Research Article

# Evaluation of Immunogenicity of a Commercially Available Live Attenuated Vaccine for Dogs Containing Canine Distemper Virus and Canine Parvovirus in African Wild Dog (*Lycaon pictus pictus*)

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**Abstract** | African wild dog (*Lycaon pictus pictus)* is an endangered species and captive populations are important for conservation. Canine distemper virus (CDV) and canine parvovirus (CPV) are two infections threatening the survival of this species. Based on a limited number of animals, live attenuated vaccines have been suggested to provide protective immunity in the African wild dog; however, their use has been hampered by the fear of vaccine-induced disease. Kolmården Wildlife Park in Sweden has used a live attenuated vaccine without any apparent cases of vaccine-induced disease, but no studies to evaluate immune response after vaccination have been performed. The objective of this study was to gain more knowledge about immune response following vaccination of the African wild dog, in order to improve husbandry in zoos and help wildlife conservation. In total, 146 serum samples from 47 individuals were analyzed. Serum neutralization test was used to determine presence of CDV antibodies, and an indirect IgG ELISA was used to detect CPV antibodies. Neutralizing antibodies was induced after CDV vaccination and persisted up to 3.9 years. Most animals had high IgG antibody titers to CPV prior to vaccination and vaccination did not result in increased titers. A decline in CPV antibody titer with increasing age was observed regardless of immunization status. Our results suggest that vaccination of African wild dogs with a live attenuated domestic dog vaccine may provide adequate protection against CDV, and could therefore be an important tool for the conservation of this endangered species.

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## Introduction

African wild dog (*Lycaon pictus pictus)* is a member of the Canidae family. Current estimates suggest that there are only 3000-5500 African wild dogs left in the wild (McNutt et al., 2012). Hence, the species is listed by the International Union for Conservation of Nature (IUCN) as endangered and with a population decline (IUCN, 2012). The greatest threats to the African wild dog are conflicts with humans and infec-

tious diseases, such as canine distemper virus (CDV) and canine parvovirus (CPV) (McNutt et al., 2012). There are over 500 wild dogs in zoos worldwide and the captive population is important for the conservation of this endangered species.

CDV is an RNA-virus of the genus *Morbillivirus* and family *Paramyxoviridae* (Greene and Appel, 2006). A wide range of mammals is considered to be susceptible to CDV infection (Appel and Summers, 1995; Philippa, 2010), but canids are the main host and believed to act as a reservoir for infection in wildlife (Cleaveland et al., 2000). Several outbreaks of canine distemper have been documented in African wild dogs; for example, in 2007 in Serengeti National Park, several animals in a pack died after showing signs of ataxia, weakness, dehydration and diarrhea (Goller et al., 2010). CDV-infection was confirmed by immunohistochemistry, and RT-PCR provided the first molecular characterization of CDV in free-ranging African wild dogs. Subclinical infections of CDV based on serological studies have also been suggested (Goller et al., 2010).

CPV is a DNA-virus of the family *Parvoviridae*, most likely causing natural infection in all species within the Canidae family (McCaw and Hoskins, 2006). Exotic animals show similar clinical signs as domestic dogs, including acute deaths, hemorrhagic gastroenteritis, dehydration, anorexia and vomiting (Wallach and Boever, 1983). A correlation between smaller pack sizes and increased CPV antibody titers in African wild dogs was observed in a game reserve in Tanzania, probably due to increased pup mortality during the time the pups were still in the den (Creel et al., 1997).

An effective way to protect domestic dogs against CDV and CPV infections is to vaccinate (Greene and Schultz, 2006). Currently available commercial vaccines are either killed or live attenuated and can include strains of both viruses, among others. Killed vaccines do not give adequate protection in wild canids (van de Bildt et al., 2002; van Heerden et al., 2002), but studies of live attenuated vaccines, using limited number of animals, reported increasing antibody titers (Spencer and Burroughs, 1992; van Heerden et al., 2002), which correlates well with protection against disease in domestic dogs (Schultz, 2006). The concerns of vaccine-induced disease have, however, limited the use of live vaccines in captive endangered canines (Appel and Summers, 1995; Loots et al., 2016; McCormick, 1983; van Heerden et al., 1989).

The aim of this study was therefore to investigate effects of vaccination with a commercial live attenuated vaccine on the development of antibody titers to CDV and CPV.

