Title: Feasibility and efficacy of oral rabies vaccine SAG2 in endangered Ethiopian wolves

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Claudio Sillero-Zubiri[1,2,3 *], Jorgelina Marino[1,2], Christopher H. Gordon[1,4], Eric Bedin[1,2], Alo Hussein[2], Fekede Regassa[5], Ashley Banyard[6] and Anthony R. Fooks[6,7]

*Corresponding Author: Jorgelina Marino, jorgelina.marino@zoo.ox.ac.uk

Author Affiliations:

[1] Wildlife Conservation Research Unit, Zoology Department, University of Oxford. The Recanati-Kaplan Centre, Tubney House, Tubney, OX13 5QL, UK.

claudio.sillero@zoo.ox.ac.uk, jorgelina.marino@zoo.ox.ac.uk, eric.bedin@zoo.ox.ac.uk

[2] Ethiopian Wolf Conservation Programme, P.O. Box 215, Bale-Robe, Ethiopia.

alo.hussein@ethiopianwolf.org

[3] IUCN SSC Canid Specialist Group

[4] Zoological Society of London, Kenya Country Programme (footnote: Present address)

chris.gordon@zls.org

[5]Ethiopian Wildlife Conservation Authority, Kera, Addis Ababa, Ethiopia.

rfekede@yahoo.com

[6] Animal and Plant Health Agency, Woodham Lane, New Haw, Addlestone, Surrey, UK.

Tony.Fooks@apha.gsi.gov.uk

[7] Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

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Abstract

Diseases are a major cause of population declines in endangered populations of several canid species. Parenteral vaccination efforts to protect Ethiopian wolves (*Canis simensis*) from rabies have targeted the domestic dog reservoir, or the wolves themselves in response to confirmed outbreaks. Oral vaccination offers a more cost-efficient, safe and proactive approach to protect Ethiopian wolves and other threatened canids from rabies. Field trials of the oral vaccine *Rabigen® SAG2Dog* were undertaken in the Bale Mountains of southeastern Ethiopia. Four different bait types and three delivery methods were tested in twelve Ethiopian wolf packs, and the oral vaccine (using the preferred bait) was trialled in three packs. Vaccine uptake and immunization rates were measured through direct observations and in live-trapped animals through the assessment of biomarker levels and serological status. Commercial baits were never taken by wolves; goat meat baits had the highest uptake, compared to rodent and intestine baits. Targeted delivery from horseback and nocturnal delivery within a pack's territory performed favourably compared to random bait distribution. Bait uptake by non-target species was lowest during the nocturnal blind distribution. Of 21 wolves trapped after vaccination, 14 were positive for the biomarker iophenoxic acid (i.e. ingested the bait and most likely pierced the sachet with the vaccine). Of these, 86% (n=12/14) had levels considered sufficient to provide protective immunity to wildlife (≥0.20 IU/ml), and 50% (n=7/14) demonstrated antibody titres above the universally recognised threshold (≥0.5 IU/ml) -the baseline average was 0.09 IU/ml (n=12 wolves). All but one of the wolves vaccinated in 2014 were alive 14 months later. Our trials confirm the potential for SAG2, delivered in a goat meat bait, to effectively protect Ethiopian wolves against rabies, supporting the initiative for a more efficient and proactive approach to manage and eventually eliminate rabies in Ethiopian wolf populations.

Keywords

Bait; biomarker; sero-conversion; vaccine; wildlife rabies.

1 Introduction

Diseases transmitted by domestic dogs threaten many wild canids, due to their shared receptivity of numerous pathogens and increasing proximity in natural and human-dominated landscapes [1-3]. Rabies is a common cause of disease outbreaks in endangered canids, leading to dramatic population declines in African wild dogs (*Lycaon pictus*) [4, 5], Blanford's foxes (*Vulpes cana*) [6] and Ethiopian wolves (*Canis simensis*) [7-10]. In the Bale Mountains of southern Ethiopia, recurring epizootics are seriously jeopardizing the survival of Ethiopian wolves, the world's rarest canid (500 adults and subadults, with more than half in Bale) [11]. Because all extant Ethiopian wolf populations are susceptible to extinction as a result of epizootics [12, 13], any policy to secure their long-term future would necessitate proactive disease control management [14-16].

