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## The effect of protected areas on pathogen exposure in endangered African wild dog (*Lycaon pictus*) populations

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### ABSTRACT

Infectious diseases impact African wild dogs (*Lycaon pictus*), but the nature and magnitude of this threat likely varies among populations according to different factors, such as the presence and prevalence of pathogens and land-use characteristics. We systematically evaluated these factors to assist development of locally appropriate strategies to mitigate disease risk. Wild dogs from 16 sites representing five unconnected populations were examined for rabies virus, canine distemper virus (CDV), canine parvovirus, canine coronavirus, and *Babesia* spp. exposure. Analyses revealed widespread exposure to viral pathogens, but *Babesia* was never detected. Exposure to CDV was associated with unprotected and protected-unfenced areas where wild dogs likely have a high probability of domestic dog contact and, in the case of protected-unfenced areas, likely reside amongst high wildlife densities. Our findings also suggest that domestic dog contact may increase rabies and coronavirus exposure risk. Therefore, domestic dogs may be a source of CDV, rabies and coronavirus, while wildlife may also play an important role in CDV transmission dynamics. Relatively high parvovirus seroprevalence across land-use types suggests that it might persist in the absence of spillover from domestic dogs. Should intervention be needed to control pathogens in wild dogs, efforts to prevent rabies and coronavirus exposure might be directed at reducing infection in the presumed domestic dog reservoir through vaccination. If prevention of CDV and parvovirus infections were deemed a management necessity, control of disease in domestic dogs may be insufficient to reduce transmission risks, and vaccination of wild dogs themselves may be the optimal strategy.

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### 1. Introduction

The African wild dog (*Lycaon pictus*) is one of the world's most endangered carnivores with <8000 animals, in <800 packs, remaining in the wild (IUCN/SSC, 2007, 2008). While habitat loss, reduced

prey base and persecution were the major causes of historical decline and continue to be important threats to wild dog conservation (Woodroffe et al., 2007), evidence suggests that infectious disease may have also contributed to these declines (Woodroffe and Ginsberg, 1997). Currently, most wild dog populations are reduced to small numbers ( $\leq 8$  packs), and pathogens may now pose an even greater threat to long-term population viability due to stochastic extinction events (Ginsberg et al., 1995; Woodroffe and Ginsberg, 1997). Pathogens, such as rabies virus (Alexander et al.,

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2010; Gascoyne et al., 1993b; Hofmeyr et al., 2000) and canine distemper virus (CDV: Alexander et al., 1996; Goller et al., 2010; van de Bildt et al., 2002), have been associated with die-offs and population declines. If pathogen impacts on wild dogs are similar to those seen in related species, other pathogens may also be of concern as they might undermine population viability by causing pup mortality (e.g., canine parvovirus (Mech and Goyal, 1995)), by acting as a co-pathogen that increases the severity of, or susceptibility to, other infections (e.g., *Babesia* spp. (Munson et al., 2008)) or by decreasing general health and hence ability to survive (e.g., canine coronavirus (McCaw and Hoskins, 2006)). Identifying the presence and prevalence of these pathogens in wild dog populations is a first step to evaluating the degree of exposure of different populations and the nature of potential disease threats. Further, identification of risk factors associated with pathogen exposure may help managers evaluate whether preventive measures are needed to reduce these risks, to determine what these measures may be, and to assess the suitability of potential wild dog reintroduction sites. For conservation managers, evaluating the degree of disease threat is challenging and must address both the likelihood of pathogen introduction into an endangered population and its potential impact on population viability. In this paper, we focus primarily on the probability of pathogen exposure as an important starting point, while recognizing that the effect of pathogens on population viability is the more important consideration.

African wild dogs live at low density, including those inhabiting fenced reserves, and contact between packs is infrequent (Woodroffe and Donnelly, 2011); hence pathogens that cannot survive long outside of their hosts and require direct contact for transmission, such as CDV and rabies virus (Greene and Appel, 2006; Greene and Rupprecht, 2006) or contact with fresh infectious material, such as coronavirus (McCaw and Hoskins, 2006), may be spread rapidly within a pack but may rarely be transmitted to other packs. In addition, pathogens such as rabies virus and CDV that cause a high degree of mortality and/or induce life-long immunity are likely to go extinct within a pack once all susceptibles have been infected.