## Materials and Methods

### *Animals and sampling*

Kolmården Wildlife Park (Kolmården, Sweden) has kept African wild dogs during the period 2001 and 2014, and in 2003 breeding animals were moved to Kolmården from various zoos in Europe. The animals live in a pack of approximately 12 individuals. An alpha couple has had one litter of 8-12 puppies per year since 2005. Routine monitoring of disease and clinical signs of infectious diseases was performed. Blood samples were drawn when the animals were immobilized for other reasons and collected over several years (2001-2012). Serum samples were therefore collected at different ages, at different times since vaccination, and not all animals were sampled before vaccination. Samples were centrifuged as soon as possible after collection and stored at -24°C. Serum samples of sufficient quantities (>0.25 ml) and belonging to individuals with complete medical records, were analyzed for the presence of antibodies against CDV and CPV, as described below. In total, there were 146 serum samples from 47 individuals and the sample distribution per year was as follows: 2001 (n=7), 2002 (n=1), 2003 (n=7), 2004 (n=13), 2005 (n=0), 2006 (n=10), 2007 (n=11), 2008 (n=42), 2009 (n=14), 2010 (n=6), 2011 (n=32) and 2012 (n=3).

### *Vaccine and vaccinations*

A commercial domestic dog vaccine (Nobivac DHP Live vet., Intervet, Sollentuna, Sweden) containing freeze-dried, living, avirulent strains of CDV (≥$10^4$ TCID50 per dose of the Onderstepoort strain), CPV (≥$10^7$ TCID50 per dose of strain 154) and adenovirus (CAV2) (≥$10^4$ TCID50 per dose of the Manhattan LPV3 strain) were used. The vaccine was administered subcutaneously using the same dose regardless of age and size of the animal. Animals were vaccinated (n=41) when immobilized for other reasons with no routines for revaccination. Some animals were never vaccinated. Of the 11 animals moved to Kolmården Wildlife Park, 8 animals had unknown

vaccination status, 1 animal had been vaccinated with an ISCOM-based vaccine (De Vries et al., 1988) and 2 had not been vaccinated.

*Serum neutralization test for detection of CDV antibodies*

Serum neutralization test (SN) is considered to be the gold standard for CDV antibody detection (Greene and Schultz, 2006). Heat inactivated (+56°C for 30 minutes) serum samples were titrated in 2-fold dilutions from 1:4 to 1:256 in Eagle's minimal essential medium (EMEM, National Veterinary Institute, Uppsala, Sweden) on a 96-well microtiter plate. Each titration was performed in duplicate. To each well, 100 TCID50 of CDV (Bussel strain) were added. After an incubation of 60 minutes at +37°C and 5% $CO_2$, Vero cells and EMEM were added to the wells. The plates were then incubated as above for an average of 4 days. Each test serum had a control well without virus to check that the serum itself did not cause cytopathogenic effects (CPE). On a separate control plate, virus dilution, cells, and a positive control were confirmed.

The plates were checked for CPE daily. All wells with CPE were counted as virus infected, and therefore without antibody protection. The final reading time (on average 4 days) was when all wells with virus titration $10^{-1}$ on the control plate displayed CPE. The plates were checked at least one additional day after the final reading to verify that no additional CPE had occurred.

*ELISA (enzyme-linked immunosorbent assay) for the detection of CPV antibodies*

For detection of CPV antibodies an indirect IgG ELISA was used, and samples were analyzed by the European Veterinary Laboratories (EVL, Woerden, The Netherlands) according to their standard procedures. Briefly, test sera were diluted 3-fold from 1:50 to 1:1350. An HRP-conjugated antibody directed against domestic dog IgG was used for detection of IgG bound to the CPV-antigen.

*Statistical analyses*

The effect of age at sampling on the titer of CPV antibodies was investigated with a linear mixed regression model, with animal included as a random effect to account for the repeated observations for some animals. The possibility of a curvilinear association was investigated by introducing a quadratic term and of different effects between home-born and immigrant individuals by introducing an interaction term between age and "source". The residuals were investigated and were found to be approximately normally distributed.

