

Title

Oral bait preferences for rabies vaccination in free-ranging black-backed jackal (*Canis mesomelas*) and non-target species in a multi-site field study in South Africa

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Abstract

Black-backed jackals (*Canis mesomelas*) are small meso-predators that occur in the wild and around cities and towns in southern Africa and have been associated with the spread of rabies in South Africa. Oral bait rabies vaccine has been used in Europe and the USA for the control of rabies in reservoir species. The effectiveness of an oral vaccination strategy depends not only on the efficacy of the vaccine but on the uptake of the bait in the target species. This study evaluated factors associated with the uptake of oral bait by free ranging jackal and other wildlife species in a multi-site field study in Gauteng Province, South Africa. Three different baits were offered: commercial fishmeal polymer, pieces of red meat and chicken heads. Bait uptake was observed using camera traps and patterns of uptake assessed by multiple correspondence analysis and Cox proportional hazards models. In general, all the baits were well accepted with an uptake of 91%. Median consumption time of bait for jackal was 18 hours (IQR: 8-21 hours; range 7-66 hours) and for all other species it was 21 hours (IQR: 4-44, range 1-283). In species other than jackals there was a faster uptake in the winter months when less food was available, and the vegetation was sparse, whereas jackal showed no seasonal preference. Jackal consumed 20% of baits placed and took all three bait types but showed a clear preference for chicken heads if available (Hazard ratio (HR)=3.41; 95%CI: 1.16-9.99; p=0.025). Species other than carnivores preferred fishmeal polymer or red meat. Jackals showed no preference for time of day whereas herbivores and other species clearly preferred day; other carnivores preferred either day or night but not both, depending upon species. This study showed that chicken heads may be the preferred bait type for oral vaccination of black-backed jackal in this area, and that consideration should be given to placing bait during summer and at dusk, in order to minimize uptake by non-target species.

Key Words

black-backed jackal, *Canis mesomelas*, oral bait, rabies, vaccination

Introduction

Black-backed jackals (*Canis mesomelas*) are found throughout southern Africa in a variety of habitats, but are more often associated with open terrain (Skinner and Chimimba, 2005). Due to their adaptability to different habitats and their exploitation of human introduced food sources, they are often found in close proximity to cities and towns (Ferguson et al., 1983; Kuhn, 2014). Immature jackals (<3 years of age) move over large areas; the average distance travelled varies depending upon the age of the jackal, with juveniles traveling further, up to 30 km having been recorded in one night (Ferguson et al., 1983). Once a territory is established mature animals tend to cover greater distances compared to immature animals but spend more time looping within their territory. The home range varies within different areas of South Africa with one individual having a home range of 244 km² over the winter months in the North West province (Ferguson et al., 1983). The average home range in the North West was about 100 km²; this is in contrast to Suikerbosrand Nature Reserve, south of Johannesburg, where 10 km² was the average territory size (Ferguson et al., 1983). The territory size is closely dependent on food availability and is usually occupied by a breeding pair, often with helpers from the previous litter. There is considerable overlap between territories in some areas (Ferguson et al., 1983; Hiscocks and Perrin, 1988).

Despite continued persecution, black-backed jackals continue to thrive in peri-urban environments around Johannesburg and Pretoria, the largest cities in Gauteng. The greater Johannesburg Metropolitan area has an estimated population of 10.5 million people and greater Pretoria 2.1 million people (United Nations, 2014). The Cradle of Humankind (a UNESCO world heritage site) lies approximately 50 km northwest of Johannesburg within the Magaliesberg Biosphere Reserve. The Cradle of

Humankind consists of approximately 47,000 hectares (180 square miles) of privately-owned lands. The John Nash and Malapa Reserves are situated within the Cradle of Humankind (Figure 1).

There have been 3 outbreaks associated with jackal rabies in Limpopo in 1993/94, 1998 and 2006. In North West province there have also been 3 outbreaks in 1997/98, 2003 and 2016/17 in jackal. There have been 2 major rabies outbreaks in Gauteng in the last 10 years. One outbreak was in dogs in the South of Johannesburg in the Soweto area in 2010/2011 and the other outbreak in 2016 centered around jackals in the Cradle of Humankind. Feral dogs are responsible for most reported human rabies cases in KwaZulu-Natal province, whereas in the rest of South Africa wild carnivores are the most important factors in the transmission of rabies (Gummow et al., 2010). Yellow mongoose (*Cynictis penicillata*), bat-eared foxes (*Otocyon megalotis*), aardwolf (*Proteles cristata*) and jackals are the most common wildlife reservoirs in South Africa, with black-backed jackal being the most important host in Limpopo and North West provinces (Gummow et al., 2010).

From January 2007 to October 2018, 127 cases of rabies were reported in Gauteng, of which 43 cases (34%) were in wildlife. The black-backed jackal was not a major component in these outbreaks until the most recent outbreak in 2016; in this outbreak, 48 cases were reported of which 28 (58%) were in jackal, 25 of which occurred in the Cradle of Humankind (Geertsma, 2016). The majority of cases were observed between June and August 2016, consistent with previous studies which reported a higher incidence of rabies in wildlife in the winter months (Gummow et al., 2010).

The relative importance of wildlife in rabies transmission has increased with the effective control of rabies in the dog population by massive parenteral vaccination

campaigns. However, vaccination of domestic dogs on their own is ineffective in preventing rabies outbreaks if jackal together with other carnivores constitute a maintenance population for rabies independent of domestic dogs (Haydon et al., 2002). The 2016 Gauteng outbreak resulted in a total of 34 people being exposed to rabies from jackal, either directly or indirectly via infected livestock, and requiring post exposure prophylaxis (Directorate Animal Health, 2016). This highlights the importance of controlling rabies in the jackal population in and around Gauteng.

Diseases that persist in wildlife are often challenging to control. Controlling the disease is especially important if it poses a risk to public health (e.g. rabies) or livestock and wildlife production (e.g. tuberculosis) (Cross et al., 2007; Bhattacharya et al., 2011). Culling has been used to control wildlife diseases such as tuberculosis, although the relationship between disease and density of vectors is not linear (Woodroffe et al., 2008). However, due to the large geographic spread of rabies in meso-carnivores, culling strategies have only been effective in the short term (Cross et al., 2007) or not effective at all, as there is no simple relationship between rabies incidence and host population density (Morters et al., 2013).