Although wild carnivore species such as bat-eared foxes (*Otocyon megalotis*) and black-backed jackals (*Canis mesomelas*) are thought to act as rabies virus reservoirs in southern Africa (Bingham, 2005; Hofmeyr et al., 2000), research suggests that, in most of Africa, domestic dogs (*Canis familiaris*) are the principal reservoir for rabies virus (Lembo et al., 2008; Prager et al., in preparation) and possibly CDV (Alexander et al., 1996; Cleaveland et al., 2000; Gowtage-Sequeira et al., 2009), from which these pathogens can spill over into wild carnivore populations. Proximity to domestic dog populations may therefore pose a significant exposure risk to African wild dogs. By contrast, pathogens such as parvovirus can survive in the environment for months (McCaw and Hoskins, 2006); thus opportunities for between-pack transmission are greater, and, once introduced, might be maintained in a wild dog population in the absence of an external reservoir. Similarly, pathogens with complex lifecycles involving invertebrate vectors, such as *Babesia* spp. (Taboada and Lobetti, 2006), might be able to persist in low-density host populations (Lloyd-Smith et al., 2005) such as those of wild dogs. Domestic dogs can transmit all of these pathogens to wild dogs, either through close contact (rabies virus, CDV), via feces (coronavirus, parvovirus), or through shared ectoparasites (*Babesia* spp.), and might therefore be the original source of infection to susceptible wild dog packs; however, once introduced into an ecosystem certain pathogens may be able to persist without subsequent domestic dog-to-wild dog transmission events. Nevertheless, contact with or proximity to domestic dogs, or other species that harbor these pathogens, may increase the level of exposure even when the pathogen is endemic.

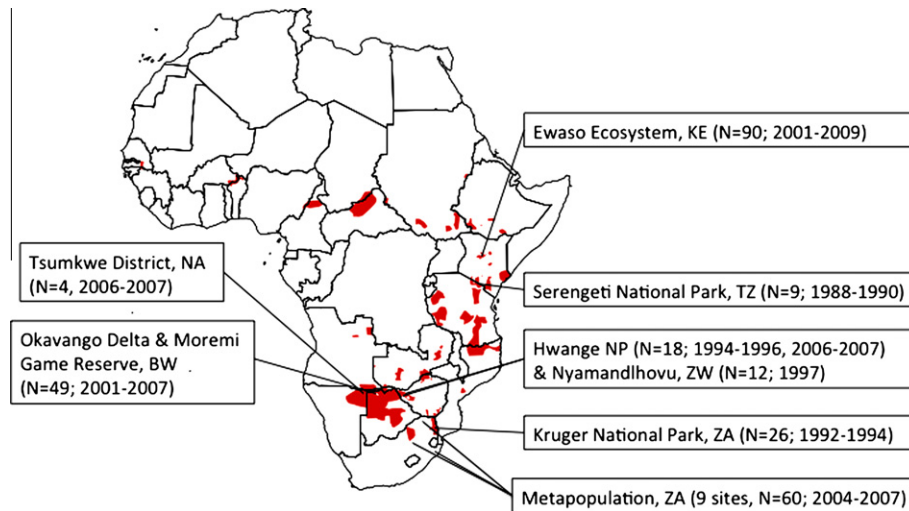
Land-use characteristics of areas inhabited by wild dogs, such as fencing and protected status, are likely to influence wild dogs' probability of exposure to domestic dogs and may therefore be used as predictors for domestic dog contact. "Predator proof" fences separate wildlife from domestic animals in some areas, thus limiting domestic dog contact with the wildlife contained within. Wildlife species from unfenced protected areas are likewise expected to have a relatively low probability of contact with domestic dogs where dogs are actively excluded by park staff. However, domestic dogs may live at high densities on lands adjoining protected areas, thus creating a perimeter zone where opportunities for pathogen transmission from domestic dogs may be high (Butler et al., 2004). Protected areas may also allow wildlife to reach greater densities than those on unprotected areas where the threats of poaching and persecution may be greater. In the absence of fences, these high wildlife densities may facilitate pathogen transmission from high domestic dog densities at the periphery to wild dogs and other wildlife at the center of a reserve.

Our goal was to determine the presence and prevalence of four viral pathogens of concern (rabies virus, CDV, canine parvovirus and canine coronavirus) and one protozoal pathogen of interest (*Babesia* spp.) in African wild dog populations across much of their range. While exposure to some of these pathogens has been examined previously in a number of wild dog populations across Africa (Alexander et al., 1993a,b, 2010; Creel et al., 1997; Gascoyne et al., 1993b; Laurenson et al., 1997a,b; Van Heerden et al., 1995), differences between the serological methods and laboratories used precludes direct comparison of results across sites. We sought to reliably and comparably screen samples across multiple sites and populations to identify risk factors for pathogen exposure among wild dogs. In particular, we sought to understand whether large-scale land-use management characteristics, associated with varying probabilities of contact with domestic dogs, influenced infectious disease exposure in wild dogs. We hypothesized that, as the probability of contact with domestic dogs increased, exposure to directly transmitted pathogens would increase. In contrast, we hypothesized that pathogens with environmental persistence or complex lifecycles might be able to persist in wild dog populations in the absence of an external reservoir, such that wild dog exposure might not be as influenced by land-use type and probability of contact with domestic dogs. Data collected from wild dog populations across 16 sites in sub-Saharan Africa allowed us to test these hypotheses.

