Survey of Disease Prevalence in Free-Ranging Domestic Dogs and Possible Spill-Over Risk for Wildlife

A Case Study from the Great Indian Bustard Sanctuary, Maharashtra state - India

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Executive Summary

Domestic dogs are the world's most common carnivore, and yet the ecology of free-ranging dogs has rarely been investigated. Many dog populations are subsidized by humans resulting in high population density, which presumably also have contact rates above the critical threshold that allows diseases to persist enzootically within the population. Domestic dogs are the reservoir of infectious diseases that have led to epidemics of rabies, canine distemper and canine parvovirus in several wild carnivore species, yet little is known about the disease ecology of domestic dogs in India, and the potential for spill-over risk to wildlife. This project was carried out to assess the disease exposure and health status of free-ranging domestic dogs in the vicinity of a wildlife reserve in central India. We carried out a sero-prevalence survey of canine distemper virus (CDV), canine parvovirus (CPV) and rabies in the dog population surrounding the Great Indian Bustard Sanctuary in Nannaj, Maharashtra. We sampled 74 dogs from four villages surrounding the wildlife reserve and obtained blood samples for testing for IgM and IgG antibodies to CDV and CPV. We also collected salivary swabs from 31 dogs to test for rabies antigen. Antibodies to CPV were detected in 93.3% of the sampled dogs, while 90.7% of dogs had evidence for recent or past exposure to CDV. This suggests that these diseases are enzootic in the dog population, and may pose a significant threat to wild carnivores if disease control measures are not implemented. Rabies was also detected in 2 (6%) of the dogs tested, raising human health concerns as well. We recommend a comprehensive vaccination and dog population control policy as well as an education campaign on responsible dog ownership among villagers.

INTRODUCTION

The world's most common carnivore is not typically viewed as a wildlife species. Rather, it is a commensal of humans: the domestic dog (*Canis familiaris*). Dogs have been introduced wherever humans have settled and when not properly managed these animals thrive in human-dominated ecosystems (Daniels & Bekoff 1989, WHO/WSPA 1990, Wandeler et al. 1993). Domestic carnivores and, dogs, in particular, are the known or suspected reservoir of infectious diseases that have led to epidemics in a variety of wild carnivore species such as African wild dogs, lions, wolves, and seals (Creel & Creel 1998, Sillero-Zubiri et al. 1996, Roelke-Parker et al.1996, Funk et al. 2001). Domestic dogs act as ideal reservoirs of diseases that may potentially infect wild carnivores; they may travel large distances into wildlife habitats, and in some areas high population densities of feral and/or unvaccinated animals allow even very virulent pathogens to persist in broader carnivore populations. Despite this, investigations of disease incidence in domestic dogs and likelihood of spill-over to wild carnivores have not been extensively carried out, and therefore risks to wildlife are largely unknown.

In much of the developing world, and particularly in India, dog populations in rural areas are large and free-ranging. That is, while they may be owned by individuals or affiliated with specific human habitations, much of their daily activity pattern involves free-ranging behaviour, and during the course of this ranging these animals have numerous opportunities to interact with wildlife at multiple levels, including as as predators, prey, and competitors (Kruuk & Snell 1981, Boitani et al. 1995, Butler & Bingham 2000, Butler & DuToit 2002, Butler et al. 2004). Furthermore, dogs may accompany people when they move through wildlife habitat while hunting or herding livestock (Fiorello et al. 2006, Vanak et al. personal observation; Fig. 1). As most wildlife reserves in India are surrounded by human habitation (Fig. 2) with livestock-based economies, the frequency and intensity of interactions between dogs and wild carnivores is likely to be high.

