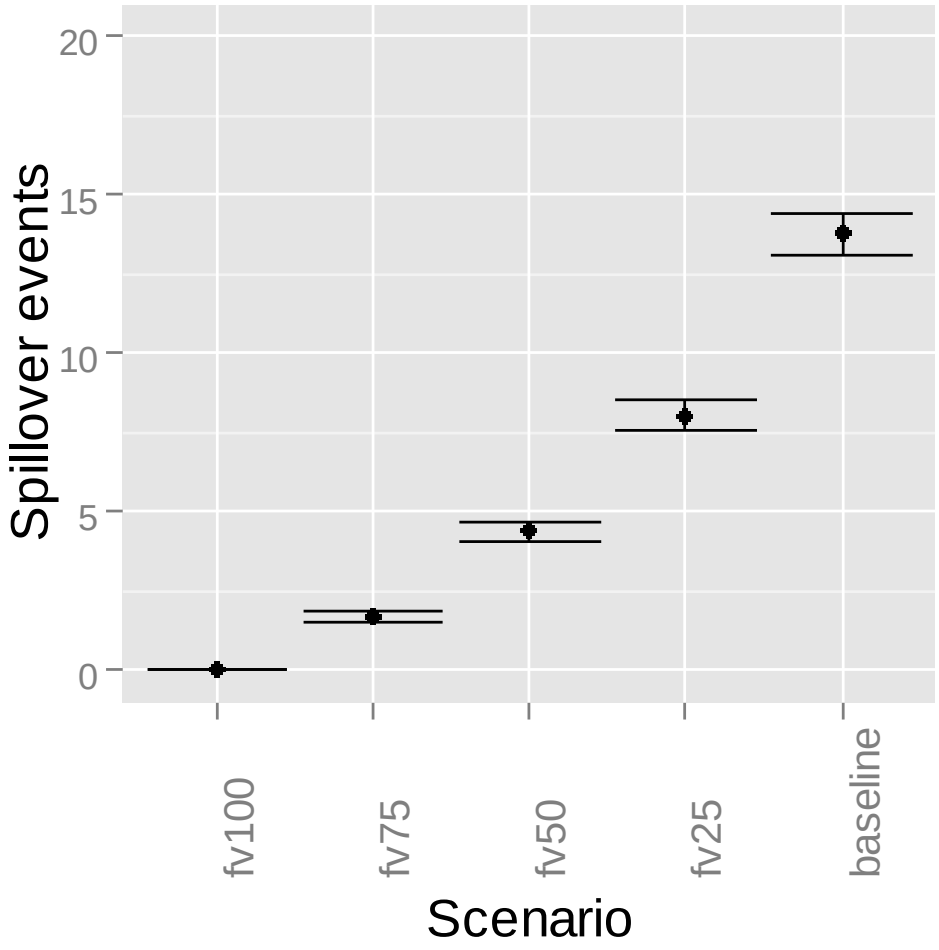


Figure 5. Mean output parameter (number of spillover events) with error bars for four scenarios of fox vaccination coverage [25% vaccination coverage (fv25), 50% vaccination coverage (fv50), 75% vaccination coverage (fv75), 100% vaccination coverage (fv100)] and the baseline scenario.



SYNOPSIS

The two main objectives of this research were 1) to assess the conservation threats posed by pathogens of free-ranging domestic dogs; and 2) to contrast potential disease management interventions in free-ranging dog populations. Demographic surveys indicated that dogs occurred at high densities in the study villages (> 526 dogs per km^2), and the village dog populations were comprised principally of adult animals. Epidemiological surveys indicated that dogs were exposed to three pathogens of conservation concern, viz. canine parvovirus (CPV), canine distemper virus (CDV), and canine adenovirus (CAV). The exposure rates of adult dogs were significantly higher than those of juveniles, suggesting that the pathogens actively circulate in the study populations and a continued potential exists for initial or repeated exposure. Such large populations of free-ranging dogs therefore pose a significant threat to susceptible wild carnivore species in the region. The finding that foxes with ongoing or recent CDV infection had a high mortality rate further supports this conclusion.

The high exposure rates of adult dogs also indicated survival following early natural exposure to these pathogens. Evidence of exposure to all three pathogens was documented in juvenile dogs, suggesting that such natural exposure occurs at an early age. Dogs that recover from natural infection due to CPV, CDV or CAV are known to develop a lifelong immunity to these pathogens.