In spite of extensive parenteral vaccination of domestic dogs, the principal reservoir for rabies[17], four major rabies epizootics have been documented in the Bale wolf population since 1990 [7-10]. Acknowledging that domestic dog vaccination by itself is not a suitable solution, oral vaccination was recommended as a priority in the National Action Plan for the conservation of Ethiopian wolves [15]. Oral vaccination offers a more efficient approach to the reactive parenteral vaccinations of wolves that were implemented in Bale in 2003, 2008/09, and 2014/15 in response to rabies outbreaks [14, 18]. While these interventions limited the spread of rabies across the wider wolf population [14], they are costly and involve laborious capture and handling of wolves. Oral wildlife rabies vaccination campaigns

have enabled elimination of the disease from terrestrial wildlife species across Western Europe and much of North America [19]. If proved feasible, extensive oral vaccinations could eliminate the need for parenteral vaccination of Ethiopian wolves, allowing wolves to be vaccinated proactively and cost-effectively, whilst reducing unnecessary handling of a substantial proportion of a very rare and threatened species.

From an oral vaccine perspective, current legislation in Ethiopia excludes the use of genetically modified recombinant vaccines, including the Vaccinia-rabies glycoprotein (VRG) vaccine that proved successful in vaccinating wildlife carnivores in Europe and North America [20, 21]. As a result, the oral *Rabigen® SAG2Dog* vaccine, a modified live attenuated virus vaccine manufactured by *Virbac* (Carros, France), was used to assess its suitability for direct delivery and its post-vaccinal efficacy following Ethiopian wolf vaccination. The species' endangered status and the lack of a captive population meant that trials could only include a small number of wolves.

The SAG2 vaccine has demonstrated good tolerance and has been confirmed as effective for the vaccination of more than 30 target and non-target species, including carnivores, primates, rodents and birds [22, 23]. SAG2 is recommended by WHO specifically for oral vaccination [24]. A review of twenty years of SAG2 use [19] highlights its efficacy in accordance with EU requirements (tested for the red fox *Vulpes vulpes* and raccoon dog *Nyctereutes procyonoides*) and its role in eliminating rabies in Estonia, France, Italy and Switzerland. No safety issues have been reported, and in particular no vaccine-induced rabies cases have been diagnosed, after the distribution of more than 20 million SAG2 baits in Europe [19]. The vaccine has also been tested in African carnivores [25, 26], including the endangered African wild dog [27].

In order to determine whether oral vaccination is feasible in Ethiopian wolves, trials were conducted in the Bale Mountains to determine bait preferences and vaccine delivery options for a range of commercial and locally available baits. The preferred bait option and delivery method were then used to test the effectiveness of the oral vaccine *Rabigen®* SAG2Dog in three Ethiopian wolf packs. Success would provide a basis for using full-scale oral vaccination campaigns to protect Ethiopian wolves from rabies, as part of a nationwide conservation strategy for the species.

2 Methodology

2.1 Study area and wolf population

The Bale Mountains National Park harbours the largest population of Ethiopian wolves, estimated at 300 adult and sub-adult wolves. Oral vaccines were trailed in two sub-populations, Web Valley and Sanetti Plateau, separated by 20 km of less suitable habitat, and where wolves occur at high densities (>1 sub-adult or adult /km²)[28]. Both subpopulations are surrounded by settlements around or within the borders of the Bale Mountains National Park, and by a numerous population of domestic dogs. Rabies and canine distemper virus are prevalent in the domestic dog population, and outbreaks can occur throughout the year.

In Bale, Ethiopian wolves are diurnal, feed almost exclusively upon rodents, and are easy to observe on foot or horseback. Long-term monitoring of the packs involved in these trials offered a detailed knowledge of their sex/age composition, breeding success, and

territoriality. Ethiopian wolves form phylopatric groups of up to 18 animals and all members defend a common territory and contribute to raise the pups of the dominant pair. For a review of the species behaviour and ecology see [29].