## 2. Methods

### 2.1. Samples

Blood samples, and associated background data, were collected between 1988 and 2009 from 268 individual African wild dogs distributed across 16 sites in five countries (Fig. 1, Table S1) (Gascoyne et al., 1993a; Osofsky et al., 1996; Rasmussen and Macdonald, 2012; Spiering et al., 2009; Van Heerden et al., 1995; Woodroffe, 2011). Where individual animals were sampled repeatedly, data from only a single sampling date were included in statistical analyses. This date was chosen by ordering individuals by ID and then alternately choosing the first, or second, sampling date to avoid collection bias. The majority of wild dog samples were tested between 2008 and 2010. All samples and data were collected in the course of wild dog monitoring projects. These 16 sites represented five unconnected wild dog populations; nine sites in South Africa were managed as a single connected metapopulation; Kruger National Park in South Africa was considered a single population, four sites fell within a very extensive connected population covering eastern Namibia, southern Angola, southern Zambia,



**Fig. 1.** Map of Africa with the location of the wild dog populations included in this study. The managed metapopulation in South Africa consists of subpopulations in nine geographically separate sites that are all managed as part of a single breeding population. The red shaded areas are where resident wild dog populations exist. The time periods during which samples were collected for each location are noted in parentheses. This map is a modification of a map from a Kenya Wildlife Service's proposal for inclusion of species on the appendices of the convention of the conservation of migratory species of wild animals (2010). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

northern Botswana and western Zimbabwe; and the populations from Kenya and Tanzania were each considered unconnected to others in the study (Fig. 1). Each of the 16 sites could be categorized into one of three broad land-use types, used as a proxy for the probability of contact with domestic dogs in the absence of empirical data on domestic dog densities. These types were (i) protected areas surrounded by game fencing likely to exclude domestic dogs, termed “protected-fenced”; (ii) protected areas where domestic dogs were not permitted, but without game fencing, termed “protected-unfenced”; and (iii) unprotected areas (Table 1). These types were expected to have low, moderate and high probability of contact with domestic dogs, respectively. Protection status

was consistent over the period of sampling for each site. Fencing is designed to keep large game and medium to large predators from crossing; however, smaller predators such as jackals may cross some of these fence lines. Breaches of the fence line can also occasionally occur by animals the size of domestic or wild dogs due to the difficulty of maintaining a long fence line or due to certain landscapes (rocky areas, cliffs and watercourses) that are difficult to fence. However, although these fences are occasionally breached, they will still act as a deterrent. In addition, active exclusion by park rangers occurs and domestic dogs within the fenced reserves are usually shot on sight; therefore we expect relatively fewer domestic dogs in fenced protected reserves than unfenced

**Table 1**

Viral seroprevalence in individual African wild dogs by site and land-use type: protected-fenced “PF”, protected-unfenced “PU” and unprotected “U”. The populations are abbreviated as follows: KNP is Kruger, M is Metapopulation, S is Serengeti, HO is Hwange–Okavango, and E is Ewaso. Exact 95% confidence intervals (CI) for binomial probabilities are included.

Country	Population	Site	Land use	Rabies virus		Distemper virus		Coronavirus		Parvovirus	
				n	Prevalence (CI)	n	Prevalence (CI)	n	Prevalence (CI)	n	Prevalence (CI)
ZA	KNP	Kruger	PF	26	0 (0–0.13)	25	0.08 (0.01–0.26)	25	0.12 (0.03–0.31)	25	0 (0–0.14)
			PF	5	0 (0–0.52)	11	0 (0–0.28)	11	0 (0–0.28)	11	0.27 (0.06–0.61)
			PF	0	–	5	0 (0–0.52)	5	0 (0–0.52)	5	0 (0–0.52)
			PF	0	–	2	0 (0–0.84)	2	0 (0–0.84)	2	0.50 (0.01–0.99)
			PF	10	0 (0–0.31)	10	0 (0–0.31)	10	0 (0–0.31)	10	0.90 (0.55–1)
			PF	0	–	1	0 (0–0.98)	1	0 (0–0.98)	1	0 (0–0.98)
			PF	6	0.17 (0–0.64)	6	0 (0–0.46)	7	0 (0–0.41)	7	0 (0–0.41)
		Subtotal	47	0.02 (0–0.11)	60	0.03 (0–0.12)	61	0.05 (0.01–0.14)	61	0.21 (0.12–0.34)	
TZ	S	Serengeti	PU	9	0 (0–0.34)	9	0.11 (0–0.48)	9	0 (0–0.34)	9	0.33 (0.07–0.70)
ZW	HO	Hwange	PU	18	0.06 (0–0.27)	18	0.44 (0.22–0.69)	14	0.07 (0–0.34)	18	0.33 (0.13–0.59)
BW	HO	Moremi	PU	14	0.07 (0–0.34)	14	0.07 (0–0.34)	14	0.14 (0.02–0.43)	14	0 (0–0.23)
		Subtotal	41	0.05 (0.01–0.17)	41	0.24 (0.12–0.40)	37	0.08 (0.02–0.22)	41	0.22 (0.11–0.38)	
ZA	M	Marakele <sup>a</sup>	U	3	0 (0–0.71)	3	0.33 (0.01–0.91)	3	0 (0–0.71)	3	0 (0–0.71)
			U	7	0 (0–0.41)	7	0.71 (0.29–0.96)	8	0 (0–0.37)	8	0.13 (0–0.53)
			U	34	0.21 (0.09–0.38)	35	0.14 (0.05–0.3)	35	0.09 (0.02–0.23)	35	0.2 (0.08–0.37)
			U	4	0 (0–0.60)	9	0.22 (0.03–0.60)	11	0.09 (0–0.41)	11	0.45 (0.17–0.77)
			U	2	0 (0–0.84)	2	0 (0–0.84)	2	0.5 (0.01–0.99)	2	0 (0–0.84)
ZW	HO	Nyamandhlovu	U	12	0 (0–0.26)	12	0.25 (0.05–0.57)	12	0.08 (0–0.38)	12	0.25 (0.03–0.57)
KE	E	Ewaso	U	73	0.12 (0.06–0.22)	90	0.17 (0.10–0.26)	86	0.24 (0.16–0.35)	60	0.21 (0.15–0.31)
		Subtotal	135	0.12 (0.07–0.19)	158	0.20 (0.14–0.27)	157	0.18 (0.12–0.25)	161	0.22 (0.16–0.29)	
		Total:	223	0.09 (0.05–0.13)	259	0.17 (0.11–0.2)	255	0.13 (0.09–0.18)	263	0.22 (0.17–0.27)	