Both parental and oral vaccination strategies have been used in the past as an alternative to culling strategies for control of infectious diseases in wildlife populations. Oral vaccination strategies allow large-scale distribution of vaccine to wildlife reservoir or hosts. Use of oral bait vaccine in the control of rabies has been highly effective in Europe, where the red fox (*Vulpes vulpes*) is the main wildlife reservoir, and has resulted in the eradication of rabies from some European countries (Müller et al., 2015). The red fox in Europe occupies a similar ecological niche to jackals in southern Africa (Rhodes et al., 1998).

The uptake of bait by non-target species is dependent on density of target and non-target species, habitat, food availability and type and presentation of the bait. Several oral bait vaccines have been used over time for the control of rabies in meso-carnivores such as feral dogs, foxes, raccoon (*Procyon lotor*) and coyote (*Canis latrans*) (Cross et al., 2007; Steelman et al., 2000; World Health Organisation, 2007).

All oral rabies vaccines used currently are either modified live or recombinant live vaccines. The World Health Organization (WHO) currently recommends two vaccines for oral use: Raboral-R-VG (Boehringer Ingelheim, previously Merial, USA) and SAG-2 (Virbac, Carros, France) (Office International Des Epizooties, 2008).

Raboral R-VG is a recombinant vaccine containing a vaccine vector with a rabies glycoprotein, is registered in the USA and Europe (Maki et al., 2017), and has been used in golden jackals (*Canis aureus*) in Israel. The SAG-2 vaccine is a modified live vaccine which has been genetically modified from the SAD vaccine (Virbac, Carros, France) by a change in two base pairs associated with virulence to make it safer.

The SAD (Berne) strain of rabies vaccine has been used effectively to orally vaccinate both black-backed and side-striped (*Canis adustus*) jackals in captivity, producing adequate titres (>0.5 IU/ml) for up to 12 months. Oral rabies vaccines are routinely used in wildlife in North America and Europe but there has also been renewed interest in its use in dogs to improve overall cover in a population where parental vaccines have not been completely effective (Cliquet et al., 2018).

The effectiveness of an oral bait vaccine depends on two factors: the efficacy of the vaccine in the target species and the uptake of the bait by the target species (Knobel et al., 2002). When bait was presented in fishmeal polymer a 40-90% acceptance by red foxes and golden jackals was noted in the first night (Yakobson et al., 2006). Oral bait preference has been investigated in the Ethiopian wolf (*Canis simensis*) (Sillero-Zubiri et al., 2016); the wolves were offered a liver bait matrix (SAG-2Dog, Merial), goat meat, boiled goat intestine or grass rats (*Arvicanthis blicki*), their main prey,

containing the rabies vaccine. There was a significant difference in uptake between the different baits, with the commercial bait not being taken and goat meat being the preferred bait (Sillero-Zubiri et al., 2016). Knobel et al (2002) found chicken heads to be the preferred bait in African wild dog (*Lycaon pictus*). Fish meal polymer was well accepted by golden jackal in Israel with an acceptance rate of 100% (Linhart et al., 1997). Chicken heads have been effective baits in black-backed jackal in Zimbabwe with 70% taken within the first night (Linhart et al., 1993). Meat blocks and intestine have been used to deliver poison to black-backed jackals in the past (Linhart et al., 1993). However, to our knowledge, bait preference has not previously been evaluated in black-backed jackals.

The objectives of this study were to evaluate the preference of free-ranging black-backed jackals in the Cradle of Humankind, Gauteng, for chicken heads and meat compared to the commercial fishmeal polymer bait, to assess the relative uptake of the different bait formulations by non-target species, and to identify factors associated with bait uptake in the various species.

Materials and Methods

Ethical approval:

This study was approved by the Animal Ethics Committee of the University of Pretoria (V127-16).

Study Area:

The John Nash and Malapa Reserves are situated in the center of the Cradle of Humankind UNESCO world heritage site and contain 76.3 km² of protected area within the Magaliesberg Biosphere Reserve in Gauteng province, South Africa. The topography is hilly, with open, rocky grassland and scattered trees and bushes, and

thickly vegetated gullies and ravines. Rainfall occurs in summer, mainly between October and March, whereas winters are cool and dry. The reserves have been under study via camera traps continuously since 2009 (Kuhn, 2014), which showed that the reserves are home to numerous black-backed jackals.

Study design:

This placebo trial was conducted at 12 camera locations on the John Nash and Malapa Reserves from November 2016 to August 2017. Lynx Optics Ranger Trail Cameras (Lynx Optics, Bromhof, South Africa) were used to monitor the locations (Figure 2). Each camera location was baited with a fishmeal polymer and another randomly selected bait type, either a piece of red meat (beef or horse) or a chicken head, to compare preference. Cameras were placed at each location and were checked weekly during the trial periods. Each camera was triggered with an infra-red motion sensor: 3 pictures were taken in quick succession with a 5 second delay before the camera would take the next potential three-picture burst.

Baits were placed in front of the camera traps at least 60 cm apart from each other, in clear view of the camera trap, and no further than 5 m from the camera trap to ensure visibility during nighttime. All baits were placed around midday. Each camera location was baited with either meat or chicken on alternative weeks and the other bait was always fishmeal polymer. The same number of baits were distributed at each camera location. Baits were not placed if one of the cameras was malfunctioning at the time of placement and could not be repaired or replaced at the time.

Each camera location was baited 1 day per week over 5 weeks during the summer months (November to January) and 1 day per week for 6 weeks during the winter months (June to August). Due to the inaccessible terrain in many part of the study area, camera locations could not be evenly distributed, but were selected based on proximity to roads and known jackal occurrence at the camera location (Kuhn, 2014).

The camera locations were checked every 7 days and the presence or absence of bait was recorded; if bait was still in place it was left in situ, otherwise a new bait of random type was placed. Location, season, date, time period until the bait was taken, time of day, species that consumed the bait and kind of bait taken were recorded based on the data captured by the camera trap and analyzed.