Collectively the findings suggest that most adult dogs in the study populations are immune to pathogens like CPV, CDV and CAV, and play no current or future role in the maintenance or transmission of these pathogens. This could have important implications for dog disease control programs, especially in settings where dog populations are large and free-ranging, and pathogens like CPV, CDV and CAV are enzootic.

Canine vaccination has been recommended as a mechanism for reducing the prevalence and incidence of viral diseases in dogs, and also used as a tool for conservation management of wild carnivore populations. But the rationale for vaccinating adult dogs in such settings is questionable as a large proportion of adult dogs in the population are already protected due to seroconversion following early natural infection. Additional vaccination of these dogs will not improve their collective immune status, nor contribute to the herd immunity. Results of the vaccination experiment undertaken along with the epidemiological surveys supported this conclusion. Vaccination failed to increase the proportion of adult dogs with protective antibodies against CPV, CDV or CAV in the treatment group (dogs immunized against CPV, CDV and CAV) compared to the control group, as much of the effort was put into vaccinating dogs that were already antibody positive. A simple, deterministic model indicated that in settings where the natural exposure rates to pathogens like CPV, CDV or CAV are high, even high levels of vaccination contributed relatively little additional benefit. For instance, in these study populations, the model indicated that only 5-10% of the vaccinated dogs actually benefitted from the mass vaccination program for each

of the pathogens. Such vaccination programs therefore seem unnecessary, and would result in an escalation of the cost-benefit ratio of dog disease control programs as well as conservation management programs. These findings underscore the importance of investigating the population pattern of pathogen exposure before planning mass vaccination programs for free-ranging dog populations. It is likely that similar conclusions could be drawn for other domestic animal taxa as well.

Further research specifically investigating epidemiological features of pathogens in free-ranging dog populations, like the age at which dogs get infected and the duration for which the infected dogs shed the pathogens, could help in identifying appropriate disease management. For instance, such data could indicate the suitability of pup vaccination as a disease control intervention in free-ranging dog populations. Nevertheless, managers will have to deal with substantial uncertainty about the disease dynamics, mostly due to the complex ecological and epidemiological interactions of pathogens and their free-ranging hosts. This work highlights the use of an approach combining data from ecological and epidemiological studies with model explorations to deal with this uncertainty, understand the disease threats, and identify potential disease management interventions.

