DISTEMPER VACCINATIONS IN AN AFRICAN WILD DOG (*LYCAON PICTUS*) BREEDING PROGRAM

Artis, **Amsterdam Zoo, the Netherlands**

2002-2004

Aart Visee, D.V.M.
Mark Hoyer, D.V.M.

AN ENDANGERED SPECIES PROGRAM UNDERTAKEN BY:
The African Wild Dog Foundation, the Netherlands
Artis, Amsterdam Zoo, the Netherlands

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Aart M. Visee. D.V.M.
The African Wild Dog Foundation
Gaspeldoornlaan 17, 3956DN Leersum, The Netherlands
Tel.: +31 343 769081
E-mail: aartvisee@gmail.com

INTRODUCTION

The African wild dog is an extremely endangered species. Diseases, large predators (lions), and man, threaten their existence. In areas where game is scarce, wild dogs tend to *approach* human settlements. As a consequence they are snared, poisoned, and may come into close contact with domestic dogs. As the African wild dog is susceptible to diseases also found in domestic dogs (distemper, rabies and parvovirus), cross infection may occur--leading, in some cases, to high mortality.

The George Adamson Wildlife Preservation Trusts, (joined in 2000 by the African Wild Dog Foundation), were invited in 1988 by the Tanzanian Government, to establish a rehabilitation program for the African wild dog *(Lycaon pictus)* involving capture, captive breeding and re-introduction. The breeding part of the program would be established in Kisima camp, Mkomazi Game Reserve, Tanzania. The breeding program could not be started until 1995. Before the program was started the question had already arisen: how to protect the dogs against infectious diseases? This report concerns efforts made to provide protection against canine distemper.

The African wild dog appears to be extremely susceptible to diseases like canine distemper. Therefore it was decided to vaccinate the dogs. However, little was known about the effectiveness of vaccinating wild dogs against this disease. Vaccinating the wild dogs would serve two purposes. First to attempt to protect the dogs from exposure to the canine distemper virus, and secondly to study the efficacy of the vaccinations. It was decided that only inactivated (i.e. killed) vaccines were to be used. This would prevent both the possibility of vaccine induced disease and the introduction and spread of viruses in the environment.

In 1995 there was no commercial inactivated distemper vaccine available and this is still the case to-day. At the Erasmus University, Institute of Virology, Rotterdam, a CDV-Iscom vaccine was developed for use in seals in the Waddenzee following the distemper outbreaks amongst the seals (OSTERHAUS et al, 1989, VISSER et al, 1989). This vaccine was kindly donated by the Institute, and from 1995 to 2001 this vaccine was used to vaccinate the dogs at Mkomazi.

For 5 years the program developed well and prospered. By the end of 2000, there were a total of 52 dogs, and plans for a re-introduction into the wild were well underway. However, just before Christmas 2000, fate struck. The breeding program was hit very hard by a canine distemper virus infection. The Mkomazi African wild dog population was almost completely wiped out and in 2001 had to be started all over again with the three survivors as a basis for a new population (VAN DE BILDT et al, 2001, VISEE et al, 2001).

Obviously the CDV-Iscom vaccinations had not provided the desired immunity. Although antibody titers were present after three vaccinations these were rather low just as the seals. (VAN DE BILDT, pers.com.). This raised the obvious question: was the heavy dog mortality due to vaccine failure or a different immunity response in the African wild dog compared to that in domestic dogs and some other species?

By mutual agreement and after careful consideration with Prof. Osterhaus June 2001, it was decided to initiate research in an attempt to find answers to these questions. However, the breeding program in Mkomazi was not at that time in a position to provide many pups for the first couple of years following the distemper outbreak. Therefore assistance was sought from the Dutch Zoos, Artis and Safari Beekse Bergen, which both have African wild dogs in their collection. Both agreed fully recognizing the importance of the research for the future of free living wild dogs.

The research on the African wild dogs at Artis is reported here:-

Method Proposed

The following trial was devised at a meeting held sixth of June 2001 at the Erasmus University:

a. If the last surviving Mkomazi female had a litter, the pups would take part in a new vaccine trial. A subsequent litter would also participate.
b. Should wild bred pups, join the breeding program in Mkomazi, they would also participate in the vaccine trial.
c. Attempts would be made to get pups born in Artis and Beekse Bergen available for inclusion in the vaccine trial.
d. The aim was to have at least 20 African wild dogs and 20 domestic dogs in the trial to compare the immune responses in the two species.
e. Two types of vaccines were to be used CDV-Iscom (Institute of Virology, Erasmus University, Rotterdam) and Purevax™ (Merial).
f. The pups would be vaccinated for the first time at the age of approximately four months and a second time at the age of five months. Simultaneously blood samples would be taken, and again one month after the last vaccination. One year after the last vaccination the dogs would receive a booster vaccination and a blood sample would be taken.

g. Humoral antibodies titers would be determined and cell mediated immunity assessed by means of proliferate T-cell response. Both tests to be performed at the Institute of Virology, Erasmus University.

Material and method

Based on the points a-g above the following procedures were carried out:

a. In Mkomazi a litter of seven pups (born 7-7-2001) was vaccinated. The schedule chosen was: - first vaccination at the age of four months, second vaccination at five months. Blood samples were taken simultaneously and three months after the second vaccination. One year after the second vaccination these dogs a received a booster vaccination and a blood sample was taken.
   A second litter of four pups (born 18-4-2002) was vaccinated three times: one month between the first and second vaccination and one month between the second and the third. Blood samples were taken at the time of the first vaccination and one year later at the time of the booster vaccination and after the third vaccination.
   Vaccine used in all the Mkomazi dogs was Purevax™.
b. Two full-grown wild bred female dogs joined the breeding program in Mkomazi in 2001. The vaccination of one of these dogs followed the same procedure as for the first litter (a. above). The other dog was not used in the trial due to her pregnancy.
c. In the Artis Zoo two litters participated. Litter I (born 2-11-2001) contained eight pups. Four pups were vaccinated with Purevax™, the other four with CDV-Iscom. All were vaccinated three times: three weeks between the first and second and three weeks between the second and third vaccination. Blood samples were taken at the same time plus one at three months and one a year after the last vaccination. All the dogs received CDV-Iscom as a booster vaccination after one year.

