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DISTEMPER, RABIES AND PARVOVIRUS VACCINATIONS IN A CAPTIVE-BREEDING PROGRAMME FOR THE AFRICAN WILD DOG *(LYCAON PICTUS)* IN NORTHERN TANZANIA

By A. M. Visee

Introduction

In 1995 the George Adamson Wildlife Preservation Trusts (USA and UK), the African Wild Dog Foundation (Netherlands) and the Wildlife Preservation Trust Fund (Tanzania), at the invitation of the Tanzanian Government, initiated an African Wild Dog captive-breeding programme in the Mkomazi Game Reserve, Tanzania. The ultimate goal of this breeding programme is to re-establish viable populations of African Wild Dogs in protected areas in which the species was formerly resident, using captive bred animals of similar genetic stock.

The African Wild Dog (*Lycaon pictus)*, endemic only to Africa, is a highly endangered species with currently probably less than 3000 free-living adults (>1 year old).

Throughout Africa loss of habitat and human persecution are the main threats to the survival of the remaining free-living populations. However, locally, disease has been shown to be the cause of high mortality in some packs of free living African Wild Dogs. Rabies decimated study packs in the Serengeti ecosystem of East Africa (GASCOYNE et al., 1993; KAT et al., 1995). In southern Africa rabies caused high mortality in both re-introduced packs (SCHEEPERS et al., 1995; HOFMEYR et al., 2000) and in a captive pack in Zimbabwe .

High exposure (50-100%) of adult African Wild Dogs to canine distemper virus (CDV) has been confirmed in four free living populations, including Selous Game Reserve, South East Tanzania, but not in the Serengeti ecosystem (CREEL et al., 1997; WOODROFFE et al., 1997). Despite high exposure in some areas, CDV related mortality in free living unvaccinated wild dogs has been confirmed in only one pack in Botswana (ALEXANDER et al., 1996).

Due to the frequent, close social interactions between individuals in an African Wild dog pack, transmission of pathogens can be rapid. It is often claimed that the African Wild Dog is extremely susceptible to disease, particularly canine distemper and rabies. As human populations and their domesticated dogs encroach on the remaining African Wild Dog ranges, the growing possibility exists that pathogens, common in domestic dogs, may be transmitted to wild animal populations. In this way common canid pathogens are potentially becoming an increasing threat to the survival of the African Wild Dog in the wild. The African Wild Dog captive-breeding programme in the Mkomazi Game Reserve was conceived and run as a management programme rather than a purely scientifically study. Our first management aim was and is to keep the dogs in the utmost health, both physically and mentally. When funds are limited problems, that arise, must be solved in the most practical cost effective way.

In a captive-breeding programme, even in an area remote from human habitation and their domestic animals, such as the Mkomazi Game Reserve and in the compounds were the African Wild Dogs are kept in relatively close proximity, contagious disease can spread easily. A decision therefore was taken to attempt to protect the Mkomazi African Wild Dogs both during their captivity and post release.

Rabies, canine distemper and canine parvovirus infection (CPV) were perceived to be the major pathogens threatening the breeding programme.
Therefore a small-scale preventive medicine research programme was started, in an attempt to discover the best way to protect our dogs; i.e. to develop safe, effective protocols for their vaccinations against viral pathogens. Measures to protect the dogs from endo- and ectoparasites were taken also.
As attenuated live vaccines may, in the African Wild Dog, induce the disease, from which they are supposed to protect (DURCHENFELD et al., 1990; Van HEERDEN et al., 1989), it was decided that only inactivated vaccines should be used. An extra reason for using only inactivated vaccines is to prevent spreading viruses in the wild.
However, of paramount consideration throughout the programme was the welfare of the pack and not the individual.
Little is known and even less published concerning the effectiveness of any commercially available vaccine in protecting African Wild Dogs from challenge by common canid pathogens. The efficacy of the use of inactivated rabies vaccines, developed for domestic dogs or other species distantly relate to the African Wild Dog, from challenge is unknown. The same applies to the significance or persistence of any rabies specific neutralizing antibody (RSA) titre levels induced post vaccination.
It is hoped that experience gained during such an investigation, both positive and negative, might not only enhance the chances of ultimate success in this and other captive-breeding programmes, but also provide important data possibly providing insight into the reasons for the extinction of two free living study populations in the Serengeti ecosystem between 1985-91 (BURROWS et al., 1995).
This paper presents the results of a 5-year vaccination programme on the Mkomazi African Wild Dogs. For the first time data are presented showing that both wild and some captive bred pups had pre-vaccination titre levels to CDV. Data are also for the first time presented on levels of sero-conversation post rabies vaccination, using different protocols, and the persistence of titres induced, over time. Vaccinations of wild and captive-bred African Wild Dogs against CDV and CPV are reported; titre levels induced and their persistence are presented.