## Results

*Routine monitoring of disease and clinical signs of infectious diseases*

No disease outbreaks or direct signs of infectious diseases in the pack were recorded, indicating that no cases of vaccine-induced disease in the 41 animals vaccinated with the live attenuated vaccine have occurred during the observation period (2001 – 2014). Several pups in the 2006 litter were euthanized due to fractures proven to be related to calcium deficient feed.

*Neutralizing antibodies to CDV*

Titers before and after vaccination, for animals with both samples drawn (n=28), are shown in Table 1 (for all animals, see Supplementary **Table** 1). All animals but one developed neutralizing antibodies to CDV, and had a 4-fold increase in titer indicating a seroconversion following vaccination (Coyne, 2000; Tizard, 2009). None of the animals had neutralizing antibody titers ≥ 20 before vaccination, and for all samples where there was an indication of neutralizing antibodies prior to vaccination, the results could have been affected due to bacterial contamination (Table 1).

The youngest pups were 73 days old at vaccination (n=7). These vaccinations resulted in induction of neutralizing antibody titers (titer < 20 pre-vaccination and ≥ 256 post-vaccination). The dam of these pups did not have antibody titers ≥ 20 at the time of parturition and suckling, and she was not vaccinated until 6 months after parturition. Nine pups from another litter were vaccinated at 80 days of age and eight of them also developed neutralizing antibody titers (range: 178 - ≥256, mean: 246). Animals vaccinated under 120 days of age did not have significantly shorter durations of antibody titers ≥ 20 compared to animals vaccinated at an age over 120 days (**Figure** 1).

Eighteen individuals were vaccinated at least twice and their blood sampled after each vaccination. If the first vaccination induced neutralizing antibody titers ≥ 20, there was no detectable increase in titers after the second vaccination. However, if the first vaccination did not result in seroconversion (individual 25), a revaccination (924 days later) resulted in an increased titer.

**Table 1:** *Antibody titers to canine distemper virus (CDV) in African wild dogs at pre- and post-vaccination using a serum neutralization test (SNT).*

| ID | CDV titer pre-vaccination | Age at vaccination (days) | CDV titer post-vaccination | Days between vaccination and sampling |
|---|---|---|---|---|
| 1 | < 4 | 456 | ≥ 256 | 156 |
| 2 | < 4 | 864 | ≥ 256 | 434 |
| 3 | 6* | 73 | ≥ 256 | 80 |
| 4 | 4* | 73 | ≥ 256 | 80 |
| 5 | 11* | 73 | ≥ 256 | 80 |
| 6 | < 4* | 73 | ≥ 256 | 80 |
| 7 | < 4 | 864 | ≥ 256 | 521 |
| 9 | < 4 | 80 | ≥ 256 | 407 |
| 10 | < 4 | 77 | ≥ 256 | 349 |
| 11 | < 4 | 77 | ≥ 256 | 349 |
| 13 | < 4 | 80 | 178 | 924 |
| 16 | < 4 | 858 | ≥ 256 | 199 |
| 17 | < 4 | 858 | 256 | 199 |
| 19 | < 4 | 80 | ≥ 256 | 23 |
| 20 | < 4 | 458 | ≥ 256 | 290 |
| 24 | < 4 | 77 | ≥ 256 | 349 |
| 25 | < 4 | 80 | < 4 | 407 |
| 27 | < 4 | 80 | ≥ 256 | 924 |
| 28 | < 4 | 80 | ≥ 256 | 924 |
| 29 | < 4 | 80 | ≥ 256 | 924 |
| 30 | < 4 | 80 | ≥ 256 | 924 |
| 31 | < 4 | 80 | ≥ 256 | 924 |
| 32 | < 4 | 458 | ≥ 256 | 553 |
| 35 | < 4 | 858 | ≥ 256 | 1066 |
| 36 | < 4 | 77 | ≥ 256 | 349 |
| 37 | 6* | 73 | ≥ 256 | 80 |
| 38 | 8* | 73 | ≥ 256 | 80 |
| 39 | 8* | 73 | ≥ 256 | 80 |

*All animals included were sampled at the same day as being vaccinated (pre-vaccination titer). The sample collected closest in time to vaccination was used to indicate post-vaccination titer. * indicates serum samples that could have affected the SNT due to bacterial contamination.*

The duration of neutralizing titers ≥ 20 was up to 3.9 years (n=11, mean: 1107 days, range: 1066 – 1434 days). No blood samples longer than 3.9 years post-vaccination were available, hence it cannot be excluded that the duration of neutralizing titers ≥ 20 is even longer.