APPENDIX

The code for dog-fox-CDV transmission model formulated in NetLogo:

```
globals
[
  fox-carcap
  village-size
  dog-population
  inf-foxes
  fox0 ;(ini-foxpop)
  final-foxpop
  ro
  cumscc
  cuminff
  d                    ;(counter that resets every 365 days)
  td                   ;(counter that resets every 30 days)
  pre
  m
  s
  prosus
  prorec
  infdog0
  dog0
  CDVdogincrate
  CDVfoxincrate
  cdd
  cumsdc
  cumsdcf
  inffox0
  year
```

```
  famCDV                    ;(fox annual mortality due to CDV)
  month
  infdog0m
  infdog0c
  yinfdog0
  yinffox0
  ycumsdc
  ycumsdcf
  CDVincratedog
  CDVincratefox
  spoeve
  probspo
  soe                       ;(spillover event)
  ddd
]

breed [dogs dog]
breed [foxes fox]

dogs-own
[
  home-patch
  susceptible?
  infected?
  infectious?
  recovered?
  ip
  ddt
  iddt
  scc
  rd?
  sdc                       ;(susceptible-days-counter)
]
```

```
foxes-own
[
  home-patch
  susceptible?
  infected?
  infectious?
  immune?
  scc                    ;(secondary case counter)
  lid                    ;(counter for disease progression in foxes)
  sdc                    ;(susceptible-days-counter)
]

to setup
  clear-all
  setup-landscape
  reset-ticks
end


to setup-landscape
set village-size 5
  ask (patch-set patch -14 14 patch -14 -14 patch 14 -14 patch 14 14) [
    ask n-of village-size patches in-radius 2 [
      set pcolor red
      ask patches in-radius 2 with [pcolor = black] [
        set pcolor brown + 1
      ]
    ]
    ask patches in-radius 9 with [pcolor = black] [
      if (random-float 100 < 21) [
        set pcolor green
      ]
    ]
```

```
 ]

ask patches with [pcolor = red] [
   sprout-dogs round (village-dog-density * 0.04) [
                                   ;(700 per km sq~28 per patch, 350 per sq km ~ 14 per
                                   patch)
     set home-patch patch-here
     set shape "dog"
     set size 1.5
     set color orange
     set ip round (17 + random-normal 13 (13 / 4))
     set infected? false
     set infectious? false
     set susceptible? true
     set recovered? false
     set ddt 1
     ifelse (random 100 < prop-roam)
     [set rd? true]
     [set rd? false]
     ]
   ]

ask patches with [pcolor = green] [
   if (random-float 100 < 50) [
     sprout-dogs 2 [
       set home-patch patch-here
       set shape "dog"
       set size 1.5
       set color orange
     set ip round (17 + random-normal 13 (13 / 4))
     set infected? false
     set infectious? false
     set susceptible? true
```

```
      set recovered? false
      set ddt 1
      ifelse (random 100 < prop-roam)
      [set rd? true]
      [set rd? false]
      ]
    ]
  ]

set dog-population count dogs

set fox-carcap round (count patches with [pcolor = black] / 16.6)
                                    ;(fox density 1.5 per km sq)
   ask n-of (fox-carcap / 2) patches with [pcolor = black] [
     ifelse (count dogs-on neighbors = 0)
     [set pcolor yellow
       sprout-foxes 2 [
         set home-patch patch-here
         set shape "wolf 7"
         set color brown
         set susceptible? true
         set infected? false
         set infectious? false
         set immune? false
         set lid 0
         set scc 0
       ]
     ]
     [ask one-of neighbors [
         if (pcolor = black) and (count dogs-on neighbors = 0) [
           set pcolor yellow
           sprout-foxes 2 [
             set home-patch patch-here
```

```
            set shape "wolf 7"
            set color brown
            set susceptible? true
            set infected? false
            set infectious? false
            set immune? false
            set lid 0
            set scc 0
          ]
        ]
    ]
    ]
  ]
end

to go
  if (ticks = 7301) [
    stop
  ]
  if count foxes < 1 [
    print "foxes extinct"
  ]
  if (ticks > 3649) [
    if (d = 0) [
      ask n-of (vaccdog * count dogs) dogs [
        if (susceptible?) [
          set susceptible? false
          set recovered? true
          set ddt 5000
        ]
      ]
      ask n-of (vaccfox * count foxes) foxes [
        if (susceptible?) [
```

```
          set susceptible? false
          set immune? true
          set cumsdcf (cumsdcf + sdc)
        ]
      ]
    ]
  ]

  set d (remainder (ticks) 365)
  set td (remainder (d) 30)

if ((remainder (d) freq-cdv) = 0) [
    if (count dogs with [susceptible?] > 0) [
      ask n-of 1 dogs with [susceptible?] [
        set susceptible? false
        set cumsdc (cumsdc + sdc)
        set infected? true
        set color blue
        set ddt 4000
        set iddt 1
