Transmission ecology of canine parvovirus in a multi-host, multi-pathogen system

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Abstract: (200 words)

Understanding multi-host pathogen maintenance and transmission dynamics is critical for disease control. However, transmission dynamics remain enigmatic largely because they are difficult to observe directly, particularly in wildlife. Here, we investigate the transmission dynamics of canine parvovirus (CPV) using state-space modelling of 20-years of CPV serology data from domestic dogs and African lions in the Serengeti ecosystem. We show that, although vaccination reduces the probability of infection in dogs, and despite indirect enhancement of population seropositivity as a result of vaccine shedding, the vaccination coverage achieved has been insufficient to prevent CPV from becoming widespread. CPV is maintained by the dog population and has become endemic with ~3.5-year cycles and prevalence reaching ~80%. While the estimated prevalence in lions is lower, peaks of infection consistently follow those in dogs. Dogs exposed to CPV are also more likely to become infected with a second multi-host pathogen, canine distemper virus. However, vaccination can weaken this coupling raising questions about the value of monovalent versus polyvalent vaccines against these two pathogens. Our findings highlight the need to consider both pathogen- and host-level community interactions when seeking to understand the dynamics of multi-host pathogens and their implications for conservation, disease surveillance and control programmes.

Keywords: State-space models, longitudinal serology, coinfection, domestic-wildlife interface, maintenance host, vaccine shedding

Introduction

Pathogens that can be transmitted between multiple host species pose challenges for disease control [1,2]. For example, multi-host pathogens can persist in ecologically complex multi-species metapopulations that may serve as either maintenance or non-maintenance populations, contributing differentially to pathogen persistence, and requiring different approaches to control [1,3]. Identifying the maintenance host population of a pathogen and unravelling the transmission dynamics among different populations is therefore critical for effective control. However, our understanding of multihost pathogens, particularly in wildlife, is often poor because epidemiological processes such as infection, clinical progression, morbidity and mortality are difficult to observe, necessitating less direct sources of inference. When it is possible to isolate pathogen genetic material from infected hosts, phylodynamic analyses can provide a rich source of insight (e.g. as for canine and bat rabies [4–6]). However, obtaining pathogen genetic material can be difficult, especially for pathogens that infect wildlife, for those that cause short-lived infections or are associated with low mortality rates that may therefore go undetected. A common alternative source of information is serological data. Serology can be used to infer if an individual has been exposed to infection [7]. However, using this type of data to make inferences about within- and between-species dynamics is challenging and requires sophisticated statistically rigorous methodology (e.g. as for canine distemper and rinderpest viruses [8,9]). Here, we use serological data to identify the maintenance population and reveal the transmission dynamics of canine parvovirus (CPV) in a complex multi-host, multi-pathogen environment [10–14].

CPV is a highly contagious DNA virus of the family *Parvoviridae* [15] that can cause vomiting, bloody diarrhoea, fever and anorexia in dogs, often leading to death. It was first isolated in the late 1970s [16] but has since become a widespread virus infecting domestic and wild canids worldwide [17–19]. CPV maintenance is enhanced by multiple factors, likely including multiple hosts capable of being infected and transmitting virus through different routes [22]. It is mainly transmitted by the faecal-oral route [23], but direct contact or environmental contamination may also play a role [22].

CPV is believed to have evolved from the variant Feline Panleukopenia Virus (FPV) [18,24,25]. The main consequence of this emergence was a host shift from felids to canids [26] with CPV subsequently establishing in dog populations worldwide [17,19,27]. The virus is now maintained in many domestic dog populations, often at very high prevalence, e.g. 90% in Korea [28], 72% in rural Chile [29]. In domestic dog populations, mortality rates following

infection can be as high as 90% in unvaccinated pups [11] but as low as 10% in unvaccinated adults [30].

While the domestic dog is now considered to be a primary maintenance host species for CPV, the consequences of short-lived epidemic 'spill overs' into smaller wildlife host populations pose a conservation concern [31,32]. For example, high prevalence has been observed in wild canids of vulnerable status such as wolves (*Canis lupus*) in Spain (62%) [20] and less vulnerable status such as red foxes (*Vulpes vulpes*; 79%) in North America [33]. In North American wolves, seropositivity was found to decrease with increasing population sizes and proportion of the population that were pups each year [34,35]. Further conservation concerns relate to felids. Although CPV may have initially lost its ability to infect and replicate in felids [36,37], recent studies have shown that it can now spread efficiently in domestic [38] and wild felids including African lions (*Panthera leo*) [39], puma (*Puma concolor*), bobcat (*Lynx rufus*) and lynx (*L. pardinus*) [40]. The cross-species transmission ability of CPV is well known mostly from laboratory infection and molecular studies [26,40,41]. However, the dynamics of CPV in different hosts and the role of each species in the local maintenance of CPV in natural systems remains unclear, especially in environments where a range of susceptible species co-exist.