Materials and Methods

It was imperative that the initial potential breeding stock be obtained from a non-conservation area where there was a perceived threat to their survival. In 1995 such a situation was identified and subsequently three African Wild Dog dens, suitable for the collection of pups were located on the Masai Steppe of Tanzania. According to the location of the dens from which the pups were obtained, the groups of pups became known as the Lendanai (1.3), the Llondirrigiss (7.1) and the Najo (7.6).
The 25 pups captured, ranged from 3 to 5 weeks old and originating from 3 different packs. Significant genetic variation was found between the groups of pups, so reducing any potential future problems of close inbreeding (VAN DE ZANDE, 1997) during the period of the captive- breeding programme.
For identification purposes, at the age of 5 months, each pup was immobilized and fitted subcutaneously on the left side of the neck with a transponder. At the same time photographs were taken of both sides of each individual.
At 18 months the dogs were divided into 3 potential breeding packs. Packs composition was based on the fact that in free living East African Wild Dog packs all females emigrate from their natal packs as yearling (12-24 months old) and form a new pack when they encounter a group of males from another pack. The offspring of such a new pack are protected and fed by their parents, assisted by all pack relatives of both sexes.
The 3 different breeding packs at Mkomazi were housed in separate outdoor enclosures (55m x 35m), with the packs in vocal but not physical or visual contact.

The breeding pair (alpha dogs) were not sedated or bled during the period when the female was pregnant, nor were pups less than 3 months old immobilized. Vaccinating of pups was, for safety reasons, delayed until the age of 12 weeks.

Anesthesia

To facilitate antibody testing individuals were blood-sampled following sedation using medetomidine hydrochloride (Domitor®, Pfizer, Neth., 1 mg/ml) and ketamine hydrochloride (100 mg/ml). As an antidote for medetomidine, atipamazole hydrochloride (Antisedan®, Pfizer, Neth., 5 mg/ml) was used. To reduce stress the individuals were temporarily restrained in a wire mesh tunnel regularly used by the pack to gain access to their food. The drugs were administered intramuscularly into the hindquarters of the stationary animals by blowpipe. In general the dogs received 1 mg medetomidine per 10 kg bodyweight, topped up with 10 mg ketamine per dog, given independent of their bodyweight. This combination proved to be very satisfactory. The dogs were well sedated for the purpose of taking blood-samples, applying transponders, and any necessary minor surgery.

As a rule the dogs received half the dosage of medetomidine as atipamazole after 30 minutes of sedation to prevent any side effects from the ketamine and completely recovered within 10 minutes.

Serum was immediately separated from the blood samples and sent to the Erasmus University, Rotterdam, Institute of Virology, for antibody testing.

Vaccinations

Canine Distemper Vaccination (CDV)

No inactivated distemper vaccine is commercially available, however, the Institute of Virology, Erasmus University, Rotterdam, developed and kindly donated such a vaccine, CDV-ISCOM Vaccine, described elsewhere (VISSER et al., 1989; VISSER et al., 1992). This vaccine must be kept frozen until used.

Individuals were vaccinated three times in the first year; with a 14 day interval between the first and second vaccination and a 30 day interval between the second and third vaccination. The 3 vaccinations were followed by a single annual booster vaccination.

The regime adopted:

1. All (n=25) pups were first vaccinated and blood-sampled following sedation on 19/12/1995 when approximately 5 months old.
2. A second vaccination was carried out on 03/01/1996 by blowpipe without sedation and no blood-samples were taken.
3. A third vaccination on 14/02/1996 again by blowpipe without sedation and no blood- samples were taken.
4. A fourth vaccination as the first annual booster was given by hand following sedation on 09/03/1997 blood-samples were taken also.
5. Blood-samples were taken but no vaccination was given on 15/10/1997.
6. Blood-samples were taken on 15/02/1998 and a further vaccination by hand was carried out using a new batch of vaccines.
7. In 1998 the annual February booster vaccination was replaced by a similar vaccination in October (04/10/1998). This meant that the dogs were vaccinated twice in 1998 again using the new vaccine batch.
8. In March 1999, 5 dogs only were tested.
9. In November 1999 the annual booster vaccination was given and blood-samples taken.
10. In March 2000, 9 dogs only were tested.