2.2. Oral baits and vaccine

The vaccine Virbac *Rabigen® SAG2Dog* is produced as a sachet within a commercial liver-based bait matrix, designed to maximise uptake in domestic dogs. We tested this commercial bait against three locally-sourced baits: goat meat (successfully used for trapping wolves [30]), boiled goat intestines [31] and grass rats *Arvicanthis blicki* (main Ethiopian wolf prey). For the bait preference tests, a placebo sachet was inserted into the rats' thorax (a grass rat weights approximately 100gr), a piece of goat meat (80-100grs), or a section of intestine, with the openings sealed using absorbable sutures (Figure 1).

Virbac *Rabigen® SAG2Dog* vaccines were imported from France (batches D|159 and 4F7R, for 2004 and 2001 trials respectively), with titre levels of $10^{8.6}$ $DlCT_{50}$/ml and 1.75 ml of solution per sachet. The vaccines were stored at -30°C until the week of the trials, and then at between -8 and -18°C in a portable fridge. Fresh baits were prepared each day for delivery using goat meat, the preferred bait. A serum biomarker, iophenoxic acid (product number 361046, from Sigma Aldrich), was injected into the meat bait surrounding the vaccine sachet, as used previously in other field trials [32]. The biomarker was dissolved in 20% ethanol, with 3 ml of the solution injected into each meat bait.

2.3. Bait preference and delivery trials

144 Uptake rates of various bait types were compared using two delivery methods within the
145 territories of eleven packs (approximately 80 km^2), between June and September 2011.
146
147 In the *random delivery method*, 336 baits were sequentially distributed over 116 random
148 locations (one bait per location, at an approximate density of 1.5 baits /km^2). Each time, one
149 of the four bait options (grass rat, goat meat, goat intestine or commercial bait) was chosen
150 at random and placed in the open within 5m of a wooden marker. Baits were located at
151 least 200m apart to reduce the likelihood that the scent of one bait would attract the target
152 animal to another bait. An observer, located less than 500m away, recorded the responses
153 of animals passing within 20m of the bait, for four hours or until all baits had been
154 consumed (unconsumed baits were removed on the day).
155
156 In the second method, the *targeted delivery method*, baits were directly delivered to
157 Ethiopian wolves encountered along transects (1-3 km long) travelled by 2-3 people on
158 horseback. Wolves were approached to within 20m and presented a randomly-selected bait
159 (commercial baits were excluded after poor uptake on the random delivery trial). The
160 person presenting the bait then retreated and, from a distance of 50m, another observer
161 recorded the wolf's reaction to the bait. In 172 km, surveyed over 40 h in 83 transects, a
162 total of 68 wolves were spotted and baits were delivered to 44 of them.
163
164 A third method, the *nocturnal delivery method,* was tested during the oral vaccination trial
165 of the Genale pack in August 2014, with the preferred bait (goat meat). Twenty eight baits
166 were distributed indiscriminately within the pack's territory, early in the evening, over 5
167 nights (4-8 baits per night), separated at least by 200 m from each other. A patch of soil was

cleared of vegetation around the bait and covered by fine, filtered soil particles. Baits were checked early the next morning and footprints were identified to deduce visits by target and non-target species. It was possible to differentiate with confidence the footprints of Ethiopian wolves (imperfect footprints were not considered), spotted hyenas (*Crocuta crocuta*), honey badger (*Mellivora capensis*), smaller carnivores, and rodents (Murinae rats). While the presence of Ethiopian wolf footprints cannot confirm consumption of a bait –a wolf might have only inspected the bait or displaced without eaten it- it is unlikely that other, non-target species would've removed the bait without leaving tracks.

2.4 Oral vaccination trials

Oral vaccinations were conducted in three packs in the Web Valley (**Table 1** summarizes the vaccination trials). During Trial I, in 2011, oral vaccines were distributed using the targeted delivery method within the territory of the Tarura pack (8 wolves), including at a rendezvous area near the den. This was followed by wolf captures to test for rabies sero-conversion. Trial II, in 2014, covered three packs in the Web Valley: Bowman (10 wolves) and Genale (9) using the targeted delivery method, and Tarura pack (10) using the nocturnal delivery method. Wolf captures to collect blood samples were carried out before and after targeted vaccination; but only post-vaccination in the nocturnal delivery trial.

Blood samples were taken approximately 2-3 weeks after delivery of the vaccine. The samples were left to rest for 24 hs after collection and the clarified serum, separated by centrifugation, was shipped for analysis in the United Kingdom. The sera was analysed for rabies-neutralising antibody titre levels and serum biomarker levels.

