

## ORIGINAL ARTICLE

# Epidemiology of Viral Pathogens of Free-Ranging Dogs and Indian Foxes in a Human-Dominated Landscape in Central India

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*Canis familiaris*; *Vulpes bengalensis*; serosurvey; canine adenovirus; canine distemper; canine parvovirus

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**Summary**

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 percentage of dogs and 87% of 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 earlier natural infection. The high prevalence of exposure to these pathogens across the sampling sessions, significantly higher exposure rates of adults compared with 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 serologic 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 earlier 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 have potentially important implications for dog disease control programmes 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 multihost pathogens infect a wide range of mammalian carnivore species, they may constitute an important threat for many populations of conservation concern (Knobel et al., 2014). 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 et al.,

2004; Vanak and Gompper, 2009b; Gompper, 2014). This latter 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*), lions (*Panthera leo*), Caspian seals (*Phoca caspica*) and Lake Baikal seals (*P. sibirica*) 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). Dogs have also been implicated as a source of CPV contributing to mortality in 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). Although dogs are typically assumed to be the reservoir of pathogens influencing wildlife, rarely are such assumptions closely examined (Knobel et al., 2014).

There has been a general lack of research on infectious diseases of dogs and wildlife in Asia, and even measures of the seroprevalence 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, 2014). Further, many wild carnivore species are known to persist in the human-dominated landscapes that these dogs inhabit, such as wolves, lions, leopards (*P. pardus*), snow leopards (*Uncia uncia*) 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., 2014). 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, a species threatened by rapid habitat loss and poaching throughout its range (Johnsingh and Jhala, 2008; Vanak et al., 2008), has been known to undergo large population fluctuations, and although disease has been

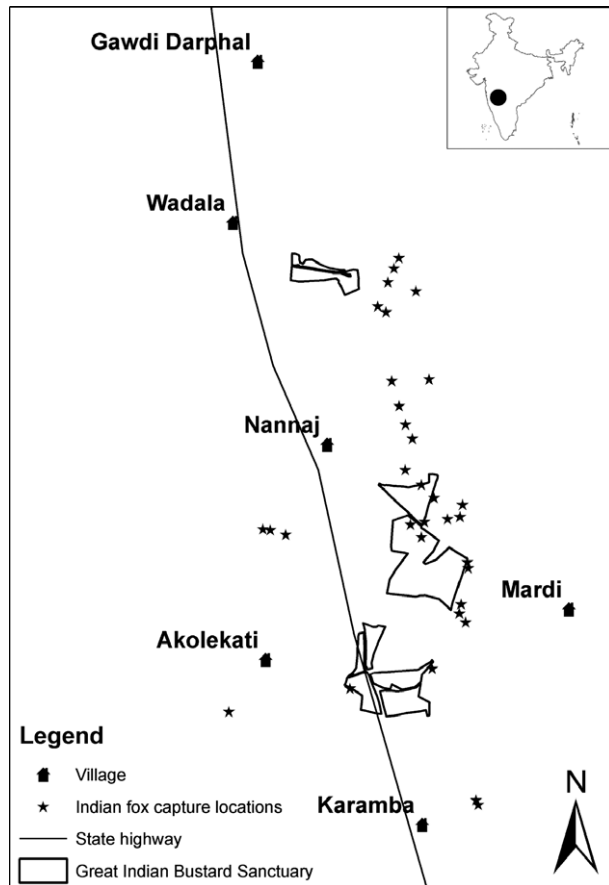
suspected, it has never been properly investigated (Manakadan and Rahmani, 2000; Vanak and Gompper, 2009b).

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, 2010). A pilot study was undertaken around the GIB WLS to determine the prevalence of exposure to CPV and CDV in dogs (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 the 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 programmes and to assess the impact of such programmes. 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<sup>2</sup> protected area, the focal study portion of the sanctuary consists of six protected grassland patches totalling approximately 6 km<sup>2</sup>, which is embedded in a human-dominated landscape that includes the study villages (Fig. 1). Combining the protected area and the village lands surrounding the sanctuary resulted in a focal study region of ~51 km<sup>2</sup>. 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). Dogs in the study villages were surveyed on multiple occasions before and during this study using a photographic mark-recapture method (Belsare and Gompper, 2013). A detailed database of village dogs was compiled using the photographs and other relevant details (sex, age, colour,



**Fig. 1.** Map of the study area showing the Great Indian Bustard Wildlife Sanctuary, six study villages and locations where Indian foxes were captured.

markings, reference person) that were documented for every photographed dog. We used this database to determine the identity of dogs during the study, especially to ensure that no dogs were resampled during a session. 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<sup>2</sup> and the median human:dog ratio was 6 (range 5–8) (Belsare and Gompper, 2013). The activity and movements of dogs, and the interactions of dogs and wildlife in this region have been the subject of detailed study; dog ranging brings them into contact with wildlife within and outside the sanctuary (Vanak et al., 2007, 2009; Vanak and Gompper, 2009a, 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*), house cat (*F. catus*), Asian palm civet (*Paradoxurus hermaphroditus*) and gray mongoose (*Herpestes edwardsi*).