Knowledge of the mechanisms of long-term disease maintenance is essential to optimize control strategies [1,3]. Vaccination is currently the most effective way to control CPV in dogs [11], with 95% survival rates of vaccinated adult dogs following infection challenges [42,43]. In the last decade, several dog vaccination programs have been implemented worldwide [44,45]. However, in resource-limited areas, vaccination coverage is typically very low. For example, in dog populations of Chile, vaccination coverage was reported to be 42% in urban areas but only 8% in rural areas [29]. In the Serengeti ecosystem of Tanzania, dog vaccination campaigns have been limited to villages in close proximity to wildlife protected areas, covering only 5% of the regional dog population in 1996 and reaching a maximum of ~ 30% coverage in 2008 [9]. It has been suggested that CPV vaccine in dogs can be shed in the faeces and the vaccine virus acquired by susceptible individuals. This has been only recently confirmed by a study in the Serengeti demonstrating the presence of vaccine strain sequences in unvaccinated dogs [46]. The role of faecal shedding of vaccine in providing unvaccinated animals with protection indirectly, potentially increasing herd immunity and the overall efficacy of vaccination programmes, is rarely, if ever, assessed. This is largely because serological

methods are unable to disentangle natural exposure from indirectly acquired immunization (i.e., seropositivity from vaccine shedding).

Well-developed frameworks to conceptualize the epidemiology of multi-host pathogens exist, but only recently have we begun to consider the joint epidemiology of multiple pathogens that co-occur within the same host [47,48]. Co-infections by multiple pathogens are common [49–51] and their interactions may be antagonistic or beneficial to both the host and pathogen by modifying transmission efficiency and virulence or by removing hosts from a shared susceptible pool [52]. Theory predicts that co-infections can have major consequences for both within- and between-host disease dynamics [51,53]. Empirically, this remains challenging to prove. Several studies report that CPV co-occurs with other canine pathogens, in particular canine distemper virus (CDV) in domestic dog [29,54] and wild canid populations [20,55]. Co-circulation of these pathogens is a major reason for the widespread use of polyvalent vaccines, for example against CPV, CDV and rabies. However, few studies investigate the dynamics of co-circulating canine pathogens.

Previous studies in the Serengeti ecosystem have shown that both CPV and CDV are present in domestic dogs [9,56] and lions [39,57]. The dynamics of CDV at the domestic–wildlife interface in this system has received attention [9], but little is known about CPV nor its interaction with CDV. To unravel these processes, this study uses: a) a large-scale longitudinal dataset comprising 29 years (1984-2012) of CPV serology in African lions in the Serengeti National Park (SNP) and domestic dogs adjacent to SNP; b) data on regional and village-level vaccination against CPV targeting domestic dogs since 1996 [9,58]; and c) CDV serological data for the same domestic dogs to investigate CPV and CDV co-exposure dynamics. We use Bayesian state-space modelling to integrate these datasets and overcome the challenges of using indirect sources of information (i.e. serological data). Ultimately, we aim to confirm the maintenance population and identify the transmission dynamics of natural CPV infection by (i) disentangling natural from indirectly acquired immunization (vaccine shedding) (ii) characterizing the within-host dynamics of natural infection in dogs and lions, and quantifying the impact of the dog vaccination programs on those dynamics; (iii) determining the cross-species transmission dynamics in dogs and lions; and (iv) investigating the co-infection dynamics between CPV and CDV in these communities.

Methods

Data collection

Unvaccinated domestic dogs in the northwest of the Serengeti ecosystem (Fig. 1a) were sampled from 1992 to 2012. This resulted in a 20-year time-series of domestic dog CPV (n=1728) and CDV (n=2368) serological data (Fig. 1b). Because of the possibility of CPV vaccination shedding, the dog samples in this study were restricted to villages where vaccination coverage was known (n=147 villages). Proxies for vaccination coverage were quantified at two levels: a) Regional vaccination coverage (Fig. 1b) estimated annually for the region as the proportion of dogs vaccinated in each village averaged across the northwest Serengeti ecosystem to the edge of Lake Victoria (including non-vaccinated villages); b) Village vaccination coverage (Fig.2a) estimated annually for each village as the proportion of dogs vaccinated only in sampled villages. Year of sampling and birth of each dog were also available.

Data from lions included CPV serology from individuals sampled between 1984 and 2012 (n=460) as part of SNP management and research operations (Fig 1). Year of sampling and birth were available for each lion. Further sampling details provided in Supplementary Information (SI) and [9].

Serological assays

Lion and dog CPV serology samples collected until 2007 was carried out using Haemagglutination Inhibition tests (HAI) and those from 2008 to 2012 were tested using in-house ELISAs (IgG). CDV neutralisation assays were carried out as previously described [9]. For consistency with previous studies, a cut-off titre equivalent to a 1:32 [39] and 1:16 [9] dilution was used to define prior exposure to CPV and CDV, respectively.

Statistical analysis

Here, we estimate the dynamics and drivers of population-level annual probability of natural infection using a Bayesian state-space modelling (SSM) approach. SSMs are useful because they comprise two interlinked parts: an observation and a biological process. The former deals with the individual-level data (i.e. serology data) to account for both known or suspected biases in the data-collection process (e.g. caused by diagnostic testing errors and vaccine shedding).

Since serology provides only the immune status at the time of sampling, the timing of infection can only be estimated from the serological data in combination with birth and sampling dates of each individual. The timing of infection describes the infection dynamics at the population level through a linearized predictor comprising several covariates (e.g. time-lags, cross-species transmission and vaccination coverage).

Here, the observation process modelled serology data as a Bernoulli trial adjusted with sensitivity and specificity terms to account for test diagnostic errors [as in 9]. Further details are provided in SI. Critically, because the probability of a dog being seropositive is made up of both 'natural infection' and the vaccine shedding effect, to disentangle these two processes, the logit probability of a dog *i* being seropositive with CPV (but not CDV) at time *t*, $Xp_{CPV,dogs}(i,t)$ was modelled as a function of the vaccine shedding rate (φ):

$$Xp_{CPV,dogs}(i,t) = p_{CPV,dogs}(t) + \varphi V_v(i,t) + \varepsilon_{CPV,dogs}(i,t) \quad (1)$$

The annual probability of CPV natural infection, i.e. that transmitted from unvaccinated dogs, $p_{\mathbf{CPV,dogs}}(t)$, corresponds to the natural infection at the population level, while φ represents the rate parameter governing the impact of the village-level vaccination coverage (V_v) on the probability of a dog becoming seropositive, i.e. shedding. This prior is based on [46]. The error term ε enables additional individual uncertainty at time t. All priors are provided in Table S2 (SI).