<sup>a</sup> Although Marakele and Thanda are fenced reserves, these animals all have a history of either roaming outside of the parks or originating from outside of the park (North and South Marakele) before ultimately residing within the park. They are therefore categorized as coming from “unfenced” areas.

protected reserves. As the probability decreases that a domestic dog uses the same areas used by wild dogs, so too will the probability of contact between domestic and wild dogs decrease.

A known subsample of the wild dogs living in protected-fenced areas had a history of ranging on ranch land outside the protected-fenced area or had been imported from unprotected areas, and were thus grouped with wild dogs from unprotected areas ( $n = 24$ ; see Table 1). In addition, although most of the Okavango Delta was categorized as unprotected, the Moremi Game Reserve, an area within the delta, is protected but unfenced and the wild dogs from this reserve were classified accordingly (Table 1). The Tsumkwe district of Namibia was classified as unfenced unprotected; however the four wild dog samples from this site were only appropriate for *Babesia* spp. analyses and are therefore not included in Table 1. Finally, reserve size was also initially considered in defining the different land-use types but was ultimately not included because only one fenced site (Kruger) differed in size from the others, and it was not possible to define the boundaries and sizes of the sites that were not fenced.

## 2.2. Serologic testing

The Centers for Disease Control and Prevention, Atlanta, GA, performed a rapid fluorescent focus inhibition test (RFFIT) to identify antibodies against rabies virus in the wild dog serum (Smith et al., 1996). A RFFIT titer  $>0.05$  IU/ml was considered indicative of prior exposure and an “aborted infection” or non-fatal exposure. Such aborted infection probably explains the observation of antibodies against rabies virus in unvaccinated free-ranging African wild dogs (Gascoyne et al., 1993b; Prager et al., in preparation), domestic dogs (Cleaveland et al., 1999; Prager et al., in preparation), black-backed jackals, *C. mesomelas*, (Alexander et al., 1994; Prager et al., in preparation), Ethiopian wolves, *Canis simensis*, (Sillero Zubiri et al., 1996), and spotted hyenas, *Crocuta crocuta*, (East et al., 2001; Prager et al., in preparation), even though established infection is known to be fatal in all these species. Serologic analysis is therefore expected to provide useful information on exposure.

The Animal Health Diagnostic Center at Cornell (Ithaca, New York, USA) performed serum neutralization (SN) tests to measure antibodies against CDV using the Onderstepoort virus strain, and antibodies against coronavirus using the canine coronavirus strain S378/6 and A-72 indicator cells (Appel and Robson, 1973). The same laboratory performed hemagglutination inhibition (HAI) tests to measure antibodies against canine parvovirus (Carmichael et al., 1980). A CDV or coronavirus SN titer  $\geq 1:8$  and a parvovirus HAI titer  $\geq 1:20$  were considered indicative of prior exposure. Although this cut-off titer for CDV is relatively low, we chose it to maximize the sensitivity of the test.

We refer to the proportion of animals with detectable serum antibodies against a given pathogen as “pathogen seroprevalence” and interpret seroprevalence as an indicator of pathogen exposure in a population. Seropositivity data provides information only on

those animals for which exposure results in pathogen transmission and for which a detectable immune response is mounted and the individual survives. Not all samples were sufficient to contribute data for all pathogens, due to volume or toxic cell culture reactions. In cases of insufficient serum quantity, CDV and rabies virus serology were prioritized.