Bait description:

The placebo was a plastic sachet containing 2 ml sterile water, measuring approximately 0.5 cm x 2 cm x 6 cm and was presented either in the original fishmeal polymer, or in a piece of meat, or in a chicken head and neck. The fishmeal polymer measured approximately 3.3 x 3.3 x 2.1 cm and weighed 23 g. For the meat bait, the plastic sachet was placed in a piece of red meat (horse or beef) approximately 10 x 5 x 5 cm in size, which was closed with absorbable suture material to ensure the sachet did not fall out. For the chicken heads, obtained from Barlet Poultry, Muldersdrift, a sachet was inserted into the mouth of the chicken and pushed into the neck area to ensure that it did not dislodge easily (Figure 3). Selection of bait types was based on reports that fish meal polymer has been well accepted in golden jackal and chicken heads were well accepted in black-backed jackal in Zimbabwe (Bingham et al., 1995; Linhart et al., 1997). Intestines were not used as bait due to the poor acceptance of it by jackal at vulture restaurants close to the study area (unpublished data).

Statistical analysis:

Species was classified as jackal, other carnivores, herbivores and others; season was either summer (November-January) or winter (June to August); and bait type was fishmeal, chicken head or meat. Time of day was classified as day, night or twilight and calculated on the sunset and sunrise in Gauteng during the study period. In winter twilight hours are shorter (total 2 h 50 min) compared to summer (total 4 h

50 min). Categorical variables were cross-tabulated, and proportions were compared using Fishers' exact test. Median time to bait uptake was compared between species and bait types using the Mann-Whitney U test. Using all observations for which the species taking the bait could be identified, multiple correspondence analysis was used to identify patterns amongst variables (bait type, species, time to taken, season and location) and scatterplots of the normalized coordinates of variable categories in two dimensions were produced; for this, time until bait was taken was categorized into quartiles (<5 hours, 5-20 hours, >20-40 hours and >40 hours). Kaplan-Meier survival curves were generated for each species category, including a combined non-jackal species category, to show their relative preference for the different baits, indicated by their rate of bait uptake. Cox proportional hazards regression models were used for each species category to estimate the associations of bait type, season and camera location with time to bait uptake, while controlling for confounding amongst the three variables. Stata 14 (StataCorp, College Station, TX, U.S.A.) was used for statistical analysis; significance was assessed at $p < 0.05$.

Results

A total of 195 baits were placed, of which 186 were monitored via camera traps; nine recordings were lost when cameras were destroyed during a veldt fire. Overall there were 102 fish polymer, 32 chicken heads and 52 meat pieces placed. All camera locations were baited with each bait type. 100 baits were placed over 5 weeks in the summer months and 86 baits over 6 weeks in the winter months. The overall uptake of bait was 87% (170/195) if the baits destroyed in fire are included. 25% of baits were taken within 4 h of bait placement, 50% were taken within 21 h and 75% were taken within 44 h. Of the 186 placements it was possible to identify the species eating the bait in 105 cases (55%) (Table 1).

Warthogs, jackals and primates were responsible for the most bait consumption (

Table 1). Jackal consumed 21 (20%) of the total baits identified, with other carnivores eating 19 (18%) and the remainder being taken by herbivores, primates and other species. The uptake was fastest when taken by pied crows, which would consume bait within an hour of placement. The median time to consumption for jackal was 18 h, not significantly different from the median for other carnivores (16 h) ($p=0.577$). Of the 21 baits consumed by jackal (Table 2), chicken heads (38%) and fish polymer (38%) were taken equally despite three times as many fish polymers being placed, and meat was consumed less often (24%). Chicken heads were taken only by scavengers and carnivores, including jackals, with the exception of one taken by a warthog.

The majority of baits were taken during the day, followed by the night and fewest were taken at twilight (Table 3). Jackal consumed bait throughout the day and night, with no period associated with increased consumption. Other carnivores took bait either during the day or during the night, reflecting the circadian habits of the individual species, and herbivores and other scavengers consumed bait mainly during the day. Most competitors (largely warthog, baboons and crows) took bait during the day (Table 3) and at night and twilight jackal took 43% of the bait.

The multiple correspondence analysis (Figure 4) showed jackals grouping with night and twilight in the first dimension (x-axis) and with chicken heads in the second dimension (y-axis). Jackals were most commonly found at the plains site (PL), the camera location in the center of the combined reserves (Figure 2), while herbivores were common at various camera locations, particularly RD. For season, winter tended to group with shorter uptake times and summer with longer uptake times.

When comparing survival times, jackals showed a clear preference for chicken heads (Figure 5) whereas other species showed no clear preferences (Figure 6), except

that herbivores did not consume chicken heads (Figure 6 B). The Cox proportional hazards models (Table 4) showed that jackals clearly consumed chicken heads more readily than fishmeal (Hazard ratio (HR)=3.41; $p=0.025$), although in comparison to meat the difference was not significant (HR=2.3; 95%CI: 0.7-7.6; $p=0.174$). Season was not associated with consumption of bait by jackal ($p=0.574$), but other species showed increased rate of consumption of bait in winter (Table 4).

Discussion

To our knowledge this is the first published study of oral bait uptake and preference in black-backed jackals, an important vector of rabies in southern Africa. Placing monitored bait stations scattered throughout the habitat of the target species is expected to provide a good indication of bait uptake by that species. In this study the baits were well accepted overall, with 20% being taken by jackals, 18% by other carnivores and 62% by other species. Several factors associated with bait uptake were identified which may help in designing baiting strategies to target jackals for oral rabies vaccination while reducing uptake by non-target species. In a high number of cases the species taking the bait could not be identified due to camera failure, or movement of camera by baboons, veldt fire and storms. This negatively affected the power of the study by reducing the effective sample size; however, it should not have introduced bias, with the possible exception that consumption by baboons may have been slightly underestimated if they moved the camera prior to taking the bait.

The problem of non-target species consuming bait was investigated when widespread use of oral bait was initiated in Europe for the control of rabies in red foxes (Brochier et al., 1989). Bait uptake by non-target species is usually associated with carnivores and scavengers (Knobel et al., 2002). In trials in the USA no evidence of uptake of fishmeal polymer by white-tailed deer was observed (Hanlon et

al., 1989), which is in contrast to the present study in which 10% of baits were taken by herbivores. The higher uptake by herbivores in this study may be due to the John Nash and Malapa Reserves being managed and fenced game reserves. Whereas jackal, small carnivores and warthog will commonly pass under fences, ungulates do not, therefore maintaining a stable high herbivore density, and potentially leading to food scarcity during the dry winter season. This is supported by the fact that rate of bait uptake by herbivores was strongly associated with season, occurring almost exclusively during winter. Warthog were the largest consumer of bait; this might be related to their abundance, with an estimated 300 warthog in the reserve. Warthogs are a common species in large parts of South Africa, even outside protected areas, and therefore our results may also be applicable outside protected areas.