Two distinct biological process models were then implemented to describe the dynamics of CPV natural infection only, i.e. $p_{CPV,dogs}(t)$ in equation 1:

(i) To estimate the annual probability of CPV natural infection in dogs ($p_{CPV,dogs}(t)$) and lions ($p_{CPV,lions}(t)$), the logit probability of CPV infection at time *t* was described as follows:

$$p_{CPV,dogs}(t) = [\textit{linear predictor}] + v_1 V_r(t-1) + \Omega_{LD}.p_{CPV,lions}(t-1) \quad (2a)$$

$$p_{CPV,lions}(t) = [\textit{linear predictor}] + \Omega_{DL}.p_{CPV,dogs}(t-1) \quad (2b)$$

where $[\textit{linear predictor}] = b_0 + b_1.t + a_1.p_{CPV}(t-1) + a_2.p_{CPV}(t-2)$

The linear predictor describes the baseline dynamics of natural infection, where the coefficient b_0 represents the intercept and b_1 the linear trend over time. The parameters a_1 and a_2 are autocovariates representing time-lags of 1 and 2 years, respectively, and enable the infection probability to cycle. The term v_1 represents the impact of regional vaccination coverage (V_r) lagged by 1 year. The coefficient Ω_{LD} governs the 1-year lagged transmission from lion-to-dog and Ω_{DL} from dog-to-lion.

(ii) To investigate the co-exposure pattern between CPV and CDV in dogs we restricted the serology time series from 1992 to 2008, as after 2008 all tested samples were CDV negative. For the biological process, we first estimated the logit probability of CPV ($p_{CPV,dogs}(t)$, as above) and CDV ($p_{CDV,dogs}(t)$) exposure independently and then together ($p_{both,dogs}(t)$) as follows:

$$p_{CPV,dogs}(t) = [\text{linear predictor}] + v_1 V_r(t-1) + \delta.p_{CDV,dogs}(t-1) \quad (3a)$$

$$p_{CDV,dogs}(t) = [\text{CDV\_linear predictor}] + v_2 V_r(t-1) + \lambda.p_{CPV,dogs}(t-1) \quad (3b)$$

$$p_{both,dogs}(t) = \Gamma_{both}.(p_{CPV,dogs}(t).p_{CDV,dogs}(t)) + \Gamma_{CPV}.(p_{CPV,dogs}(t).p_{CDV,dogs}(t-1)) + \Gamma_{CDV}.(p_{CPV,dogs}(t).p_{CDV,dogs}(t-1)) \quad (3c)$$

The linear predictors, v1 and v2 are defined as in eq.1. The coefficient δ governs the influence of CDV on CPV exposure and λ governs the influence of CPV on CDV exposure. In eq. 3c, if the two diseases have independent dynamics, then the probability of an individual being exposed to both is the product $p_{CPV}(t).p_{CDV}(t)$. Then, the probability of being co-exposed $p_{both,dogs}$ is described by three parameters: a) Γ_{both} which governs the effect of both diseases being independent, b) Γ_{CPV} which governs the effect of CPV on the probability of co-exposure, and c) Γ_{CDV} which governs the effect of CDV on the probability of co-exposure.

The influence of each covariate on co-exposure was also estimated by comparing the full prediction with predictions obtained when either the parameter governing the impact of CDV on the co-exposure or the parameter governing the impact of CPV on the co-exposure was set to zero.

The priors for all parameters are defined in SI (Tables S2-3). All models were fitted in JAGS and details of model set-up, convergence and diagnostic assessments are provided in SI.

Results

The observed mean CPV seroprevalence in unvaccinated domestic dogs was ~55% across the entire time series (Fig. 1b). However, it varied greatly over time with peaks sometimes reaching 80% seropositivity. The mean CPV seroprevalence observed in lions was similar at ~57% (95% CI=0.20-1.00, Fig.1b and Fig. S2 in SI), though the sample size was smaller.

The SSMs used to analyse and quantify specific characteristics of CPV infection dynamics converged and fitted well to the data supporting the inferences made. Prior and resultant posterior distributions for estimated parameters shown in SI.

Dynamics of CPV and effects of vaccination in domestic dogs

Our results show that the probability of natural CPV infection in domestic dogs increased at an average of ~5% per year since the mid-90's, with large peaks of infection occurring every ~3.5 years (Fig. 2a and Fig. S7 in SI). The increasing probability of natural infection over time, together with the absence of exposure-free periods, suggests that CPV is likely to be maintained in the domestic dog population. Note that dog sampling started in 1992 but, albeit with high uncertainty, our model back-predicts the probability of infection from 1984, when the oldest dog was born.

Vaccination coverage determined from the number of dogs vaccinated provided an underestimation of the true proportion of dogs in the population exposed to CPV vaccine. Our model estimated that post-2003, when vaccination coverage was highest, vaccine shedding contributed an average of 10.6% to the probability of domestic dogs being seropositive (Fig. 2a, red line). This increase corresponded to ~19.3% of the village vaccination coverage (Fig. 3a).