192 Wolves were captured following detailed standard trapping protocols [9, 18], using sets of
193 two rubber-lined *Soft Catch*™ leg-hold traps (Woodstream Corporation, PA, USA), checked
194 every 2–3 hours, day and night. Trapped wolves were immediately covered with a blanket
195 to induce passivity and subsequently immobilized using a combination of Medetomidine
196 (Domitor, dose 0.4 to 0.7 ml depending on body size) and Ketamine (0.2 ml), injected intra-
197 muscularly. Once sedated, 5-10 ml of blood were taken from each individual from the
198 cephalic vein in the foreleg in a vacutainer. All captured wolves were ear-tagged for
199 identification during subsequent monitoring. Atipamezole reversal was applied intra-
200 muscularly (Antisedan, at dosage equivalent to the Domitor injected). The reversal occurred
201 between 10 and 15 minutes. Post-capture, animals were closely observed to monitor their
202 health.
203
204 Rabies-neutralizing antibody titres of sera samples were assessed using the fluorescent
205 antibody virus neutralization (FAVN) test [33]. A constant volume of rabies virus (CVS-11,
206 100 $TCID_{50}$/50µl) was added to serial dilutions of serum in quadruplicate. The 50% endpoint
207 dilution, where neutralization of the virus ceased, was calculated with the Spearman-Kärber
208 method. The virus dose was checked by back-titration on every test and results were
209 rejected if virus dose was outside pre-determined limits. Serum titres were converted into
210 international units (IU/ml) by comparison with a standard control serum. The threshold for
211 adequate response to rabies vaccination is internationally recognised to be 0.5 IU/ml [34]
212 but levels recognised as effective against experimental challenge in cats and dogs were 0.1
213 IU/ml and 0.2 IU/ml, respectively [35]; see also[22]. Such lower threshold for protection for
214 wildlife is widely acknowledged, although never truly tested.
215

In order to detect iophenoxic acid (IPA) and confirm bait ingestion, a detection method was developed using an Agilent 6410 Triple Quadrupole mass spectrometry system equipped with Agilent 1200 nanoHPLC system and HPLC chip. Calibration standards were developed based on human serum spiked with known quantities of IPA, ranging from 6 ng-6 µg/ml serum (α-Ethyl-3-hydroxy-2,4,6-triiodohydrocinnamic acid, 361046 - 97%, SigmaAldrich). Each serum sample was processed in triplicate (100 µl). Samples were prepared as previously described [36-38]. Briefly, to each 100 µl serum aliquot, 0.6 ml of acetonitrile and 0.2 ml of 0.33 M sulphuric acid were added, followed by 0.2 ml 10% sodium tungstate. Samples and standards were left to stand for 15 minutes at room temperature then frozen overnight at -20˚C. Samples were then centrifuged and 1 ml of supernatant from each sample was transferred to a clean tube. Supernatants were re-centrifuged to remove any remaining particulate matter. From each supernatant, 10 µl was taken and mixed with 30 µl 5 mM ammonium formate in an autosampler vial. From the resulting mixtures, 1 µl was injected onto the HPLC chip for IPA detection by mass spectrometry. Data were analysed using MassHunter Quantitative Analysis Version B.03.01, and IPA concentrations in samples were calculated by extrapolation from the calibration standards using Prism 5 for Windows v5.01.

Statistical Analysis

Statistical analyses were run in SPSS 14.0. Contingency tables were used to test the significance of observed differences between observed consumption of baits and species. Wald chi-squared values were calculated for testing the effect of bait and species on bait consumption (a binary response) using logistic regression.

