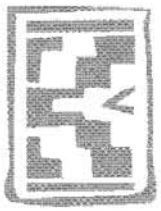

RESEARCH COMMUNICATION

Seroconversion in captive African wild dogs (*Lycaon pictus*) following administration of a chicken head bait/SAG-2 oral rabies vaccine combination

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ABSTRACT

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This study determined the proportion of captive juvenile and adult African wild dogs (*Lycaon pictus*) that developed protective titres of rabies neutralising antibodies following ingestion of a chicken head bait/SAG-2 oral rabies vaccine combination. A single chicken head containing 1.8 mℓ of SAG-2 vaccine ($10^{8.0}$ $TCID_{50}$/mℓ) in a plastic blister was fed to each of eight adult and three juvenile wild dogs. Bait ingestion resulted in a significant rise in serum neutralising antibody titres. Overall seroconversion rate was eight out of 11 (72.7 %), and all the puppies and five out of eight (62.5 %) adults showed potentially protective levels of antibodies on day 31. The mean post-vaccination neutralising antibody titre was within the range reported to be protective against challenge with virulent rabies virus in other species.

Keywords: *Lycaon pictus*, oral vaccination, rabies, SAG-2

INTRODUCTION

Rabies has been responsible for the decline of several populations of African wild dogs (*Lycaon pictus*) in southern and eastern Africa (Gascoyne, King, Laurenson, Borner, Schildger & Barrat 1993; Kat, Alexander, Smith & Munson 1995; Scheepers & Venzke 1995; Woodroffe, Ginsberg & Macdonald 1997; Hofmeyr, Bingham, Lane, Ide & Nel 2000). Small and/or declining wild dog populations seem particularly vulnerable and the disease has proven an impediment to the establishment of a metapopulation of this endangered species in South Africa (Hofmeyr *et al.* 2000; Mills, Ellis, Woodroffe, Maddock, Stander, Rasmussen, Pole, Fletcher, Bruford, Wildt, Macdonald & Seal 1998). Previous control measures have focused on the use of parenteral vaccination techniques; however, these methods are associated with logistical difficulties (including the inability to vaccinate animals younger than about 5 months) and have proven unsuccessful in several instances (Kat *et al.* 1995; Scheepers & Venzke 1995; Hofmeyr *et al.* 2000). Current research is aimed at developing an effective oral vaccination technique to protect wild dog populations under threat from rabies (Knobel, Du Toit & Bingham 2002). This research is based on the successful

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use of modified live, attenuated virus vaccines for the oral immunization of red foxes (*Vulpes vulpes*) against rabies in Europe (Aubert, Masson, Artois & Barrat 1994), a technique which has been extrapolated to a variety of other species (Farry, Henke, Beasom & Fearneyhough 1998; Roscoe, Holste, Sorhage, Campbell, Niezgoda, Buchannan, Diehl, Rupprecht & Niu 1998; Steelman, Henke & Moore 1998; Bingham, Schumacher, Hill & Aubert 1999; Hammami, Schumacher, Cliquet, Tlatli, Aubert & Aubert 1999).

Van Heerden, Bingham, Van Vuuren, Burroughs & Stylianides (2002) demonstrated that the SAG-2 (Street Alabama Dufferin mutant Gif) oral rabies vaccine induces a humoral immune response in African wild dogs when administered by direct installation into the oral cavity. The SAG-2 vaccine is a modified live rabies virus, a double mutant of the SAD_{Berne} strain obtained by selective growth in the presence of specific monoclonal antibodies (Schumacher, Coulon, Lafay, Bénéjean, Aubert, Barrat, Aubert & Flamand 1993). The vaccine has also been tested and found to be effective in domestic dogs *Canis familiaris* (Schumacher *et al.* 1993; Fekadu, Nesby, Shaddock, Schumacher, Linhart & Sanderlin 1996), red foxes (Schumacher *et al.* 1993; Lambot, Blasco, Barrat, Cliquet, Brochier, Renders, Krafft, Bailly, Munier, Aubert & Pastoret 2001) and side-striped and black-backed jackal, *Canis adustus* and *Canis mesomelas* (Bingham *et al.* 1999).

Previous work (Knobel *et al.* 2002) has shown that chicken heads can potentially be used to deliver oral rabies vaccine to wild dogs. Trials using a topical tissue marker (rhodamine B) as a vaccine placebo resulted in 72.7 % ($n = 11$) of animals showing significant staining of the oral mucosa following ingestion of a chicken head containing the marker. The objective of the current study was to determine the efficacy of the chicken head bait/SAG-2 vaccine combination in inducing seroconversion in adult and juvenile African wild dogs.