   Litter II (born 24-11-02) contained 12 pups. All pups were vaccinated twice with, three weeks between the first and second vaccination. Blood samples were taken at vaccination and two months and 14 months after the second vaccination. No booster vaccination was given.
   The alpha pair and a subordinate male were twice sedated for other reasons. The first time they were vaccinated with CDV-Iscom and blood samples taken. Approximately one year later further blood samples were taken.
   Unfortunately Safari Beekse Bergen Zoo had little luck in their African wild dog breeding program and so had no pups to participate in the trials.
d. In total 12 Mkomazi pups and 20 pups from Artis Zoo took part in the trials but as no domestic dogs were vaccinated any differences in their response to the vaccinations could not be measured.
e. Humoral antibody testing was carried out by the Elisa and Virus Neutralization tests, although the Elisa test results were not obtained on all occasions. To date (March 2005) the planned cell mediated immunity research could not be undertaken due to technical problems in growing cells needed for this research.

Results-humoral immunity (see Appendix II + III)

A titer of ≥20 is considered to provide protection in seals after use of CDV-Iscom vaccine (Osterhaus, pers.com.) This figure is used as reference point in this report for the CDV-Iscom vaccinated dogs as well as for the Purevax™ vaccinated dogs to allow comparison of results.

Virus Neutralizing antibody Titers (VNT)

1. Pre-vaccination titers: For unknown reasons four pups of Litter II had a pre vaccination titer of 20 – 40, probably due to maternal immunity.
2. Post-vaccination titers: Litter I: All four dogs vaccinated with Purevax™ showed a positive titer three weeks after the second vaccination, three weeks after the third vaccination and one year after the third vaccination. Of the four dogs vaccinated with CDV-Iscom only one dog had a positive titer after each of the vaccinations.
   Litter II: Four of the twelve dogs showed a positive titer three weeks after the first vaccination and after two months after the second vaccination. However 15 months after the second vaccination no dog had a positive titer.
3. Four adult dogs were tested on different occasions. One two year old female was sedated for an unrelated reason and although never vaccinated showed a titer of 640. The alpha pair and a subordinate male were tested twice. The alpha female and the subordinate male showed a positive titer but the alpha male was negative on both occasions.

Elisa antibody Titers

1. Pre-vaccination titers: Four pups of Litter II showed titers for unknown reasons but probably due to maternal immunity.
2. Post-vaccination titers: Litter I: Three weeks after the first vaccination one dog, CDV-Iscom vaccinated, had a positive titer however three weeks after the second vaccination all dogs had a high, >160, titer as was the case one year after the third vaccination.
   Litter II: Three weeks after the first vaccination all twelve dogs showed a positive titer and after two months after the second vaccination only six dogs showed a positive titer. One year later, 15 months after the second vaccination, six of the ten dogs had a positive titer.
3. The alpha pair and a subordinate male were tested once, all of them had a high, >160, titer without being vaccinated.
4. Some dogs of Litter II received double dosage CDV-Iscom vaccine on some occasions The results were no better than in the dogs that received single dosage.

Unfortunately no Elisa results were received from the blood samples collected on:
24-07-02 and thus no results could be incorporated in the tables and figures below.

Table 1: Virus neutralizing antibody titers in African Wild Dogs during a vaccination trial with CDV-Iscom vaccine Litter I

Number vaccinations at time of testing	Time-lapse between first vaccination and date of testing in days (D)	Number of dogs VNT-titer ≥20*	%	Number of dogs VNT-titer <20	%	Total number of dogs	Average titer
0	0	0	0	4	100	4	0
1	21	1	25	3	75	4	5
2	42	1	25	3	75	4	20
3	144	1	25	3	75	4	5
3	400	1	33	2	67	3	13

* ≥20 considered to protect against challenge

Table 2: Elisa antibody titers in African Wild Dogs during a vaccination trial with CDV-Iscom vaccine litter I

Number vaccinations at time of testing	Time-lapse between first vaccination and date of testing in days(D)	Number of dogs Elisa titer ≥20*	%	Number of dogs Elisa titer <20	%	Total number of dogs	Average titer
0	0	0	0	4	100	4	0
1	21	1	25	3	75	4	10
2	42	4	100	0	0	4	1040
3#	144	-	-	-	-	-	-
3	400	3	100	0	0	3	160

* ≥20 considered to protect against challenge
# No results received

Figure 1: Mean titers after vaccination with a CDV-Iscom vaccine in the African wild dog Litter I

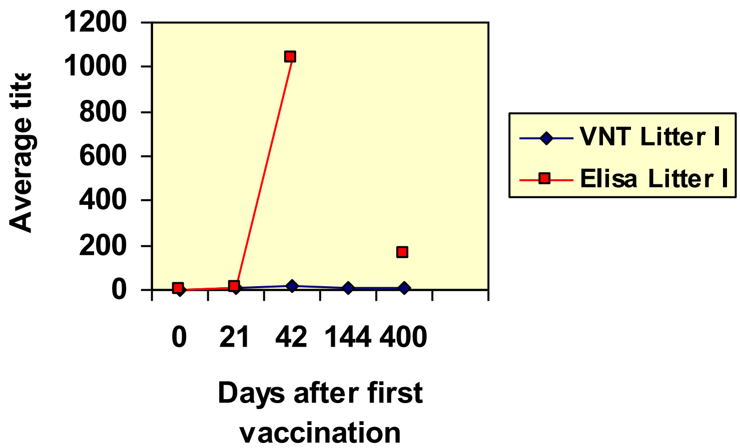


Table 3: Virus neutralizing antibody titers in African Wild Dogs during a vaccination trial with CDV-Iscom vaccine Litter II