240 **3 Results**

241

242 3.1 Bait preference and delivery trials

243 During the random delivery trial, bait consumption was affected both by bait type (Wald χ^2_3
244 (N=95) = 13.77, p<0.005) and by the species encountering the bait (Wald χ^2_2 (N=95) = 15.47,
245 p<0.005) (**Table 2**). The goat meat bait had the highest encounter-to-consumption rate, and
246 the commercial bait the lowest; there were also significant differences between the
247 consumption of goat meat and grass rat baits (Wald χ^2_1 (N=34) = 5.72, p<0.05). Of a total of
248 21 baits eaten, wolves consumed 8, dogs 7 and raptors 6. Raptors consumed the most baits
249 per encounter, while wolves consumed the least (Wald χ^2_1 (N=73) = 11.41, p<0.005). Wolves
250 showed a preference for meat baits (Wald χ^2_3 (N=62) = 7.31, p=0.06) and never consumed
251 the commercial bait. Half of the eight wolves that walked within 20m of a meat bait
252 consumed it; 2 consumed rat baits and 2 intestine baits. In the latter, the two wolves
253 dropped the placebo sachet un-pierced (also two of the five dogs that consumed intestine
254 baits). Of a total of 9 meat baits consumed, 4 were eaten by Ethiopian wolves, 3 by raptors
255 and 2 by dogs.

256

257 During targeted delivery of baits to wolves, 31 of the 44 baits offered were approached
258 within 5m and 17 were consumed. Meat baits had the highest rate of approach (77%) and
259 successful uptake (53%) (**Table 3**). Wolves consumed meat baits more frequently than rodent
260 baits (χ^2_1 (N=29) = 3.93, p<0.05), but the evidence was weak for differences in uptake rates
261 across all bait types (χ^2_2 (N=44) = 3.9, p=0.14). Of the 11 wolves that approached intestine
262 baits, 6 ate them, but one spat out the placebo sachet. The two wolves that approached grass
263 rat baits consumed them.

264

265 The method of nocturnal delivery of baits was tested during the oral vaccination of one
266 pack, using goat meat as bait. In comparison with the random and targeted methods, the
267 nocturnal delivery was comparatively more efficient in terms of uptake rates: of 28 baits
268 delivered, 93% were consumed. Footprints indicated that Ethiopian wolves approached and
269 possibly consumed 20 baits (other 6 missing baits were visited by either wolves or dogs;
270 footprints were poor or incomplete); only three baits were apparently consumed by non-
271 target carnivores **(Table 4).**

272

273 3.2 Oral vaccine trials

274 Of 36 wolves targeted in four packs, 15 where observed to eat at least one bait and
275 seemingly chewed the sachet (excluding animals that discarded the sachet without piercing
276 it) (**Table 5**); it was not possible to determine how many wolves eat baits during the
277 nocturnal trial in the Genale pack. In total, 21 wolves were trapped after vaccinations, of
278 which 14 were biomarker-positive (and therefore consumed a bait) (**Table 5**). Two to three
279 weeks after vaccination, seven (50 %) of these 14 wolves had sero-converted to at least 0.5
280 IU/ml, and 12 (86%) had levels above ≥ 0.20 IU/ml; the mean neutralizing antibody titre was
281 1.49 (SE 0.36) IU/ml and 1.01 (SE 0.26) IU/ml respectively. Baseline titre values of 12 wolves,
282 captured from the study packs before the vaccination, averaged 0.09 (SE 0.01) IU/ml
283 (highest 0.13 IU/ml). This measure excluded one wolf from Tarura pack (TAR02), vaccinated
284 in November 2011, who still showed sero-conversion (0.38 IU/ml) when captured in July
285 2014 (as this individual was not trapped in 2014, it didn't affect the results presented here).
286 **Table 6** shows the individual results from pre and post vaccination tests. Noticeably, there
287 was no correlation between the number of baits apparently consumed and VNA levels; this

likely a result of whether the bait was chewed allowing the bait matrix to be pierced and the vaccine to be released and taken up by M-cells in the oral mucosa.

4 Discussion

The rapid and successful delivery of oral vaccines to free-ranging large carnivores requires a bait suitable for the target species. Our field trials indicated that goat meat was the best medium for delivering the oral rabies vaccine *SAG2* to Ethiopian wolves; this was preferred over rodent or intestine baits, and Ethiopian wolves did not consume the commercial liver-based matrix of *Rabigen® SAG2Dog*. Goat meat was probably the most attractive bait in terms of scent and taste; intestines were the second preferred bait but on repeated occasions wolves removed the vaccine sachet from the intestine bait without piercing it, limiting its suitability to successfully deliver the vaccine. A total of 38 bait consumption events were recorded during the baiting studies; this is a relatively small sample but the results were consistent across delivery methods.