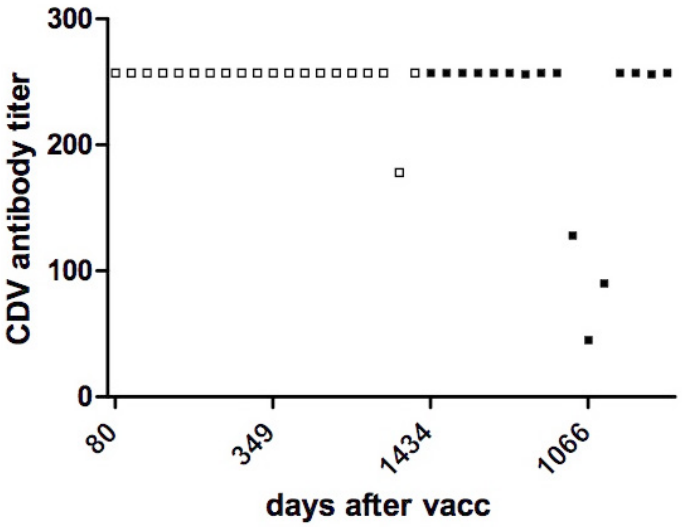


**Figure 1:** *Duration of antibody titres to canine distemper virus (CDV) depending on age at time of vaccination. Unfilled boxes are individuals vaccinated <120 days of age, and filled boxes are individuals vaccinated >120 days of age. Titres ≥ 1:256 are quoted as 257.*

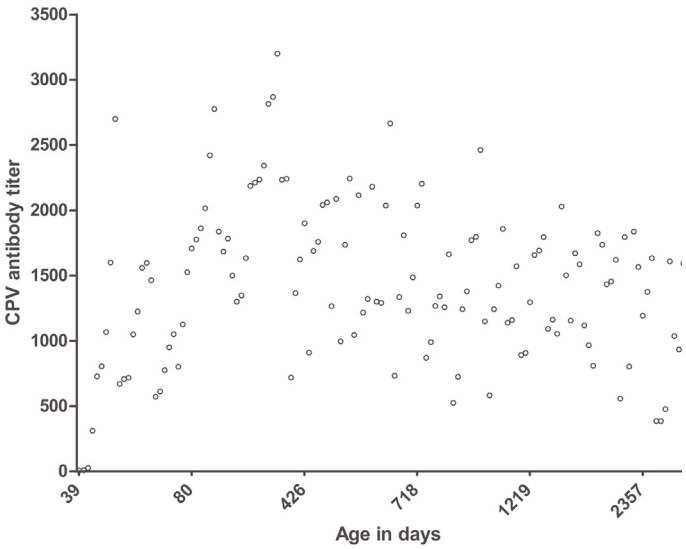


**Figure 2:** *Antibody titres (y-axis) to canine parvovirus (CPV) at different ages in days (x-axis).*

### *Antibodies to CPV*

In contrast to CDV, where no or very low neutralizing antibody titers were detected prior to vaccination, high antibody titers to CPV were detected in the sera of most animals before vaccination (61 samples from 44 individuals, mean: 1:1900, range 1:6-1:3200). Young individuals (53 samples, age: <1500 days, range: 1:6-1:3200) generally had higher titers than older individuals (8 samples, age: >1500 days, range: 1:386-1:1825). In 13 of 26 vaccinated animals, the CPV antibody titer increased after vaccination (mean increase in titer: 101%, range: 39-233%), whereas 5 animals had decreased titers (mean decrease in titer: 35%, range: 31-49%; Table 2). Eight of the vaccinated

animals did not have any substantial changes in CPV antibody titers. No animal had a fourfold increase in titer after vaccination, indicating that no seroconversion had taken place despite vaccination (Coyne, 2000; Tizard, 2009).

