

Progress Report III



Patagonian wild foxes warning against poisoning by Conditioned Taste Aversion

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Proposal executive summary

Poisoning is a widespread non selective predator control method throughout Patagonia. Our aim is to develop a field protocol to aware two fox species from being poisoned, through conditioned taste aversion. Our results should be used inside protected areas, but also in sheep ranches, to protect the small, harmless grey fox. Initially, we want to test our ability to generate taste aversion against meat, fish or dog food based baits, so it could later be generated against other non selective poison substrates, frequently used by ranchers, like chicken eggs. These will contribute to stop the uncontrolled elimination of top predators, those raptors and mammalian carnivores responsible of biodiversity conservation by top-down regulation in Patagonia and all over the world.

Introduction

Sheep ranching in Santa Cruz province, often characterized by an overload of animals in the fields, has caused irreversible changes in the steppe environment (Oliva et al. 1995). The consequent reduction in the field receptivity and the economic and climatic factors, led to a gradual abandonment of cattle throughout the province. Ranchers have been generally very inactive and apathetic to the incorporation of new technologies (Andrade 2005). Instead, they have concentrated their efforts on fight to which they identify as the principal responsible for their failure, wild sheep predators as Culpeo fox (*Pseudalopex culpaeus*) and the Cougar (*Puma concolor*) (Travaini et al. 2000, García Brea 2007). To reduce losses attributed to predation, and against current recommendations for selective control (Burns et al. 1996, Sacks et al. 1999) restricted to problem individuals (Blejwas et al. 2002, Jaeger 2004, Williams et al. 2003) or even based on non lethal techniques (Andelt and Hopper 2000, Bromley and Gese 2001, Shivik 2004), they have used and continue to do so, poison based non selective methodologies (Travaini et al. 2000, García Brea 2007). Among the most affected non target species, is the grey fox (*Pseudalopex griseus*), which coexists in sympatry with Culpeo foxes. Also many birds of prey are affected, like "Jote cabeza negra" (*Coragyps atratus*), "Caranchos" (*Caracara plancus*) or "Águilas Mora" (*Geranoaetus melanoleucus*).

The Culpeo Fox, apparently a solitary species, is distributed throughout the foothills of the Andes, from northern Ecuador to the South of Chile and Argentina. It inhabits the Patagonian steppe, including areas of Neuquén, Río Negro, Chubut, Santa Cruz and Tierra del Fuego. It has an adult weight of 6-12 kg. The smaller Grey Fox, with an adult weight of 2-5 kg, inhabits plains and mountains on both sides of the Andes, from northern Chile to Tierra del Fuego. Both sizes and size differences between these species increase southward. Both canids are opportunistic predators that feed mainly on small mammals but frequently consume lagomorphs and livestock or its carrion, when this becomes abundant. They also feed on birds, lizards, insects and fruit (MacDonald, D.W. and Sillero-Zubiri, BC 2004).

To protect non target species like the grey fox, or both foxes inside protected areas against illegal poisoning, one possible tool is to make teach animals not to consume food potentially poisoned. This is called Conditioned Taste Aversion (CTA).

Conditioned Taste Aversion develops when an animal associates the taste of a particular food with illness and avoids consuming that food in subsequent encounters (García et al. 1974). Learned aversion to bait is sometimes referred to as 'bait shyness' and occurs when an animal ingests a sub-lethal dose of a toxin added to the bait and thereafter refuses to consume the same type of bait (Gustavson CR 1974, Morgan et al. 1996, Massei et al. 2003, Cagnacci F et al. 2005). Bait shyness may arise either from the acquisition of a conditioned taste aversion (CTA) or from secondary repellence. The difference between CTA and *secondary repellence* lies in the ability of an animal to detect the illness-inducing compound added to the bait. If this compound is undetectable, the animal will form an aversion to the bait itself and will avoid consuming it even if the CTA agent is present (Cagnacci et al. 2005).

Many substances are known to induce severe, short-term illness in a wide range of animals. Nevertheless, only those that can be successfully hidden in baits retaining the taste and smell of target food, are able to produce CTA among free-ranging consumers. To be successful, the substance should produce severe short-term illness in consumers; the effective (illness-producing) dose of the substance should be much less than the lethal dose; should be physically stable and so survive intact in baits in the field; and should be undetectable to consumers when present at the appropriate concentration in the baits (Nicolaus LK et al. 1989).