Jackals showed a clear preference for chicken heads, indicated by their faster rate of uptake of chicken heads compared to fishmeal polymer and meat, although the latter was not significant, likely due to inadequate sample size. This is consistent with the findings of Knobel et al (2002), where chicken heads were the preferred bait in African wild dog compared to fish or liver flavoured wax blocks or chicken mince. Linhart et al (1993) reported an uptake of up to 70% of chicken heads by jackal during the first night of placement although no comparison with fishmeal polymer was made in this study. Chicken heads may therefore be better for targeting jackals as there is also less loss of bait to non-target species; this is supported by our finding that both red meat and fishmeal polymer were far more likely to be taken by competitor species than were chicken heads. However, chicken heads are more labour intensive as they need to be prepared individually and freshly while the fish polymer is more hygienic and easier to handle and store.

Design of an oral rabies vaccination strategy should consider the period of vaccine viability in relation to the expected rate of bait uptake by the target species. In this

study the uptake of the bait was generally good with most bait being consumed within 40 hours of placement. The SAG-2 and V-RG vaccines have been reported viable at 37°C for up to 42 hours and 168 hours respectively (Maki et al., 2017; Massen et al., 1996). The stability of the vaccine to direct sunlight has also to be considered in South Africa, as the SAG-2 matrix has been shown to melt in direct sunlight, in contrast with the V-RG vaccine (Yakobson et al., 2006). However, the vaccine itself should not be exposed to direct sunlight when it is put into baits such as chicken heads or meat.

Timing of vaccine placement can assist with greater uptake of vaccine by target species. It has been suggested that bait uptake will be increased in winter months when less food is available to carnivores (Maki et al., 2017). Loveridge and MacDonald (2001) suggested vaccination of jackals before the mating season (April to June) and before the pups become independent (October to January). Maternally derived antibodies in domestic dogs will protect pups up to 42 days of age, but pups are susceptible to rabies after that time period, unless protection is boosted via vaccination (Chappuis, 1998). In the Cradle of Humankind jackal pups are usually born around mid-September to the beginning of October (Snyman, personal communication) which may make August and September appear to be suitable months for vaccine distribution, as pups are not independent and food is still not as abundant and bait therefore more readily consumed. However, our study indicates that uptake by non-target species is increased in winter months whereas uptake of bait by jackal is not significantly affected by season, suggesting that bait placement during summer months may be more efficient for targeting jackal. There is a risk of pups not being exposed to vaccine as the vaccine strain is usually destroyed by gastric acid in regurgitated food provided by their parents (Papatheodorou et al., 2018). The pups need to be old enough to consume bait themselves (Bruyère et al.,

2000). This again suggests that vaccination during summer, once pups become independent, may be more effective.

In Europe baiting twice annually is advocated but research has shown that as long as one individual eats one bait and seroconverts, and 50 to 70% of target population is immunized, it will prevent a rabies outbreak (Rosatte et al., 2007). Ideally vaccines should be distributed initially twice yearly to ensure a maximum protection of target species (Maki et al., 2017) but obviously this is more labour-intensive and costly than once annually. When considering the cost of a vaccine campaign versus its effectiveness, our study would suggest that, in our study area, a single annual vaccination may be most effective if given in late December/January. Clearly, further research, including studies of duration of vaccine-induced immunity in jackals, is needed to determine the optimum timing and frequency of vaccination.

In this study only three bait types were compared, whereas there may be other bait types that might be more attractive to jackal. In Africa there will always be numerous carnivorous and omnivorous competitor species, making it unlikely that a bait type will be found that is attractive only to jackals. Nevertheless, more research may be needed into bait development and placement strategies to minimize uptake by non-target species. However, it is worth noting that all mammals, including most competitor species in this study, are susceptible to rabies and may also benefit from oral rabies vaccine.

Another limitation of this study is that, for logistical purposes, all baits were placed at around the same time of day, at midday; therefore, the effect of bait placement at different times of day could not be assessed. To our knowledge this has also not been investigated in previous studies; although a loss of 10% of bait to beetles and millipedes has been noted if bait was not consumed by jackal during first night of

placement (Linhart et al., 1993). The effect of time of day on bait consumption by non target species will vary between regions and seasons depending on the particular non-target species present in the area. In this study there were clear differences between species in the time of day when the baits were consumed. Numerically the biggest competitors for bait uptake were warthogs, baboons and crows, all of which invariably consumed the baits during the day, whereas jackals showed little preference. Therefore, this study suggests that placing bait at dusk or at night may largely eliminate these species as competitors and more effectively target jackals. This would still leave the other carnivores as competitors; however, they occurred in lower numbers, and in addition most of them may also be involved as potential rabies vectors and could therefore also be considered target species in a rabies vaccination campaign.

Another limitation of this study, leading to loss of statistical power, was that a substantial amount of data was lost, firstly due to loss of cameras because of fires and flooding, and secondly, in many instances the species consuming the bait could not be identified. The latter could have either been due to camera failure, loss of image due to movement of cameras by baboons, or consumption of bait by small animals, such as rodents, that did not trigger the camera.

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References

- Bhattacharya, D., Bensaci, M., Luker, K.E., Luker, G., Wisdom, S., Telford, S.R., Hu, L.T., 2011. Development of a baited oral vaccine for use in reservoir-targeted strategies against Lyme disease. *Vaccine* 29, 7818–7825.
<https://doi.org/10.1016/j.vaccine.2011.07.100>
- Bingham, J., Kappeler, A., Hill, F.W.G., King, a a, Perry, B.D., Foggin, C.M., 1995. Efficacy of Sad (Berne) Rabies Vaccine Given by the Oral Route in 2 Species of Jackal (*Canis-Mesomelas* and *Canis-Adustus*). *J. Wildl. Dis.* 31, 416–419.
<https://doi.org/10.7589/0090-3558-31.3.416>
- Brochier, B., Blancou, J., Thomas, I., Languet, B., Artois, M., Kieny, M.P., Lecocq, J.P., Costy, F., Desmettre, P., Chappuis, G., ., 1989. Use of recombinant vaccinia-rabies glycoprotein virus for oral vaccination of wildlife against rabies: innocuity to several non-target bait consuming species. *J.Wildl.Dis.* 25, 540–547. <https://doi.org/10.7589/0090-3558-25.4.540>
- Bruyère, V., Uillaume, P., Cliquet, F., Aubert, M., 2000. Oral rabies vaccination of foxes with one or two delayed distributions of SAG2 baits during the spring. *Vet. Res.* 31, 339–345.
- Chappuis, G., 1998. Neonatal immunity and immunization in early age: Lessons from veterinary medicine. *Vaccine* 16, 1468–1472. [https://doi.org/10.1016/S0264-410X\(98\)00110-8](https://doi.org/10.1016/S0264-410X(98)00110-8)
- Cliquet, F., Guiot, A.L., Aubert, M., Robardet, E., Rupprecht, C.E., Meslin, F.X., 2018. Oral vaccination of dogs: A well-studied and undervalued tool for achieving human and dog rabies elimination. *Vet. Res.* 49, 1–11.
<https://doi.org/10.1186/s13567-018-0554-6>
- Cross, M.L., Buddle, B.M., Aldwell, F.E., 2007. The potential of oral vaccines for disease control in wildlife species. *Vet. J.* 174, 472–480.
<https://doi.org/10.1016/j.tvjl.2006.10.005>
- Directorate Animal Health, 2016. Animal Disease Reporting, Department of