**Table 2:** *Antibody titres to canine parvovirus in African wild dogs at pre- and post-vaccination using an IgG-ELISA.*

| Individual | CPV titer pre-vaccination | Age at vaccination (days) | CPV titer post-vaccination | Days between vaccination and sampling |
|---|---|---|---|---|
| 2 | 1257 | 864 | 2029 | 434 |
| 3 | 718 | 73 | 1301 | 80 |
| 4 | 1050 | 73 | 1634 | 80 |
| 5 | 671 | 73 | 2236 | 80 |
| 6 | 1560 | 73 | 2212 | 80 |
| 7 | 1663 | 864 | 1155 | 521 |
| 9 | 1525 | 80 | 2115 | 407 |
| 10 | 573 | 77 | 1900 | 349 |
| 11 | 1052 | 77 | 720 | 349 |
| 13 | 802 | 80 | 726 | 924 |
| 16 | 1341 | 858 | 1423 | 199 |
| 17 | 1268 | 858 | 1243 | 199 |
| 19 | 1708 | 80 | 1684 | 23 |
| 24 | 776 | 77 | 1623 | 349 |
| 25 | 1125 | 80 | 1046 | 407 |
| 27 | 1862 | 80 | 1771 | 924 |
| 28 | 2016 | 80 | 1379 | 924 |
| 29 | 1776 | 80 | 1797 | 924 |
| 30 | 2776 | 80 | 2462 | 924 |
| 31 | 2421 | 80 | 1242 | 924 |
| 32 | 1736 | 458 | 1149 | 553 |
| 35 | 991 | 858 | 1434 | 1066 |
| 36 | 950 | 77 | 1365 | 349 |
| 37 | 707 | 73 | 2186 | 80 |
| 38 | 1225 | 73 | 2342 | 80 |
| 39 | 1597 | 73 | 2815 | 80 |

*All animals included were sampled at the same day as being vaccinated (pre-vaccination titer). Only individuals born at Kolmården Wildlife Park are included in the table. The sample collected closest in time to vaccination was used to indicate post-vaccination titer.*

Since vaccination did not affect the serum antibody levels, statistical analysis was performed to determine if difference in titers were linked to age. A decrease in titer with age was evident (regression coefficient = -0.239; Standard Error ± 0.050; Fig. 2), and the regression analysis showed a highly significant association (p-value <0.0001). The titer thus decreases by 0.239 units per day of increasing age. The quadratic term was not significant, i.e. no indication that the association was not linear, and there was no statistical difference between home-born and immigrant individuals (data not shown).

Pups from the litter born at the end of 2006 (n= 5) had the lowest antibody titers to CPV. Titers of 1:1502 and 1:1454 were observed in the mother's serum in July 2006 and March 2008, respectively. At 39 days of age, the average antibody titer of the pups was 1:88. Four of the pups from the same litter were sampled at 69 days of age, with average titers of 1:1543. A greater than 4-fold increase in titer indicates that a seroconversion had occurred despite the pups not being vaccinated.

## Discussion

In this study, we show that African wild dogs develop neutralizing antibody titers ≥ 20 to CDV after vaccination with a live attenuated domesticated dog vaccine. Kolmården Wildlife Park has not had any apparent cases of vaccine-induced disease in their vaccinated African wild dogs, despite using live attenuated vaccines that have been shown to induce disease (McCormick, 1983; van Heerden et al., 1989). In most reports describing such disease in African wild dogs, the afflicted individuals were frail or in some way affected by disease (McCormick, 1983; van Heerden et al., 1989). The lack of such cases in Kolmården Wildlife Park may be due to the vaccine strain used (Onderstepoort strain), as this strain is considered to be one of the safest (Appel and Summers, 1995), and/or that only healthy individuals were vaccinated.

There are no studies indicating the cut-off for neutralizing antibody titers needed for protection to CDV infection. In one study, a neutralizing antibody titer of ≥ 20 was observed in an African wild dog prior to a CDV outbreak that the dog survived (van de Bildt et al., 2002); however, it was not documented whether that dog actually was exposed to virus. In domestic dogs, neutralizing antibody titers of ≥ 20 are considered as a cut-off for protective immunity to CDV infection (Carmichael, 1999). Whether the same holds true for African wild dogs are still unknown.

