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Cover: Dorsal view of Mantis Shrimp *Cloridina ichneumon* (Fabricius, 1798) & *Gonodactylellus demanii* (Henderson, 1893). © Fisheries Research Station, Junagadh Agricultural University, Sikka.

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COMMUNICATION

Serosurvey of viral pathogens in free-ranging dog populations in the high altitude Trans-Himalayan region

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Abstract: Dogs, as reservoir hosts, have been implicated in the decline of carnivore populations across the globe. We conducted a serosurvey of free-ranging dog populations to assess the population level exposure rates to three viral pathogens, canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV) in a Trans-Himalayan landscape in India that is home to the endangered Snow Leopard. A total of 97 dogs were sampled across six villages as a part of a surgical sterilization campaign during the study period. Samples were tested for IgG antibodies using a table top ELISA kit. Exposure rates to the three viral pathogens in the dog populations was high; 100% for CPV, 54% for CDV and 66% for CAV, with high positive immunoglobulin titer values for CAV and CPV, and low to moderate values for CDV. Overall conservation efforts for native carnivores need to address the role of free-ranging domestic dogs in disease transmission.

Keywords: *Canis lupus familiaris*, canine distemper, canine parvovirus, canine adenovirus, commensal, epidemiology.

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Author contributions: Study conception and design: CH and ATV; Funding: CH raised funds for research; Data collection in field: CH and AB; Logistic support for data collection: AB and YVB; Analysis and interpretation of result: CH and ATV; Manuscript preparation: CH with inputs from AB, YVB and ATV. All authors reviewed the results and approved the final version of the manuscript.

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INTRODUCTION

Infectious diseases in wildlife are an important conservation concern, particularly with increasing evidence of human-induced escalations in incidence rates (Daszak et al. 2001). Changes such as habitat loss, biodiversity loss, homogenization of ecosystems, and a rapidly changing domestic-wildlife interface have been associated with increased disease risks in wild species (Funk et al. 2001; Van De Bildt et al. 2002; Morgan et al. 2006; Pongsiri et al. 2009; Murray & Daszak 2013). Domestic animals in particular are reservoirs as well as vectors for pathogens such as distemper virus, *Trypanosoma*, *Echinococcus,* and *Brucella*. A majority of the pathogens of domestic animals are multi-host pathogens (Cleaveland et al. 2001). Many pathogens of conservation concern are transmitted by populations of free-ranging domestic animals, such as cats and dogs. Domestic dogs have been implicated in carnivore population declines across the globe (Funk et al. 2001; Knobel et al. 2005; Acosta-Jamett et al. 2011). Pathogens such as canine parvovirus (CPV) and canine distemper virus (CDV) have been reported to cause disease in several wild carnivores (Truyen et al. 1998; Cleaveland et al. 2000; Fiorello et al. 2007; Acosta-Jamett et al. 2015), resulting in death (Seimon et al. 2013; Belsare et al. 2014) and subsequent severe population declines (Osterhaus et al. 1997; Randall et al. 2006). Viral pathogens such as CDV are highly immunizing and require a large host population for persistence (Acosta-Jamett et al. 2010; Almberg et al. 2010). Large dog populations facilitate the transmission and maintenance of such pathogens within the ecosystem.

The global population of domestic dogs is close to a billion, and they are ubiquitous in most terrestrial landscapes (Gompper 2014). Dogs have been globally known to threaten 188 species, with greatest impacts in global biodiversity hotspots (Doherty et al. 2017). With a population of ~60 million (Gompper 2014), domestic dogs have emerged as an important conservation problem for native wildlife in India (Home et al. 2018). With the exception of a few studies (Hiby et al. 2011; Belsare & Gompper 2013;Tiwari et al. 2018), there is a significant lack of information on dog demography in both urban and rural areas, considering that they co-occur with several other native carnivores in human-dominated landscapes (Vanak & Gompper 2010; Vanak et al. 2014). High sero-prevalence of antibodies for CPV, CDV and canine hepatitis (CAV) has been documented for rural dog populations in Central India (Belsare & Gompper 2013). The study not only detected pathogens in an endemic canid, Indian Fox *Vulpes bengalensis* but also reported high mortality in foxes putatively infected with CDV (Belsare et al. 2014). The sero-prevalence of antibodies against these three viral pathogens was also high among domestic dogs sampled around Ranthambore National Park (Sidhu et al. 2019) and Kanha Tiger Reserve (Chaudhary et al. 2016). The recent deaths of Asiatic Lions *Panthera leo persica* in Gujarat, putatively due to CDV infection <https://in.reuters.com/article/india-wildlife-lions/more-asiatic-lions-in-india-test-positive-for-virus-after-23-deaths-idINKCN1MK22A>, highlights the importance of gathering long term epidemiological surveillance data for these pathogens in both wild and domestic carnivores.