### Capture and handling

Indian foxes were captured in and around the GIB WLS between April 2006 and May 2007 (Fig. 1). 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 a 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 and released after obtaining a blood sample. During Session 3, dogs in the study villages were vaccinated as a part of a village-level vaccination experiment 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 (Belsare, 2013; Belsare and Gompper, in review). All dogs were vaccinated against rabies because of the public health risks posed by dog-transmitted rabies in the region. Additionally, dogs in the villages of Akolekati, Mardi and Wadala were vaccinated with Canigen DHPPi/L (Virbac Animal Health), 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*. Vaccinated dogs were not resampled in the subsequent session.

We classified dogs as pups (0–4 months), juveniles (5–12 months) and adults (>12 months) based on body size, allometry (visual estimate of head size and leg length compared with the body size) and behaviour (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 (Protocol #4262 and #7049).

### Estimation of antibody seroprevalence

For both foxes and dogs, Vacuette 4-ml serum tubes with clot activating factor (Greiner Labortechnik, Frickenhausen, Germany) were used to collect blood samples by venipunc-

ture 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 stored at 4°C for up to 48 h. The sera samples were transported on ice to a -20°C freezer at the Serum Institute of India, Pune, for storage.

We used commercially available dot-ELISA kits for serological assessments of the samples. For dog sera samples collected between 2005 and 2007 (Session 1 and Session 2), IgG titres against CPV and CDV were determined using ImmunoComb® dot-ELISA kits (Biogal-Galed Laboratories, Kibbutz Galed, Israel), while Canine VacciCheck Antibody test kits (Biogal-Galed Laboratories) were used for sera samples collected between 2011 and 2012 (Session 3 and Session 4). Canine VacciCheck Antibody test kit additionally includes tests for determining IgG antibodies against CAV. For fox serum samples, IgG titres against CPV and CDV were determined using ImmunoComb® dot-ELISA kits, and IgM titres against CPV and CDV were also determined using the ImmunoComb® CPV and CDV IgM kit. We used Canine VacciCheck Antibody test kits to test some of the stored fox sera samples for IgG antibody titres against CAV.

These antibody test kits are based on solid phase immunoassay technology. Each kit consists of a comb-shaped plastic card and a multicompartiment developing plate. The concentration of antibodies in serum samples is measured using the colour-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 titre by virus neutralization test (VN) for CAV, 1:80 titre for CPV by HI test 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. The sensitivity and specificity values of these kits for detecting antibody titres in dog serum have been provided by the manufacturer: CPV IgG sensitivity = 97%, CDV IgG sensitivity = 95%, CPV IgG specificity = 100% and CDV IgG specificity = 100% (ImmunoComb® dot-ELISA kits and Canine VacciCheck Antibody test kits); CAV IgG sensitivity = 95%, CAV IgG specificity = 86% (Canine VacciCheck Antibody test kits); CPV IgM sensitivity = 91.4%, CDV IgM sensitivity = 93.1%, CPV IgM specificity = 90.8%, CDV IgM specificity = 95.5% (ImmunoComb® CPV and CDV IgM kit).

We also assessed Canine VacciCheck Antibody test kit cross-reactivity in the fox by testing serial dilutions of fox

sera. Three fox sera samples with high anti-CDV IgG titres ( $\geq S5$ ) were used, and dilutions prepared using normal saline. The solutions were thoroughly mixed, and the S scores for each dilution were obtained using VacciCheck kits.

The prevalence of exposure to a pathogen was calculated as percentage of sampled animals with detectable IgG antibodies against the pathogen ( $\geq S1$ ). 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  $\chi^2$  or Fisher's exact tests for statistical comparisons of prevalence by sampling sessions, age class and sex. Prevalence data were stratified by age class, and Cochran-Mantel-Haenszel (CMH) chi-square tests were used to assess patterns in prevalence due to sex.