Rabies vaccination

In December 1995, at the age of approximately 5 months, the dogs received their first single rabies vaccination. However due to lack of seroconversion (see results) in 1997 the vaccination schedule

was changed and the dogs were vaccinated three times; with the second and third vaccination given at 1 and 5 months respectively after the first. Antibody testing was carried out approximately 1 month, 5 months and 12 months after the third vaccination. One month after the third vaccination (October 1997) antibodies were tested in 22 dogs (see table 3). Due to shortage of vaccines, 9 dogs had received only 2 vaccinations; 13 dogs received the full course of 3 vaccinations.
In 1995 and 1997 Dohyrab® (Solvay Duphar) was given, but was replaced in 1998 by Rabdomun® (Schering-Plough). Both Dohyrab® and Rabdomun® are inactivated vaccines. According to Solvay Duphar and Schering-Plough these vaccines protect domestic dogs from rabies for up to 3 years provided the dog is more than 12 weeks of age at the time of vaccination.

Parvovirus vaccination (CPV)

An inactivated vaccine Dohyvac I-LP® (Solvay Duphar) against parvovirus and leptospirosis was used. In the first year the dogs were vaccinated twice, with one month interval. This was followed by an annual booster vaccination. Following the manufacturers advise for domesticated dogs older than 12 weeks; 2 vaccinations with a 4 week interval followed by an annual, single, booster vaccination.
In March 1997 antibody testing for parvovirus infection on an annual basis ceased (see discussion), but the annual, single, booster vaccination was continued.

Results

Canine Distemper Vaccination CDV)

1. Pre-vaccination titres to CDV: seventy-five percent (n=12) of the wild born pups of 1995 were sero-positive for CDV when first vaccinated at approximately 5 months of age on 19-12-95 (see table 1). A groupof (n=4) captive bred unvaccinated pups (Sangito pack) were seropositive for CDV on 04/10/1998 when first vaccinated with titre levels of 80 (1x3) and 20 (x1).
2. Post-vaccination titre levels from 29-2-96 to 30-03-2000 (see table 1).

Tab. 1: Virus neutralizing antibody titres in African Wild Dogs during a vaccination trial with a CDV-ISCOM vaccine.

Date	Number of dogs NT-titer ≥20	%	Number of dogs NT-titer <20	%	Total number of Dogs
19-12-1995*	9	75	3	25	12
29-02-1996	22	88	3	12	25
09-03-1997	5	26	14	74	19
15-10-1997**	0	0	22	100	22
15-02-1998***	14	78	4	22	18
04-10-1998****	24	100	0	0	24
30-03-1999	5	100	0	0	5
02-11-1999	13	87	2	13	15
30-03-2000	6	67	3	33	9

NT-titre ≥20 are considered protective.

* pre-vaccination titre

** not vaccinated on this occasion

*** a new vaccine batch was used in February 1998

**** in 1998 the annual booster vaccination was replaced from February to October, as a consequence the dogs were vaccinated twice that year

One year after the annual booster vaccination of 1998, 87% of the tested dogs had antibody-levels >20. March 2000, 5 months after the booster vaccination of November 1999, 67% of the tested dogs had antibody-levels >20.

Rabies vaccination

1. Single vaccination.
   In February 1996, 2 ½ months after the single vaccination at the age of approximately 5 months, blood-samples from all dogs were collected. Not a single dog developed sufficient antibodies (>0.5 I.U./ml).
2. After 3 vaccinations sero-conversion occurred in 1997 (see table 2).

Tab. 2: Rabies antibody titres in African Wild Dogs after vaccination with inactivated commercial rabies vaccines: Dohyrab® (Solvay Duphar) in 1995&1997 and Rabdomun® (Schering-Plough) from 1998 and onwards.

Months after third rabies vaccination	Number of dogs >0.5 I.U./ml*	%	Number of dogs <0.5 I.U./ml	%	Total number of dogs
1	12	92	1	8	13
5	13	100	0	0	13
12 **	17	85	3	15	20
24	6	60	4	40	10

* a Rabies Specific VNA titre of >= 0.5 International Units (I.U./ml) is considered to be the minimum titre likely to provide protection against challenge.

** received booster vaccination at the time of blood-sampling.

Of the tested dogs 85% had sufficient antibodies (>0.5 I.U./ml) one year after the third vaccination (1998). One year after the annual booster vaccination (1999) the result dropped to 60% when only half the number of dogs was tested compared to previous years.
From the 9 dogs, which received 2 vaccinations, only one showed more than 0.5 I.U./ml (5 dogs and 4 dogs, respectively 6 months and 2 months after the second vaccination).