Irrespective of human population densities, high dog densities facilitate a favorable environment for pathogen spillover, which can put native species at risk (Vanak & Gompper 2009). The Trans-Himalayas in India support amongst the lowest density of humans in the subcontinent, but domestic dogs in this region pose a threat to humans and wildlife. Domestic dogs contribute to a majority of the livestock losses in this landscape (Suryawanshi et al. 2013; Home et al. 2017) and interact with wildlife as predators and competitors (Pal 2013; Ghoshal et al. 2016; Home et al. 2017). Such interactions can result in pathogen spillover into native carnivores, in particular, the globally threatened Snow Leopard, an apex predator of the high altitude Himalayan landscapes.

There is a general dearth of information on the disease ecology of free-ranging dogs in the Himalaya. We undertook a serosurvey of free-ranging dog populations in the upper Spiti landscape in the Indian Trans-Himalaya to assess sero-positivity levels to three viral pathogens, CPV, CDV, and CAV. An understanding of the population level exposure to these viruses is important to evaluate the risks posed by dogs to wild carnivores, and to help in planning mitigation programs in the landscape.

MATERIALS AND METHODS

Study area

The study was conducted in the Upper Spiti Landscape (32–32.700 ^{0}N & 77.617–78.500 ^{0}E), in Lahaul and Spiti district, a high altitude region in the state of Himachal Pradesh, India (Figure 1). This landscape has the lowest densities of humans and is comprised mainly of agro-pastoralists. The area supports endangered wild herbivores and large predators such as Snow Leopard *Panthera uncia*, and Tibetan Wolf *Canis lupus chanco*, medium to small-sized predators (Red Fox