MATERIALS AND METHODS

Study animals

Fourteen captive-bred African wild dogs from three separate packs were used in the study. Pack A consisted of six adult (> 1 year) individuals, four males and two females. Pack B comprised four subadult (nine-month-old) males and pack C consisted of four ten-week-old pups (two males, two females). For the purpose of this study the subadults were considered mature animals. Packs A and C were held at the Lion Park near Johannesburg. Pack A was housed in a 1 ha camp surrounded by a wire-mesh fence, while the pups were kept in a smaller enclosure with a concrete kennel for shelter. Pack B was housed at the De Wildt Cheetah and Wildlife Trust, under similar conditions as pack A. Adult animals were fed fresh meat five to six times a week while pups received the same diet twice daily. Water was provided *ad libitum*. None of the study animals had previously been vaccinated against rabies, although the pups were derived from vaccinated dams.

Rabies vaccine

SAG-2 vaccine (batch no. RB2TO3) was obtained from Virbac Laboratories, Carros, France. The liquid vaccine was pre-packaged in Virbac blisters, with each blister containing 1.8 mℓ of vaccine. Blisters were stored at –60 °C until use. Stability of the titre of the vaccine was verified upon receipt. A mean titre of $10^{8.0}$ median tissue culture infectious doses ($TCID_{50}$)/mℓ was obtained. Before each experiment, blisters were thawed and immediately stapled under the skin of fresh chicken heads with the foil side outwards, against the skin. The bait/vaccine combinations were then kept on ice until use (<30 min).

Experimental design

The 14 wild dogs were separated into two groups according to age—one group of ten adults and one group of four pups. All animals were bled two days prior to vaccination (day –2) to determine pre-vaccination rabies-neutralising antibody levels. Adults were immobilised with a combination of 40 mg of ketamine hydrochloride ("Anaket-V", Bayer Animal Health) and 900 µg medetomidine hydrochloride ("Domitor", Novartis SA Animal Health) and blood was collected from the lateral saphenous vein. Pups were manually restrained while blood was collected from the cephalic vein.

On day 0 each animal was offered a single chicken head/vaccine combination. The response of the animal to the bait, as well as the number of chews on the bait and the fate of the vaccine blister was noted. Blood was again collected on day 31 for determination of post-vaccination antibody titres.

Serology

Sera were stored at –20 °C until testing. Prior to testing sera were heat inactivated for 30 min at 56 °C.

TABLE 1 Neutralising rabies antibody titres (IU/mℓ) of 11 wild dogs that each ingested a chicken head containing 1.8 mℓ of $10^{8.0}$ $TCID_{50}$/mℓ SAG-2 rabies vaccine on day 0. Seropositive titres (≥0.5 IU/mℓ, WHO 1992) are marked with an asterisk

Individual	Age group	Neutralising antibody titre (IU/mℓ)		Number of chews
		Day -2	Day 31	
LM2	Adult	0	1.55*	13
LM3	Adult	0	0	9
LM4	Adult	0	1.55*	12
LF1	Adult	0	0.5*	12
DM1	Adult	0	0.3	13
DM2	Adult	0	0	7
DM3	Adult	0	0.6*	12
DM4	Adult	0	0.5*	7
LPM1	Pup	0	0.5*	> 30
LPM2	Pup	0	0.9*	> 30
LPF1	Pup	0.2	0.7*	> 30

Antibody titres were determined using the method described by Cliquet, Aubert & Sagne (1998). A level of 0.5 IU/mℓ was adopted as a threshold for seropositivity (World Health Organization 1992).

RESULTS

Two adult animals and one pup did not ingest the proffered bait and were excluded from the trial. The remaining animals (eight adults and three pups) immediately picked up and chewed the chicken head containing the vaccine. Mean number of chews per bait was 10.6 ± 0.91 for the adults and >30 for the pups. Only one vaccine blister, from that of bait offered to a pup, was recovered. The blister had been perforated and no fluid remained. All other vaccine blisters were swallowed.

All animals were seronegative on day –2 (Table 1). The analysis of post-vaccination neutralising antibody titres showed a significant difference between pre- and post-vaccination titres (paired t-test: t = 3.98, d.f. = 10, $P < 0.01$). The mean neutralising antibody titre 31 days after bait ingestion was 0.65 ± 0.16 IU/mℓ. Eight out of 11 (72.7%) animals seroconverted (neutralising antibody titre ³ 0.5 IU/mℓ). The proportion of animals seroconverting was 100% (3/3) amongst the pups and 62.5 % (5/8) amongst the adults. There was no relationship between the neutralising antibody titres on day 31 and the number of chews on the bait.