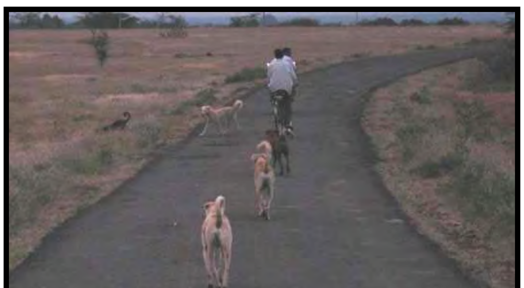


Fig.1. A pack of dogs heads off into the reserve from a village road

In general there has been a lack of research on infectious diseases of wildlife in India. Nonetheless, it is likely that several populations of endangered carnivores have suffered severe population declines due to disease spill-over without the problem being identified. For example, the Indian fox

(*Vulpes bengalensis*) has been known to undergo large population fluctuations, and though disease has been suspected it has never been properly investigated (Manakadan and Rahmani 2000). Similarily, wolves which attack humans and livestock are often found to be rabid, with such infection likely the result of wolf contact with infected dogs (A. V. Belsare, personal observation). Even in the absence of direct interactions between dogs and wild carnivores, the ability of some pathogens (e.g. canine parvovirus) to persist in the environment for long periods of time may allow for disease transmission.

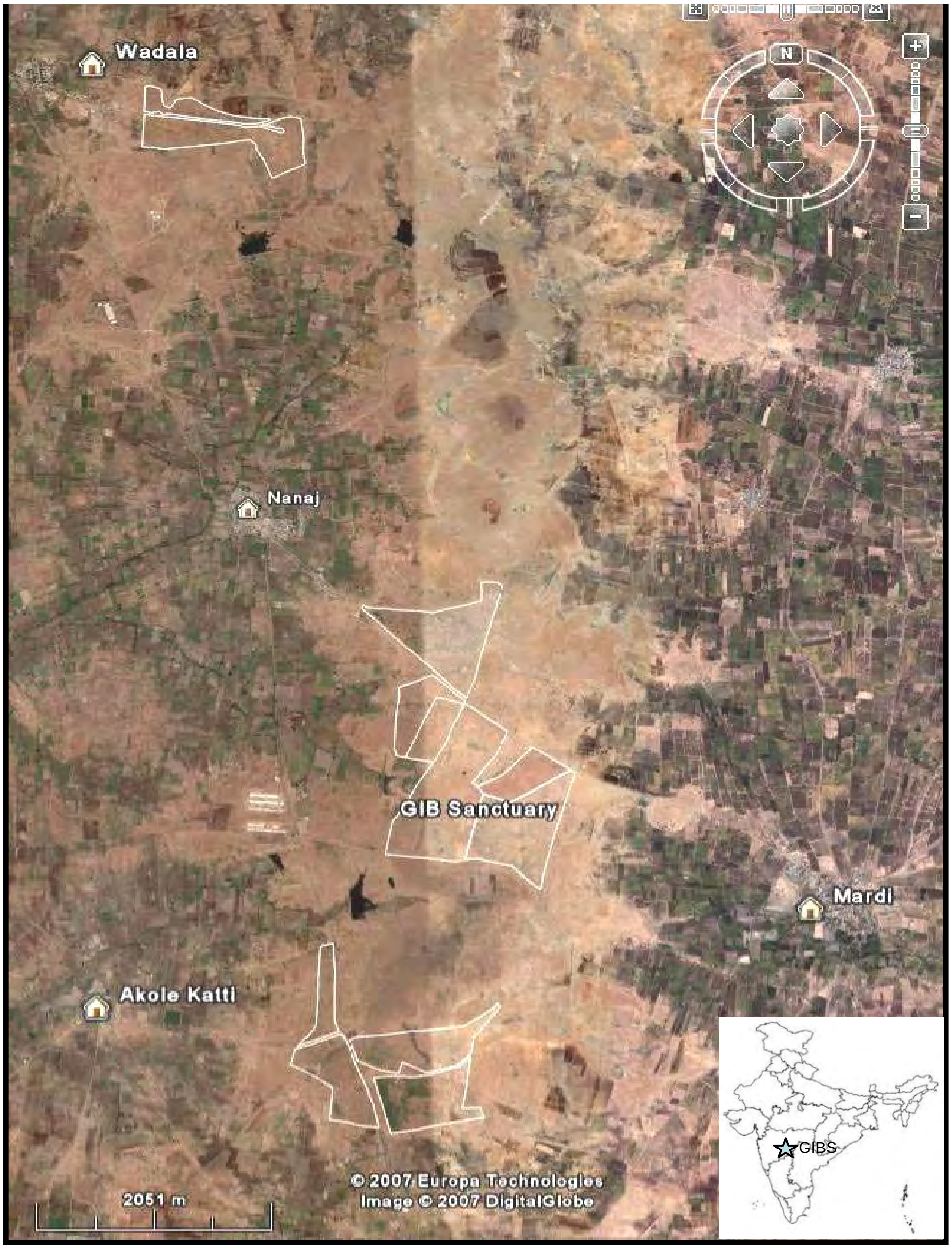
The objective of this study was to determine the prevalence of exposure to select infectious diseases and parasites of free-ranging domestic dogs in the vicinity of the Great Indian Bustard Sanctuary, Nannaj, Maharashtra, India. Specifically we tested for canine distemper virus (CDV), canine parvovirus (CPV), and rabies virus, all of which are important causes of illness and deaths in domestic dogs and have been the cause of several epidemics in wild carnivores of conservation concern. The design of vaccination programs has sometimes stemmed the risk of spillover of disease to wild populations (Haydon et al. 2006). Prior to such management actions, which are often expensive, logistically difficult, and controversial, a full knowledge of disease exposure and transmission potential involving dogs living in close proximity to wild carnivores is essential.