The nocturnal delivery of the preferred bait produced the higher uptake rate (93%, including 71% uptake by wolves); in turn, targeted delivery of baits from horseback was more successful than the random delivery. Whilst uptake rates at night time could have been affected by one or a few bait-hungry individuals, the proportion of trapped wolves biomarker-positive was similar after targeted and nocturnal delivery. It is also worth noticing that most of the baits presented near a den during the daytime delivery were consumed by a few young wolves. Night time vaccination resulted in a lower uptake by non-target species, most notably by raptors, although it attracted some nocturnal carnivores

such as hyaenas but at very low frequency. Extended inocuity studies could bring light into responses in these non-target species - such studies have demonstrated the safety of the SAG2 vaccine for various wild non-target species in oral vaccination campaigns for jackals in Zimbabwe [34]).

Attenuated rabies viruses, such as SAG2, replicate in local tissues of the oral cavity, and can be cleared relatively quickly, leading to protective immunity [39]. It is possible that some Ethiopian wolves swallowed the vaccine sachet without piercing it, or did not chew the bait enough as to puncture the sachet, in which case the vaccine is not released and taken up by cells in the areas suitable for virus attachment and absorption. This may explain why individuals that returned biomarker levels confirming successful consumption of the bait, did not always show increased titres in blood after vaccination, or that rabies neutralizing antibody levels did not correlate with the observed numbers of baits consumed by wolves. Overall, bait efficacy was 82%, out of 11 biomarker-positive wolves.

It is essential that any vaccine proposed for immunising a threatened wild carnivore must meet a rigorous safety protocol. The success of extensive oral vaccinations over the past few decades, focusing upon the red fox, was achieved when technical improvements occurred in vaccine quality and production, including the design of recombinant viruses, as well as in the ease of mass distribution of millions of edible baits over large geographical areas [40-42]. Our study, limited to small samples sizes, was a proof-of-principle experiment to demonstrate that Ethiopian wolves can be vaccinated using oral rabies vaccines (the Ethiopian government imposes strict limitations on the handling of Ethiopian wolves, an endemic species with no more than 500 individuals and no captive populations). The trials

demonstrated the SAG2 rabies vaccine is safe for Ethiopian wolves. All the wolves that consumed SAG2 in 2011 were still alive and healthy more than 18 months later. All, but one, identified wolves that consumed SAG2 during the 2014 trial were alive and healthy 14 months later, and all three vaccinated packs were breeding successfully between October and December 2014.

We explored the efficacy of the SAG2 vaccine in inducing sero-conversion in fourteen vaccinated Ethiopian wolves. Because a protective titre has not yet been defined for this and numerous other wild species, a threshold for sero-conversion was considered as an evidence of response to vaccination. This is arbitrarily accepted as 0.5 IU/ml, but rabies challenges have indicated that lower titre responses in domestic dogs may still confer protective immunity against rabies [22, 35]. Half of the Ethiopian wolves that consumed baits developed titre levels considered to offer robust protective immunity against rabies in humans (≥0.5 IU/ml), and 86% developed a response at the reduced threshold of ≥0.20 IU/ml. Proving this is a sufficient protection against rabies infection is impractical, as delivering a rabies challenge to Ethiopian wolves is clearly not an option. Similar experimental studies in threatened wild canids are rare, but cautious comparisons from other studies in canids indicate that the antibodies levels recorded after oral vaccination with $10^{8.6}$ $DICT_{50}$/ml SAG2 could potentially confer adequate protection to Ethiopian wolves. The closest example is a test of sero-conversion in captive wild dogs following the administration of SAG2 vaccines using chicken heads as bait at similar doses ($10^{8.0}$ $DICT_{50}$/ml, 1.8 ml per blister) [27]. Thirty one days after the vaccination, the mean neutralizing antibody titre of the eleven animals that ingested the vaccine was 0.65 (SE 0.16) IU/ml, and 73% had sero-converted (i.e. >0.05 IU/ml); this is comparable to 0.88 (SE 0.24) IU/ml 2-3 weeks after

vaccination in fourteen Ethiopian wolves and 50% sero-conversion. In laboratory conditions, five side-stripped jackals (*Canis adustus*) that ingested baits with 1.8ml of SAG2 at $10^{8.0}$ $DICT_{50}$/m, had mean titres of 0.80 (SE 0.54) IU/ml and 2.79 (SE 1.08) IU/ml 14 and 31 days later, and all five resisted a lethal rabies challenge [25]. It is also worth noticing that titre levels increased after the second week, suggesting the possibility of a more pronounced response in Ethiopia wolves, which we tested 2-3 weeks after vaccination.