        set infdog0 (infdog0 + 1)
        ]
      ]
  ]

  if (d = 0) [
    set year (year + 1)
    set infdog0 0
    set infdog0m 0
    set inffox0 0
    set dog0 count dogs
    set fox0 count foxes
    set cumsdc 0
```

```
  set cumsdcf 0
  set famCDV 0
  ]

if (d = 364) [
  set CDVdogincrate precision (infdog0 / (cumsdc / 30)) 2
                                ;(annual CDV incidence rate in dogs)
  if (cumsdcf > 0) [
    set CDVfoxincrate precision (inffox0 / (cumsdcf / 30)) 2
                                ; (annual CDV incidence rate in foxes)
  ]
  ;file-open "CDVincidencerateannualdogs.txt"
  ;file-write CDVdogincrate
  ;file-close
  ;file-open "CDVincidencerateannualfoxes.txt"
  ;file-write CDVfoxincrate
  ;file-close
  ]

;if (td = 0) [
  ;set infdog0c infdog0
  ;set month (month + 1)
  ;if (month > 12) [
    ;set month 0
 ;]
  ;]
;if (td = 29) [
  ;ifelse (month = 1)
  ;[set infdog0m infdog0]
  ;[set infdog0m (infdog0 - infdog0c)]
  ;print infdog0
  ;print infdog0m
  ;set pvd precision (count dogs with [ddt > 3999] / count dogs) 2
```

```
    ;file-open "CDVmonthwiseprevalence dogs.txt"
    ;file-write month file-write pvd
    ;file-close
    ;file-open "epizooticcurve.txt"
    ;file-write month file-write infdog0m
    ;file-close
  ;]

 if (ticks > 4013) [              ;(for recording inc rate between 11-20 years use
ticks>4013)
    if (d = 364) [
    if (inffox0 > 0) [
      set spoeve (spoeve + 1)
    ]
    set yinfdog0 (yinfdog0 + infdog0)
    set ycumsdc (ycumsdc + cumsdc)
    set yinffox0 (yinffox0 + inffox0)
    set ycumsdcf (ycumsdcf + cumsdcf)
  ]
  ]

if (ticks = 7300) [
    set CDVincratedog (yinfdog0 / (ycumsdc / 30))
    set CDVincratefox (yinffox0 / (ycumsdcf / 30))
    set probspo (spoeve / 10)
  ]

set prosus (count dogs with [susceptible?] / count dogs)
set prorec (count dogs with [recovered?] / count dogs)

 ask dogs [
    disease-risk
    dogs-roam-infect
```

```
  dogs-reproduce
  dogs-die
  ]

ask dogs with [ddt = 4000] [
  dog-disease-progression
]

ask dogs with [susceptible?] [
  if (d = 0) [
    set sdc 1
  ]
  if (d > 0) [
    set sdc (sdc + 1)
  ]
  if (d = 364) [
    set cumsdc (cumsdc + sdc)
  ]
]

ask foxes [
  foxes-roam
  foxes-die
]
ask foxes with [susceptible?] [
  if (d = 0) [
    set sdc 1
  ]
  if (d > 0) [
    set sdc (sdc + 1)
  ]
  if (d = 364) [
    set cumsdcf (cumsdcf + sdc)
```

```
      ]
  ]
   ask foxes with [infected?] [
    fox-disease-progression ;(infectious-phase)
  ]
  ask patches [
    update-fox-numbers
  ]
  let x (count foxes with [lid = 1])
  set inf-foxes (inf-foxes + x)
   if (count foxes > 0) [
    set pre (count foxes with [infected?] / count foxes)
  ]
  ;file-open "proprecdogs.txt"
  ;file-write precision (prorec) 2
  ;file-close
  tick
end

to disease-risk                    ;(dogs procedure)
 if (ddt < 3999) [
    set ddt ddt + 1
  ]

if (ddt = 0) [
      set susceptible? true
      set sdc 1
      ifelse (random 100 < prop-roam)
      [set rd? true]
      [set rd? false]
    ]
end
```

```
to dogs-roam-infect            ;(dogs procedure)
  pd
  rt random 360
  ifelse (not rd?)
  [fd round random-poisson .2]
  [fd round random-poisson 3]
  if (infectious?) [
    dog-infect-foxes
    dog-infect-dogs
  ]

  if (susceptible?) [
    dog-gets-infected
  ]
  pen-erase
  move-to home-patch

  if (infectious?) [
    dog-infect-foxes
    dog-infect-dogs
  ]

  if (susceptible?) [
    dog-gets-infected
  ]
end

to dog-infect-foxes
  ask foxes in-radius .5 [
    if (susceptible?) and (random-float 1 < dog-fox-transmissibility) [
      set susceptible? false
      set cumsdcf (cumsdcf + sdc)
      set infected? true
```

```
      set inffox0 (inffox0 + 1)
      set color red
      if (ticks > 3650) [
        set soe (soe + 1)
      ]
    ]
  ]
end

to dog-infect-dogs
  ask dogs in-radius .5[
      if (susceptible?) and (random-float 1 < dog-dog-transmissibility) [
        set susceptible? false
        set cumsdc (cumsdc + sdc)
        set infected? true
        set color blue
        set ddt 4000
        set iddt 1
        set infdog0 (infdog0 + 1)
        ]
      ]
end

to dog-gets-infected
  if any? dogs with [infectious?] in-radius .5[
      if (random-float 1 < dog-dog-transmissibility)[
        set susceptible? false
        set cumsdc (cumsdc + sdc)
        set infected? true
        set color blue
        set ddt 4000
        set iddt 1
        set infdog0 (infdog0 + 1)
```

```
      ]
    ]
end

to dog-disease-progression ;(dogs procedure)
  set iddt iddt + 1
  if (iddt = 7) [
    set infectious? true
    set color red
    ]