## 2.3. Molecular analyses

Quantitative real-time polymerase chain reaction (qPCR) was performed on whole blood or red blood cells to determine the presence of *Babesia* spp. and related protozoan pathogens (*Theileria*, *Cytauxzoon* and *Hepatozoon*) at the Molecular Diagnostic Laboratory, University of Illinois Zoological Pathology Program using previously published methods (Munson et al., 2008). Samples were screened for infection with *Babesia* spp. and related protozoan pathogens using density gel gradient electrophoresis, and the species was determined by direct sequencing of PCR products. Suitable samples were available from 154 wild dogs from seven of the 16 sites in four of the five countries – Botswana, Namibia, South Africa (Hluhluwe-iMfolozi, Madikwe, Pilanesberg and Venetia) and Kenya (Ewaso).

## 2.4. Statistical analyses

A generalized linear mixed model (GLMM) approach with a logit link and with wild dog pack as a random effect (Brostrom, 2009) was used to evaluate associations with pathogen seroprevalence using R (R Development Core Team, Vienna, Austria). This method fits the GLMM with a random intercept by maximum likelihood and numerical integration via Gauss-Hermite quadrature. The random intercept was used to account for the correlation likely to arise due to non-independence of individuals from the same pack (which can transmit infection to one another and also share the same exposure history). Associations between pathogen seroprevalence and age (months), sex (male or female) and land-use type (protected-fenced, protected-unfenced, unprotected) were evaluated by calculating odds ratios (OR) and their 95% confidence intervals (CI) using the GLMM. Because older animals have a greater opportunity to have been exposed, age, which was calculated based on known or suspected birth dates, was included as a potential explanatory variable in the models. Sex was included because it may influence behavioral patterns associated with exposure risk. All samples included in the study were in storage for as few as 2 months and up to 21 years prior to being tested. With increased time in storage, the quality of a serologic sample may deteriorate and antibody levels may decline due to freeze-thaw cycles or other storage problems. Since the time between sample collection and screening might be negatively associated with antibody detectability and therefore seropositivity, we included the variable of “time since collection” in all of the GLMMs regardless of the statistical significance, i.e., we forced time since collection into the GLMMs

**Table 2**

Best fitting models for rabies virus and canine distemper virus were chosen by backward stepwise selection using the likelihood ratio method including time since sample collection without regard for contribution to model fit. All samples were from African wild dogs. Pack was included in the model as a random effect. Sample size (N), seroprevalence (SP), odds ratios (OR) with their associated 95% confidence intervals (CI), and *P*-values for the overall contribution of a variable calculated using a likelihood ratio test (LR) were reported where appropriate.

Pathogen	Variable	N	SP	OR (CI)	<i>P</i> -value (LR)
Rabies virus	Age (in months)	211	–	1.04 (1.01–1.06)	<0.010
	Time since sample collection (in years)	211	–	0.85 (0.71–1.03)	0.065
CDV	Protected-fenced	57	0.035	Reference	
	Protected-unfenced	32	0.281	17.55 (2.27–135.61)	
	Unprotected	153	0.196	12.27 (1.49–100.77)	
	Overall contribution of land-use				0.005
	Time since sample collection (in years)	242	–	1.07 (0.90–1.27)	0.44



for each pathogen. Exact 95% CIs for binomial probabilities were calculated for seroprevalence results using the Hmisc package in R (Harrell and users, 2010).

Initially, univariable analyses (including pack as a random effect) were performed for each pathogen to identify potential predictors of pathogen seroprevalence. Multivariable GLMMs were then selected by backward stepwise selection (with time since collection forced into all models) using a likelihood ratio test to compare models. Strengths of association were evaluated using ORs and their 95% CIs. Age data were not available for the nine animals from Serengeti; therefore results from these individuals were excluded from statistical analyses. Hence, the seroprevalence for each land-use reported for the entire dataset (Table 1) differed slightly from that reported for the statistical analyses (Table 2 and statistical results section). A small subset of animals ( $n = 15$ ) had a history of vaccination against rabies virus and/or CDV; these were excluded from statistical analyses evaluating associations between risk factors and exposure to the pathogen(s) against which they were vaccinated.

### 3. Results

#### 3.1. Serologic results

The proportions of African wild dogs from each site with evidence of exposure to each pathogen are shown in Table 1. For all the viral pathogens (i.e., all pathogens but *Babesia* spp.), evidence of exposure was detected among the wild dogs in this study, although not all pathogens were detected at all sites (Table 1).

#### 3.2. Molecular results

*Babesia* spp. DNA was not detected in any of the 154 wild dog blood samples. However, using the same method, *Hepatozoon canis* DNA was amplified and sequenced in 148 of these samples. No statistical analyses were conducted on these data due to insufficient variation in pathogen prevalence.

#### 3.3. Rabies virus

The model that best explained variation in rabies seroprevalence included age of animal and time since sample collection (the latter included without regard to improvement of model fit (forced); Table 2). Wild dogs were more likely to be seropositive as they increased in age (measured in months; OR = 1.04, 95% CI = 1.01–1.07). Although land-use did not contribute significantly to the final model ( $P = 0.23$ ), the values reported from basic seroprevalence (SP) calculations suggest greater seroprevalence in wild dogs from unprotected areas (SP = 0.12) than in wild dogs from protected-unfenced (SP = 0.06) or protected-fenced (SP = 0.02) areas. Sex did not improve the fit of this model ( $P = 0.48$ ).