Agriculture, Forestry and Fisheries, South Africa.

Ferguson, J.W.H., Nel, J.A.J., Wet, M.J., 1983. Social organization and movement patterns of Black-backed jackals. *J. Zool.* 199, 487–502.

Gertsma, P., 2016. GDARD Rabies Outbreak Gauteng, 2016.

Gummow, B., Roefs, Y. a a, de Klerk, G., 2010. Rabies in South Africa between 1993 and 2005--what has been achieved? *J. S. Afr. Vet. Assoc.* 81, 16–21.

Hanlon, L., Hayes, D.E., Hamir, A.N., Snyder, D.E., Hable, P., Rupprecht, E., 1989. Proposed Vaccine Selection , and for Evaluation Species Trials of a Rabies Lotor): Characteristics , Recombinant Site Raccoons (Procyon Target Placebo. *J. Wildl. Dis.* 25, 555–567.

Haydon, D.T., Cleaveland, S., Taylor, L.H., Laurenson, M.K., 2002. Identifying Reservoirs of Infection : A Conceptual and Practical Challenge. *Emerg. Infect. Dis.* 8, 1468–1473.

Hiscocks, K., Perrin, M.R., 1988. Home range and movements of black-backed jackals at Cape Cross Seal Reserve, Namibia. *South African J. Wildl. Res.* 18, 97–100.

Knobel, D.L., du Toit, J.T., Bingham, J., 2002. Development of a bait and baiting system for delivery of oral rabies vaccine to free-ranging African wild dogs (*Lycaon pictus*). *J. Wildl. Dis.* 38, 352–362. <https://doi.org/10.7589/0090-3558-38.2.352>

Kuhn, B.F., 2014. A preliminary assessment of the carnivore community outside Johannesburg , South Africa. *South African J. Wildl. Res.* 44, 95–98.

Linhart, S.B., Kappeler, A., Windberg, L.A., 1993. A Review of Baits and Bait Delivery Systems for Free-Ranging Carnivores and Ungulates, in: Terry J. Kreege (Ed.), *Contraception in Wildlife Management*. Washington. DC: US Government Printing Office, pp. 69–132.

Linhart, S.B., King, R., Zamir, S., Naveh, U., Davidson, M., Perl, S., 1997. Oral rabies vaccination of red foxes and golden jackals in Israel: preliminary bait evaluation.

- Rev. Sci. Tech. l'OIE 16, 874–880. <https://doi.org/10.20506/rst.16.3.1076>
- Loveridge, A.J., Macdonald, D.W., 2001. Seasonality in spatial organization and dispersal of sympatric jackals (*Canis mesomelas* and *C. adustus*): implications for rabies management. *J. Zool.* 253, 101–111.
<https://doi.org/10.1017/S0952836901000097>
- Maki, J., Guiot, A.L., Aubert, M., Brochier, B., Cliquet, F., Hanlon, C.A., King, R., Oertli, E.H., Rupprecht, C.E., Schumacher, C., Slate, D., Yakobson, B., Wohlers, A., Lankau, E.W., 2017. Oral vaccination of wildlife using a vaccinia – rabies – glycoprotein recombinant virus vaccine (RABORAL V – RG ®): a global review. *Vet. Res.* 48, 1–26. <https://doi.org/10.1186/s13567-017-0459-9>
- Massen, E., Aubert, M.F.A., Barrat, J., Vuillaume, P., 1996. Comparison of the efficacy of the antirabies vaccines used for foxes in France. *Vet. Res.* 27, 255–266.
- Morters, M.K., Restif, O., Hampson, K., Cleaveland, S., Wood, J.L.N., Conlan, A.J.K., 2013. Evidence-based control of canine rabies: A critical review of population density reduction. *J. Anim. Ecol.* 82, 6–14. <https://doi.org/10.1111/j.1365-2656.2012.02033.x>
- Müller, T.F., Schröder, R., Wysocki, P., Mettenleiter, T.C., Freuling, C.M., 2015. Spatio-temporal use of oral rabies vaccines in fox rabies elimination programmes in Europe. *PLoS Negl. Trop. Dis.* 9, 1–16.
<https://doi.org/10.1371/journal.pntd.0003953>
- Office International Des Epizooties, 2008. Rabies, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds, and bees).
<https://doi.org/10.1016/j.ncl.2008.03.010> (accessed 1 May 2017)
- Papatheodorou, D.P., Tasioudi, K.E., Korou, L.-M., Ge, V.L., Markantonatos, G., Iliadou, P., Kirtzalidou, A., Katsifa, A., Ztrivas, S., Bechtsi, G., Tzani, M., Chondrokouki, E., Mangana-vougiouka, O., 2018. Efficacy of oral rabies vaccination in individual age groups of juvenile red foxes. *Vet. Microbiol.* 226,

