Clinical and serological response of wild dogs (Lycaon pictus) to vaccination against canine distemper, canine parvovirus infection and rabies

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ABSTRACT

Wild dogs (Lycaon pictus) were vaccinated 4 times against canine distemper (n = 8) (initially with inactivated and subsequently with live attenuated strains of canine distemper) and canine parvovirus infection (n = 8) over a period of 360 days. Four of the wild dogs were also vaccinated 3 times against rabies using a live oral vaccine and 4 with an inactivated parenteral vaccine. Commercially-available canine distemper, canine parvovirus and parenteral rabies vaccines, intended for use in domestic dogs, were used. None of the vaccinated dogs showed any untoward clinical signs. The inactivated canine distemper vaccine did not result in seroconversion whereas the attenuated live vaccine resulted in seroconversion in all wild dogs. Presumably protective concentrations of antibodies to canine distemper virus were present in all wild dogs for at least 451 days. Canine parvovirus haemagglutination inhibition titre were present in all wild dogs prior to the administration of vaccine and protective concentrations persisted for at least 451 days. Vaccination against parvovirus infection resulted in a temporary increase in canine parvovirus haemagglutination inhibition titre in most dogs. Administration of both inactivated parenteral and live oral rabies vaccine initially resulted in seroconversion in 7 of 8 dogs. These titres, however, dropped to very low concentrations within 100 days. Booster administrations resulted in increased antibody concentrations in all dogs. It was concluded that the vaccines were safe to use in healthy subadult wild dogs and that a vaccination protocol in free-ranging wild dogs should at least incorporate booster vaccinations against rabies 3–6 months after the first inoculation.

Key words: canine distemper, canine parvovirus, Lycaon pictus, rabies, vaccination, wild dogs.


INTRODUCTION

The wild dog (Lycaon pictus) is a highly endangered canid species that occurs in fragmented pockets throughout its former range. Various reasons for its decline have been postulated and include habitat destruction, persecution, competition with other predators, lack of genetic heterozygosity and disease.

The potential threat of infectious disease to free-ranging populations of carnivores, especially metapopulations, is increasingly recognised. Rabies, caused by a virus in the family Rhabdoviridae, and canine distemper, caused by a virus of the genus Morbillivirus in the family Paramyxoviridae, are possibly the 2 most important viral diseases of carnivores that may impact significantly on free-ranging populations.

Rabies was responsible for the death of 21 of 23 wild dogs, and probably responsible for the subsequent disappearance of 8 wild dogs in Kenya. Rabies in wild dogs has also been reported from Tanzania, Namibia and from a captive pack in Zimbabwe (Veterinary Research Laboratory, Harare, unpubl. data). It is also suspected to have occurred in the Central African Republic and Zambia. In Madikwe, South Africa, Hofmeyr et al. witnessed the decimation of a pack of wild dogs from 24 to 3, directly or indirectly as a result of rabies. Some of the dogs that died were vaccinated against rabies (Pfizer Animal Health), more than 2 years before the outbreak. Wild dog packs appear to acquire infectious disease through contact with rabid domestic dogs (Canis familiaris) or jackals (Canis mesomelas) although the source of infection cannot always be confirmed. It is not known whether commercial rabies vaccine (oral or injectable) intended for the vaccination of domestic dogs, given at appropriate intervals, would generate a protective antibody response in wild dogs. Seroconversion after vaccination of wild dogs with commercial dog vaccines, has, however been reported (J van Heerden, unpubl. obs., 1994; D. G. A. Metzer, unpubl. obs., 1994).

Despite the fact that canine distemper virus has never been isolated from either free-ranging or captive wild dogs, clinical, histopathological and serological evidence indicates that wild dogs are susceptible to canine distemper virus infection. Clinical signs suggestive of canine distemper have been observed in free-ranging wild dogs in the Serengeti, the Kruger National Park, and the Hluhluwe-Umfolozi Park (G Andreka and J van Heerden, unpubl. obs., 1994). Ten free-ranging wild dogs died of canine distemper in northern Botswana. Serological evidence of exposure of free-ranging wild dogs to the distemper virus has been demonstrated in wild dogs in the Hluhluwe-Umfolozi Park (G Andreka and J van Heerden, unpubl. obs., 1994), in Northern Botswana, in the Selous Game Reserve and in the Tsumkwe District of Namibia. Vaccination of captive wild dogs, especially pups, with live attenuated vaccines intended for use in domestic dogs, induced distemper-like disease in a number of animals. Captive wild dogs at the De Wildt Cheetah Research Centre have, however, been vaccinated with live canine distemper commercial dog vaccines (Pfizer Animal Health) on numerous occasions, without any untoward effects (J van Heerden, unpubl. obs., 1993). Recently, vaccine-induced distemper was observed in 3-and-a-half month old wild dog puppies vaccinated with canine distemper vaccine (Vanguard Puppy 5, Pfizer Animal Health) (R E J Burroughs, unpubl. obs., 2001). Limited investigations into the use of domestic dog distemper vaccines in wild dogs, however, has been reported (J van Heerden, unpubl. obs., 1994).