#### 3.4. Canine distemper virus

The model that best explained variation in CDV seroprevalence included land-use and time since collection (the latter forced; Table 2). Seroprevalence was over 12 times higher among wild dogs from unprotected (95% CI = 1.49–100.77) and 18 times higher for protected-unfenced areas (95% CI = 2.27–135.61) than from protected-fenced areas (Table 2). Increased age ( $P = 0.16$ ) and sex ( $P = 0.58$ ) did not improve the fit of this model.

#### 3.5. Canine coronavirus

There were no statistically significant predictors of coronavirus seroprevalence compared to the model with only time since collection included. However, although land-use did not contribute significantly to the model ( $P = 0.08$ ), as with rabies virus, the values reported from basic seroprevalence calculations suggest greater seroprevalence in wild dogs from unprotected areas (SP = 0.18) compared to those from protected-fenced areas (SP = 0.03) and protected-unfenced (SP = 0.11).

#### 3.6. Canine parvovirus

There was a negative association between parvovirus seroprevalence and time since collection ( $P = 0.008$ ) indicating that antibodies may have become more difficult to detect with increased time samples spent in storage. No other variables improved the fit of this model. In particular, land-use did not contribute significantly to the model ( $P = 0.64$ ), and calculated parvovirus seroprevalences were similar across the three land-use types: unprotected (SP = 0.21) protected-unfenced (SP = 0.19), and protected fenced (SP = 0.22).

### 4. Discussion

This study represents the most comprehensive analysis of pathogen exposure in African wild dog populations to date: it spanned viral pathogens of major concern, as well as an important protozoal potential co-pathogen (*Babesia*); it included the majority of the well-studied wild dog populations across Africa; and it used the same serologic methods and laboratories for each pathogen, ensuring consistency and enabling direct comparison of results between sites and populations. However, because samples were collected over a period of more than 20 years, some samples were necessarily old at the time of testing and may have deteriorated relative to the newer samples, making antibody detection more difficult. In addition, detection of antibodies against a pathogen provides information only on those animals for which exposure leads to pathogen transmission, a detectable immune response and survival. Exposure to the pathogens of concern was widespread in wild dog populations across Africa, with one notable exception: *Babesia* infection was not detected in any of the populations examined. Seroprevalence patterns varied substantially between sites, and not all pathogens were detected at all sites (Table 1). Patterns of exposure differed by pathogen: the highest levels of exposure to rabies virus and coronavirus were found in wild dogs from unprotected areas; relative to protected-fenced areas, significantly higher levels of exposure to CDV were found in wild dogs from both unprotected and protected-unfenced areas; and relatively high levels of parvovirus exposure were detected in all three land-use types.

Canine distemper virus exposure was relatively high in both protected-unfenced and unprotected areas. Opportunities for contact with domestic dogs should be fewer in protected-unfenced areas than in unprotected areas; therefore the observed pattern of CDV exposure suggests that factors other than direct contact with domestic dogs may also strongly influence CDV transmission to wild dogs. These findings are consistent with those of other recent studies (Woodroffe et al., 2012) suggesting that wildlife play an important role in maintaining CDV infection (Craft et al., 2008; Prager et al., in preparation). In this study, the wildlife management status of protected-unfenced areas means that these areas probably supported higher densities of other wild carnivores such as bat-eared foxes, jackals, spotted hyenas and lions (*Panthera leo*) than did the unprotected areas. These wild carnivores may

play a significant role in the transmission dynamics of CDV (Craft et al., 2008; Prager et al., in preparation): as wild carnivore densities increase, contact between these animals and both domestic and wild dogs will increase, thereby increasing opportunities for CDV transmission from domestic dogs to wild carnivores and from wild carnivores to wild dogs. With the combination of a robust wild carnivore population and moderate levels of domestic dog contact, CDV transmission to wild dogs may occur in protected-unfenced areas at levels as high as those in unprotected areas with greater opportunities for domestic dog contact.

The calculated seroprevalence for rabies and coronavirus in wild dogs appeared to be greatest in unprotected areas with the highest opportunity for contact with domestic dogs. Although these results were not statistically significant, the findings are consistent with those of prior work suggesting that domestic dogs are a likely source of rabies for African wild dogs (Lembo et al., 2008; Prager et al., in preparation) and may indicate that domestic dogs are also a source of coronavirus infection. Rabies serology is complicated by the fact that rabies antibodies may be short-lived in the host, as rapid post rabies vaccination titer decline has been shown to occur in some domestic dogs (Kennedy et al., 2007; Sage et al., 1993; Tepsumethanon et al., 1991), and thus seroprevalence is likely to be lower than that of other pathogens for which wild dogs have the same degree of exposure. The statistical power to detect significant differences is decreased by both low seroprevalence values and low sample size, both of which are characteristics of our study (Table 1). Such a lack of statistical power to detect differences in exposure due to small sample size may provide one explanation for the lack of statistical significance in some of the patterns observed in the serological results for the pathogens included in this study, despite this data set representing the most extensive one compiled for analysis to date on pathogen exposure in African wild dogs. Other approaches, including higher-resolution data on risks of domestic dog contact (such as those in Woodroffe et al. (2012)), phylogenetic analyses and temporospatial analyses of case-incidence data are likely to be required to demonstrate conclusively the potential epidemiologic links between domestic dogs and African wild dogs.