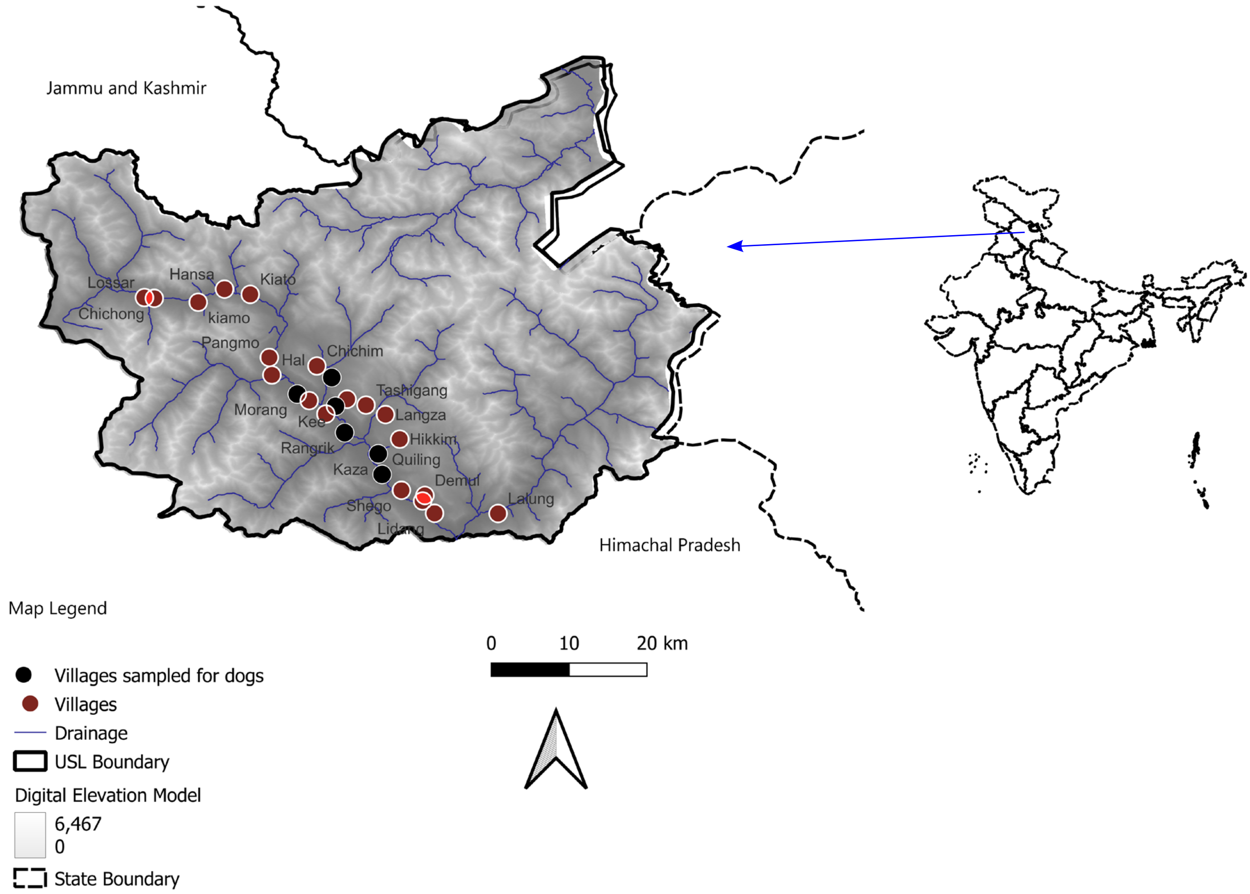

Figure 1. Map of the upper Spiti landcape. The villages sampled for dogs as a part of the sterilization camp are are marked in black circles.

Vulpes vulpes and other mustelid species) (Anonymous 2011). Domestic dogs are ubiquitous in the landscape. The last two decades have seen a rapid increase in the tourism infrastructure of the Spiti valley, particularly the township of Kaza and the largest village Rangrik. As a consequence of the increased availability of garbage and other resources, the domestic dog population in the study area has increased rapidly (Home et al. 2017, 2018). As dogs have spread to neighboring upland villages, there has been a concomitant increase in negative interactions with wildlife (Pal 2013; Hennelly et al. 2015; Ghoshal et al. 2016) as well as a severe problem of livestock depredation by free-ranging dogs (Home et al. 2017; Home 2018).

Capture and handling

We collected blood samples from dogs that were opportunistically captured during two surgical sterilization camps conducted in six villages in the study area in October 2013 and June–July 2014. The township of Kaza, which had the highest number of dogs, was sampled in both years. Dogs associated with households were brought on leash while free-ranging dogs were baited and captured using nets. Dogs were immobilized for sterilization using a combination of xylazine hydrochloride-ketamine hydrochloride, and blood was collected by venipucture of the cephalic vein. Blood was stored in vacutainers (BD vacutainer, 5 ml) with clot activating factor. The serum was decanted in 2 ml vials (Tarsons) and stored in a -20 ˚C freezer in Kaza that was hired on monthly basis till the samples were transported. The samples were transported on ice and stored in a -20˚C freezer in Bengaluru prior to analysis.

Ethics approval for this study was granted by Ashoka Trust for Research in Ecology and the Environment Animal Ethics committee (AAEC-IRB/ACA/0015/CH/2009X). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. The participation of owners who brought their dogs to the sterilization camps was voluntary.