Number vaccinations at time of testing	Time-lapse between first vaccination and date of testing in days(D)	Number of dogs VNT-titer ≥20*	%	Number of dogs VNT-titer <20	%	Total number of dogs	Average titer
0	0	4	33	8	67	12	8
1	21	4	33	8	67	12	7
2	90	3	25	9	75	12	5
2	460	0	0	10	100	10	13

* ≥20 considered to protect against challenge

Table 4: Elisa antibody titers in African Wild Dogs during a vaccination trial with CDV-Iscom vaccine Litter II

Number vaccinations at time of testing	Time-lapse between first vaccination and date of testing in days(D)	Number of dogs Elisa titer ≥20*	%	Number of dogs Elisa titer <20	%	Total number of dogs	Average titer
0	0	4	33	8	67	12	7
1	21	12	100	0	00	12	40
2	90	6	50	6	50	12	17
2	460	6	60	4	40	10	274

* ≥20 considered to protect against challenge

Figure 2: Mean titers after vaccination with a CDV-Iscom vaccine in the African wild dog Litter II

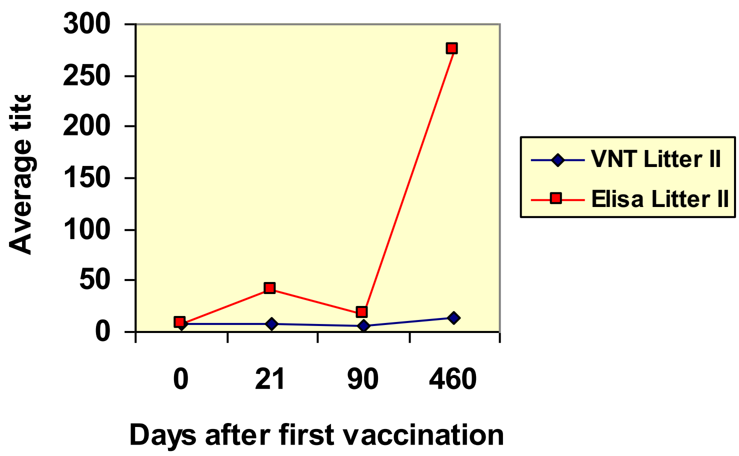


Table 5: Virus neutralizing antibody titers in African Wild Dogs during a vaccination trial with Purevax™ vaccine Litter I

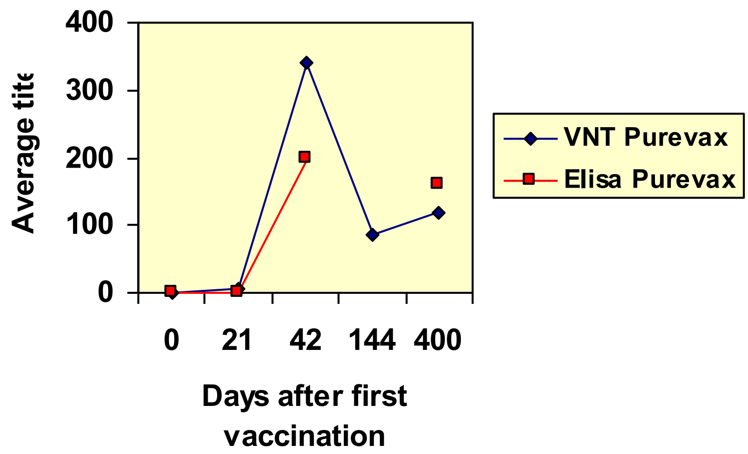
Number vaccinations at time of testing	Time-lapse between first vaccination and date of testing in days(D)	Number of dogs VNT-titer ≥20	%	Number of dogs VNT-titer <20	%	Total number of dogs	Average titer
0	0	0	0	4	100	4	0
1	21	1	33	3	67	4	5
2	42	4	100	0	0	4	340
3	144	4	100	0	0	4	85
3	400	4	100	0	0	4	120

Table 6: Elisa antibody titers in African Wild Dogs during a vaccination trial with Purevax™ vaccine Litter I

Number vaccinations at time of testing	Time-lapse between first vaccination and date of testing in days(D)	Number of dogs Elisa titer ≥20	%	Number of dogs Elisa titer <20	%	Total number of dogs	Average titer
0	0	0	0	4	100	4	0
1	21	0	0	4	100	4	0
2	42	4	100	0	0	4	200
3#	144	-	-	-	-	-	-
3	400	4	100	0	0	4	160

 # No results received

Figure 3: Mean titers after vaccination with Purevax™ in the African wild dog



Discussion

CDV-Iscom

Litter I: (Table 1 and 2, Figure 1): On each occasion when tested (except some of those when tested pre-vaccination) only one of four dogs had, what is considered to be protective, titer of ≥20 (Osterhaus, pers.com.). The percentage rise 25- 33% is due to the death of one of the dogs during the trial. However, the Elisa test revealed a different picture. On two occasions, Day 42 and 400, all (100%) of the dogs showed a protective titer. The mean titers were very high, 1040 and 160 respectively, in contrast to the mean titers in the VNT test 20 and 13. According to the VNT test the dogs were not protected but according to the Elisa test the dogs were protected. This large unexplainable difference needs further investigation.

Litter II: (Table 3 and 4, Figure 2): At the time of the first vaccination only the Elisa test results of Litter I were known. As these results indicated a good response to the CDV-Iscom vaccinations it was advised to vaccinate the dogs of Litter II twice in stead of three times (OSTERHAUS pers.com.). Results were dramatic, very low mean post vaccination VNT titers (5-13) and a percentage of 25 to 33 % of dogs with a titer ≥20. The same percentage as in Litter I. As in Litter I post vaccination Elisa titers differed dramatically from the VNT titers. At Day 21 100% of the dogs had an Elisa titer ≥20, which dropped to 50% at Day 90 and 60% one year later, still high compared to the VNT titers. Once again the same unexplainable occurrence as in Litter I.

