The HANDLING-STRESS HYPOTHESIS

And its Importance for the Conservation and Survival of the African Wild Dog

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The feature common to all the wild dog study packs that died post 1985 was 'handling' and an hypothesis (Burrows 1992) later known as '**The handling-stress hypothesis**', **was an attempt to explain the selective extinction of all the 'handled' study packs whilst an 'unhandled' non-study population of wild dogs survived.**

To date, this is the only hypothesis that fits the available data

Between 1986 – 1991 the entire wild dog study population comprising 14 packs containing approximately 200 individuals died/disappeared from the two study areas in the Serengeti-Mara ecosystem. The extinction of all the study packs followed the commencement, in 1985, of invasive research involving anaesthetization, radio-collaring, tissue sampling and, post 1988, vaccinations against rabies. Such invasive research is collectively known as 'handling'.
Despite the extinction of all the handled study packs a non study, unhandled population of wild dogs persisted in and around the study areas formerly occupied by the now extinct study packs and persists to date.

The African wild dog (*Lycaon pictus)*, one of the most endangered large carnivores in Africa, has been subject to increasing use of invasive conservation research since 1985 in Serengeti National Park and Ngorongoro Conservation Area in Tanzania (Serengeti) and since 1987 in the Masai Mara region of Kenya (Mara). These regions together form part of the Serengeti-Mara Ecosystem with the wild dogs within the ecosystem and adjacent areas forming one breeding population linked via yearling and adult emigration.

Between June 1985 and August 1990 in the Serengeti, **5 unvaccinated study packs died within 4 months of the radio-collaring of some individuals,** with rabies confirmed in one Serengeti study pack in 1990. Other study and non study packs, some with home ranges overlapping that of the extinct packs, survived each study pack death. This was prior to the mass vaccination of Serengeti study packs which took place in September/October 1990.

Prior to 1986 no whole pack death was known other than packs shot by game wardens in Serengeti as 'vermin'(wild dogs were thought to 'disturb the game'), a practice that ceased in 1973.

By September 1989, the then only Mara (Kenyan) study pack (the 'Aitong') died from rabies, **a disease not previously confirmed in a free-living wild dog in Africa.** This pack included 2 individuals handled by researchers for radio-collaring and vaccination against rabies in 1988 and 4 individuals, including 3 ten month old pups, that were radio-collared and vaccinated against rabies earlier in 1989.

This was the first pack in Africa in which free-living wild dogs were rabies vaccinated AND the first such pack in which the cause of death was confirmed to be rabies in both vaccinated and unvaccinated individuals.

Following these losses, an attempt was made in 1990 to vaccinate the remaining study packs in both sectors of the ecosystem using an inactivated rabies vaccine delivered either directly by hand following anaesthetization or remotely via air pressurized darts.

All vaccinated study packs died or disappeared between 1990 -1991 in both sectors of the ecosystem within a year of vaccination with rabies confirmed in a Mara (Kenyan) Pack, in the only tissue sample taken from a dead vaccinated wild dog pack in either study area. Although no known outbreak of rabies occurred at this time in other wildlife in Serengeti sector of the ecosystem, where no tissue samples were collected from the 5 study packs that died, rabies was suspected.

Following the extinction of all the handled study packs, unhandled packs **i.e. not vaccinated and not radio collared still existed in or near both study areas in both sectors of the ecosystem.**

The feature common to all the wild dog study packs that died post 1985 was 'handling' and an hypothesis (Burrows 1992) later known as **'The handling-stress hypothesis'**, **was an attempt to explain the selective extinction of all the 'handled' study packs whilst an 'unhandled' non-study population of wild dogs survived.**

Many mammalian species carry latent viruses, which can be reactivated by stress in some cases. In raccoons it was suspected that latent rabies infections may be reactivated by stress (McLean 1975 , Johnston & Beuregard , 1960).

Both subclinical rabies, and a 'carrier' state in domestic dogs has been recognized in Africa, where it is known as 'OULOU FATO' , and in Asia and this replaces the classical form of rabies.