The promising outcome of these trials, and the lack of any apparent negative impact of vaccine bait consumption on the Ethiopian wolves, opens the way for the implementation of periodic vaccination campaigns in the Bale Mountains and elsewhere in Ethiopia. Oral vaccination of Ethiopian wolves will enable a shift in the current rabies management paradigm to protect the last remaining wolf populations from extinction, as the current reactive response could be replaced by proactive approaches and result in higher wolf survival**.**

The work presented in the article has been carried out in an ethical way. The University of Oxford's Local Ethical Review Process (Zoology ERC) approved the animal care and use protocols for the ethical handling of Ethiopian wolves in this study under case number ZERC040905. The animal care and use protocols adhere to the UK's ASPA regulations (1986). Furthermore, all animal handling protocols were approved by the Ethiopian Wildlife Conservation Authority.

Conflicts of interest: One of the co-authors, Dr Anthony R. Fooks, acts as an Associate Editor for Vaccine.

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Tables

Table 1 Summary of oral vaccination trials

	Delivery method	Dates	Packs
Oral vaccination trial I	Targeted delivery	3-8 Nov 2011: bait delivery to wolves individually 21-24 Nov 2011: post-vaccination captures	Tarura
Oral vaccination trial II	Targeted delivery	24-26 Jun 2014: captures for antibodies baseline 27 Jul - 09 Aug 2014: baits delivered to wolves individually 2-6 Sep 2014: re-captures to test sero-conversion	Bowman, Tarura
	Nocturnal delivery	15-21 Aug 2014: baits delivered during five nights 7-10 Sep 2014: captures to test sero-conversion.	Genale

Table 2 Results of bait preference trial using four types of bait and the random delivery method. The table shows numbers of animals that approached within 20m of a bait ('Approach'); that approached a bait but did not react to its presence ('Indifferent'); that approached the bait and sniffed it, but did not consume it ('Sniff bait') -does not apply to raptors; and numbers of animals that approached the bait and consumed it ('Eat bait').

		Type of bait			
		Commercial (n=86)	**Intestine** (n=86)	**Meat** (n=86)	**Grass rat** (n=78)
Ethiopian wolves	*Approach (< 20m)*	*6*	*7*	*8*	*9*
	Indifferent	5	0	4	6
	Sniff bait	1	5	0	1
	Eat bait	0	2	4	2
Domestic dogs	*Approach (< 20m)*	*2*	*5*	*2*	*2*
	Indifferent	0	2	0	1
	Sniff bait	1	0	0	0
	Eat bait	1	3	2	1
Raptors	*Approach (< 20m)*	*1*	*2*	*3*	*2*
	Indifferent	1	0	0	1
	Eat bait	0	2	3	1
Hours observing each bait type:		330.4	324.6	313.2	293.5

Table 3 Results of bait preference trial using three bait types (commercial bait excluded) and the targeted delivery method. The table shows the numbers of Ethiopian wolves that approached within 5m of the bait offered ('Approach') –others ran away; that approached but did not react to the bait ('Indifferent'); that approached the bait and sniffed it, but did not consume it ('Sniff bait'); and animals that approached the bait and consumed it ('Eat bait').

	Type of bait			**Total**
	Intestine (n=15)	**Meat** (n=17)	**Grass rat** (n=12)	
Approach <5m	*11*	*13*	*7*	*31*
Indifferent	2	2	2	6
Sniff bait	3	2	3	8
Eat bait	6	9	2	17

Table 4 Uptake of goat meat baits distributed at night time within the territory of the Genale pack, and identification of the footprints on soil traps built around each bait.