Adult dogs: Three of the four adult dogs showed a high pre-vaccination VNT titer (320 - >1280), in contrast to the fourth one, the alpha male, who had no titer. From the results of the Elisa test the alpha male had a positive pre-vaccination titer. The odd thing is that all four dogs had never before been vaccinated to the best of our knowledge. The results suggested that three of them had recently survived a distemper infection, but the results from the other dogs at the same day appear to suggest this was unlikely.

Purevax™ (Table 5 and 6, Figure 3) In contrast to the CDV-Iscom vaccinated dogs, all four dogs (100%) after the second vaccination had satisfactory VNT titers; mean 85 to 340. Like the CDV-Iscom vaccinated dogs in Litter I the Purevax™ vaccinated dogs had satisfactory Elisa titers of 200 and 160.

Conclusion

All groups of CDV-Iscom vaccinated dogs, i.e. half Litter I and Litter II had discrepancies between the VNT and Elisa results, i.e. low VNT titers and high Elisa titers compared with the Purevax™ vaccinated dogs which had both high VNT and Elisa titers.

Although the number of dogs in this trial was small (especially for dogs vaccinated with Purevax™), our results indicate that vaccination with Purevax™ (rather then CDV-Iscom) is the most likely to protect African wild dogs against canine distemper.

Anesthesia

In order to take blood samples the dogs had to be anesthetized. A combination of medetomidine (Domitor®*) and ketamine*** with atipamezole (Antisedan®**) as an antidote for Domitor®, proved to be a safe way of sedating the dogs. During this trial 91 sedations (see table below) have been performed.

Table Number sedations 2002-2004

Date	Number sedations
27-02-02	7
20-03-02	8
10-04-02	8
24-07-02	9
13-03 03	12
02-04-03	22
11-06-03	12
21-06-04	13
8 occasions	**Total 91**

The drugs were administered by blowpipe, intramuscularly, in the hindquarters. For that purpose, the dogs were separated from the pack; two or three dogs a time to avoid confusion at the darting process.

In general for adult dogs (25-30 kg) a dosage of 1 ml Domitor® and 1 ml ketamine was used, which means 40 – 33,3 μg Domitor® and 4,0 – 3,3 mg ketamine per kg bodyweight.

Pups (10-12 kg) received 1 ml Domitor® and 0,1 ml ketamine , 100 – 83 μg Domitor® and 1,0 – 0,8 mg ketamine HCL per kg bodyweight. Except the pups of Litter II; they received 0,4 ml Domitor® and 0,2 ml ketamine HCL, 40 μg Domitor® and 2,0 mg ketamine per kg at the time of an average bodyweight of approximately 10 kg.

Induction time varied from 5 to 15 minutes. The combination Domitor® and ketamine proved

to be very satisfactory for purposes such as taking blood samples, applying transponders, measuring their bodyweight, etc. There appeared to be no difference in effectiveness between the different ratios of Domitor® and ketamine.

The smaller dogs (<15 kg) received Antisedan® approximately 30 minutes after the veterinary procedures involving them ended and the larger dogs received their Antisedan® at least an hour after they were finished, due to the larger amount of ketamine they had received. Side effects observed after application of Antisedan® involved gazing in the distance and ataxia which disappeared usually within 30 minutes. Dosage of Antisedan® was in general half the dosage of Domitor® in volume.

* Domitor® (medetomidine hydrochloride 1 mg/ml), Pfizer Animal Health B.V.
** Antisedan® (atipamezole hydrochloride 5 mg/ml), Pfizer Animal Health B.V.
*** ketamine hydrochloride 100 mg/ml