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per vaccines in wild dogs have been conducted\textsuperscript{40,41}. Canine parvovirus infection is a potentially important cause of mortality in domestic dog puppies\textsuperscript{4}. Antibodies to the virus have been found in some free-ranging wild dog populations\textsuperscript{33}. It is, however, not known whether parvovirus persists in wild dog populations or whether wild dogs are infected through contact with infected domestic dogs. Seroconversion following the administration of canine parvovirus vaccine has been described in wild dogs\textsuperscript{35}. The aim of this investigation was to monitor the safety and efficacy (in inducing antibodies) of commercial rabies, canine distemper and canine parvovirus domestic dog vaccines in wild dogs.

**MATERIALS AND METHODS**

Ten captive-bred wild dogs (Numbers 1 to 10), individually identified by inserted transponders, 2 males and 8 females 11 months of age were randomly allocated to 1 of 3 treatment groups (Table 1). Group 1 consisted of 2 control wild dogs that only received inactivated canine distemper vaccine. Group 2 consisted of 4 animals that received live attenuated canine distemper vaccine, canine parvovirus vaccine, and rabies vaccine intramuscularly, and Group 3 consisted of 4 animals that received live canine distemper vaccine, canine parvovirus vaccine, and oral rabies vaccine (Table 1). Before administration of the live distemper and parvovirus vaccine, all dogs were vaccinated with an inactivated canine distemper vaccine. All animals were in an apparently healthy physical condition. The dogs were held in 2 adjoining camps, approximately 50 m\textsuperscript{2} in size, each with its own facilities for capturing, handling and feeding of the animals.

The body masses of the 2 males were respectively 20 and 22 kg at the beginning of the experiment and 25 and 31 kg at the end of the experiment. The body masses of the females ranged from 17 to 19 kg at the beginning of the experiment and ranged from 21 to 25 kg at the end of the experiment. Dogs were immobilised by intramuscular administration of a total dose of medetomidine (Domitor, Novartis) ranging from 80 to 90 µg in combination with a total dose of ketamine hydrochloride (Anestesin, Centaur Labs) ranging from 20 to 25 mg. The anaesthetic drugs were administered with a pole syringe while the wild dogs were temporarily restrained in a crush. Following collection of specimens, weighing of the dogs and administration of the respective vaccines, anaesthesia was reversed with the intramuscular administration of atipamezole hydrochloride (Antisedan, Novartis) at a dose ranging from 0.8 to 0.9 mg.

The inactivated vaccine used was a formaldehyde-inactivated Rockborn strain of canine distemper virus, manufactured for non-commercial purposes (Lot Number 930601, Akzo Nobel, Intervet South Africa). The attenuated distemper vaccine used was a commercial vaccine intended for use in dogs, containing a total dose ranging from 0.8 to 0.9 mg. The inactivated virus used was a formaldehyde-inactivated Rockborn strain of canine distemper virus (Snyder Hill strain, modified by adaptation to NL-DK-1 cells) and canine parvovirus (NL-35-D strain) (Vanguard puppy 5, Pfizer Animal Health). This vaccine also contains the Manhattan strain of canine adenovirus type 2 and the NL-CPI-5 strain of canine parainfluenza virus. The rabies vaccine used was a commercial inactivated rabies vaccine of cell culture origin (Paris strain (PV-4)); each dose contains at least 10\textsuperscript{5.5} FID\textsubscript{0} of rabies virus before inactivation) (Defensor\textsuperscript{5}, Pfizer Animal Health) intended for use in domestic animals, and a live oral vaccine (an escape mutant live rabies vaccine\textsuperscript{6}, Virbac Laboratories, France) diluted in tissue culture medium with 10% foetal calf serum to give a titre of 8.0 log\textsubscript{10} median tissue culture infectious doses per ml (TCID\textsubscript{50}/ml). The titre of the oral vaccine was determined by titre in BHK-21 cells. Live attenuated distemper vaccine (given subcutaneously in the neck area) and rabies vaccine were administered on 3 occasions to Group 2 and 3 experimental wild dogs (Table 1). The inactivated rabies vaccine was given intramuscularly in the gluteal muscle and the oral vaccine was administered by deposition of 1 ml of SAG-2 vaccine into the oral cavity by syringe.