Our results show that vaccination can decrease the probability of natural CPV infection in domestic dogs, but efforts during the study period were not high enough to eliminate infection even considering the indirect additional exposure provided by vaccine shedding. By comparing the predicted annual probability of infection from the full model with that predicted without vaccination (Fig. 3b), our results show that there was no measurable impact of dog vaccination on the probability of infection when regional vaccination efforts were patchier (pre-2003). However, following intensification of the vaccination effort in 2003, dog vaccination decreased the predicted annual probability of infection. For example, in 2008 when vaccination effort

was the highest (~30%), the predicted annual probability of infection was ~10% lower than that predicted without vaccination. Since there was a continued increase in CPV prevalence over time, these results indicate that vaccination was likely working to slow down the rate of CPV prevalence increase.

CPV dynamics at the domestic-wildlife interface

The estimated mean annual probability of CPV infection in lions was low (~9%; Fig. 2b). We note that this estimate was considerably lower than the observed mean seroprevalences (Fig. 1b) because it takes into account sample size and the timing of infection of each individual, which the observed seroprevalence does not.

The time between peaks of CPV infection in dogs and lions was ~3.5 years (Fig. 2 and S8 in SI) resulting in a pattern of peaks of infection in dogs preceding those in lions by one year. In support of this observation, our model showed a strong positive influence of the probability of natural infection in dogs on that in lions, as estimated from the dog-to-lion cross-species transmission parameter (mean 1.04 and 95% CI = [0.021, 3.78]; Fig. 4). In contrast, the model estimated only a negligible influence of the probability of infection in lions on that in dogs (mean 0.11 and 95% CI = [0.0030,0.40], Fig. 4). However, sample sizes in lions were small and the credible intervals on the associated predictions were too wide to accurately quantify the factual increase in the probability of infection in lions driven by dogs (Table S1).

CPV infection in lions does not seem endemic since lions had at least four distinct population-wide peaks of infection between 1980 and 2012, each following a period when infection in the lions was absent (Fig. 2b). These observations are supported by age-seroprevalence curves (Fig. S8 in SI). Together with the lagged but coupled peaks of infection in lions, our findings suggest that domestic dogs are integral to the maintenance of CPV infection in the Serengeti ecosystem and that lions alone do not maintain CPV.

CPV and CDV co-exposure patterns in domestic dogs

We found evidence of CPV and CDV co-exposure in domestic dogs. From the 1728 dogs for which we had a serology result for both CPV and CDV, 51.3% (n=887) were positive for CPV and 7.4% (n=128) for CDV. This is in agreement with the average seroprevalence (Fig.1b) observed and is consistent with previous studies on CDV in the same system [9]. The 110 dogs that were co-exposed to CPV and CDV correspond to 6.4% of the total number of individuals, as shown in the Venn diagram (Fig. 5a). However, if infection from these two diseases occurs

independently, the probability of co-exposure by both viruses - estimated as the product of the estimated probabilities for each one of them - would be 3.8%, which corresponds to 68 dogs, i.e. we observe 1.62 times more co-exposed dogs than expected by chance. This overrepresentation of dogs infected by both viruses suggests that CDV and CPV infection patterns are not independent of each other as confirmed by a chi-squared independence test (p-value=$8.3e^{-16}$).

Our model results provide further evidence of an interaction between CPV and CDV in domestic dogs and indicates that CPV infection might increase the probability of CDV infection. Fig. 5b shows the estimated posterior distribution of the parameters describing the probability of co-exposure (p_{both}). The posterior for Γ_{both}, which quantifies the independence of CPV and CDV on the probability of being co-exposed, was estimated to be 71% (95% CI= [-4.50,7.05]) above zero (Fig. 5b). This supports the observation that exposure to these two pathogens is not occurring purely independently. In contrast, the parameters Γ_{CDV}, which quantifies the influence of being infected with CDV and Γ_{CPV}, which corresponds to the influence of CPV on the probability of being co-exposed, were estimated as 87% (95% CI= [-1.30,8.17]) and 99.9% (95% CI= [0.67,9.04]) positive, respectively (Fig. 5b). This suggests that co-exposure could be driven by either pathogen but it is more likely that CPV infection makes an individual more prone to CDV infection.

Another line of evidence comes from the difference between the predicted dynamics of p_{both} without the influence of CDV (or CPV) and the full prediction (Fig. 6) in domestic dogs. If CDV or CPV drive co-exposure this difference will always be negative. Our results show that until ~2003 the difference in both cases is generally negative. However, from 2003, the difference between predicted dynamics with and without CPV or CDV increases substantially (Fig. 6). This suggests that the increase in vaccination effort which occurred at this time, might have acted to decouple the co-infection processes.

Discussion

This study used Bayesian state-space models to investigate the transmission ecology of CPV in the Serengeti ecosystem, a multi-pathogen system at the domestic-wildlife interface. We found endemic CPV circulation with a mean probability of infection at least 2 times higher in dogs than in lions. Dog vaccination reduced the rate of increase of CPV prevalence in dogs,

but the vaccination coverage achieved did not eliminate or stabilise CPV circulation in the western part of the Serengeti ecosystem, even with additional "protection" of unvaccinated dogs occurring as a result of vaccine virus shedding. Our findings further suggest that cross-species transmission of CPV largely occurs in one direction, from dogs to lions, and that peaks of CPV incidence in lions follow those in dogs. This is consistent with the hypothesis that dogs are a maintenance population and a source of CPV infection for lions. Periodic outbreaks of other canine viruses such as CDV have been previously reported in this complex system [9]. Here, we found that CPV infection might have made dogs more prone to CDV infection, but that more recent (higher) vaccination levels could have weakened this coupling.