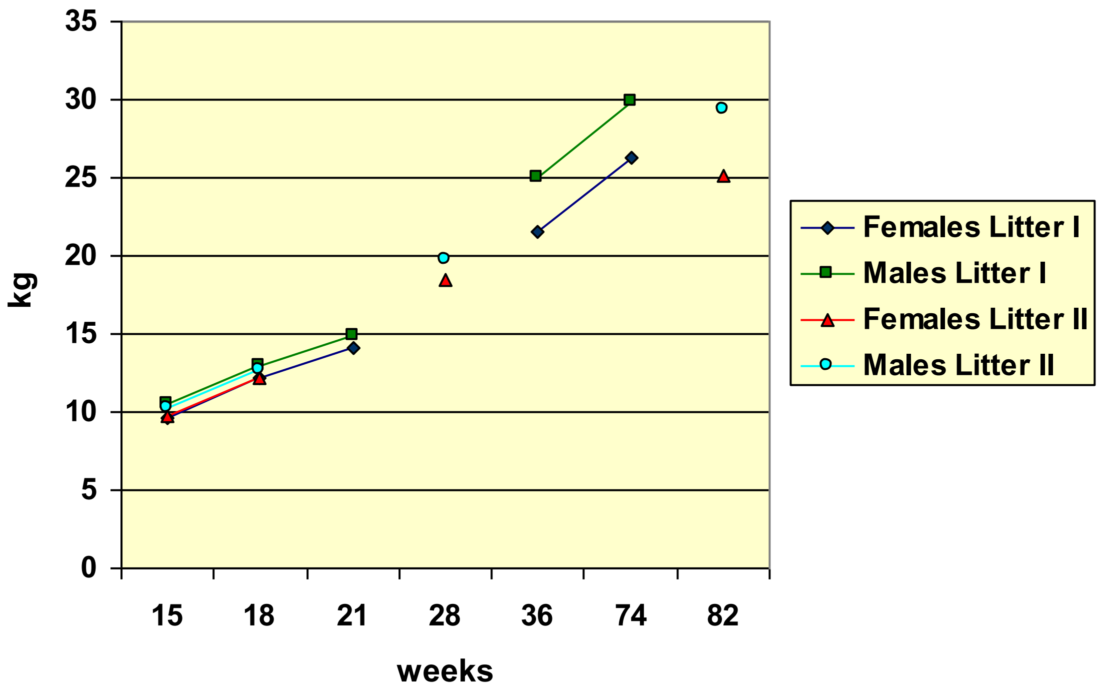
Identification

The first time the dogs were anesthetized they received a transponder dorsal subcutaneously in the neck. On every other occasion of sedation the transponder was checked to identify the dog. Three times the transponder did not return the transmitted signal of the reader and an extra transponder was applied. For numbers see the appendices. As an extra way of identification pictures were taken both sides of the dog in lateral recumbency. This way it was possible to identify the dogs without sedation, which proved to be helpful in case of not responding transponders and to identify dogs from a distance.

Bodyweight (See appendix I)

Every time a dog was anesthetized bodyweight was measured. This way it was possible to follow the increase in bodyweight during growing up of two litters. In figure 4 the bodyweights of the dogs are reflected in a diagram, distinguished in litter and gender. As the diagram shows there is hardly a difference between the litters, the pups grew up at the same pace. Apparently they reach their mature bodyweight at the age of approximately 1 ½ years and appeared to be 25 kg for females and 30 kg for males which was in accordance with the bodyweight of the alpha pair.

Figure 4: Average bodyweight in the course of time per litter and per gender



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Daphne Valk, animal technician, for her help and overall assistance and keeping track of the ID numbers.
Keepers of the African wild dogs in Artis for their help and assistance.

Summary

Following a serious canine distemper outbreak in an African wild dog breeding program, Mkomazi Game Reserve, Tanzania, in December 2000, a small vaccine research trial was carried out involving two litters of African wild dogs at Artis, Amsterdam Zoo. Two different vaccines, CDV-Iscom and Purevax ™, were used in the trials and their results compared. It appeared that dogs vaccinated with CDV-Iscom developed very few antibodies according to the VNT test but for unexplainable reasons performed much better according to the Elisa test. Dogs vaccinated with Purevax ™ showed satisfactory titers according to both the VNT and Elisa test.

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Appendix I AFRICAN WILD DOGS Artis

Bodyweight in kilogrammes (kg)								
			Date	27-02-02	20-03-02	10-04-02	24-07-02	02-04-03
Identification		Sex	of Birth	kg	kg	kg	kg	kg
624029D		F	02-11-01	9,0	12,0	14,0	21,0	26,0
61D37D2		F	02-11-01	10,0	12,5	15,0	23,0	27,0
6241274		F	02-11-01	9,5	12,0	13,5	21,0	26,0
6160708*		F	02-11-01	10,0	12,5	14,0	21,5	
Average bodyweight females:				9,6	12,2	14,1	21,6	26,3
Age in weeks:				15	18	21	36	74
Age in days:				106	127	148	253	516
61D48E6	615D5BF	M	02-11-01	10,0	12,0	14,5	24,5	29,0
61D3E03	61D2B53	M	02-11-01	10,0	13,5	15,0	26,0	30,5
61D4E58		M	02-11-01	10,0	12,5	14,5	23,5	28,5
6160A26	61601E0	M	02-11-01	12,0	13,5	15,5	26,0	31,5
Average bodyweight males				10,5	12,9	14,9	25	29,9
Age in weeks:				15	18	21	36	74
Age in days:				106	127	148	253	516
			Date	12-03-03	02-04-03		11-06-03	21-06-04
631C70D		F	24-11-02	9,6	12,0		16,5	
631EAFF		F	24-11-02	10,1	12,5		19,0	
6321FD4		F	24-11-02	9,7	11,5		18,0	23,4
630D5F4		F	24-11-02	9,8	12,5		18,5	24,1
631FD0E		F	24-11-02	9,4	12,0		19,0	26,8
631FE29		F	24-11-02	10,3	12,5		19,5	26,0
Average bodyweight females				9,8	12,2		18,4	25,1
Age in weeks:				15	18		28	82
Age in days:				108	129		199	575
63181BF		M	24-11-02	10,3	13,0		20,0	31,0
631E215		M	24-11-02	10,5	13,0		19,5	27,7
631E389		M	24-11-02	10,5	12,5		20,0	28,9
6317A94		M	24-11-02	9,7	12,0		19,5	30,8
631CAAB		M	24-11-02	10,6	13,5		21,5	30,0
631829F		M	24-11-02	9,9	12,5		17,5	27,3
Average bodyweight males				10,2	12,7		19,7	29,3
Age in weeks				15	18		28	82
Age in days				108	129		199	575
126B22D	Alpha	M	29-11-94		45,5			34,4
00F78503	Alpha	F	29-10-95		26,0			24,5
01D3843F		M	11-04-00		31,0			30,4