The duration of CDV neutralizing antibody titers ≥

20, though based on a few individuals, appears to be at least up to 3.9 years. One vaccination at a young age was enough to trigger an immune response, and since a sterile immunity was achieved, there seems to be no reason to submit the animals to a second vaccination until at least 3 years have passed. A previous study on African wild dogs reported a persistence of CDV antibodies for at least 451 days (1.2 years) (van Heerden et al., 2002). The long duration observed in this study is in good agreement with studies of domestic dogs, where the persistence of protective neutralizing antibodies was at least 5 years in a challenge study and even longer based on in vitro analyses of antibody titers (Schultz, 2006). Since no blood samples were taken more than 3.9 years after vaccination, it is possible that the duration of these antibodies in African wild dogs is as long as observed in domestic dogs.

In contrast with the clear induction of CDV neutralizing antibodies by the live attenuated vaccine commonly used in domestic dogs, we did not observe an increase in CPV antibody titer after immunization. The African wild dogs in our study most likely had sterile immunity to CPV before vaccination, interfering with the live attenuated CPV strain. Another explanation for the lack of increasing CPV antibody titers could be interference between the three different virus strains included (CDV, CPV and CAV), resulting in less efficient virus replication and immunization of CPV. Such interference has, to our knowledge, not been reported in domestic dogs, making this explanation less likely.

In this study, the true vaccine efficacy regarding CPV could not be demonstrated. To fully evaluate the vaccine there is a need for either a challenge study, which is not possible in this endangered species, or for serum samples from vaccinated animals with no previous exposure to the virus which can be analyzed to determine antibody titers. Here, there were too few samples from individuals with low CPV titers (without sterile immunity) before immunization in order to confirm that vaccination triggered a humoral immune response. A previous study has argued that vaccination of African wild dogs with a live attenuated CPV vaccine strain was successful (van Heerden et al., 2002), but this study was based on few individuals (n=8) and the detected antibody titers were often not increased fourfold, indicative of seroconversion (Coyne, 2000; Tizard, 2009). In fact, the titer variation between samples in non-vaccinated individuals was similar to the titer increase in post-vaccination samples (van Heerden et al., 2002). Thus, the efficacy of CPV vaccination in African wild dogs is still an open question.

The antibody titers to CPV observed in this study before vaccination could reflect natural immunity contributed to virus in the surroundings. Parvoviruses are extremely durable and can survive for a long time in the environment, and they are resistant to most disinfectants (McCaw and Hoskins, 2006). CPV is highly contagious and is transmitted primarily by oro-nasal contact with infected feces, but also humans, insects or rodents can spread the virus (McCaw and Hoskins, 2006). In accordance with our study, CPV antibody titers were seen in all African wild dogs regardless of vaccination status (van Heerden et al., 2002).

The first African wild dogs to arrive at Kolmården Wildlife Park were between 6-12 years old on arrival. Most of these animals were seropositive with low CPV antibody titers on arrival at the park. Two years later, low CPV antibody titers were still detectable. This could be because there was less parvovirus in the environment during the early period and no natural boosting of the humoral immunity occurred. The African wild dog litter born in 2006 had low CPV antibody titers at 39 days of age, but had seroconverted only 30 days later. This seroconversion coincided with the time when pups had begun to leave the den and therefore increase the risk of exposure to contaminations in the environment.

Regardless of vaccination status there is a significant decrease in CPV antibody titer with increasing age. The immune system undergoes changes as animal ages, so-called immunosenescence (HogenEsch and Thompson, 2010). It has been proven that older domestic dogs can maintain protective titers against parvovirus (Schultz et al., 2010). However, some studies of domestic dogs show higher antibody titers in sera of a greater proportion of young individuals (reviewed in McCaw and Hoskins, 2006), while protective titers are not detected in a greater proportion of older individuals (Taguchi et al., 2011). Whether vaccination of older African wild dogs with low antibody titers would increase the level of CPV antibodies is not known and should be explored.