In the Serengeti ecosystem, it is unclear whether CPV was circulating in dogs until mid'90s but it has since rapidly increased to become widespread and endemic in dogs. This suggests a high force of infection and raises questions about the role of the multiple possible transmission routes of CPV. In addition to the common faecal-oral route of transmission from fresh faeces [23], it has also been suggested that the virus can be transmitted after some time from the environment [22,60]. However, the lack of stable endemic infection, as evidenced by periodic peaks of infection in dogs and periods when CPV infection is absent in lions, as well as the coupling of infection in lions with infection in dogs are not entirely consistent with long-term environmental persistence. If environmental persistence does not occur, and with little evidence for an external force of infection in this system, control of CPV through vaccination could be possible. Although our findings show that CPV vaccination can reduce the probability of infection in dogs, consistent and higher coverage is needed to counteract the high prevalence observed.

Cross-species transmission of CPV between domesticated and wild animals is a widespread phenomenon [41,62]. We show for the first time that CPV infection cycles in lions might be coupled with those in dogs, but lag by one year. Together with evidence for unidirectional cross-species transmission from dog to lions, this strongly suggests that dogs act as a source of infection for lions and that lions are not maintaining CPV infection independently of dogs. Although domestic dogs and lions rarely have direct interactions, there are numerous other carnivore species that could act as intermediate hosts [63]. Phylogenetic information about CPV in the Serengeti ecosystem might help disentangle long-term cross-species jumps and clarify the origins of CPV in this system.

Additional complexity arises because CPV and FPV are antigenically very similar [64,65], which makes these two pathogens difficult to distinguish serologically. It is therefore possible that some of the lion CPV seropositives in this study arise from infection with FPV. Recent genetic analysis indicated that there is an independent FPV cycle in the Serengeti lions that was not dependent on domestic dogs [46] and did not detect CPV DNA in any of the lions tested (6 FPV positives from 44 samples). It is possible that, compared to FPV which seems to remain as a persistent infection within individuals, cycles of infection of CPV in wildlife are short-lived with virus being cleared or host animals dying as a result of disease. Our hypothesis that there is a CPV cycle in lions is supported by the coupling of peaks in seropositivity in lions and dogs, which suggests that infection processes are linked, with dogs a likely source of CPV infection for lions. Although it is possible that this temporal relationship is coincidental, if FPV infection was the only explanation for the observed CPV seropositivity in lions, more stable patterns of lion seropositivity would be expected across time, yet distinct peaks of infection are evident.

The lack of coupling of CDV with CPV peaks of infection in dogs suggests that the two viruses can circulate independently from one another. This is not surprising as both viruses are widespread with different routes of transmission [29,54]. Nonetheless, we found evidence of interactions between these two pathogens, although it remains unclear which pathogen facilitates co-infection dynamics in dogs. The mechanisms driving co-exposure to CDV and CPV are unknown, however we suggest three reasons that might explain the dynamics observed: First, there could be different survival rates of hosts infected by CPV or CDV, rather than contemporaneous coinfection. However, in non-equilibrium systems, the mortality rates associated with these diseases are difficult to infer. Second, it is well established that these diseases weaken the immune system [66] leaving individuals more susceptible to other viruses. Third, these viruses could have common risk factors. For example, CPV can cause brain pathology, especially in puppies [67], leading to behavioural changes such as anxiety or aggression triggered by fear, which could increase the probability of high-risk contacts with other dogs. There may not be a single explanation for the co-exposure patterns we observe and potential interdependencies could be difficult to disentangle. However, these hypotheses provide intriguing avenues for future research.

Targeting the maintenance population can be an effective way to manage disease with potential for elimination [1,3]. Dog vaccination could help limit CPV transmission within the Serengeti ecosystem but elimination is likely to be challenging. Village vaccination coverage reached at

most 56%, but the regional vaccination coverage was ~30%. This level of coverage has not been sufficient to prevent an increase in CPV circulation in this system, even with the potential increase in coverage from immunity acquired from vaccine shedding. However, this level of coverage could have altered the disease dynamics through decoupling CDV from CPV dynamics. Further research is needed to confirm disease decoupling driven by vaccination and whether targeting a single disease would have been sufficient for this decoupling. Studies from other systems have shown that vaccination can alter dynamics of co-infections either negatively, for example by decreasing the immune reactivity to a vaccine against a second disease [68], or positively, by increasing the efficacy of the vaccine against a second disease [69]. Our co-exposure time series ends at the peak of vaccination effort. Further studies will be required to fully understand the role of vaccination in CPV and CDV coinfection dynamics and the importance of this decoupling to this system or for other co-infections. This highlights the importance of considering the joint dynamics of multiple pathogens.