  if (iddt = 17) [
    if (random 100 < 25) [
      ;set cdm cdm + 1
      die
      ]
  ]
  if (iddt = ip) [
    set infected? false
    set infectious? false
    set recovered? true
    set susceptible? false
    set ddt 5000
    set color white
    set iddt 0
    ]
  end

to dogs-reproduce
  if (d > 30) and (d < 150) [
    ifelse (m < (dog-population * 0.4))
    [let avbirths ((dog-population * 0.4) / 120)
      let abt (random-normal avbirths avbirths / 4)
      if (random 100 < abt) [
```

```
        hatch 1 [
          set home-patch patch-here
          set shape "dog"
          set size 1.5
          set color orange
          set ip round (17 + random-normal 13 (13 / 4))
          set infected? false
          set infectious? false
          set recovered? false
          set susceptible? false
          set ddt random -90
          ]
        set m m + 1
        ]
      ]
    [stop]
    ]
  if (d = 151) [
    set m 0
    ]
 end

to dogs-die
  if (d = 364) [
    set s 0
  ]
  let mdog (1 / ((dog-population * 0.4) / 365))
  let dd (remainder (d) mdog)
  set ddd (ddd + dd)
  if (ddd > 1) [;if (dd > 1) [
    ifelse (s < (count dogs * 0.4))
    [if (random (count dogs) < 2)
      [set s s + 1
```

```
        if (susceptible?) [
          set cumsdc (cumsdc + sdc)
          ]
        die
        set ddd (ddd - 1)
      ]
    ]
    [stop]
  ]
end

to foxes-die
  let mfox 1 / ((fox-carcap * 0.2) / 365) ; (fox population turnover)
  let dd round (remainder (d) mfox)
  if (dd = 1) [
    if (random (count foxes) < 1)[
      ifelse (infected?)
      [set famCDV (famCDV + 1)
        set cumscc cumscc + scc
        set cuminff cuminff + 1
        if (cuminff = 1) [
        set ro (cumscc / cuminff)
        die]
    ]
      [set cumsdcf (cumsdcf + sdc)
        die]
    ]
  ]

  ask patches with [pcolor = yellow] [
    let fhere count foxes-here
    if (fhere = 0) [
      set pcolor black
```

```
    ]
  ]
end

to update-fox-numbers
  if (d = 358) [
    let fr (fox-carcap - count foxes)
    ask n-of round (fr / 2) patches [
      if (pcolor = black) and (count dogs-on neighbors = 0)
      [set pcolor yellow
        sprout-foxes 2 [
          set home-patch patch-here
          set shape "wolf 7"
          set color brown
          set susceptible? true
          set infected? false
          set infectious? false
          set immune? false
          set lid 0
          set scc 0
          set sdc 1
        ]
      ]
    ]
  ]
end

to fox-disease-progression          ;(infectious-phase foxes)
  set lid (lid + 1)
  if (lid = 7) [
    set infectious? true
    set color green
  ]
```

```
  if (lid = 21) [
    set cumscc cumscc + scc
    set cuminff cuminff + 1
    if (cuminff = 1) [
      set ro (cumscc / cuminff)
    ]
    ifelse (random-float 100 < (immune-foxes))
    [set infected? false
      set infectious? false
      set immune? true
      set scc 0]
    [set famCDV (famCDV + 1)
      die]
  ]
end

to foxes-roam
  pd
  rt random 360 fd round (random-poisson 1.5)
  let ddogs dogs in-radius .5 with [infectious?]
  if (susceptible?) and (any? ddogs) [
    fox-gets-infected
    ]
  if (infectious?)[
    fox-infects-foxes
  ]
  pen-erase
  move-to home-patch
  if (susceptible?) and (any? ddogs) [
    fox-gets-infected
    ]
   if (infectious?)[
    fox-infects-foxes
```

```
  ]
end

to fox-gets-infected
  if (random-float 1 < dog-fox-transmissibility) [
      set susceptible? false
      set cumsdcf (cumsdcf + sdc)
      set infected? true
      set inffox0 (inffox0 + 1)
      set color red
      if (ticks > 3650) [
        set soe (soe + 1)
        ]
      ]
end

to fox-infects-foxes
  let inff count foxes in-radius .5 with [infected?]
    ask foxes in-radius .5 [
      if (susceptible?) and (random-float 1 < fox-fox-transmissibility)[
        set susceptible? false
        set cumsdcf (cumsdcf + sdc)
        set infected? true
        set inffox0 (inffox0 + 1)
        set color red
      ]
    ]
    let pif count foxes in-radius .5 with [infected?]
    set scc (scc + (pif - inff))
end
```

VITA

Aniruddha Belsare obtained his B.V.Sc & A.H. from the Bombay Veterinary College in 1996. He worked as a small animal veterinarian in India until 2008. Aniruddha also worked as a zoo veterinarian in the Pune Zoo (2001-2004), and as a wildlife veterinarian associated with several wildlife research projects in India. During the course of these projects, he developed an interest in disease ecology. Aniruddha began his doctoral work in 2008 and will continue to work as a Postdoctoral Associate at the University of Missouri.