DISCUSSION

Several studies have examined the humoral immune response following ingestion of baits containing SAG-2 oral vaccine. The proportions of animals seroconverting (as measured on or around day 30) varies widely: Bingham *et al.* (1999) obtained a seroconversion rate of 100 % (n = 5) in side-striped jackal after feeding chicken head baits containing $10^{8.0}$ $TCID_{50}$/mℓ SAG-2, while none of 12 beagles demonstrated seroconversion following ingestion of baits containing $10^{8.3}$ $TCID_{50}$ of lyophilized SAG-2 virus vaccine (Orciari, Niezgod, Hanlon, Shaddock, Sanderlin, Yager & Rupprecht 2001). Two other papers reported 60 % seroconversion at day 30 using baits with lower vaccine titres [$10^{7.0}$ $TCID_{50}$/mℓ in side-striped jackal (Bingham *et al.* 1999); $10^{7.5}$ $TCID_{50}$/mℓ in domestic dogs (Fekadu *et al.* 1996)]. An important point to note, however, is that seroconversion appears to be a poor predictor of resistance to rabies virus infection. Numerous studies have reported cases of animals that failed to seroconvert developing an anamnestic response and surviving following challenge with rabies virus (Aubert 1993; Fekadu *et al.* 1996; Bingham *et al.* 1999; Hammami *et al.* 1999; Orciari *et al.* 2001). This suggests the involvement of protective mechanisms other than antibodies, as demonstrated by Lambot *et al.* (2001). Seroconversion is therefore a conservative estimate of protection against infection.

The mean post-vaccination neutralising antibody titre in this study was significantly lower (Student's t-test: t = 5.93, d.f. = 13, $P < 0.0001$) than that obtained by Van Heerden *et al.* (2002), 27 days after direct installation of 1 mℓ $10^{8.0}$ $TCID_{50}$/mℓ SAG-2 in four captive wild dogs [8.45 ± 2.30 IU/mℓ (Van Heerden *et al.* 2002)]. This suggests a lower efficacy of the vaccine when delivered by bait. The results of our trial fall within the range of mean neutralising

antibody titres reported in studies on other species approximately 30 days (range 28–33 days) after ingestion of baits containing similar or higher doses of SAG-2 vaccine [*C. adustus*: 0.82 IU/mℓ, n = 5 (Bingham *et al.* 1999); *C. familiaris*: 0.50 IU/mℓ, n = 7 (Hammami *et al.* 1999); *C. familiaris*: < 0.1 IU/mℓ, n = 12 (Orciari *et al.* 2001)]. These studies also reported the proportion of animals surviving after challenge with a virulent strain of rabies virus. There appears to be no relation between the mean neutralising antibody titres of the group and the proportion of animals surviving challenge. Bingham *et al.* (1999) reported a 60 % (3/5) survival rate, while 83.3 % (10/12) of animals survived in the study by Orciari *et al.* (2001).

Logistical and ethical considerations precluded the use of challenge experiments in this investigation. Although comparisons between trials are confounded by differences in species, vaccine titres, and method of application, cautious extrapolation from other studies (as discussed above) indicates that the antibody levels obtained in this study after oral vaccination with $10^{8.0}$ $TCID_{50}$/mℓ SAG-2 could potentially confer adequate protection to wild dogs against rabies infection. However, our results also support the recommendations made by previous authors (Hofmeyr *et al.* 2000, Van Heerden *et al.* 2002) that booster doses of rabies vaccine will probably be necessary shortly after primary vaccination.

One of the major difficulties experienced with the parenteral vaccination technique in free-ranging wild dogs is the potential for injury to juvenile pack members. Individuals can only be safely darted at around 4–5 months of age, by which stage the pack has often left the den and is difficult to locate. This results in a high proportion of susceptible individuals under one year of age. A rabies outbreak in such a scenario, resulting in high juvenile mortality and low pack recruitment, can have serious consequences for pack survival, as experienced in Madikwe Game Reserve, South Africa (Hofmeyr, Hofmeyr & Bingham 2001).

The success of the chicken head bait/SAG-2 vaccine in pups in this study, and the ready ingestion of chicken heads by free-ranging pups (Knobel *et al.* 2002) offer a non-invasive mechanism for vaccination of free-ranging wild dogs as young as 10 weeks. Further controlled studies are needed to examine the effect of maternally-derived antibodies on the active immune response in these young animals.

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