MATERIALS AND METHODS:

Description of study site

This study was conducted in the Great Indian Bustard Sanctuary (GIBS) at Nannaj, Maharashtra. This is one of the few wildlife reserves in the country that offers protection to the dry short grassland ecosystem, which is generally underrepresented in the Protected Area network of India (Rodgers et al. 2000). The landscape has a flat to gently undulating topography and typically consists of a matrix of sugarcane fields, seasonal crops, communal grazing lands, and, protected grasslands and forestry plantations. The intensive study area of approximately 50 km^2 comprises of 4 villages (Nannaj, Akole Katti, Wadala and Mardi) surrounding the protected grasslands of Nannaj (Fig 2). The economy of this region is based on agro-pastoralism, and has a high human population density of 1300/ km^2 (http://solapur.gov.in/htmldocs/dgraphy.htm accessed on 13/05/07)

Fig. 2. Google Earth Imagery of the study area showing the main villages and reserve boundary



Sample and data collection and analysis

To assess the sero-prevalence of CDV, CPV and rabies virus in domestic dogs, we carried out surveys from November 2005 to April 2007 within the intensive study area. Domestic dogs in this region can be roughly divided into three categories: owned free-ranging sheep dogs, owned free-ranging farm dogs and un-owned free-ranging village dogs. There are no completely feral dogs in this area as all dogs are associated with human habitations and dependent on humans for resources. With the permission of the owners we chemically immobilised dogs using a mixture of ketamine hydrochloride (8mg/kg estimated body weight) and xylazine hydrochloride (1mg/kg estimated body weight). We captured free-ranging un-owned dogs using box traps and throw nets or by darting them using a blow pipe (Fig 3). During anesthesia we monitored respiration, heart rate and body temperature every 10 minutes until the animal showed signs of recovery. Age was assessed on the basis of incisor wear and body size. Animals were marked with ear-tags and selected individuals were fitted with radio-collars (Advanced Telemetry Systems, California). Capture and handling procedures were approved by the Animal Care and Use Committee of the University of Missouri-Columbia (Protocol #4262).

Fig. 3. A farm dog being darted using a blowpipe

Fig. 4. An anesthetised dog being processed, while the owners look on.