Parvovirus vaccination (CPV)

Fourteen days after the second vaccination (February 1996) all (n=25) dogs had ig G>20.
One year later (March 1997 the ig G of all tested dogs (n=18) was >20.

Discussion

Canine Distemper Vaccination (CDV)

For the first time is reported and confirmed sero-positivity in unvaccinated African Wild Dog pups, both wild caught and in captivity. As pups of 4-5 weeks of age are confined to the area of the den and exposure to CDV was confirmed in 75 % of the pups from the three litters obtained from three different dens, it is likely that they carried maternally derived antibodies. This suggests that the adults in the three packs, from which the wild captured pups were obtained, had been exposed to CDV in the Masai Steppe.
The presence of CDV titres in the African Wild Dog population of the Masai steppe is similar to the situation in the Selous (approximately 400 km south of the area from which the pups were obtained), where 59 % of adults immobilized for radio-collaring were sero-positive. Here the evidence presented

suggested they were not the result of surviving an epidemic (CREEL et al., 1997). In the absence of domestic dogs from both Selous and Mkomazi a wildlife reservoir and vector of CDV appears to be a possibility.
The presence of antibodies in the captive born pups, as with the wild born pups, suggests maternally derived antibodies.
Although initially the titre levels achieved after three vaccinations appeared to be satisfactory, a year later (1997) the titre levels had declined below the safe level in the majority of the individuals. Apparently the failure of the CDV vaccinations was due to the batch used. The way the vaccines are stored might also have a negative influence; ideally the vaccines should be stored in a freezer at minus 70 °C, but 'in the bush' it is impossible to reach that temperature in a freezer dependant mainly on solar energy.
After a new batch was used, results improved dramatically.

Rabies vaccination
Three vaccinations in the first year (second vaccination one month after the first and third vaccination 6 months after the first) followed by one single annual booster vaccination was successful.

Parvovirus vaccination (CPV)
Fourteen days after the second vaccination (February 1996) all 25 dogs were well-protected (ig G >20). One year later (march 1997) the ig G of all dogs tested (18) was still above 20. This excellent result was reason enough to stop antibody testing for parvovirus infection on an annual basis, but the annual vaccination was continued.

Conclusions

Canine Distemper (CDV)
To vaccinate the dogs three times in the first year (14 days between the first and the second vaccination and 30 days between the second and third vaccination) followed by a single annual booster vaccination appeared to be successful in providing a protective titre level ($\geq$20). All the dogs tested appeared to have one year after the third vaccination (1998) antibody-titres that are considered to be protective (OSTERHAUS pers.com.).
However, much to our surprise and horror, in December 2000 a serious outbreak of canine distemper occurred. At the time of writing we can not foresee the consequences.Further research will be undertaken and published at a future date.

Rabies
From the data presented it may be concluded that 3 vaccinations with an inactivated vaccine, according to the above mentioned schedule, result in sufficient antibodies production, but a single vaccination of such a vaccine, as recommended for domestic dogs, or even two vaccinations (the second one month after the last vaccination) do not provide adequate titre levels.

Parvovirus infection (CPV)
The first year two vaccinations at a 30 day interval followed by one single, booster vaccination proved to be successful: 100% of the tested dogs had sufficient antibodies one year after the second vaccination.

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Summary

Distemper, rabies and parvovirus vaccinations in a captive-breeding programme for the African wild dog (Lyacon pictus) in Northern Tansania

In 1995 the George Adamson Wildlife Preservation Trusts and The African Wild dog Foundation, through the Ministry of Natural Resources and Tourism and the Department of Wildlife, Tanzania, started a breeding programme for the African Wild Dog in Mkomazi Game reserve, Tanzania.

In order to protect the dogs from infectious diseases they are vaccinated for distemper, rabies, parvovirus. To establish the effectiveness of the vaccinations, antibody testing was performed.

Distemper vaccination (CDV-ISCOM) according to the schedule three vaccinations the first year (14 days between the first and second vaccination and 30 days between the second and third vaccination) followed by an annual booster, proved to be satisfactory until December 2000. Despite the vaccinations a serious outbreak of distemper occurred. Research will continue.

Three rabies vaccinations the first year, one month between the first and second and five months between the second and the third, followed by an annual booster, gave a satisfactory quantity of antibodies.

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