The question remains why there are no clinical signs of CPV infection in African wild dogs in zoos, when

CPV in the environment seems to trigger a humoral immune response. It is believed that all members of the family *Canidae* are susceptible to parvovirus (McCaw and Hoskins, 2006), but no cases in African wild dogs have been confirmed by virus isolation or direct detection of the virus. The presence of antibodies has been described in the African wild dog, both in the wild (Woodroffe et al., 2012) and in captivity (van Heerden et al., 2002). In domesticated dogs, there are both acute cases with sudden death and subclinical cases with no or very mild clinical signs (Steinel et al., 2001). CPV requires rapidly replicating cells such as intestinal epithelial cells to proliferate (McCaw and Hoskins, 2006). Young growing animals are therefore most severely affected by the virus, and especially pups less than 12 weeks of age, due to lack of protective immunity and larger amounts of dividing cells. In African wild dogs, CPV is the suspected cause of increased pup mortality, although dead pups can often not be recalled for pathological examination and virus isolation (Creel et al., 1997). Kolmården Wildlife Park has only had video surveillance of the African wild dog den on 1-2 occasions and occasional deaths of pups before leaving the den would most likely not be detected. One explanation could be circulation of less virulent strains of CPV or similar viruses inducing cross-protective antibodies.

## Conclusions

Knowledge regarding vaccination of African wild dogs is lacking, especially concerning at what age pups can be successfully immunized without interference of maternal antibodies and duration of immunity. More knowledge about the efficacy of vaccination can lead to better husbandry in zoos and help this species to survive in the wild. In this study, we show that a live attenuated domestic dog vaccine induced neutralizing antibodies to CDV in the African wild dog. Healthy pups vaccinated as early as 11 weeks of age developed CDV neutralizing antibodies. Since the presence of CDV neutralizing antibody titers ≥ 20 persisted at least 3 years, vaccination is probably not needed to be repeated during that time, avoiding the frequent use of vaccines and saving the animals from stress and other risks associated with sedation and vaccination. Even though the effect of CPV vaccination could not be concluded in this study, recommended vaccination procedures would be the same as for CDV, because the vaccine currently in use contains vaccine strains of both viruses.

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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.hv/2018/5.3.26.34

## Author's Contribution

LW conceived and designed the study, performed laboratory work, analyzed and interpreted the data, and drafted the manuscript. UE conceived and designed the study, analyzed and interpreted the data, and drafted the manuscript. TM conceived and designed the study, interpreted the data and drafted the manuscript. JJW conceived, designed and coordinated the study, analyzed and interpreted the data, and drafted the manuscript. All authors approved the final version of the manuscript.

## References

- Appel MJ, Summers BA. 1995. Pathogenicity of morbilliviruses for terrestrial carnivores. Veterinary Microbiology 44(2-4):187-191. https://doi.org/10.1016/0378-1135(95)00011-X
- Carmichael LE. 1999. Canine viral vaccines at a turning point--a personal perspective. Advances in Veterinary Medicne 41:289-307. https://doi.org/10.1016/S0065-3519(99)80022-6
- Cleaveland S, Appel MG, Chalmers WS, Chillingworth C, Kaare M, Dye C. 2000. Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. Veterinary Microbiology 72(3-4):217-227. https://doi.org/10.1016/S0378-1135(99)00207-2