Approximately 8-10 ml blood was collected to serum separator and EDTA tubes by venepuncture of the femoral or saphenous vein (Fig. 4). 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 -20 C freezer at the Serum Institute of India, Pune for storage. The EDTA blood was kept cool before transportation to the Joshi Pathology laboratory, Pune for haematology and blood chemistry analysis. We also bled a peripheral vein, and immediately prepared several smears for examination of blood parasites. We collected salivary swabs from 25 randomly selected individuals to test for rabies. Another 6 animals that showed bite wounds, symptoms of rabies or general signs of illness were also tested for rabies. To assess endoparasite load we collected faecal samples from the rectum and stored it in 10% formalin. We also examined each animal for ectoparasites and collected specimen samples were stored in 10% formalin.

Testing procedures

We tested for CDV and CPV IgG and IgM immune response antibodies using Biogal Immunocomb dot ELISA assay kits (Biogal, Galed Labs, Israel). These are semi-quantitative tests which indicate a high/medium/low response to antibodies present in the sample (Fig. 5). These tests have been truthed for dogs; Waner et al. (2003, 2004) have found that Immunocomb derived titers strongly correlate with results derived from immunofloressence assays (IFAs). Interpretations of the test results were based on manufacturer's recommendations. For CDV and CPV IgG, a value of S5 or greater was considered as high positive, between S3 and S5 as medium and values between S1 and S4 as low and S1 and below as negative. As per the manufacturer's information, S3 represents a positive response of 1:80 titer (H.I. test for anti CPV antibodies and V.N. for anti CDV antibodies). For CDV and CPV IgM, values of S3 or greater were considered as high positive, S2 as medium and S1 as low positive. Values lower than S1 were considered negative.

Serological assays are commonly used for epidemiological studies (Greiner & Gardner 2000), even though they cannot distinguish between active and previous infection, or infection and disease (Barr 1996, Evermann & Eriks 1999). This hinders the value of seroprevalence data for addressing spillover risk. However, the use of IgM and IgG diagnostic tests facilitates modelling spillover risk. The absence of IgM antibodies combined with elevated IgG suggests an earlier exposure, survival of acute-phase infection, and current immunity. In contrast, elevated levels of IgM and low levels of IgG suggest recent or active infection. This allows us to go beyond simply identifying exposure of the populations, but also quantify the proportion of the population that is susceptible (IgM-, IgG-), infected (IgM+, IgG-), and recovered and immune (IgM-, IgG+), which can form the basis for epidemiological models. Using the combination of IgG and IgM levels for CDV and CPV we categorised each dog into 5 classes of health status (Table 1).

Table 1: Health status of domestic dogs based on a combination of Ig/G and Ig/M values.

Status	**Ig/M values**	**Ig/G values**	**Animal status**
Susceptible	No detectable Ig/M	Negative or low Ig/G	Animal has no or insufficient immunity and no recent exposure– Susceptible to disease
Active infection	Low to High Ig/M	Negative to low Ig/G	Animal has been recently exposed to the virus and may either build immunity or may die from disease
Infected-gaining immunity	Medium to High Ig/M	Medium Ig/G	Animal has started to develop immunity to recent infection
Immune	Negative or low Ig/M	Medium to High Ig/G	Animal has survived acute phased of infection and has now developed immunity
Immune-reinfected	Medium to High Ig/M	High Ig/G	Immune from previous exposure, but now re-exposed to virus

Dogs were tested for exposure to Rabies using Anigen® rabies kit (Anigen, South Korea) which is a dot ELISA test that detects rabies antigen in saliva or brain of an infected animal. This test indicates a positive or negative reaction to rabies presence in the sample (Fig 6).

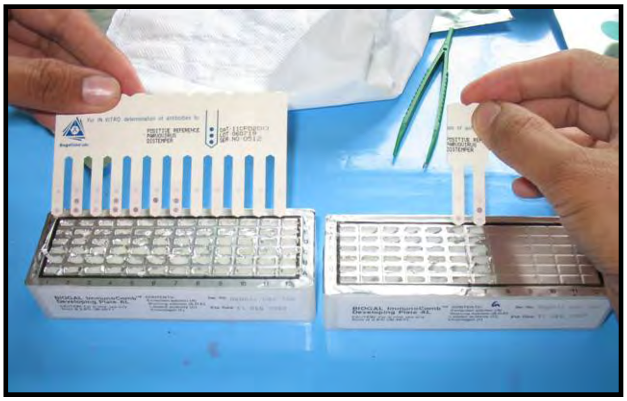


Fig. 5 Biogal Immunocomb tests. The dots indicate a positive response to CDV and CPV