* Overleden 25-10-2002

Appendix II				Distemper antibody levels (VNT) + Vaccination dates Artis															
Identification		**Date**		**27-02-02**		**20-03-02**		**10-04-02**		**24-07-02**		**13-03-03**		**02-04-03**		**11-06-03**		**21-06-04**	
Litter I		**Sex**	**Day of birth**	**D0**		**D21**		**D42**		**D144**				**D400**					
624029D		F	02-11-01	V I	<20	V I	<20	V I	80		20			V I	<20				
61D37D2		F	02-11-01	V P	<20	V P	<20	V P	640		160			V I	160				
6241274		F	02-11-01	V P	<20	V P	<20	V P	320		80			V I	80				
6160708		F	02-11-01	V I	<20	V I	20	V I	<20		<20	**Died 25-10-02**							
61D48E6	615D5B[	M	02-11-01	V I	<20	V I	<20	V I	<20		<20			V I	40				
61D3E03	61D2B5:	M	02-11-01	V I	<20	V I	<20	V I	<20		<20			V I	<20				
61D4E58		M	02-11-01	V P	<20	V P	20	V P	320		80			V I	80				
6160A26	61601E[	M	02-11-01	V P	<20	V P	<20	V P	80		20			V I	160				
Litter II												**D0**		**D21**		**D90**		**D460**	
631C70D	M02164	F	24-11-02									V I*	<20	V I*	<20		20		
631EAFF	M02165	F	24-11-02									V I	20	V I	<20		<20		
6321FD4	M02166	F	24-11-02									V I	20	V I*	20		<20		<20
630D5F4	M02167	F	24-11-02									V I	<20	V I	20		<20		<20
631FD0E	M02168	F	24-11-02									V I	40	V I*	20		20		<20
631FE29	M02169	F	24-11-02									V I*	20	V I*	<20		<20		<20
63181BF	M02158	M	24-11-02									V I*	<20	V I*	<20		<20		<20
631E215	M02159	M	24-11-02									V I*	<20	V I	<20		20		<20
631E389	M02160	M	24-11-02									V I	<20	V I	<20		<20		<20
6317A94	M02161	M	24-11-02									V I*	<20	V I*	<20		<20		<20
631CAAB	M02162	M	24-11-02									V I*	<20	V I	20		<20		<20
631829F	M02163	M	24-11-02									V I	<20	V I	<20		<20		<20
Adult dogs														**DO**				**D440**	
611B13C	M00180	F	18-12-00								640								
126B22D alpha	M99038	M	29-11-94											V I	<20				<20
00F78503 alpha	M95168	F	29-10-95											V I	320				≥ 80
01D3843F	M00088	M	11-04-00											V I	> 1280				≥ 80
V=Vaccinated; I=CDV Iscom; P=Purevax; *=double dosage																			

Appendix III				Distemper antibody levels (Elisa) + Vaccination dates Artis															
Identification		**Date**		**27-02-02**		**20-03-02**		**10-04-02**		**24-07-02**		**13-03-03**		**02-04-03**		**11-06-03**		**21-06-04**	
Litter I		**Sex**	**Day of birth**	**D0**		**D21**		**D42**		**D144**				**D400**					
624029D		F	02-11-01	V I	<20	V I	40	V I	>2560		#			V I	>160				
61D37D2		F	02-11-01	V P	<20	V P	<20	V P	160		#			V I	>160				
6241274		F	02-11-01	V P	<20	V P	<20	V P	320		#			V I	>160				
6160708		F	02-11-01	V I	<20	V I	<20	V I	320		#	Died 25-10-02							
61D48E6	615D5BF	M	02-11-01	V I	<20	V I	<20	V I	640		#			V I	>160				
61D3E03	61D2B53	M	02-11-01	V I	<20	V I	<20	V I	640		#			V I	>160				
61D4E58		M	02-11-01	V P	<20	V P	<20	V P	160		#			V I	>160				
6160A26	61601E0	M	02-11-01	V P	<20	V P	<20	V P	160		#			V I	>160				
Litter II												**D0**		**D21**		**D90**		**D460**	
631C70D	M02164	F	24-11-02									V I*	20	V I*	20		<20		
631EAFF	M02165	F	24-11-02									V I	20	V I	20		<20		
6321FD4	M02166	F	24-11-02									V I	20	V I*	20		20		<20
630D5F4	M02167	F	24-11-02									V I	<20	V I	40		20		<20
631FD0E	M02168	F	24-11-02									V I	<20	V I*	20		20		320
631FE29	M02169	F	24-11-02									V I*	<20	V I*	20		<20		640
63181BF	M02158	M	24-11-02									V I*	<20	V I*	40		<20		640
631E215	M02159	M	24-11-02									V I*	<20	V I	20		20		320
631E389	M02160	M	24-11-02									V I	<20	V I	20		<20		<20
6317A94	M02161	M	24-11-02									V I*	<20	V I*	40		<20		180
631CAAB	M02162	M	24-11-02									V I*	20	V I	20		80		<20
631829F	M02163	M	24-11-02									V I	<20	V I	80		40		640
Adult dogs														**D0**				**D440**	
611B13C	M00180	F	18-12-00								#								
126B22D alpha	M99038	M	29-11-94											V I	>160				#
00F8503 alpha	M95168	F	29-10-95											V I	>160				#
01D3843F	M00088	M	11-04-00											V I	>160				#

 # Results not received

V=Vaccinated; I=CDV Iscom; P=Purevax; *=double dosage

Genetic Analysis of African Wild Dogs (*Lycaon pictus*)
In Artis, Amsterdam Zoo
(2002)

For: **The the African Wild Dog Foundation**

Att: **Drs. A. Visee**

By: **Dr. Ir. A. Kappe**

Gendika
Industrieweg 1
9641 HM Veendam

Date: 4 November 2005

Introduction

The African wild dog (Lycaon pictus) is an extremely endangered species. In order to prevent the species for extinction a rehabilitation program was established by the George Adamson Wildlife Preservation Trusts and the African Wild Dog Foundation involving capture, captive breeding and re-introduction. As a part of the project a genetic analysis of eight African Wild dogs from Artis, Amsterdam Zoo, the Netherlands was performed. In this report the genetic analysis of the African wild dogs is presented. Also a parallel will be drawn with African Wild dogs involved in a captive breeding program with animals from three locations in the Masai Steppe, Tanzania; the Mkomazi Project.

Materials and methods

Genetic samples

Blood-samples were collected from 8 African wild dog pups, 4 females and 4 males in one litter at Artis Zoo, Amsterdam, the Netherlands. See also Appendix 1 for a complete list of samples in the Mkomazi Project.