Following administration of vaccines, the wild dogs were observed daily for clinical signs of disease. Blood specimens were collected on 10 occasions (Table 1) from all experimental animals. Clotted specimens were transported to the laboratory within 2 hours, centrifuged and stored frozen until tested for antibodies to canine distemper, canine parvovirus, and rabies viruses at the end of the experiment. Rabies antibodies were detected using the neutralisation test as described by Cliquet et al.\textsuperscript{4}. Antibodies to canine distemper virus were detected by means of a serum neutralisation test. Serum antibodies against canine parvovirus were determined by means of a haemagglutination inhibition test. The procedure was briefly as follows: sera were diluted 1:2 in phosphate buffered saline (pH 7.4) and heat-inactivated at

<table>
<thead>
<tr>
<th>Day</th>
<th>Group 1 (n = 2)</th>
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<tr>
<td>0</td>
<td>Inactivated canine distemper vaccine subcutaneously</td>
<td>Inactivated canine distemper vaccine subcutaneously</td>
<td>Inactivated canine distemper vaccine subcutaneously</td>
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<tr>
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<td>No vaccines administered</td>
<td>Live attenuated canine distemper and parvovirus vaccine subcutaneously</td>
<td>Live attenuated canine distemper and parvovirus vaccine subcutaneously</td>
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<td>No vaccines administered</td>
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<td>Rabies vaccine intramuscularly</td>
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<td>Oral rabies vaccine</td>
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<tr>
<td>363</td>
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<td>Rabies vaccine intramuscularly</td>
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<td>634</td>
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Table 1: Days on which wild dogs were immobilised and blood specimens collected as well as the vaccination regimens for the three experimental groups.
56 °C for 30 min. Duplicate serial 2-fold dilutions of the sera were made in a barbital-acetate buffer (pH 6.2). To the first dilution series, 8 HA units of CPV (reference strain CPV-2) was added, while the second set of serum dilutions contained buffer and therefore served as a control for nonspecific inhibition. After the addition of the antigen, the plates were held at room temperature for 80 min. This was followed by the addition of a 1% porcine red blood cell suspension (prepared in barbital-acetate buffer). Plates were placed at 4 °C overnight and the titre was read as the highest dilution at which 50% inhibition occurred. The positive control serum of a known antibody titre was included with each batch of tests.

Full autopsies, including histopathological examination, and direct fluorescent antibody staining for rabies virus, were conducted on all animals that died during the trial.

RESULTS

All wild dogs remained healthy throughout the experiment with the exception of 1 of the control dogs (Number 2), which developed an acute and severe watery diarrhoea on Day 50. This diarrhoea progressed and it died within 24 hours. An autopsy revealed an animal in fair condition with an empty stomach, diarrhoea and in anorexic, depressed condition. The wild dog was anorexic, depressed, the intestines were congested and flaccid. Histopathological examination of the small intestines demonstrated severe, acute, multifocal bacterial necrotic enteritis with areas of pseudomembrane formation. Small Gram-positive rods were present on the surface of the villi and Gram-negative rods in the crypts. Marked acute necrosis of the follicles in the spleen and lymph nodes was evident. The alveolar walls in the lung were thickened due to congestion and leukosiasis. A few large bronchi showed a mild acute neutrophil infiltration. The liver and kidney were severely congested. The brain and spinal cord showed severe oedema. There were no histopathological indications of canine distemper infection. The fluorescent antibody test for the detection of rabies virus yielded negative results. Electron microscopic examination of intestinal contents for the presence of parvovirus was negative. Aerobic and anaerobic culturing of tissue specimens yielded growth of Streptococcus canis, Klebsiella pneumoniae, Flavobacterium sp. and Citrobacter freundii.

Rabies serum neutralising antibody titres are given in Table 2, canine distemper serum neutralising antibody titres in Table 3, and canine parvovirus haemagglutination inhibition titres in Table 4.

DISCUSSION

The decision to use an inactivated canine distemper vaccine as the first inoculation was based on the fact that attenuated vaccines have been reported to yield adverse effects in wild carnivores. Whereas recommendations on vaccination of wild carnivores with live canine distemper virus-containing vaccines should be based on clinical trials and not extrapolation, the use of a live vaccine was not deemed to be without risk, and therefore raised ethical concerns when considering the current status of wild dogs. Although the wild dogs in this trial did not seroconvert following the use of the inactivated vaccine, a cellular response was most probably generated, and may have contributed to the strong anamnestic response after the first inoculation with an attenuated vaccine.