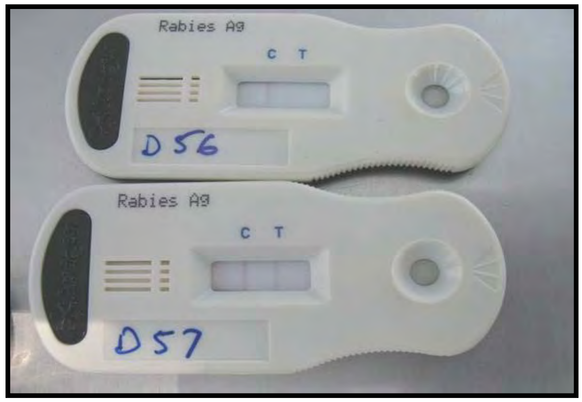


Fig. 6 Anigen rabies test. The top plate indicates a negative response, while the bottom plate shows a positive reaction for rabies

Faecal samples were examined by direct microscopic examination, and by floatation and sedimentation methods at the Bombay Veterinary College, Mumbai. Ticks, fleas and flies were identified based on external morphological characteristics. Blood smears were examined by microscopic examination for the presence of blood-borne parasites.

RESULTS AND DISCUSSION

We collected 75 sera samples from 74 dogs in 4 villages surrounding the Great Indian Bustard Sanctuary, Nannaj. None of these dogs had ever been vaccinated, and the vast majority had likely never received any kind of medical treatment. All dogs sampled were adults, except for one 1 month old pup which showed acute clinical symptoms of canine distemper and which died within 24 hours of observation.

Exposure to canine parvovirus

Canine parvovirus is an extremely virulent and contagious virus that affects dogs, wolves, foxes and other carnivores and can cause serious illness leading to death, with symptoms such as lethargy, vomiting, fever and bloody diarrhoea. CPV is spread through direct contact with infected faeces, urine, or soil and the virus can persist in the environment for months. Evidence of current or past exposure to CPV was detected in 93.3% of the dog population over all seasons for the entire study area. Of these 4% were found to have an active infection, 13.3% had an active infection, but were gaining immunity, 60% were immune and 16% were immune, but had been re-exposed to the virus (Table 2).

The maximum number of ongoing infections (Active+ gaining immunity + re-infections = 78%) were observed during the wet season (June-November), while the maximum number of immune animals were observed during summer (February-May). The number of susceptible individuals was low throughout the year indicating that that this disease is enzootic in the domestic dog population with most infection occurring in young age classes (e.g. pups). Also, no deaths of sampled dogs were observed due to disease two months after the initial sampling period indicating that while morbidity for this disease is high, mortality seems to be low among adults. Since this virus has a long persistence time in the environment, and since wild carnivores come into regular contact with the peri-domestic environment (A.T. Vanak and M.E. Gompper unpublished data), the risk of transmission of this disease to wild populations may be high.

Table 2. Prevalence of CPV in domestic dogs of the GIBS

Health Status	**Wet % (n)**	**Winter% (n)**	**Summer% (n)**	**Annual% (n)**
Susceptible	7.1(1)	6.7(2)	6.5(2)	6.7(5)
Active Infection	7.1(1)	3.3(1)	3.2(1)	4.0(3)
Gaining Immunity	21.4(3)	20.0(6)	3.2(1)	13.3(10)
Immune	14.3(2)	60.0(18)	80.6(25)	60.0(45)
Immune-reinfected	50.0(7)	10.0(3)	6.5(2)	16.0(12)

Exposure to canine distemper virus

Canine distemper is a contagious, incurable, often fatal, multisystemic viral disease that affects the respiratory, gastrointestinal, and central nervous systems of canids and many other carnivores such as felids, procyonids, and mustelids. CDV is transmitted through direct contact with body secretions and excretions of infected animals, and in particular via airborne viral particles that infected dogs exhale while in the vicinity of susceptible dogs. Once infected, dogs may continue to shed the virus for several weeks, even after symptoms disappear.