Sero-prevalence of antibodies

For serological assessments, commercially available dot-ELISA immunoassay kits (ImmunoComb ®, Biogal

Galed Laboratories, Israel) were used. These kits have previously been validated for antibody assays in domestic dogs (Waner et al. 1996, 1998). The ImmunoComb ® kits were used to determine the IgG antibody titres against canine distemper virus (CDV), canine parvovirus (CPV) and canine adenovirus (CAV), for the dogs sampled in the villages. The antibody kits are based on solid phase immunoassay technology and their concentrations in the serum are measured on a qualitative scale. The kits provide a colour-coded scale (CombScale) which scores each sample on a scale of 0–6 "S" units, where S3 corresponds to titers at which the virus is neutralized. The information provided by the manufacturer mentions that values of S3 and above suggest that the animal has protective levels of antibodies to CDV, CPV, and CAV while values below S3 but above S1 suggests that the individual has been exposed to the pathogen and has seroconverted (https://www.biogal.com/wp-content/uploads/2019/09/PI-CVV-31_03_2016-4.pdf.) As a measure of prevalence exposure, we used descriptive statistics for summarizing the information and calculated the percentage of sampled animals with detectable levels of IgG against the pathogen (> S1) stratified by age and sex.

RESULTS

We sampled a total of 97 dogs (52 males: 45 females) across six villages (Kaza; N= 38, Rangrik; N= 33, Quiling; N= 8, Kibber; N= 5, Kee; N= 10, and Morang; N= 3) (Bijoor 2016), which included 73 adults, 19 juveniles, and five pups. Nearly 73% of the samples were collected from the township, Kaza and the largest village, Rangrik, which account for 74% of the dog population in the study area (Home et al. 2017). None of the dogs had any prior vaccination as this was the first attempt for a dog sterilization program in the region.

The assay detected antibodies against CPV in all the samples collected across all the villages, while antibodies against CDV were detected in 54% (52/97; 95% CI 43 – 63%; Sterne's exact method) of the samples and against CAV in 66% (64/97; 95% CI 56 – 75%; Sterne's exact method) of the samples (Reiczigel et al. 2019) (Table 1). We observed low to medium values (Inadequate immunity) for CDV for a majority of the sampled population (Table 2).

DISCUSSION

We documented high exposure rates to the three pathogens for the free-ranging dog populations in the upper Spiti landscape. Antibody response to CPV was detected in all the dogs sampled indicating that it is circulating widely in the population. Domestic dogs in the study area have never been vaccinated, and thus the antibody titers observed can be reliably interpreted as evidence of pathogen exposure (Belsare & Gompper 2013). Given the widespread distribution of dogs in this landscape, and their frequent interactions with carnivores, our study provides evidence that dogs can pose a serious disease risk to endangered species.

All the three pathogens; CDV, CPV, and CAV are contagious viruses that infect canids as well as other carnivores, demonstrating their ability to cross species barriers. In the case of CPV, its ability to persist in the environment for months is of particular concern, since direct contact is not required for transmission (Castanheira et al. 2014). For CDV, the primary mode of infection is inhalation requiring direct contact with infected animals (Beineke et al. 2015). While CDV may or may not be fatal for dogs, they are usually fatal for the wild counterparts (Cleaveland et al. 2000; Van De

Table 1. Pathogen exposure in sampled dog populations in six villages in the upper Spiti landscape. Numbers are dogs sampled, with percentages in parenthesis.

	Canine Parvovirus	Canine Distemper		Canine Adenovirus (ICH)	
	Detected (% in parenthesis) (N= 97)	Detected (% in parenthesis) (N= 52)	Not detected (% in parenthesis) (N= 45)	Detected (% in parenthesis) (N= 64)	Not detected (% in parenthesis) (N= 33)
Adult male	39 (40)	23 (44)	16 (36)	30 (47)	9 (27)
Adult female	34 (35)	23 (4)	11 (24)	27 (42)	7 (21)
Juvenile male	(10) 10	1 (2)	9 (20)	1 (2)	9 (27)
Juvenile female	(9) 9	3 (6)	6 (13)	3 (5)	6 (18)
Pup male	3 (3)	2 (4)	1 (2)	1 (2)	2 (6)
Pup female	2 (2)	0 (0)	2 (4)	2 (3)	0 (0)

Table 2. Seropositivity scores of CPV, CDV and CAV for dog samples across six villages.