## Results

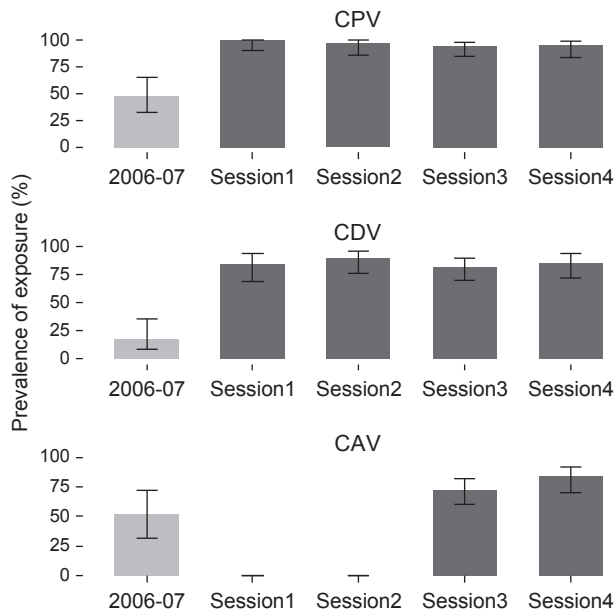
### Seroprevalence of viral antibodies in dogs

A total of 219 dogs from the study region were sampled during the study period: 34 (25 males, nine females) during Session 1, 39 (35 males, four females) during Session 2, 77 (62 males, 15 females) during Session 3 and 69 (50 males, 19 females) during Session 4. Overall, the observed prevalence of exposure to each of the three viral pathogens in adult dogs was high (>72%) across sampling sessions (Fig. 2). Although 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 ( $\chi^2 = 0.062$ ,  $P = 0.804$  for CPV;  $\chi^2 = 0.053$ ,  $P = 0.818$  for CDV, and  $\chi^2 = 0.713$ ,  $P = 0.398$  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 percentage of dogs sampled in sessions 3 and 4 were exposed to more than one of the tested pathogen species (Fig. 3). 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). There were, however, 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 with juveniles (53%).

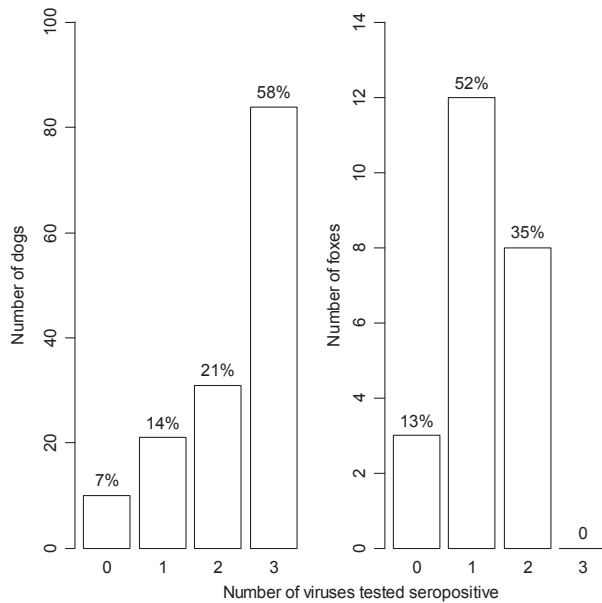
### CPV

The observed prevalence of exposure to CPV, obtained by combining data from Session 3 and Session 4, was 88% (95% CI 81–92%). The CPV exposure rate was significantly



**Fig. 2.** The observed prevalence of exposure ( $\pm 95\%$  CI) to canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV) in foxes (light bars) and dogs (dark bars) sampled around the Great Indian Bustard Wildlife Sanctuary between 2005 and 2012. Samples obtained during Session 1 and Session 2 were not tested for exposure to CAV.

greater in adults (94%) than juveniles (68%) ( $P = 0.0001$ , Fisher’s exact test), but there was no significant difference in prevalence between males (85%) and females (97%)



**Fig. 3.** 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).

( $P = 0.074$ ; Fisher’s exact test). There was also no significant pattern in prevalence of exposure to CPV when sex-prevalence data were stratified by age class (CMH statistic = 2.29,  $P = 0.130$ ). Two of the three resampled seronegative dogs acquired high titres of anti-CPV antibodies after the first sampling.

**CDV**

Combining data from Session 3 and Session 4, the observed prevalence of exposure to CDV was 72% (95% CI 64–79). The exposure rate was significantly greater in adults (83%) than in juveniles (40%) ( $\chi^2 = 26.77$ ,  $P < 0.0001$ ). CDV exposure rates were significantly greater in females (88%) than in males (67%) for Session 3 and Session 4 ( $\chi^2 = 5.84$ ,  $P = 0.016$ ). The difference in prevalence pattern when sex-prevalence data were 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 titre, while two resampled dogs remained seronegative.