An experimentally induced carrier state has been reported following the experimental inoculation of young adult beagles in captivity with a rabies strain from the saliva of an apparently healthy Ethiopian dog. In another study it was found that rabid animals may secrete virus in saliva but have no detectable virus in their salivary glands and brain after death. (Fekadu 1983)

However other researchers/publications claim that rabies does not have a dormant or 'latent' period, but that following a bite by a rabid animals the virus then replicates and travels along nerves to the brain and salivary glands a process that may take several months before symptoms appear.

The feature common to all Serengeti wild dog study packs that **died sporadically** between 1985 and August 1990 was recent 'handling' for **blood sampling and radio collaring only** of some individuals**.**

Here serum samples taken up to 2 years before vaccination, **but only examined post-vaccination**, showed that **some study packs had been exposed to rabies with some individuals carrying significant, possibly natural protective levels of rabies-neutralizing antibody**.

The handling- stress hypothesis suggests that 'handling' wild dogs may adversely affect the normal functioning of their immune system and so inhibit the natural body defenses when naturally exposed to viral infections.

It is possible that a rabies virus persists in Serengeti-Mara wild dogs in a normal host-parasite relationship with some naturally immune individuals.

This stable system could be disrupted by handling-induced stress in some individuals.

Handling-induced stress, as measured by highly elevated peripheral serum cortisol concentrations, followed immobilization/anaesthetization of some captive wild dogs, but this is disputed by other researchers. Corticosteroids tend to inhibit the body defenses in rabies carrier individuals so leading to clinical rabies with the disease spreading within, but not between, the widely separated packs, by oral social contact.

The first pack to die with rabies confirmed contained just a few rabies vaccinated individuals with the majority unhandled, but following the mass rabies vaccinations of 1990 all or most of the pack members were vaccinated.

The confirmed rabies related death of the unvaccinated Serengeti study pack in 1990 contained 2 individuals recently radio-collared - an old male and a young female that had recently produced a litter of pups. The extinction of all mass vaccinated study pack can be explained by the 'handling -stress hypothesis'; any rabies carrier dog(s) would be 'hit' during the whole pack vaccinations that took place in 1990 but only selected by chance when one or more individuals in a pack was selected for radio collaring only.

This hypothesis would also explain why in both sectors of the ecosystem and surrounding areas an un-handled, unvaccinated non-study population not only persisted pre and post 1991 but expanded their population and range. This despite a major rabies epidemic all around Serengeti National Park (SNP) that began in late 2002 and which had a devastating effect on the local human population and affected many local domestic dogs and some species of wildlife outside the protected area of SNP.

Significantly there is **no evidence that the rabies epidemic affected either the local wild dog population resident in the rabies epidemic area outside the protected area of SNP in close proximity to rabid animals, nor was it detected in any of the closely monitored wildlife species within the protected area,** despite the Bat-eared fox (*Otocyon megalotis*) population in SNP suffering a high incidence of rabies confirmed between 1986 and 1989, in the closely monitored study population near Seronera (Mass 1993).

This Bat -eared fox rabies is also reported by Cleaveland et. al in 2008 .(Serengeti 111, *Human impacts on Ecosystem Dynamic*). Here (p. 215) is it claimed . *' During the peak period of wild dog mortality (1987 and 1988), rabies epidemics also affected bat-eared-foxes in the central Serengeti, killing 60% of all adult females and 20% of males and cubs (Maas 1993).'*

This is wrong, the peak of wild dog mortality in 'Serengeti' was in 1990-91 when 6 study packs, 5 containing vaccinated members, died following the mass single rabies vaccinations given to the majority of individuals in the study packs in 1990. In 1987-88 in Serengeti only one study pack died out from unknown causes despite the pack being closely monitored by researchers.

In a recently published paper examples are given of vaccine-induced enhancement of susceptibility to virus infections (Huisman et. al. , 'Vaccine' 2009).