Directly transmitted pathogens (rabies and CDV) or pathogens with short-lived infectious stages outside the host (coronavirus), which also have short incubation and infectious periods, are unlikely to persist below a predicted population threshold or “critical community size” (CCS; Bartlett, 1960). Wild dog populations likely fall below this CCS, while many African domestic dog populations may exist well above it (Hampson et al., 2009; Kitala et al., 2001; Lembo et al., 2008). The concept of a CCS in combination with differences in carnivore species density among land-use types may explain some of the differing patterns of exposure among these land-use types. In the case of CDV, the required CCS may exist in a single large domestic dog population (Gowtage-Sequeira et al., 2009) or may involve a more complex, interconnected multi-carnivore community (Craft et al., 2008; Prager et al., in preparation).

In contrast with directly transmitted pathogens, parvovirus can persist outside of the host in the environment for months (McCaw and Hoskins, 2006); therefore the CCS for persistence, once parvovirus has been introduced into an area, may be quite low or non-existent (Lloyd-Smith et al., 2005). This may explain why parvovirus exposure was equally high in all land-use types, showing that it can persist in the absence of domestic dogs. Alternatively, parvovirus may persist because of the combination of prolonged environmental persistence and interspecies transmission events occurring in the wild carnivore community.

Although exposure to most of the pathogens was found in most of the sites, the exceptions provide interesting insights. We found no evidence of exposure to rabies virus and parvovirus in wild dogs from Kruger National Park, or of rabies virus exposure in wild dogs

from Serengeti. Sample deterioration may explain our failure to detect exposure to either pathogen in wild dogs sampled from Kruger National Park over 16 years ago, as we found a significant negative association between parvovirus seroprevalence and time since sample collection. However, our negative findings were consistent with those of Van Heerden et al. (1995), which were based on a greater number of samples tested sooner after collection than were ours. Thus a more likely explanation may be that the absence of parvovirus and rabies virus from Kruger National Park was a result of management efforts, such as electric fences and immediately shooting domestic dogs observed in the park, having prevented or substantially limited transmission into the park from domestic dogs. By contrast, deterioration of antibodies over time may explain why we failed to detect any rabies seropositive wild dog samples collected from the Serengeti over 20 years ago, while Gascoyne et al. (1993b) detected 25% seroprevalence using many of the same serum samples. However, the discrepancy between our findings and those of Gascoyne et al. (1993b) may also be due to the fact that different laboratories were used to perform the rabies serology and variation can exist between laboratories despite efforts towards consistency. Other studies, in which wild dog exposure to rabies virus has been examined, have failed to detect any seropositive animals (Alexander et al., 1993a,b; Creel et al., 1997; Laurenson et al., 1997a,b); this may be due to differences in the sensitivity of the laboratory techniques used, the difficulty of detecting exposure in a population when seroprevalence is low and sample sizes are small, or it may be due to the epidemic nature of the pathogen and the fact that a recent epidemic may not have occurred, as rabies antibodies may be short-lived (Kennedy et al., 2007; Sage et al., 1993; Tepsumethanon et al., 1991).

The absence of exposure to *Babesia* in wild dogs from the sites included in this analysis is consistent with results from other studies: Matjila et al. (2008) and Flacke et al. (2010) found no evidence of *Babesia* spp. in free ranging wild dogs from five small protected-fenced reserves in South Africa. However, this pathogen has been previously reported in wild dogs from Kruger National Park (Van Heerden et al., 1995) – a site not included in our analyses – and in captivity at De Wildt Cheetah and Wildlife Centre in South Africa (Matjila et al., 2008), indicating that although not a widespread problem across Africa, wild dogs can be infected with *Babesia* spp. and it is present in some free-ranging populations. Therefore, continued monitoring is recommended as *Babesia* has been shown in lions to be an important copathogen with the potential to cause high mortality (Munson et al., 2008). Almost all of the wild dogs sampled in our study were infected with a related protozoan parasite, *H. canis*. These results are similar to findings of Pierce et al. (1995) and Goller et al. (2010), both of whom also found a high prevalence of *H. canis* infection in wild dogs from Serengeti. *H. canis* infection in domestic dogs can vary from the more commonly seen sub-clinical or mild infection, to the less common severe and life-threatening (Allen et al., 2011); however, the wild dog health significance of infection with this pathogen is unknown.