Longitudinal serology from two host species combined with powerful statistical approaches provide insights into the within-host species disease dynamics of two pathogens and between-host species dynamics of one pathogen circulating among wildlife and domestic animals. Further, our statistical methods allowed, for the first time, analysis of CPV serological data that incorporated exposure from vaccine virus shedding. It is clear that understanding the dynamics of such complex multi-host, multi-pathogen systems is important but challenging, given the difficulties of directly measuring infection processes. Cross-species transmission and co-infection are only two of several factors affecting disease dynamics. Establishing (dis)similarities in epidemiological dynamics in single infection versus co-infection and single-host versus multiple host species scenarios offer exciting opportunities for designing improved interventions strategies. Within the context of our study system there might be implications for vaccine development as there are currently no monovalent vaccines available for CDV and CPV. Although there are no additional costs of vaccinating against CPV, the pathogen is now widespread in the Serengeti ecosystem, which raises the question of the need for continued vaccination at current levels. Nonetheless, whether increased vaccination levels might be able to reverse the observed endemic circulation in dogs remains unknown and, if confirmed, the decoupling of the two infections as a result of vaccination could justify maintaining the vaccination programme, even at current levels.

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FIGURES

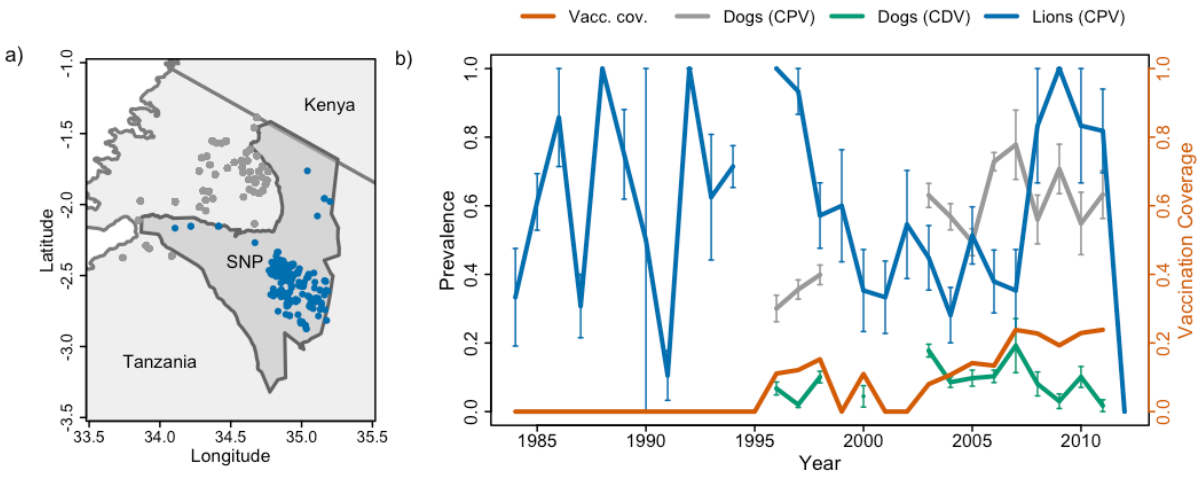

Figure 1: Data used in this study. a) Sampling locations of domestic dogs (grey circles) and lions (blue circles) in the Serengeti ecosystem. The Serengeti National Park (SNP) is shown in the dark grey shaded area. b) Observed annual seroprevalence of CPV in domestic dogs (grey) and African lions (blue) and of canine distemper virus (CDV; green) in dogs. The orange dotted line corresponds to the regional vaccination coverage. Sample sizes available in Table S1 (SI).

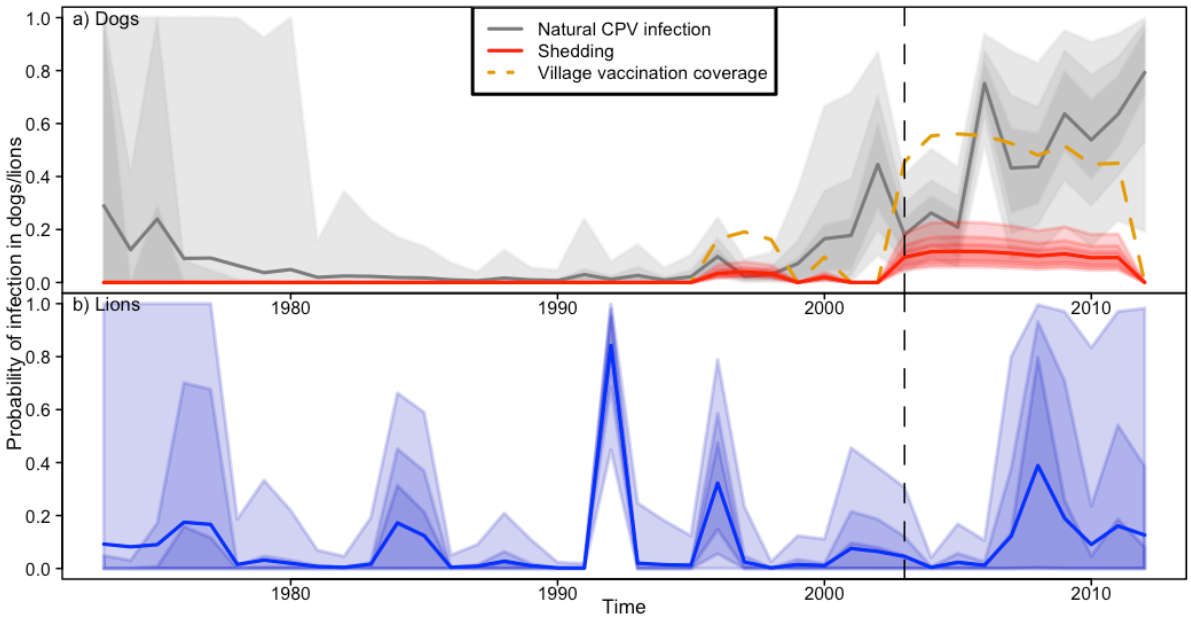

Figure 2: Estimated CPV temporal profiles. a) Mean annual probability of CPV natural infection (grey) and vaccine-shed (red) seropositivity in domestic dogs, and village vaccination coverage (orange dotted line). b) Mean annual probability of CPV infection in lions. Associated 50%, 75% and 95% credible intervals shown in increasingly lighter colour shading. Vertical dotted line corresponds to year 2003, the start of larger vaccination efforts.