There is no evidence of the widely claimed possibility of rabies from outbreaks in local domestic dogs being transmitted directly to wild dogs outside SNP nor to any wildlife within protected areas during the massive post 2002 rabies epidemic in and around the Serengeti-Mara ecosystem of Tanzania and Kenya.

There is no data-based evidence that rabies has caused mortality in any other 'handled' free living study packs in Africa. The only other wild dogs confirmed to have died from rabies were from vaccinated captive packs in Southern Africa.

The study packs in the Serengeti-Mara ecosystem were (and still are) the only free-living wild dog packs in which all or some individuals that had been **vaccinated against rabies, died with rabies confirmed** by laboratory investigation of samples.

ALTERNATIVE EXPLANATIONS GIVEN FOR THE EXTINCTION OF THE SERENGETI-MARA STUDY PACKS: NONE FIT THE AVAILABLE DATA

It is claimed that because the Serengeti-Mara study packs were vaccinated against rabies using a 'inactivated' vaccine that the vaccinations could not have been the cause of death of the study packs.

However, following rabies vaccinations of wild dogs in captive packs in Southern African some died from rabies *despite* being vaccinated with an inactivated rabies vaccine as were the rabies vaccines used in wild dog study packs in the Serengeti-Mara ecosystem. It was concluded from the South African research that a single parenteral vaccination of African wild dogs (as used in the Serengeti-Mara wild dog vaccinations) fails to protect them against rabies (Hofmeyr et al 2003). No data is available on the efficacy of a single vaccination via dart as also used in Serengeti but as quoted above (Huisman et. al. in 'Vaccine' 2009) gives examples of vaccine-induced enhancement of susceptibility to virus infections.

The only other suggested explanation for the selective loss of the radio-collared and/or vaccinated 'handled' study packs in the Serengeti-Mara ecosystem post 1985 is that the extinctions were the result of canine distemper outbreaks in local domestic dogs (Cleaveland et al 2000) .

Contra the claimed involvement of Canine Distemper in the death of wild dog study packs in the Mara in 1990-91(Alexander & Appel 1994, Alexander et al 1996) there is no serological evidence for this with between 1985-91, rabies the only confirmed cause of death of one unvaccinated Serengeti pack in 1990 (in which two members had been recently radio-collared) and two Mara packs in 1989 and 1990 both containing rabies vaccinated individuals.

The first free living wild dog pack confirmed to have died from Canine -Distemper was in 1996 in Chobe National Park in Botswana (Alexander et. al. 1996) where researchers described the wild dog population as 'common'.

As rabies vaccinations of free-living wild dogs only took place in the Serengeti-Mara ecosystem from 1988-1990 there are no data from any other free-living 'handled' study populations of wild dogs in Africa that can be used for comparative purposes.

The alternative explanations for the selective extinction of the handled study packs in the Serengeti-Mara ecosystem between 1985 and 1991 can also be shown to be highly improbable as un-handled non -study packs survived in the same or adjacent areas during the entire period when all the study packs became extinct.

These un-handled non-study free-living wild dogs packs in and around the Serengeti-Mara ecosystem, not only survived a confirmed 1993-94 canine distemper epidemic in wildlife in Serengeti-Mara ecosystem and adjacent areas but also survived the massive rabies epidemic post 2002 all around SNP (but not in any wildlife within the protected area) despite their close proximity to local domestic dogs suffering from sporadic outbreaks of rabies and Canine Distemper.

Currently the only hypothesis that fits the field observations and the serological data collected from the Serengeti-Mara wild dog study packs that died between 1985-91 is the 'Handling-Stress hypothesis' regardless of the precise mechanism(s) involved.

MORTALITY FOLLOWING HANDLING OF OTHER WILD DOG STUDY POPULATIONS:

1) KRUGER NATIONAL PARK, South Africa

Kruger National Park (KNP) **–** the most continuously, intensively and invasively studied population of wild dogs began in 1988. Between 1989 – 2005 there was a **dramatic decline** in the wild dog study population in Kruger National Park.