Our findings can help inform guidelines for mitigating pathogen exposure to wild dogs. The remaining wild dog populations in Africa differ in the risks that they face, including those related to infectious disease, and each population must be considered separately when devising pathogen control strategies. Prior studies involving some of the populations examined in this study suggest that total deaths due to any disease may be quite low (Woodroffe et al., 2007); therefore, mitigation may not always be necessary. However, pathogens such as rabies virus and CDV have been associated with major die-offs (Alexander et al., 1996; Gascoyne et al., 1993b; Hofmeyr et al., 2000; van de Bildt et al., 2002), and disease-related local extinction is a real risk for small populations sensitive to stochastic events such as those in South Africa (Hofmeyr et al., 2000) and the Serengeti (Gascoyne et al., 1993b; Ginsberg et al., 1995).



Hence mitigation may be of great conservation benefit under some circumstances, such as when wild dog populations are relatively small and local domestic dog densities are high.

Mitigation may not be necessary for pathogens such as canine parvovirus and coronavirus, which are not known to have been associated with any major wild dog population declines and their pathological impact on wild dogs remains unclear (Van Heerden et al., 1995; Woodroffe and Ginsberg, 1999). However, they may contribute to less noticeable population decline due to increased pup mortality and/or decreased general health. Given the widespread occurrence of these pathogens, it is unlikely that any major population declines caused by these pathogens would be missed, although further research might reveal more subtle impacts on wild dog populations.

Where mitigation is deemed appropriate, our results help to indicate which conservation approaches might be effective. Our results are consistent with prior work indicating that domestic dogs are the major reservoir for rabies virus throughout most of Africa; therefore management that can reduce or eliminate pathogen spillover from domestic dogs by limiting domestic dog-wild dog contact, or eliminate rabies from the domestic dog reservoir through vaccination, might substantially reduce the rabies exposure to wild dogs. Hampson et al. (2009) suggest that with adequate funding, effort and compliance, elimination of canine rabies in Africa, through vaccination of domestic dogs, is an achievable goal. In addition, our findings suggest that limiting contact between domestic and wild dogs through fencing may have some effect in limiting wild dog exposure to CDV, and perhaps to rabies and coronavirus. However, any benefits of fencing regarding pathogen control must be weighed against the costs, which can be significant and include: (i) reducing landscape connectivity for multiple wildlife species, including wild dogs, which can then reduce population viability; (ii) considerable financial investment to construct and maintain fences; and (iii) preventing the formation of herd immunity in a population due to complete lack of pathogen exposure which could thereby place a population at risk from epidemics (Woodroffe, 1999); thus vaccination, of either wild or domestic dogs, may be the better mitigation strategy.

Controlling CDV exposure may be more complicated than controlling rabies exposure because, as our own and others' results (Craft et al., 2008) suggest, wild carnivores may play a significant role in CDV transmission dynamics. This role of wild carnivores means that limiting contact between wild dogs and domestic dogs, or vaccinating domestic dogs, may not limit wild dogs' exposure to CDV. Fortunately, the potential for CDV to cause mortality in wild dogs appears to vary, and there has never been a confirmed die-off described in the wild involving more than one pack (Alexander et al., 1996; Goller et al., 2010); therefore, mitigation may not always be necessary. Should CDV pose a significant health risk to a wild dog population, such as may be the case with very small populations, vaccination of individual wild dogs may be the most effective means of protection (Prager et al., 2011). If further research confirms domestic dogs as the rabies reservoir, vaccination of some proportion of a wild dog population may also be an appropriate strategy (Prager et al., 2011) in cases where control of rabies virus in the domestic dog population is not an achievable goal and the wild dog population is very highly threatened. This strategy was successful in protecting the adult wild dogs in a small population from Madikwe Game Reserve, South Africa (Hofmeyr et al., 2004). Our data clearly show that most wild dog populations are in contact with both rabies virus and CDV, yet most have persisted over time. Therefore, pathogens must be considered when devising management strategies, especially since many populations have been reduced to a small size (Woodroffe and Ginsberg, 1997). However, pathogen management must occur in conjunction with management to mitigate other major wild dog conservation

concerns, such as deliberate and accidental killing, as well as habitat loss, and loss of prey base.

## 5. Conclusion

This study revealed evidence of widespread exposure of African wild dogs to canine pathogens. Significantly higher levels of CDV exposure were found in wild dogs from both unprotected and protected-unfenced areas, while rabies virus and coronavirus exposure was highest in wild dogs from unprotected areas. These patterns suggest that exposure to CDV, and possibly to rabies virus and coronavirus, may be associated with increased contact with domestic dogs; however, wild carnivores may also play a significant role in CDV transmission dynamics. Continued monitoring of pathogen exposure in wild dog populations is needed to determine the long-term effect of these pathogens on population persistence and could provide managers with the information needed to decide whether to intervene to mitigate pathogen exposure risk. Should intervention be needed, efforts to prevent rabies and coronavirus infection might be directed at reducing infection in the presumed domestic dog reservoir through vaccination, while vaccination of wild dogs themselves may be necessary to prevent canine distemper virus and parvovirus infections.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2012.03.005>.

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