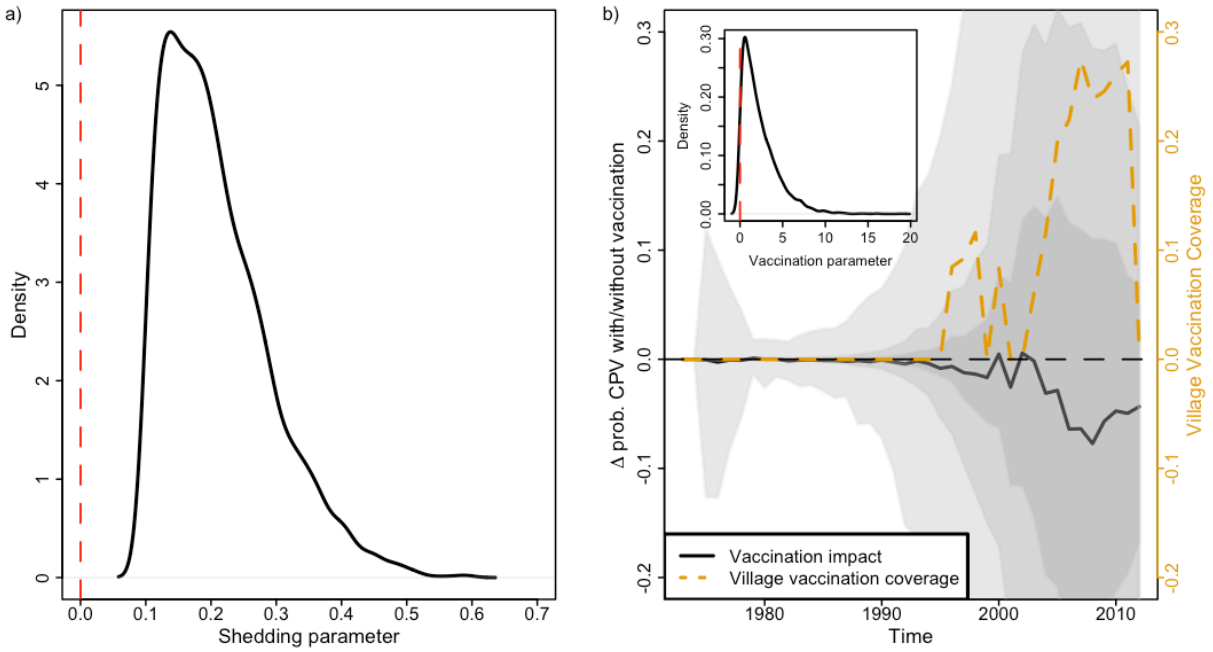

Figure 3: Effects of vaccination. a) Posterior distribution of the parameter describing the influence of shedding (φ in eq.1). b) Mean difference between the estimated prediction of the annual probability of CPV infection and that without the vaccination effect (black). This difference is negative if vaccination reduces the probability of CPV infection. Associated 50%, 75% and 95% credible intervals shown in increasingly lighter colour shading. Annual village vaccination coverage shown in orange. Inset, the posterior distribution of the parameter describing the influence of vaccination on the annual probability of dog infection (v1).

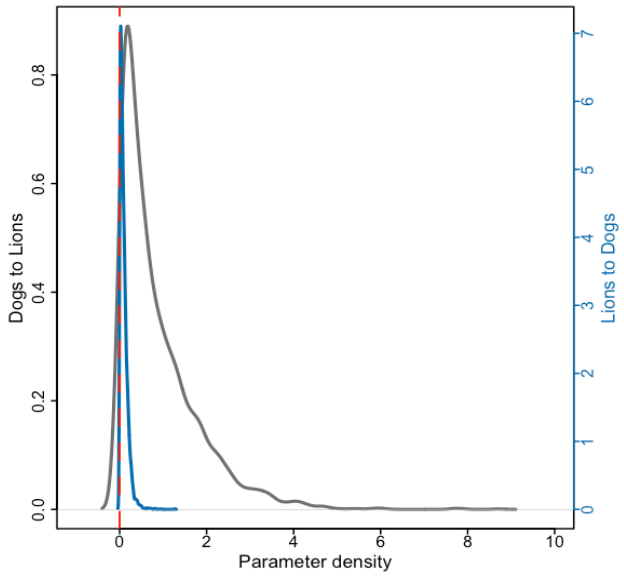

Figure 4: Cross-species transmission of CPV. Posterior distributions of the parameters describing the influence of the probability of infection of dogs on that of lions ($\mathbf{\Omega_{DL}}$; grey) and vice-versa ($\mathbf{\Omega_{LD}}$; blue).