- Coyne MJ. 2000. Seroconversion of puppies to canine parvovirus and canine distemper virus: a comparison of two combination vaccines. Journal of American Animal Hospital Association 36(2):137-142. https://doi.org/10.5326/15473317-36-2-137
- Creel S, Creel NM, Munson L, Sanderlin D, Appel MJ. 1997. Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. Journal of Wildlife Diseases 33(4):823-832. https://doi.org/10.7589/0090-3558-33.4.823
- De Vries P, Uytdehaag FGCM, Osterhaus ADME. 1988. Canine distemper virus (CDV) immune-stimulating complexes (iscoms) but not measles virus iscoms, protect dogs against CDV infection. Journal of General Virology 69(8):2071-2083. https://doi.org/10.1099/0022-1317-69-8-2071
- Goller KV, Fyumagwa RD, Nikolin V, East ML, Kilewo M, Speck S, Müller T, Matzke M, Wibbelt G. 2010. Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania. Veterinary Microbiology 146(3-4):245-252. https://doi.org/10.1016/j.vetmic.2010.05.018
- Greene CE, Appel MJ. 2006. Canine Distemper. In: Greene CE, editor. Infectious Diseases of the Dog and Cat. 3 ed. St Louis: Saunders Elsevier. p 25-41.
- Greene CE, Schultz RD. 2006. Immunoprophylaxis. In: Greene CE, editor. Infectious Diseases of the Dog and Cat. St Louis: Saunders Elsevier.
- Harder TC, Osterhaus AD. 1997. Canine distemper virus--a morbillivirus in search of new hosts? Trends in Microbiology 5(3):120-124. https://doi.org/10.1016/S0966-842X(97)01010-X
- HogenEsch H, Thompson S. 2010. Effect of ageing on the immune response of dogs to vaccines. Journal of Comparative Pathology 142 Suppl 1:S74-77. https://doi.org/10.1016/j.jcpa.2009.09.006
- [IUCN] International Union for Conservation of Nature. 2012. IUCN Red List of Threatened Species.
- Loots AK, Mitchell E, Dalton DL, Kotzé A, Venter EH. 2017. Advances in canine distemper virus (CDV) pathogenesis research: a wildlife perspective. Journal of General Virology. 98(3):311-321. https://doi.org/10.1099/jgv.0.000666
- McCaw DL, Hoskins JD. 2006. Canine viral enteritis. In: Greene CE, editor. Infectious Diseases of the Dog and Cat. 3 ed. St Louis: Saunders Elsevier.
- McCormick AE. 1983. Canine distemper in African cape hunting dogs (Lycaon pictus) - possibly vaccine induced. Journal of Zoo Animal Medicine 14(2):66-71. https://doi.org/10.2307/20094641
- McNutt JW, Mills MGL, McCreery K, Rasmussen G, Robbins R, Woodroffe R. 2012. IUCN SSC Canid Specialist Group - African Wild Dog Working Group. Lycaon pictus.
- Philippa J. 2010. Vaccination of non-domestic carnivores. Available at: http://www.eaza.net/activities/Pages/Transmissible%20Diseases%20Handbook.aspx. Accessed 21st October 2012.
- Schultz RD. 2006. Duration of immunity for canine and feline vaccines: A review. Veterinary Microbiology 117(1):75-79. https://doi.org/10.1016/j.vetmic.2006.04.013
- Schultz RD, Thiel B, Mukhtar E, Sharp P, Larson LJ. 2010. Age and long-term protective immunity in dogs and cats. Journal of Comparative Pathology 142(Suppl 1):S102-108. https://doi.org/10.1016/j.jcpa.2009.10.009
- Spencer J, Burroughs R. 1992. Antibody response to canine distemper vaccine in African wild dogs. Journal of Wildlife Diseases 28(3):443-444. https://doi.org/10.7589/0090-3558-28.3.443
- Steinel A, Parrish CR, Bloom ME, Truyen U. 2001. Parvovirus infections in wild carnivores. Journal of Wildlife Diseases 37(3):594-607. https://doi.org/10.7589/0090-3558-37.3.594
- Taguchi M, Namikawa K, Maruo T, Orito K, Lynch J, Sahara H. 2011. Antibody titers for canine parvovirus type-2, canine distemper virus, and canine adenovirus type-1 in adult household dogs. Canadian Veterinary Journal 52(9):983-986.
- Tizard I. 2009. Diagnostic applications of immunological tests. In: Tizard I, editor. Veterinary immunology: An introduction. Eighth ed. St. Louis: Saunders Elsevier. p 526-527.
- van de Bildt MW, Kuiken T, Visee AM, Lema S, Fitzjohn TR, Osterhaus AD. 2002. Distemper outbreak and its effect on African wild dog conservation. Emerging Infectious Diseases 8(2):211-213. https://doi.org/10.3201/eid0802.010314
- van Heerden J, Bainbridge N, Burroughs RE, Kriek NP. 1989. Distemper-like disease and encephalitozoonosis in wild dogs (Lycaon pictus). Journal of Wildlife Diseases 25(1):70-75. https://doi.org/10.7589/0090-3558-25.1.70
- van Heerden J, Bingham J, van Vuuren M, Burroughs RE, Stylianides E. 2002. Clinical and

serological response of wild dogs (Lycaon pictus) to vaccination against canine distemper, canine parvovirus infection and rabies. Journal of South African Veterinary Association 73(1):8-12. https://doi.org/10.4102/jsava.v73i1.541

- Wallach JD, Boever WJ. 1983. Diseases of exotic animals. Philadelphia: W.B. Saunders Company.
- Woodroffe R, Prager KC, Munson L, Conrad PA, Dubovi EJ, Mazet JA. 2012. Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (Lycaon pictus). PLoS One 7(1):e30099. https://doi.org/10.1371/journal.pone.0030099