- 59–63. <https://doi.org/https://doi-org.proxy.eap.gr/10.1016/j.vetmic.2018.10.006>
- Rhodes, C.J., Atkinson, R.P.D., Anderson, R.M., Macdonald, D.W., 1998. Rabies in Zimbabwe: Reservoir dogs and the implications for disease control. *Philos. Trans. R. Soc. B Biol. Sci.* 353, 999–1010. <https://doi.org/10.1098/rstb.1998.0263>
- Rosatte, R., Tinline, R., Johnston, D., 2007. Rabies Control in Wild Carnivores, in: Jackson, A., WH, W. (Eds.), *Rabies*. Academic Press, Elsevier, pp. 595–634. <https://doi.org/10.1016/B978-0-12-369366-2.50020-8>
- Sillero-Zubiri, C., Marino, J., Gordon, C.H., Bedin, E., Hussein, A., Regassa, F., Banyard, A., Fooks, A.R., 2016. Feasibility and efficacy of oral rabies vaccine SAG2 in endangered Ethiopian wolves. *Vaccine* 34, 4792–4798. <https://doi.org/10.1016/j.vaccine.2016.08.021>
- Skinner, J.D., Chimimba, C.T., 2005. Black backed jackal, in: *The Mammals of Southern African Subregion*. Cambridge University Press, Cape Town, pp. 486–490.
- Steelman, H.G., Henke, S.E., Moore, G.M., 2000. Bait delivery for oral rabies vaccine to gray foxes. *J. Wildl. Dis.* 36, 744–51. <https://doi.org/10.7589/0090-3558-36.4.744>
- United Nation, Department of Economic and Social Affairs Population Division. 2014 *World Urbanization Prospects: The 2014 Revision*. <https://www.un.org/en/development/desa/publications/2014-revision-world-urbanization-prospects.html> (accessed 5 March 2017)
- Woodroffe, R., Gilks, P., Johnston, W.T., Le Fevre, A.M., Cox, D.R., Donnelly, C.A., Bourne, F.J., Cheeseman, C.L., Gettinby, G., McInerney, J.P., Morrison, W.I., 2008. Effects of culling on badger abundance: Implications for tuberculosis control. *J. Zool.* 274, 28–37. <https://doi.org/10.1111/j.1469-7998.2007.00353.x>
- World Health Organisation. *Oral Vaccination of Dogs against rabies: Guidance for research on oral rabies vaccines and field application of oral vaccination of dogs*

against rabies World Health Organisation. Geneva, 2007.

Yakobson, B.A., King, R., Amir, S., Devers, N., Sheichat, N., Rutenberg, D.,
Mildenberg, Z., David, D., 2006. Rabies vaccination programme for red foxes
(*Vulpes vulpes*) and golden jackals (*Canis aureus*) in Israel (1999-2004). *Dev.*
Biol. (Basel). 125, 133–140.

Table 1: Bait consumption and median time to consumption by different species in a multi-site field study in South Africa

Species	Scientific name	No of baits taken	%	Median time to consumption (h)	IQR*
Herbivore					
Eland	<i>Taurotragus oryx</i>	2	2	9	9 -9
Greater kudu	<i>Tragelaphus strepsiceros</i>	4	4	8.5	4 - 55
Nyala	<i>Tragelaphus angasii</i>	1	1	6	6 -6
Blue wildebeest	<i>Connochaetes taurinus</i>	3	3	48	29-52
Carnivore					
Black-backed Jackal	<i>Canis mesomelas</i>	21	20	18	8 - 21
Caracal	<i>Caracal caracal</i>	1	1	16	16 - 16
Honey badger	<i>Mellivora capensis</i>	1	1	17	17 - 17
Brown hyaena	<i>Parahyaena brunnea</i>	7	7	32	8-129
Serval	<i>Leptailurus serval</i>	4	4	21.5	2 - 41
Slender mongoose	<i>Galerella sanguinea</i>	4	4	13	4 - 21
Yellow mongoose	<i>Cynictis penicillata</i>	2	2	1	1 - 1
Other					
Warthog	<i>Phacochoerus africanus</i>	27	25	22	4-30
Chacma baboon	<i>Papio ursinus</i>	13	12	48	5 - 98
Vervet monkey	<i>Chlorocebus pygerythrus</i>	1	1	1	1 - 1
Dung beetle	Scarabaeidae	2	2	38	18-58
Pied crow	<i>Corvus albus</i>	12	11	2.5	1 - 5
Total		105	100	21	4-44

* Interquartile range

Table 2: Consumption of three bait types by black-backed jackal and other species categories in a multi-site field study in South Africa

Species	Number (%) of baits consumed			
	Fish polymer	Chicken head	Meat	Total
Jackal	8 (38%)	8 (38%)	5 (24%)	21
Other carnivore	13 (68%)	2 (11%)	4 (21%)	19
Herbivore	6 (60%)	0 (0%)	4 (40%)	10
Other	27 (49%)	7 (13%)	21 (38%)	55
Total	54 (51%)	17 (16%)	34 (32%)	105

Table 3: Bait consumption in relation to time of day by different species categories in a multi-site field study in South Africa

Species	Number (%) of baits consumed			
	Day	Night	Twilight	Total
Jackal	8 (38%)	6 (29%)	7 (33%)	21
Other carnivore	8 (42%)	9 (47%)	2 (11%)	19
Herbivore	6 (60%)	3 (30%)	1 (10%)	10
Other	52 (96%)	1 (2%)	1 (2%)	54
Total	74 (71%)	19 (18%)	11 (11%)	104

Table 4: Cox proportional hazards models of associations of time to bait being taken with bait type, season and camera trap location, for black-backed jackals and other species categories in a multi-site field study in South Africa

Species category	Variable and level	Hazard ratio	95% CI (HR)	p-value
Jackal	Bait			
	Fishmeal	1*	–	–
	Chicken head	3.41	1.16, 9.99	0.025
	Meat	1.48	0.48, 4.59	0.495
	Season (Winter vs. summer)	0.75	0.27, 2.06	0.574
	Camera location (7 sites)	–	–	0.003
Other carnivore	Bait			
	Fishmeal	1*	–	–
	Chicken head	0.62	0.13, 2.90	0.544
	Meat	0.97	0.30, 3.16	0.956
	Season (Winter vs. summer)	5.74	1.86, 17.7	0.002
	Camera location (7 sites)	–	–	0.939
Herbivore	Bait			
	Fishmeal	1*	–	–
	Chicken head	0	–	–
	Meat	1.8	0.45, 7.59	0.39
	Season (Winter vs. summer)	11.19	2.05, 60.91	0.005
	Camera location (7 sites)	–	–	0.017
Other species	Bait			

	Fishmeal	1		
	Chicken head	1.02		
	Meat	2.1	0.43, 2.46	0.956
	Season (Winter vs. summer)	2.87	1.58, 5.22	0.001
	Camera location (7 sites)	–	–	0.098
All species other than jackal	Bait			
	Fishmeal	1		
	Chicken head	0.70		
	Meat	1.76	0.33, 1.49	0.357
	Season (Winter vs. summer)	3.77	2.31, 6.17	0.000
	Camera location (7 sites)			0.047

Figure 1: Map of the Cradle of Humankind showing the Magaliesberg Protected Environment, Cradle of Humankind and John Nash and Malapa Reserves, Gauteng Province, South Africa. (The map was constructed using QGIS 3.4 using country boundaries and protected area boundaries downloaded and used with permission from protectedplanet.net.)