In July 2002 Dr. G. Mills reported:- '***The number of wild dogs is down to under 200 now from over 400*** *a few years ago, and we really don't know why'*. (African Conservation Organization).

In 2005 intensive invasive studies ended and population data, previously based on intensive invasive techniques, was replaced by photographic competitions. The results of the photographic survey 2008-9 showed an increase in the reported wild dog population.

The population decline occurred in **a previously 'stable population', with high (71%) pup survival** (Reich 1981, Maddock & Mills 1994, Woodroffe et al 1997).

Between 1989-94, the majority of KNP wild dog study population was handled in some way and packs subsequently located by helicopter and frequently monitored both from the air and on the ground. The levels of stress induced in individuals (some repeat darted and blood sampled) and their packs, is unknown.

Invasive Handling of pups

The survival of pups in the KNP study area in the 1970s **before invasive handling began was 71%** (Reich 1981). In 1989 soon **after invasive research began fell to 56 %** (Fuller et al 1992,). Between 1990-93 **pup**

survival fell dramatically to 25-33% (van Heerden et al 1995) coincident with the rapid expansion of invasive handling.

Between 1990-93 nineteen pups >6 months were anaesthetized in KNP (van Heerden et al 1995) and between 1989 and 1994, 15 wild dogs of unspecified age were anaesthetized for the fitting of radio-collars, 15 for radio-implants and 28 for blood sampling (Ginsberg et al 1995).

Of the 201 pups that survived at least until 6 months old during the period between 1990 and 1/1/94, pup survival to 12 months (1990-93) **was 30%** (van Heerden et al 1995) **just 60 surviving to 12 months.**

Invasive Handling of Yearlings and Adults, including the Alpha Pair

In the KNP southern district study area in the 5 years (1989 to 1/1/94) **skin or blood samples were collected from 92 adults** (Girman et al 1997) of these **80 were handled as yearlings** (G.Mills pers. comm.).

The alpha pair, if known**,** in each pack **were selected for genetic research sampling** either by anaesthetization for blood sampling or skin sampling by biopsy dart (Girman et al 1997 and 2001).

A large adult male was usually selected for the fitting of heavy (900g.) satellite collars starting in 1989 (Gorman et. al. 1992).

Number of wild dogs immobilized in Kruger 1989-93:- 19 up to -1 year old , 17, 1-2 years old, 10 > 2 years old - total for period July 1989-March 1993 = 46

The 'handling' of, at the very least, 59 % of the adults *and* 65% of the pups between 1989-1/1/94 in the KNP must be considered as possibly causally related to the dramatic falls in pup number from 81 on 1/1/95, to 32 on 1/1/96.

The population decline post 1995 occurred in **a previously 'stable population', with high (71%) pup survival** (Reich 1981, Maddock & Mills 1994, Woodroffe et al 1997).

Creel et al (2004) referring to the decline in the KNP wild dog study population concludes ***"…. the current demography of wild dogs in Kruger leads to dynamics that are more prone to collapse"***

2) BOTSWANA - Chobe National Park & Moremi Wildlife Reserve.

In 1994 in Chobe National Park, based on histopathology, Canine Distemper is reported to have killed 10 of 12 wild dogs in a study pack (Alexander et al .1996 , Goller et. al. 2009 & 2010). In Moremi Wildlife Reserve a comment made by the researcher involved, T. McNutt, is reported by Richard Connif in National Geographic, May 1999:- ***" In our study population in 1996 five packs died in a 3 week period. "*** . The cause of death of these study packs was unfortunately not established.

OTHER EXAMPLES OF ADVERSE EFFECTS (INCLUDING MORTALITY) FOLLOWING HANDLING OF OTHER MAMMALS AND VERTEBRATES.