DNA isolation

DNA was isolated from 5 ml whole blood collected in EDTA anticoagulant vacutainers. DNA was extracted by proteinase K digestion (100 µg/ml) and DNA was isolated and purified by using Standard Gendika protocol GDKiso06.

Identification of microsatellite alleles

Wild dog samples were screened by the microsatellite technique. In the microsatellite technique highly variable microsatellites are used as genetic markers. Microsatellites, also known as simple sequence repeats or short tandem repeats, are genomic sequences that consist of di-nucleotide motif repeated in multiple tandem copies, in mammals CA-repeats are most common. The variation within the microsatellite loci has been shown to arise from variation in the number of repeat units (the number of CA-repeats). The variable appearances of a microsatellite are called alleles. Alleles present for a microsatellite are generated by PCR amplification. The lenght of the various alleles can be detected by cappilary gel electrophoresis in a Genetic Analyzer.

By examination of a set of microsatellites a specific pattern of alleles will be produced for each individual. This specific pattern of alleles is also known as a DNA fingerprint.

Wild dog samples were screened for variation in 10 $CA_{(n)}$ microsatellite loci, originally isolated from a domestic dog library, combined in a Canine 10 Plex Kit (Applied

Biosystems). Nine microsatellites were identified that consistently gave PCR product, were polymorphic in wild dogs.

Detection of microsatellite alleles from DNA was achieved by performing 35 cycles of PCR amplification in a 10 µl reaction volume using 10 ng of target DNA and 0.36 units of *Taq* DNA polymerase (Applied Biosystems). Alleles were detected by a Genetic Analyzer.

Data analysis

The similarity, which is the average of shared alleles between two animals, was calculated as

$S_{xy} = 2n_{xy} / (n_x + n_y)$,

where n_x and n_y are the number of microsatellite alleles present in individual x and indiviual y, respectively, and n_{xy} is the number of alleles shared by individuals x and y (Wetton *et al.*, 1987). The similarity between animals is presented as a percentage.

With this percentage the amount of mean similarity within a group was estimated by averaging the similarities of all pairs of individuals that belong to the group. Mean similarity between groups A and B was estimated by averaging the similarities of all pairs of individuals of which one belonged to group A and the other to group B.

Results

For all eight African wild dogs a pattern of microsatellite alleles could be scored and individual specific genotypes were identified, which means that for all animals a specific DNA fingerprint could be produced.

The patterns of alleles for the nine microsatellites of all individual animals were compared and the percentage of shared alleles, the similarity S_{xy}, was calculated between animals, see Appendix 2. The calculated similarity S_{xy} is a measure for the relationship between animals. Parents share at least 50% of the alleles with their offspring, which means that the similarity between parents and offspring will be on average 0.5 or higher. Between siblings the similarity will be higher, than the similarity between unrelated animals, normally a similarity of 0.4 - 0.6 is found between siblings and a similarity of 0.0 – 0.3 is found in unrelated animals. In small populations all similarities will be higher, more alleles are shared between the animals because of the appearance of inbreeding within small populations (Avise, 1994).

Similarity in the Artis African wild dogs

The relationship within the group of African wild dogs in was calculated and analysis of patterns yielded similarities of 0.44 - 0.83 (see Appendix 2). The average similarity for this whole group is 0.62. In table 1 the Artis animals are compared to wild dogs in the Mkomazi Project.

Table 1: Similarity of alleles, S_{xy} (%) within the Artis African wild dogs and comparison with original African wild dogs in the Mkomazi Project

Similarity		
Artis (n=8)	Within litter	**0.62**
Mkomazi project (n=25)	Unrelated animals	**0.46**
	Related animals	**0.56**
Artis-Mkomazi project	Between groups	**0.26**

The mean similarity within the Artis litter is considerably higher than the values found for the Mkomazi Project animals, both for unrelated animals and related animals.

Conclusion

From the results of the genetic analysis by DNA microsatellites can be concluded that there is considerable genetic variation in the African wild dog litter in the Mkomazi project. In the Artis wild dog litter the similarity between animals is quite high 0.62. In literature a similarity of 0.0 – 0.3 is found in unrelated animals, a similarity of 0.5 or higher is found for parents and offspring and a similarity of 0.4 - 0.6 is found between siblings. The Artis wild dog litter represents a value higher than found between siblings. This implies the conclusion that the genetic variation among these animals is much smaller than in the wild African wild dogs from Africa and a higher level of inbreeding is found in the Artis African wild dog. Between the two populations there is a very low similarity. From this result the conclusion can be taken that there is a complete different genetic basis in the Artis African wild dogs, compared to the Mkomazi Project African wild dogs.

The kinship between the different European zoos are being researched in 2005 by Brinkman, & Bijma. In this research a kinship between the different European zoos are calculated between 0.0-0.4, which is a good base of genetic variability to exchange animals between zoos.

Between the Artis wild dog litter and African wild dogs from the Mkomazi Project a comparison was made, these two populations show a very low similarity. From this result the conclusion can be taken that there is a complete different genetic basis in the Artis African wild dogs, compared to the Mkomazi Project African wild dogs.

Literature

Avise, J.C. 1994. Molecular markers, natural history and evolution. *Chapman and Hall, Inc*

Brinkman, S. & Bijma, P. 2005. Genetic variability in the African wild dog population in European zoos. *Colloquium Animal Breeding and Genetics, ABG-80436.*

Wetton, J.H., Carter, R.E., Parkin, D.T., and Walters, D. 1987. Demographic study of a wild house sparrow population by DNA 'fingerprinting'. *Nature*, **327**, 147-149.