Villages	CPV					CDV					CAV				
	0	1–2	4<5	5–6	>6	0	1–2	4<5	5–6	>6	0	1–2	4<5	5–6	>6
Kaza (N= 38)	0	2	0	24	12	19	13	6	0	0	11	4	1	7	15
Kee (N= 10)	0	0	0	10	0	8	2	0	0	0	7	1	2	0	0
Kibber (N= 5)	0	0	1	2	2	2	3	0	0	0	0	0	0	2	3
Morang (N= 3)	0	0	0	3	0	3	0	0	0	0	1	0	0	1	1
Quiling (N= 8)	0	0	0	5	3	4	4	0	0	0	3	3	0	2	0
Rangrik (N=33)	0	3	3	23	4	9	16	8	0	0	11	7	3	7	5

0—Not detected | 1–2—Low or inadequate immunity | 4<5—Positive | 5–6—High Positive | >6—Very High Positive. Total number of dogs sampled—97.

Bildt et al. 2002; Belsare et al. 2014). In a recent study in the Nepal Himalaya, CDV antibodies were not only detected in a large proportion of the dog population, but the virus was also found to be circulating in the population, as revealed through molecular analysis (Ng et al. 2019). Canine Adenovirus (CAV) can also survive in the environment for long periods and is transmitted through both excreta as well as secretions (Balboni et al. 2013). While variation in seropositivity levels across villages may be difficult to interpret due to a lower sample size in two villages (Morang & Kibber), positive values for CDV seroprevalence levels were reported only in the two largest villages Kaza and Rangrik (See Table 2). A proportion of dogs had sero-positivity scores that were lower than the "control" threshold. This could be interpreted either as non-detection (https://www.biogal.com/wp-content/uploads/2019/09/PI-CVV-31_03_2016-4.pdf), or evidence of waning immunity. Vaccination for CDV provides long term protection from CDV reinfection (Belsare & Gompper 2013). As mentioned earlier, none of the dogs were vaccinated for any of these pathogens. While it is difficult to interpret why CDV titres were low for dogs in Kaza and Rangrik, there could be possibility of future reinfections due to lower immunity. Although most adult dogs may survive a CDV reinfection, the impacts will be fatal for wild carnivores. However, this requires a better understanding for free-ranging dogs and its implications for transmission.

There is a considerable degree of movement of dogs from these high dog population clusters (Kaza & Rangrik) to other villages (Home et al. 2017) within the landscape, which could potentially facilitate pathogen circulation and persistence. Long-term research work on snow leopards in the study area has detected domestic dog movements in areas used by snow leopards <http://snowleopardblog.com/camera-reveals-dog-pack-attacking-snow-leopard/>. There is therefore a potential for pathogen spillover for the endangered snow leopards. Domestic dogs have also been observed to frequently interact with Red Foxes (Ghoshal et al. 2016) and a single occurrence of mating with the Tibetan Wolf has been observed (Hennelly et al. 2015). Since domestic dogs co-occur and interact with native carnivores in the landscape, high pathogen exposure rates in dogs could potentially pose a pathogen spill-over risk for these species especially in the context of human-mediated environmental change (Daszak et al. 2001; Foley et al. 2005; Brearley et al. 2013).

A model-based approach to understanding the spillover of infectious pathogens show that the proportion of free-ranging dogs in a population has a strong influence on CDV infections in dogs as well as spill-over events (Belsare & Gompper 2015). The adult dog population in the study area was estimated to be ~570 dogs (Home et al. 2017), and the total population of dogs including juveniles and pups could be estimated to about ~1,500 for the upper Spiti landscape. Considering that almost all dogs are free-ranging, and a proportion of the dog population is also wide ranging, moving across villages (Home et al. 2017) to areas used by Snow Leopard, Wolf, and Red Fox there is a strong potential for these pathogens to be transmitted to wild carnivores with potentially fatal consequences.

Our study has important implications for disease surveillance and monitoring for both domestic and wild carnivores within the landscape. Since the maintenance of infectious pathogens is determined by the host population, managing dog populations and restricting free-ranging movement would be imperative to prevent spillover. Conservation efforts for native carnivores should concurrently address the role of free-ranging domestic dogs in disease transmission.

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