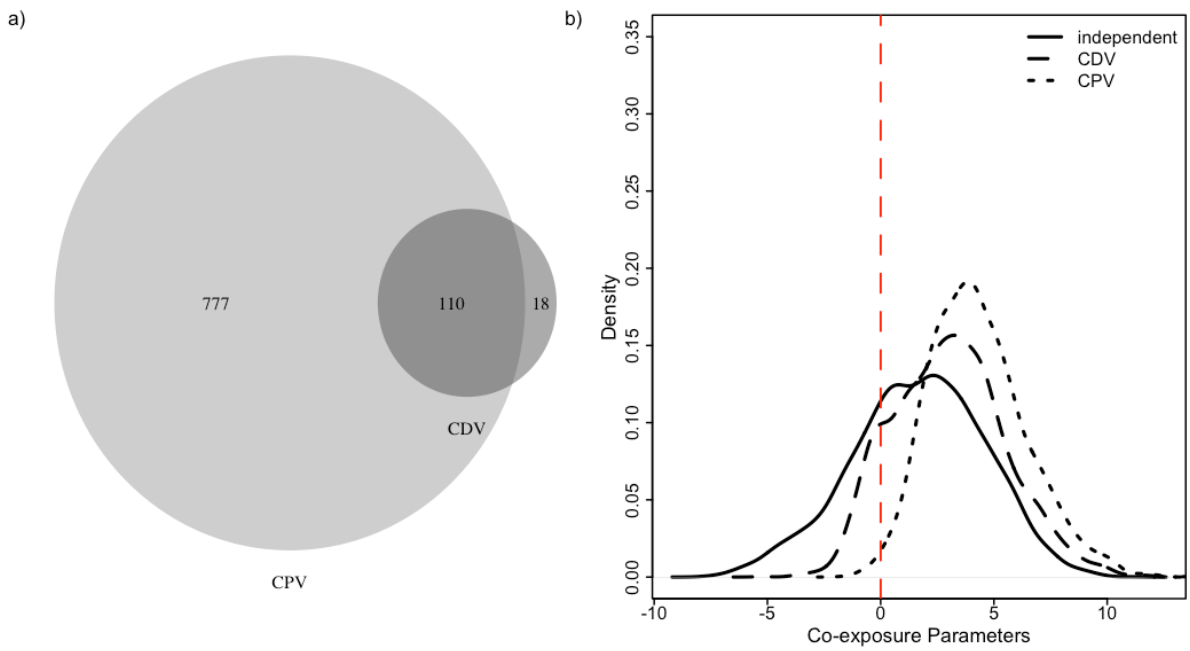

Figure 5: Co-exposure patterns. a) Venn Diagram representing the number of dogs exposed to CPV (light grey) and CDV only (intermediate grey) and co-exposed with both diseases (dark grey). b) Posterior distributions of the parameters describing the influence of CPV (dotted line) and CDV (dashed line) only, and that of both diseases if they are independent (p_{CPV}*p_{CDV}; solid line) on the co-exposure (p_{both}) dynamics.

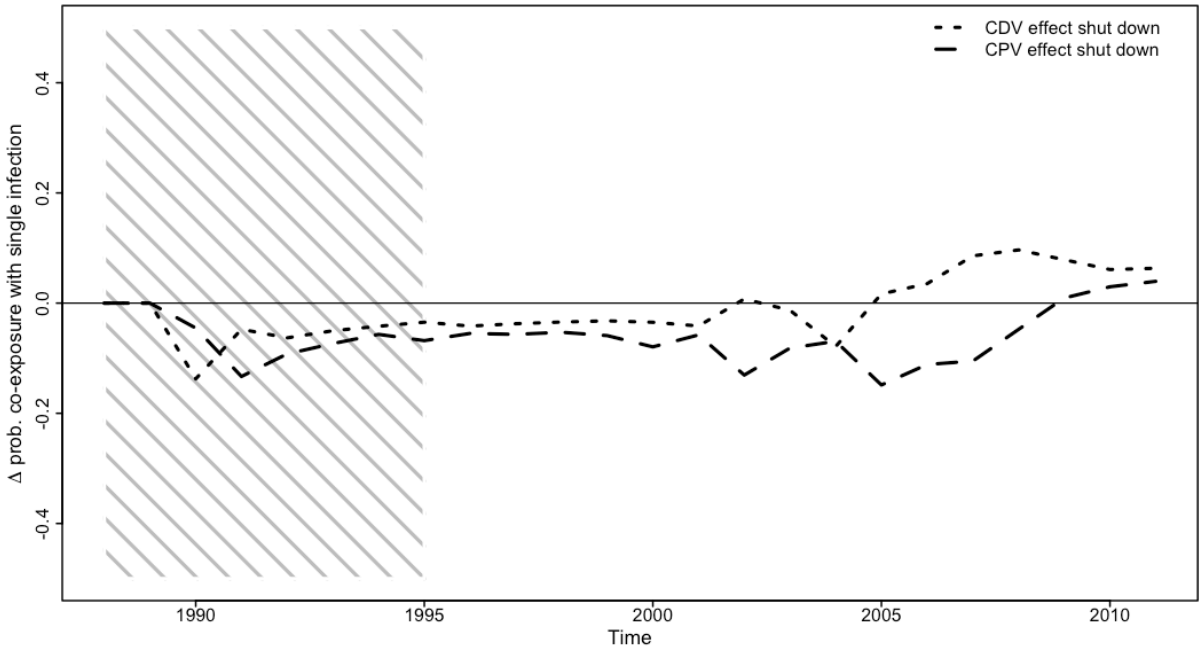

Figure 6: Influence of CPV and CDV on co-exposure. Difference between the predicted annual probability of being co-exposed to CPV and CDV, and that without the effect of CDV (dotted line) and CPV (dashed line). This difference is negative if shutting down the effect of one pathogen reduces the probability of co-exposure. Shaded area corresponds to the back-predicted period.