During the trial there was no indication of vaccine-induced disease caused by the live distemper, parvovirus or rabies vaccines. The continued healthy state of the experimental animals throughout the duration of the trial is supported by their gains in body mass. Although none of the experimental wild dogs in this investigation that received distemper vaccine developed any untoward side-effects, the vaccine cannot be recommended without reservation as safe for use in wild dogs. Most reports on vaccine-induced distemper and/or distemper-like disease in wild dogs occurred in puppies, especially in those infected with a concomitant pathogen. Vaccination of wild dogs with attenuated canine distemper vaccines should...
therefore preferably be undertaken in healthy wild dogs that are free from other infections. The exact age at which pups can safely be vaccinated has not been established, but it is possible that their state of health may be more important than their age.

The death of one of the control dogs from severe bacterial enteritis shortly after the third immobilisation of the wild dogs might have been precipitated by the stress of capturing and darting. Darting of captive wild dogs is frequently associated with excitement, running, panting and vocalisation. There was also a marked variation in behaviour between the different animals. A detailed autopsy and microbiological investigation was undertaken to exclude the possibility of death due to any of the 3 infectious diseases under investigation.

Failure to seroconvert following the use of an inactivated distemper vaccine is in accordance with the findings of other workers who found little serological evidence of protection in follow-up studies in several species. Seroconversion was obtained following administration of the attenuated vaccine and protective titres remained throughout the observation period. Booster injections in general did not result in increased antibody titres, but the frequency of sampling may have been inadequate to detect booster effects.

All wild dogs given oral rabies vaccine and all but one given the parenteral vaccine responded after the primary vaccination with marked anti-rabies neutralising antibody titres. The non-responder only showed seroconversion following the booster injection. In all dogs, the titres following the primary vaccination declined to low concentrations within less than 2 months, affording that similar to previous studies in domestic dogs vaccinated parenterally. However, other studies of parenterally and orally vaccinated dogs indicate that the primary antibody response is short in comparison with domestic dogs. As the neutralising antibodies following primary vaccination are short-lived and booster vaccinations should be given within about 3 months. Booster vaccinations were effective for stimulating high and more persistent neutralising antibody concentrations. Although both types of vaccine demonstrated a booster effect, it appeared that parenteral vaccination stimulated higher concentrations of neutralising antibodies than oral vaccination at the evaluated dose rate. This, however, does not necessarily imply that immunity was more protective or of longer duration with parenteral vaccination. Higher oral doses within safe concentration limits may well induce higher antibody concentrations.

Canine parvovirus haemagglutination inhibition titres were present in the experimental wild dogs before the administration of vaccine. The experimental dogs were housed on a facility where domestic dogs were held in close proximity, which could possibly explain the higher haemagglutination inhibition titres before vaccination. An anamnestic response was observed after administration of the first vaccine in 4 of the 8 dogs, while 5 out of 8 dogs demonstrated an increase after a booster injection on Day 360. Antibodies to canine parvovirus have been demonstrated in samples from some free-ranging populations, whereas samples from other free-ranging populations have tested negative.

No challenge studies were carried out in this investigation owing to the unavailability of sufficient naive wild dogs, the lack of suitably characterised virus strains, lack of facilities to safely house rabid animals, and ethical considerations. Absence of neutralising antibody does not necessarily indicate the absence of protection; without controlled challenge studies it would be impossible to accurately assess the significance of an undetected titre. The presence of neutralising antibody is generally well correlated with protection against rabies in other species using SAG-2 and similar vaccines. Similarly, it has been stated that serum-neutralising titres of 1:20 are protective for canine distemper. Evaluation of vaccines on the basis of antibody response alone was the most practical method of assessing efficacy. Although there may be unquantifiable protection in the absence of neutralising antibody, it is most likely that such protection would not be of long duration and therefore could, for practical purposes, be considered inadequate. We therefore considered a significant antibody titre as indicating protection and the lack thereof as an indication of probably inadequate protection.

This trial has attempted to demonstrate efficacy and safety of commercial dog vaccines against 3 important canine diseases in wild dogs. Unlike the inactivated vaccine, live distemper vaccine appears to be effective, but it should be used with care, as the risk of vaccine-induced disease with the currently available vaccines registered for domestic dogs remains. Although all wild dogs had parvovirus antibody through natural exposure at the start of the trial, the parvovirus vaccine induced an anamnestic response in Dogs 5, 6, 8 and 9 following the first vaccination. Immunity to canine parvovirus is believed to be antibody-mediated, and haemagglutination inhibition titres equal to or more than 1:80 are considered protective. Both parenteral and SAG-2 oral vaccine caused seroconversion, although the response to primary vaccination in both cases was of short duration. Using vaccines of similar potency as used in this trial, booster doses of rabies will be necessary 1-3 months after the primary vaccination. We tentatively conclude that all the vaccines used in this trial could be used in free-ranging wild dogs, with the exception of inactivated distemper vaccine, which does not appear to be effective.

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