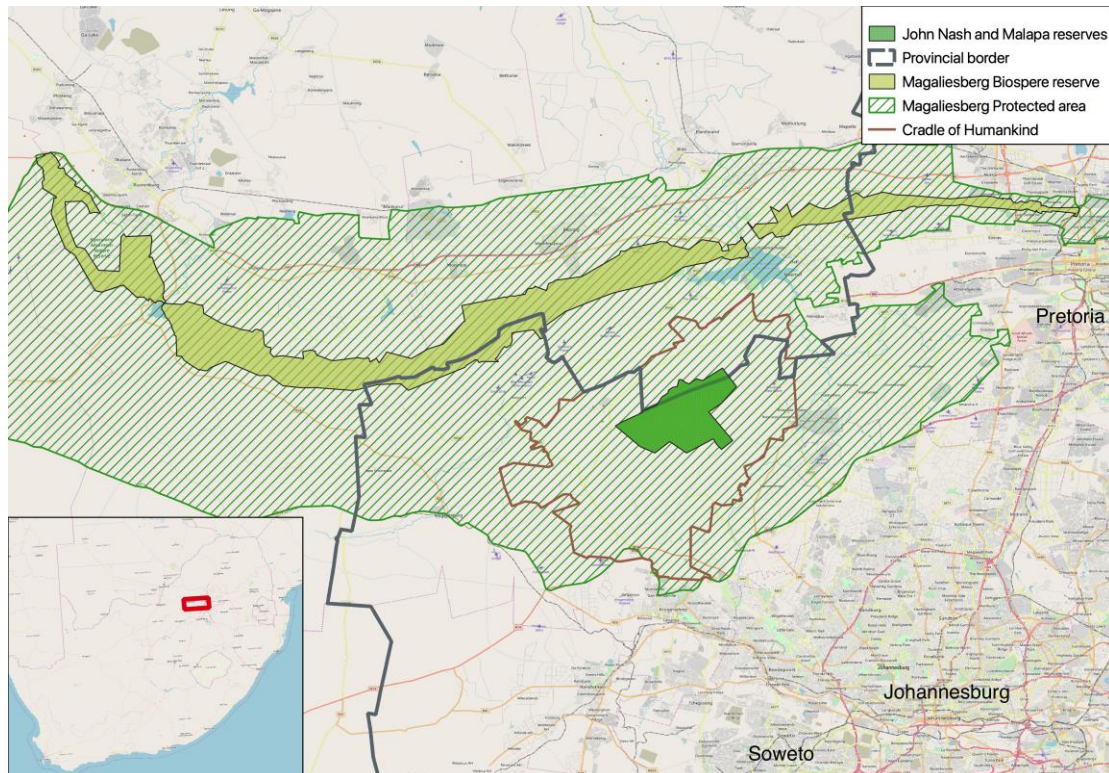


Figure 2: Camera trap locations in John Nash and Malapa Reserves in the Cradle of Humankind, Gauteng Province, South Africa. (The map was constructed using QGIS 3.4 using country boundaries and protected area boundaries downloaded and used with permission from protectedplanet.net)

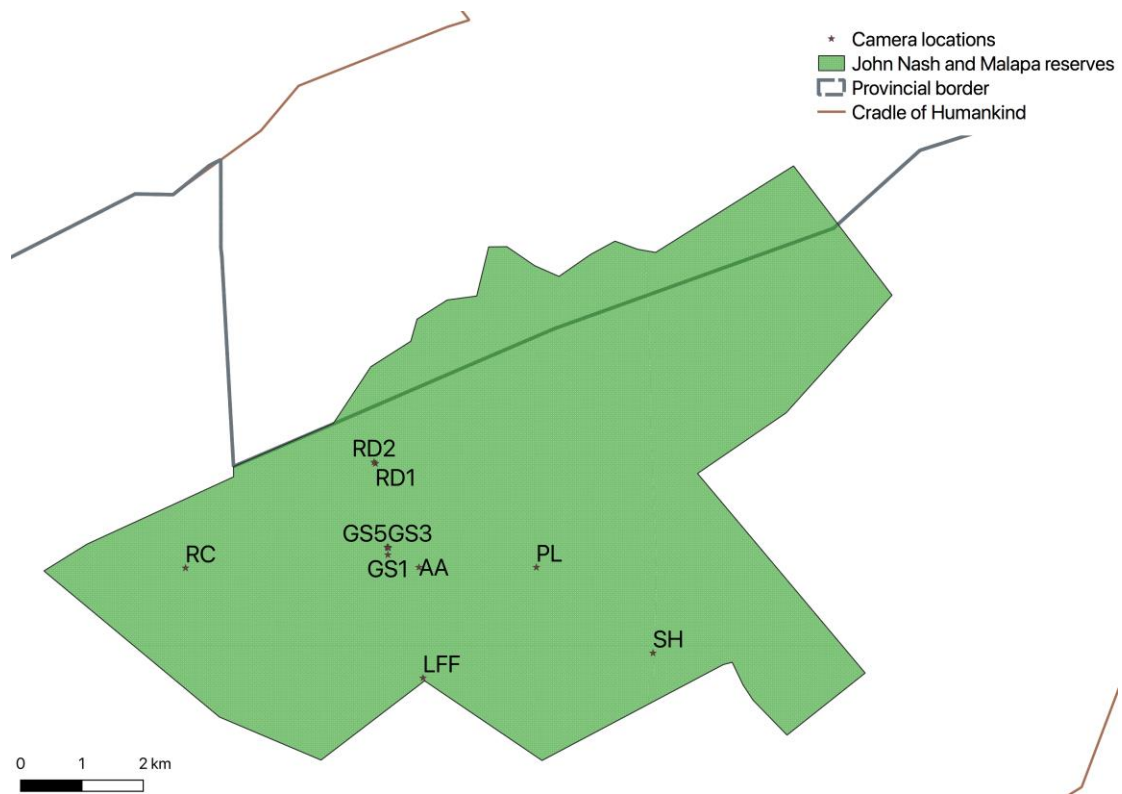


Figure 3: Types of baits offered and evidence of consumption. A: Fishmeal polymer; B: red meat; C: chicken head and neck and D: punctured sachet confirming uptake