**CAV**

Analysis of the combined data (Session 3 and Session 4) indicated that CAV exposure was significantly higher ( $\chi^2 = 7.936$ ,  $P = 0.005$ ) in adults (77%) compared with juveniles (53%), but did not differ significantly between males and females (71% for each sex) ( $\chi^2 = 0$ ,  $P = 0.995$ ). There was no significant pattern in prevalence of exposure to CAV when sex-prevalence data were 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 titres subsequently; four dogs had seroconverted with high, and one with low, IgG antibody titres.

**Seroprevalence of viral antibodies in foxes**

Sera samples obtained from 33 adult Indian foxes (18 males, 15 females) were tested for exposure to CPV and CDV. Combining the results from IgG and IgM antibody tests, 9% ( $n = 3$ ) of the foxes 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 pathogen. The observed prevalence of exposure to the three viral pathogens in foxes was calculated by combining the observations for IgG and IgM antibodies (Fig. 2). Of the 33 samples, 23 (13 males, 10 females) 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 (Fig. 3). 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).



Dilutions of fox sera samples tested with Canine VaccinCheck Antibody test kits yielded S scores that decreased with the dilution factor, indicating parallelism among dilutions.

#### CPV

The exposure rate for CPV did not differ significantly ( $\chi^2 = 0.793$ ,  $P = 0.373$ ) between males (56%) and females (40%). Twelve percentage ( $n = 4$ ) of the foxes tested had detectable levels of anti-CPV IgM antibodies, indicating recent or ongoing CPV infection. One female fox had high titres of anti-CPV IgG (S5) with concurrent anti-CPV IgM antibody titres. 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 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 titres were found in 15% ( $n = 5$ ) of foxes. Three of these foxes had concurrent high anti-CDV IgG titres and were found dead within a month of sampling.

#### CAV

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 seroconversions 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. Cleaveland et al., 2000; Bronson et al., 2008; Bryan et al., 2011; Millán et al., 2013).

We have used commercially available dot-ELISA kits in this study due to the lack of facilities in India where serological assessments via traditional methodologies (i.e. serum neutralization [SN] or haemagglutination-inhibition [HI]) can be undertaken. Also, due to the strict Indian export laws, sera samples cannot be shipped outside India. Nevertheless, diagnostic kits based on ELISA techniques are widely used for infectious disease diagnosis in veterinary

medicine and have been recommended as a standard tool for population-based serological studies (Wright et al., 1993). The kits used in this study have been verified for assay of antibodies in dogs (Waner et al., 1996, 1998, 2004). However, it should be noted that the Canine VaccinCheck Antibody test kit does not distinguish between the two types of CAV due to cross-reactivity, thus any CAV antibodies detected could be the result of exposure to either (or both) CAV types.

A possible limitation of this study is that the methodology used to assess antibody levels has been designed for dogs, and these test kits have not been fully validated for wild canid species. Our assessment using dilutions of fox sera samples indicated parallelism among dilutions, providing some support to the assumption of kit cross-reactivity in foxes. However, further validation of the kits with traditional methods would be critical to ensure the reliability of results in wild species.

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. In our study area, as in rural areas across India, most dogs are affiliated with neighbourhoods and therefore considered 'owned' by the community. Yet 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. 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 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, 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. Despite the high serocon-

version rates in adult dogs, a threshold for herd immunity is apparently not met because the pool of susceptibles (primarily pups and juveniles) in the population is rapidly replenished due to high population turnover rates.

These findings could have important implications for dog disease control programmes 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 (Belsare, 2013; Wright et al., 2013). 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., 2014). 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. Detection of CDV-specific IgM antibodies in a single serum sample using ELISA indicates current or recent CDV infection, and this technique has been validated for serodiagnosis of CDV (Blixenkron-Moller et al., 1991). During this study, three foxes with detectable titres of anti-CDV IgM antibodies died within a month of their respective sampling occasions. Although 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, nor could we undertake necropsies to confirm CDV diagnosis, as the carcasses were scavenged extensively by the time we located them. We did, however, observe clinical signs compatible with a neurologic disease like CDV in another fox (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). The

similar immune response documented in the 4 foxes that died, including the one that exhibited symptoms typical of CDV infection, provide some evidence of high CDV-related mortality in foxes and of the value of the test kits to assess CDV 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 titres 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 titres ( $\geq 54$ ; 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.

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 and not 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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