Evidence for current or past exposure to CDV was found in 90.7% of individuals. Of these 25% had an active infection, 18.7% had been infected but were gaining immunity, 22.7% were immune and 24% had previous immunity but had been re-exposed to the virus (Table 3). Active infections and total percentage of exposed individuals were highest in the wet (June-November)and winter (December-January) seasons, while in the summer the numbers of animals immune to CDV were highest. Importantly, the proportion of susceptible animals in the population was also highest in the summer possibly due to new births in the winter. This indicates a seasonal trend in the occurrence of this disease in the domestic dog population, though this would have to be corroborated with longitudinal analysis of sero-conversion by re-sampling individuals over seasons. Mortality due to this disease was observed in only one instance – that of a one month old pup that exhibited acute clinical symptoms and that died within 24 hours of first observation. Apart from this one case, no other deaths in the sampled dog population were observed within two months after the initial sampling period. This indicates that this disease is enzootic in the domestic dog population, and that there is low immediate mortality because of the disease in adult dogs.

Table 3. Prevalence of CDV in domestic dogs of the GIBS

Health Status	**Wet % (n)**	**Winter % (n)**	**Summer % (n)**	**Annual % (n)**
Susceptible	0.0 (0)	0.0(0)	22.6(7)	9.3(7)
Active Infection	71.4(10)	23.3(7)	6.5(2)	25.3(19)
Gaining Immunity	14.3(2)	30.0(9)	9.7(3)	18.7(14)
Immune	7.1(1)	3.3(1)	48.4(15)	22.7(17)
Immune-reinfected	7.1(1)	43.3(3)	12.9(4)	24.0(8)

Evidence for exposure to rabies

Rabies is a deadly viral disease that causes acute encephalitis in mammals and is transmitted through bites from an infected animal. In India, domestic dogs are the principal carriers of rabies, and there are a reported 30,000 cases of rabies infections in humans every year (WHO/WSPA 1990). There are few records of the number of rabies cases reported from wild animals mainly due to the lack of diagnostic tools and monitoring protocols in India. There is no cure for rabies, although it is preventable by vaccination.

Of the 31 dogs we tested for rabies by collecting an oral swab, 2 (6%) animals tested positive, one each in Wadala and Akole Katti villages. These were owned dogs and did not exhibit any symptoms of rabies. Following this positive result, we informed the owners, and the dogs were euthanized by a veterinarian. Given the small sample size and asymptomatic nature of these animals, this prevelance is troubling. Furthermore, there were at least 14 cases of suspected rabid dogs that were killed by villagers in Nannaj alone that we are aware of during the course of this study. We were, however, unable to obtain samples from these cases, either because information reached us several days after the incident, or the carcass had been disposed off by the villagers. Together this data suggests that rabies, like CDV and CPV, is enzootic in the dog population, and suggests the risk of spillover of rabies to wild canids is a valid concern. During the field work, we were also informed of two instances of golden jackals (*Canis aureus*) being killed by villagers after they entered a house and attacked the occupants. It is possible that this abnormal behaviour may have been due to rabies, but it is speculation. Given the relatively low density of large wild canids (jackals and wolves) in the region and the high density of dogs, it is likely that rabies in wild canids may be the result of spill-over from dogs.

Endo and ectoparasites of sampled animals

We collected faecal samples from 29 dogs. Twelve (41%) samples were positive for hookworms (*Ancylostoma* spp.) with an average load of 300 eggs/gm (n=13, ±216 SD). One sample tested positive for oocysts of *Isospora* spp. and 3 animals were infected with tapeworms (*Dipylidum caninum*). We did not find any blood-borne parasites from examination of smears from 55 animals.