Date	Baits delivered	Baits consumed	Footprints		
			wolf	others	
16/08/2014	8	8	7	1	small carnivore (mongoose?)
17/08/2014	4	4	3	1	spotted hyaena
19/08/2014	4	2	2	0	
20/08/2014	8	8	5	3	wolf or dog (imperfect footprint)
21/08/2014	4	4	3	1	honey badger
Totals	*28*	*26*	*20*	*6*	

Table 5 Results of oral vaccination trials: numbers of wolves that consumed baits (according to observations and to biomarker tests) and that sero-converted. Two thresholds for sero-conversion are presented as evidence of response to vaccination, the internationally accepted value of 0.5 IU/ml, and >0.2 IU/ml, proved to be effective against experimental challenges in dogs.

Pack	Tarura 2011	Tarura 2014	Bowman	Genale
Group size	8	10	9	9
Wolves that consumed bait				
Observed	5	5	5	-
Biomarker positive (numbers trapped)	4 (5)	4 (5)	3 (5)	3 (6)
Wolves that sero-converted				
>0.5 IU/ml	2	1	1	3
>0.2 IU/ml	3	3	2	3

Table 6 Details of wolves involved in trials and the results of pre and post vaccination tests. In grey the animals that successfully consumed baits.

Pack	ID	Age/ Sex	**Pre-vaccination tests**			Baits consumed (observations)	**Post vaccination tests**		
			Date of capture	Date of test	Titre IU/ml		Date of capture	Titre IU/ml	Biomarker IPA ug/ml
Tarura	TAR 02	AF	-			2	22/11/2011	0.29	61.2
Tarura	TAR 03	SM	-			5	21/11/2011	1.14	43
Tarura	TAR 04	SF	-			0	23/11/2011	0.06	0.2
Tarura	TAR 05	SM	-			4	22/11/2011	0.06	17.7
Tarura	TAR 07	SM	-			9	22/11/2011	0.87	75.4
Tarura	TAR 09	SM	-			1	-		
Tarura	TAR 02	AF	26/06/2014	05/02/2015	0.38	0	-		
Tarura	TAR 08	JF	26/06/2014	05/02/2015	0.10	6	05/09/2014	0.13	10.99
Tarura	TAR 09	AM	26/06/2014	05/02/2015	0.10	0	-		
Tarura	TAR 10	JF	28/06/2014	05/02/2015	0.10	0	04/09/2014	0.04	0.1
Tarura	TAR 11	JM	26/06/2014	05/02/2015	0.03	3	02/09/2014	0.38	13.7
Tarura	TAR 13	JM	26/06/2014	05/02/2015	0.10	5	03/09/2014	0.87	2.8
Tarura	TAR 15	JM	26/06/2014	05/02/2015	0.10	3	03/09/2014	0.38	2.67
Tarura	KOT 31	AM	-			3	-		
Bowman	BOW 02	AF	-			0	06/09/2014	0.1	0.23
Bowman	BOW 03	AM	24/06/2014	05/02/2015	0.10	0	06/09/2014	0.04	0.03
Bowman	BOW 09	SM	24/06/2014	05/02/2015	0.07	3	-		
Bowman	BOW 05	SM	24/06/2014	05/02/2015	0.04	0	-		
Bowman	BOW 07	SM	23/06/2014	05/02/2015	0.10	0	-		
Bowman	BOW 08	SF	-			1	-		
Bowman	BOW 10	JF	23/06/2014	05/02/2015	0.10	2	05/09/2014	0.22	0.66
Bowman	BOW 11	SM	-			1	05/09/2014	0.38	0.99
Bowman	BOW 13	JM	24/06/2014	05/02/2015	0.10	8	06/09/2014	1.97	28.6
Genale	GEN 03	AM	-			-	07/09/2014	3.42	5.45
Genale	GEN 05	AM	-			-	09/09/2014	0.04	0.04
Genale	GEN 08	SF	-			-	07/09/2014	0.66	17.4
Genale	GEN 09	SM	-			-	09/09/2014	0.04	0.05
Genale	GEN 10	JF	-			-	08/09/2014	0.1	0.03
Genale	GEN 12	AF	-			-	10/09/2014	1.5	7.94

Figures

Figure 1. Bait types tested in delivery trails. Clockwise from top left: commercially-available bait (Rabigen® SAG2Dog) containing placebo sachet; placebo sachet inserted into boiled goat intestines; into a chunk of goat meat; into a grass rat.