Figure 4: Multiple correspondence analysis of factors affecting bait uptake by black-backed jackal and other species categories in a multi-site field study in South Africa

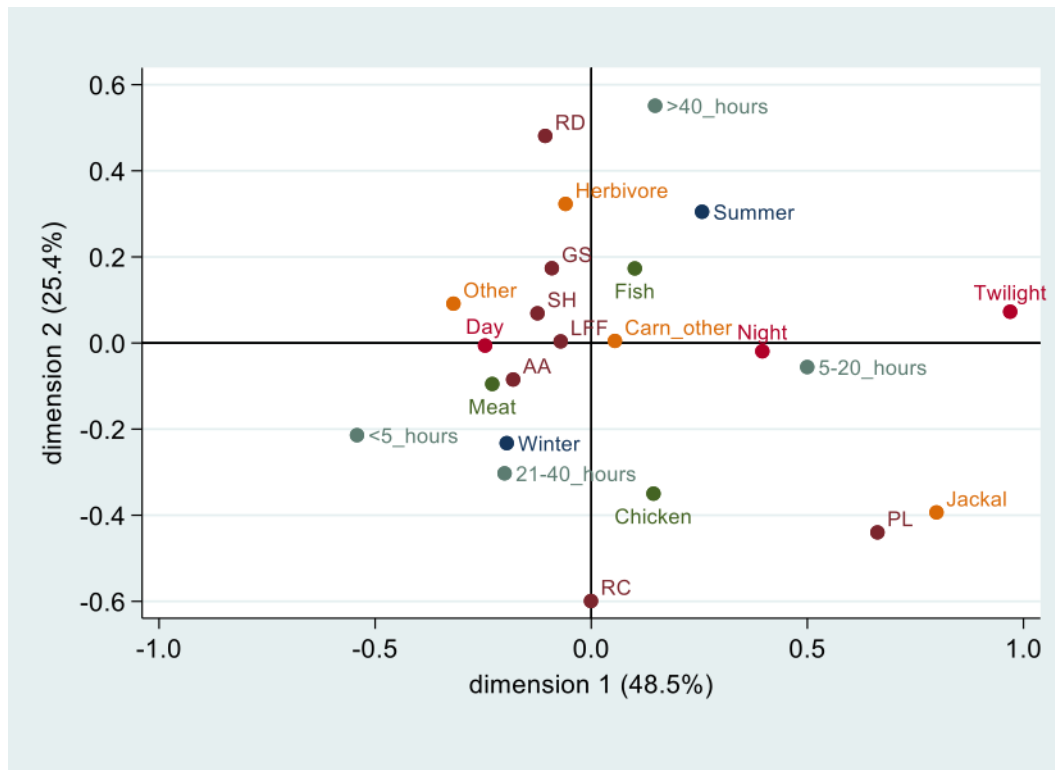


Figure 5: Kaplan-Meier survival curves showing time until different bait types were taken by black-backed jackal in a multi-site field study in South Africa

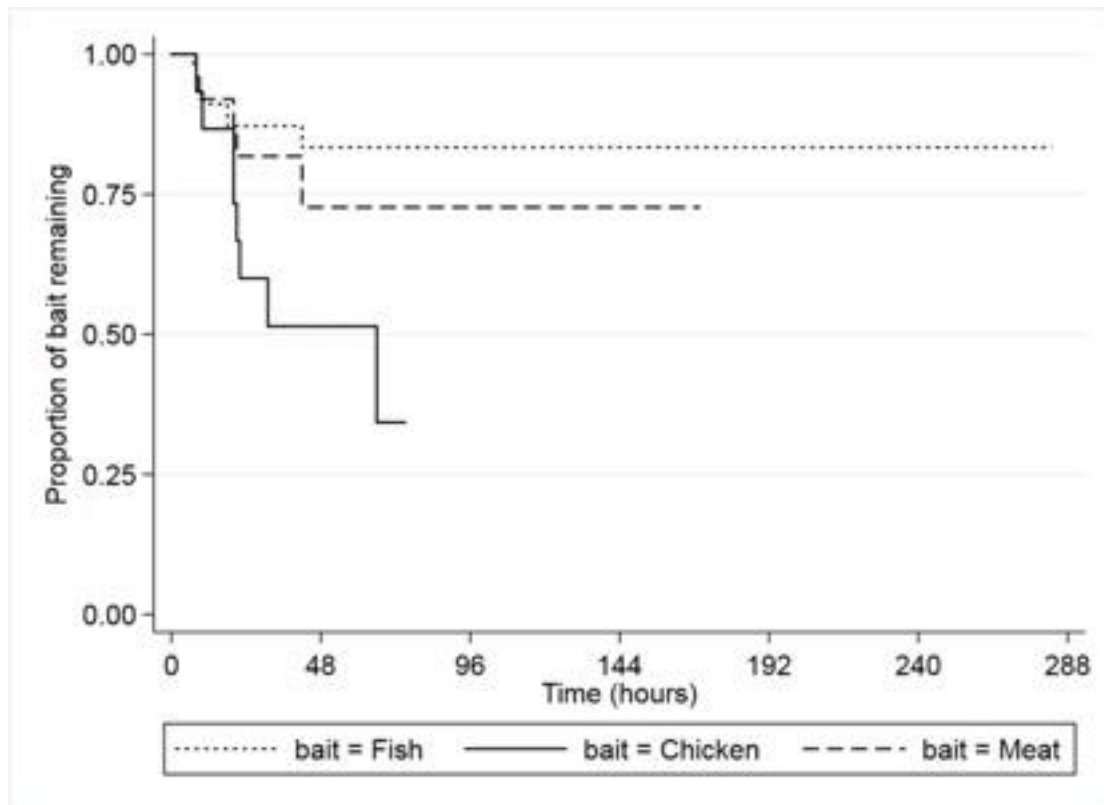


Figure 6: Kaplan-Meier survival curves showing time until different bait types were taken by species other than jackal in a multi-site field study in South Africa.

A: Other carnivores; B: Herbivores; C: Other species; D: All species other than jackal.