Of the 77 dogs examined for ectoparasites, 41% had ticks (*Rhipicephalus* spp.), 34% had fleas (*Ctenocephalides* spp.) and 16% had biting flies (*Hippobosca* spp). Two dogs (2.6%) dogs had moderate to heavy infestations of ticks. Fourteen percent (11/77) of dogs had moderate to heavy infestations of fleas and one dog had a heavy infestation of flies.

CONCLUSION AND RECOMMENDATIONS

We found significant evidence for exposure to pathogens of conservation and human health concern in the domestic dog population surrounding the Great Indian Bustard Sanctuary in Maharashtra, India. High rates of prevalence of important diseases such as CDV and CPV in the domestic dog population poses a significant risk to the health of wildlife in the region. This is more so because the diseases are enzootic in the dog population, and contagious animals form a high proportion of the population. The data on rabies is more limited, but this virus may also be enzootic in these villages surrounding the wildlife sanctuary. There is evidence from ongoing telemetry studies to indicate there is sufficient contact between dogs and wild carnivores, especially Indian foxes to allow for disease transmission (Vanak, A.T. and Gompper. M.E unpublished data). Indeed, preliminary testing of Indian foxes has shown presence of CDV and

CPV in the population, but at lower rates. Significantly, CDV positive foxes all died within one month of initial sampling (Vanak, A.T., Belsare, A.V. and Gompper, M.E. unpublished data).

A comprehensive and targeted vaccination program can stem the risk of disease being transmitted to wild carnivores (Haydon et al. 2006). This should however, be coupled with dog population control measures as well as educating the public on responsible dog ownership. To effectively control disease as well as dog populations, we recommend an 8-point program. This program is regionally focused but could be adapted to other regions of India.

- With the help of livestock development officers of the Animal Husbandry Department posted at Veterinary Dispensary, Wadala and Nannaj, owners of dogs should be given the option of vaccinating their dogs against rabies and canine distemper virus.
- Monthly vaccinations camps jointly organized by the Forest and Animal Husbandry Departments should be promoted to the public as opportunities to obtain these vaccinations.
- Vaccinated dogs should be marked with a colour coded collar that would be provided by the Forest Department.
- Dog owners should be given a window of opportunity (e.g three months) to vaccinate their dogs, and the number of vaccinated dogs should be limited to 2/household free of cost, with additional dogs vaccinated at cost.
- Following this initial vaccination period, any unvaccinated dogs (identified by the absence of colour coded collar) that are found in these villages or fields adjoining the villages should be caught and humanely put down on a regular basis. This can be done with the help of the Animal Husbandry Department, and professional dog catchers.
- Any dogs, whether owned or free-ranging, if found inside the wildlife sanctuary should be caught, identified and if necessary put down. This will also encourage owners to keep their dogs restrained.
- These steps should be regularly repeated over several months during intial vaccination efforts, then on a regular annual basis to maintain regional coverage. It is important to ensure that unvaccinated dog numbers remain low in the population, and that vaccinated dogs are not allowed to roam freely.
- The vaccination should be repeated annually with the targeted vaccination drive carried out during the monsoon season.

- Implementation of vaccination programs should co-occur with the onset of public education and population control programs, since vaccination-only measures may result in increased survival of dogs and thus larger population sizes.

Direct action as a result of this study

Based on our findings, we submitted a preliminary report to the Maharashtra state forest department who took cognisance of the implications of the results. In particular, the Chief Wildlife Warden of the state, Mr. B. Majumdar initiated rapid action and called for a meeting with the Animal Husbandry department of the state where we presented our findings. We also made a set of recommendations to the department on how to reduce the incidence of disease in domestic animals. Following this, the state forest department undertook a pilot vaccination program and 200 dogs were vaccinated with anti-rabies vaccine and Maxivac6 vaccine for prevention of CDV, CPV, Adenovirus, Parainfluenza and Leptospirosis. Following the success of this pilot program, the department plans to vaccinate an additional 800 dogs in the vicinity of the sanctuary. However, we have stressed upon them that vaccinating is not the only solution, and that villagers and dog owners have to be educated about the need for responsible dog ownership and dog population control.

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