Appendix 1: List of all Animals in Mkomazi project

nr	Soort	Code	Extra gegevens
1	Wilde hond	ANWH 101	624029D, Artis 2002
2	Wilde hond	ANWH 102	616078, Artis 2002
3	Wilde hond	ANWH 103	61D4E58, Artis 2002
4	Wilde hond	ANWH 104	6241274, Artis 2002
5	Wilde hond	ANWH 105	61D2B53, Artis 2002
6	Wilde hond	ANWH 106	6160A26, Artis 2002
7	Wilde hond	ANWH 107	61D37D2, Artis 2002
8	Wilde hond	ANWH 108	615D5BF, Artis 2002
9	Wilde hond	ANWH 297	1998
10	Wilde hond	ANWH 300	1998
11	Wilde hond	ANWH 333	1998
12	Wilde hond	ANWH 335	1998
13	Wilde hond	ANWH 336	1998
14	Wilde hond	ANWH 337	1998
15	Wilde hond	ANWH 339	1998
16	Wilde hond	ANWH 261	1999
17	Wilde hond	ANWH 305	1999
18	Wilde hond	ANWH 308	1999
19	Wilde hond	ANWH 323	1999, parents 262 (F) & 274 (M)
20	Wilde hond	ANWH 327	1999, parents 262 (F) & 274 (M)
21	Wilde hond	ANWH 336	1999, parents 297 (F) & 300 (M)
22	Wilde hond	ANWH 337	1999, parents 297 (F) & 300 (M)
23	Wilde hond	ANWH 348	1999, parents 297 (F) & 300 (M)
24	Wilde hond	ANWH 464	F 07-07-01, parents 306 (F) & 372 (M)
25	Wilde hond	ANWH 448	F 07-07-01, parents 306 (F) & 372 (M)
26	Wilde hond	ANWH 251	M 07-07-01, parents 306 (F) & 372 (M)
27	Wilde hond	ANWH 368	M 07-07-01, parents 306 (F) & 372 (M)
28	Wilde hond	ANWH 269	M 07-07-01, parents 306 (F) & 372 (M)
29	Wilde hond	ANWH 202	M 07-07-01, parents 306 (F) & 372 (M)
30	Wilde hond	ANWH 389	M 07-07-01, parents 306 (F) & 372 (M)
31	Wilde hond	ANWH 40	Kimondo F 2002, Wild caught
32	Wilde hond	ANWH 6	Zawadi F 2002, Wild caught
33	Wilde hond	ANWH 372	Chupu Chupu M 2002, parents 284 (F) & 298 (M)
34	Wilde hond	ANWH 325	2002 parents 262 (F) & 274 (M), died 2002
35	Wilde hond	ANWH 104	F 18-04-02, parents 040 (F) & 372 (M)
36	Wilde hond	ANWH 348	F 18-04-02, parents 040 (F) & 372 (M)
37	Wilde hond	ANWH 651	M 18-04-02, parents 040 (F) & 372 (M)
38	Wilde hond	ANWH 730	M 18-04-02, parents 040 (F) & 372 (M)
39	Wilde hond	ANWH 666	M 11-05-04, parents 006 (F) & 251 (M)
40	Wilde hond	ANWH 671	M 11-05-04, parents 006 (F) & 251 (M)
41	Wilde hond	ANWH 732	M 11-05-04, parents 006 (F) & 251 (M)
42	Wilde hond	ANWH 853	M 11-05-04, parents 006 (F) & 251 (M)
43	Wilde hond	ANWH 860	M 11-05-04, parents 006 (F) & 251 (M)
44	Wilde hond	ANWH 887	M 11-05-04, parents 006 (F) & 251 (M)
45	Wilde hond	ANWH 926	M 11-05-04, parents 006 (F) & 251 (M)
46	Wilde hond	ANWH 939	M 11-05-04, parents 006 (F) & 251 (M)
47	Wilde hond	ANWH 949	M 11-05-04, parents 006 (F) & 251 (M)
48	Wilde hond	ANWH 261	1995, Lendanai, RUG
49	Wilde hond	ANWH 262	1995, Lendanai, RUG
50	Wilde hond	ANWH 263	1995, Lendanai, RUG
51	Wilde hond	ANWH 264	1995, Lendanai, RUG
52	Wilde hond	ANWH 265	1995, Najo, RUG
53	Wilde hond	ANWH 273	1995, Najo, RUG
54	Wilde hond	ANWH 274	1995, Najo, RUG
55	Wilde hond	ANWH 275	1995, Najo, RUG
56	Wilde hond	ANWH 276	1995, Najo, RUG
57	Wilde hond	ANWH 284	1995, Najo, RUG
58	Wilde hond	ANWH 285	1995, Najo, RUG
59	Wilde hond	ANWH 288	1995, Najo, RUG
60	Wilde hond	ANWH 289	1995, Llondirrigiss, RUG
61	Wilde hond	ANWH 291	1995, Llondirrigiss, RUG
62	Wilde hond	ANWH 294	1995, Llondirrigiss, RUG
63	Wilde hond	ANWH 296	1995, Llondirrigiss, RUG
64	Wilde hond	ANWH 297	1995, Llondirrigiss, RUG
65	Wilde hond	ANWH 298	1995, Llondirrigiss, RUG
66	Wilde hond	ANWH 299	1995, Llondirrigiss, RUG
67	Wilde hond	ANWH 300	1995, Najo, RUG
68	Wilde hond	ANWH 303	1995, Najo, RUG
69	Wilde hond	ANWH 305	1995, Najo, RUG
70	Wilde hond	ANWH 310	1995, Najo, RUG

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Appendix 2: Similarities between African Wild dogs, Artis, Amsterdam Zoo, the Netherlands

animal	101	102	103	104	105	106	107	108
101		0,61	0,61	0,56	0,61	0,78	0,78	0,72
102			0,56	0,61	0,56	0,61	0,83	0,56
103				0,67	0,83	0,56	0,50	0,56
104					0,72	0,56	0,67	0,44
105						0,61	0,50	0,50
106							0,67	0,67
107								0,61
108								