Adverse effects following immobilization including high mortality following 'handling' of other mammal species has been reported:-

In free living **Black Rhinoceros in Africa** researchers report unacceptably high radio-collar failure rates and that an intensive immobilization regime negatively impacted black rhino fertility (Alibhai & Jewell & Towindo, 1999 & 2001, Alibhai, S.K. & Jewell, Z.C. 2002)

In the U.K. following the radio-collaring of **Water-Voles** *(Arvicola terrestris)* in a paper *'Indirect negative impacts of radio-collaring : sex ratio variation in water voles'* published in the Journal of Applied Ecology , 2005, Moorhouse & Macdonald report :-

A. *'We conclude that the observed decline in female-numbers resulted from male -skewed recruitment sex ratios due to the attachment of radio-collars to female water voles. These results question the assumption that the use of radio-collars does not fundamentally affect the biology of collared water voles*

B . *'Radio -tracking is used ubiquitously in studies of wild vertebrates, a fundamental assumption being that tagged animals do not significantly differ, behaviorally or otherwise , from untagged animals.'*

C. *' Radio -tracking may adversely affect animal populations . Withey, Bloxton & Marzluff (2001) reviewed 96 articles assessing the effects of radio-transmitters on animals. Of these 47% reported an adverse effect, including effects on reproduction , weight, condition , behaviour and survivorship* [references provided but omitted here]. *Such impacts could have profound consequences, and some discussion of them has been controversial (Burrows, Hofer & East 1994, 1995 vs. Ginsberg et al .1995;*
Woodroffe 1997).
R. Woodroffe in IUCN Species Survival Commisssion Veterinary Specialist Group Newsletter 16 Autumn 1998 reported :

'… my analysis concluded that the possible costs of vaccinating wild dogs against rabies currently outweigh the benefits (Woodroffe 1997)
and
'…. It is clear that vaccination failed to provided protection since vaccinated wild dogs are known to have died from rabies in the Mara and in Namibia (Scheepers & Venzke 1995). New data suggest that vaccination failed because the delivery protocol used was in appripriate (Woodroffe 1997).

Concerns re the use of Invasive research techniques have been voiced by some for many years:-

'If such a mess can be made of efforts to save a creature as attractive as the black-footed ferret in a country as well organized and prosperous as the United States, prospects for conservation in other parts of the world are indeed bleak'. (Robert May 'Nature' 1986).

'The majority of workers claim no effect of radio-tracking equipment on their subjects, but this claim is not always justified' and' Simple handling and blood sampling have measurable effects on hormonal correlates of stress in captive animals, yet there are few investigations of the effects of analogous treatment of wild animals'. (Colwall et. al. 1998; Gratto-Trevor et. al. 1991, Cuthill 1991.)

'Studies of carnivores, in particular, rely heavily on radio telemetry to obtain information from their elusive subjects, but little is known on how handling and tagging affects individuals' and 'It is likely, however, that in many studies any adverse effects are either unnoticed, or perhaps because they are rare, or more likely, because they are not reported ' (Laurenson 1992)

'Open discussion of past successes and failures [of interventions] is vital to encourage the necessary development of this emerging field'. Woodroffe, R., 1998 IUCN Species Survival Commission Veterinary Specialist Group Newsletter, 16.

'The adverse effects of tagging may be subtle and important, although difficult to detect' (Tuyttens Macdonald & Raddam (2003)

' . the resolution of one question remains imperfectly ragged, despite exhaustive attention, and that is whether handling or vaccinating wild dogs had inadvertently contributed to their demise in the Serengeti-Mara ecosystem' (David Macdonald, Chair of the Canid Specialist Group, in Preface to: - IUCN, Species Survival Commission, Canid Specialist Group (CSG), Status Survey & Conservation Action Plan, 'The African Wild Dog' (Woodroffe et.al.1997)

"….. from our results and PVA models presented previously (Burrows et al 1995 ,Ginsberg et al 1995b), it is difficult to determine whether the extinction of the Serengeti wild dog population is more likely to be due to chance alone (Ginsberg et al 1995b) or to invasive research methods (Burrows 1992)." (Cross and Beissinger 2001).