Levamisole induces a robust and long-lasting CTA in laboratory rats and foxes (*Vulpes vulpes*) when its taste is 'masked' either by oral gavages (for rats) (Massei and Cowan 2002), or by surrounding a treated core of meat with plain minced meat (for foxes) (Massei et al. 2003). However ferrets (*Mustela putorius*), free-living foxes and badgers (*Meles meles*) detected the levamisole when offered into food prepared by mixing levamisole with test food and therefore acquired an aversion to this chemical (repellence) rather than to the food itself (Massei 2003, Gentle 2004, Cagnacci 2005). The use of levamisole as a CTA agent is very promising as it is used in veterinary medicine and is considered safe and stable. However, the compound, which is freely soluble in water, is said to have a bitter taste. For levamisole to be successful as a CTA agent in the field, its taste must therefore be masked. The taste of levamisole can be masked through formation of a resinate using the acidic ion-exchange Amberlite® IRP-64 resin (Cotterill JV et al., 2006).

The aims of the present study was (1) identify an aversive drogue and the way to add it to the bait, so we can use it in generating Conditioned Taste Aversion; (2) generate Conditioned Taste

Aversion in both sympatric foxes to protect them from being poisoned (inside the protected areas of Patagonia or outside them), depending on the species and situation, and potentially to reduce bait monopolization in vaccination and fertility control campaigns.

Methods

Study area

The study was conducted at "Monte León National Park" (Figure 1), located on the Patagonian coast, in Santa Cruz Province, southern Argentina (PNML, 50.35° S;

69.20° W) (Figure 2). It comprises 62.700 hectares of grasslands and shrublands. Grasslands are characterized by *Junellia tridens* and *Lepidophyllum cupressiforme* whereas shrublands are characterised by *Festuca pallescens*, *Puccinellia sp.*, *Agrostis sp.* and *Poa atropidiformis* (Oliva et al. 2006). Average annual precipitation is 240 mm, occurring mostly during winter months, and mean seasonal temperature varies from 1°C in winter to 20°C in summer.



Figure 1. Study area in Santa Cruz Province, Argentine. PNML: Monte León National Park. Land view and map.



Figure 2. Coastal views of the protected area on a windy day.

Experimental design

Our experimental design to generate conditioned taste aversion consisted of one Control and two Treatments. Our experimental units were groups of six double bait stations: the transect. One bait station in the pair received a meat plain bait and the other the alternative bait, with or without the compound responsible for generating aversion (see details below). Treatment I with the levamisole included directly to the bait, and Treatment II with the levamisole masked within a resin (Amberlite IRP-64, Cotterill et al. 2006).

Unmasked aversive: Treatment I

The experiment consists of four phases (Figure 3):

Phase 1, Pre-conditioning. We placed meat baits "A" in one bait station from each pair at 5 transects for Control and 5 transects for Treatment I. During the next 3 to 6 days we checked daily each bait station, recorded the species that visit the bait station (Travaini et al. 2001) and replaced the bait when it has been consumed. This was done to attract and provide foxes the opportunity of getting used to visit the stations and establish a base of consumption of baits.

Phase 2, Conditioning. We placed and replenished for 5 to 10 days pet food-raisins baits "B" in the second station of the control transects, and the same bait "B", but treated with levamisole ("BL") in equivalent stations of Treatment I transects (Figure 3).

Prediction(a). If levamisole produces repellence or induce conditioned taste aversion (CTA), foxes in treatment I will gradually eat less baits "B" than in control.

Phase 3, Posconditioning I. We offered, from 3 to 7 days, two types of baits in both groups of transects, control and treatment I: meat bait "A" and pet food bait "B", but without the aversive (Figure 3).

Prediction(b). If the levamisole induced conditioned taste aversion to foxes, in treatment I foxes should eat less baits "B" (without the aversive) than in control, but a similar amount of baits "A".

Phase 4, Posconditioning II. This stage is identical to phase 3, only that this time the pet food baits "B" have the aversive ("BL") in treatment I transects (Figure 3).

Prediction(c). If the foxes in treatment I eat less baits with levamisole than foxes in control during phase 2 and consume also baits "B" without levamisole in phase 3, indicating that has not acquired CTA, baits consumption in phase 4 should follow a pattern similar to Phase 2. If levamisole is identified by the foxes in treated bait ("BL") and then acts as a repellent, they must eat less baits treated than no treated ("A") and also less treated bait ("BL") than no treated ("B") in the control.

Prediction(d). If levamisole generate repellence with treated baits ("BL"), in treatment transects foxes will visit stations, attracted by the smell of baits, but does not consume them once detected the presence of levamisole.

The number of stations visited and baits consumed were registered every morning during the experiment, and we assumed that consumed bait was by the species of fox whose traces were within the station. This has support based on our experience of 10 years of activation of similar bait stations in Patagonia (Travaini and al. 2001, Travaini et al. 2009a and (b), in preparation). To avoid any effect of the residual smell of the bait in the station, each bait "A" and "B" were always on the same station during the experiment (Figure 3).

Masked aversive: Treatment II

The protocol to test the usefulness of mask the aversive drogue in the resin was identical to that described, only that this time the levamisole was masked with Amberlite IRP-64. The second treatment was developed in 5 transects corresponding to Treatment II after finishing the first one (Figure 3).

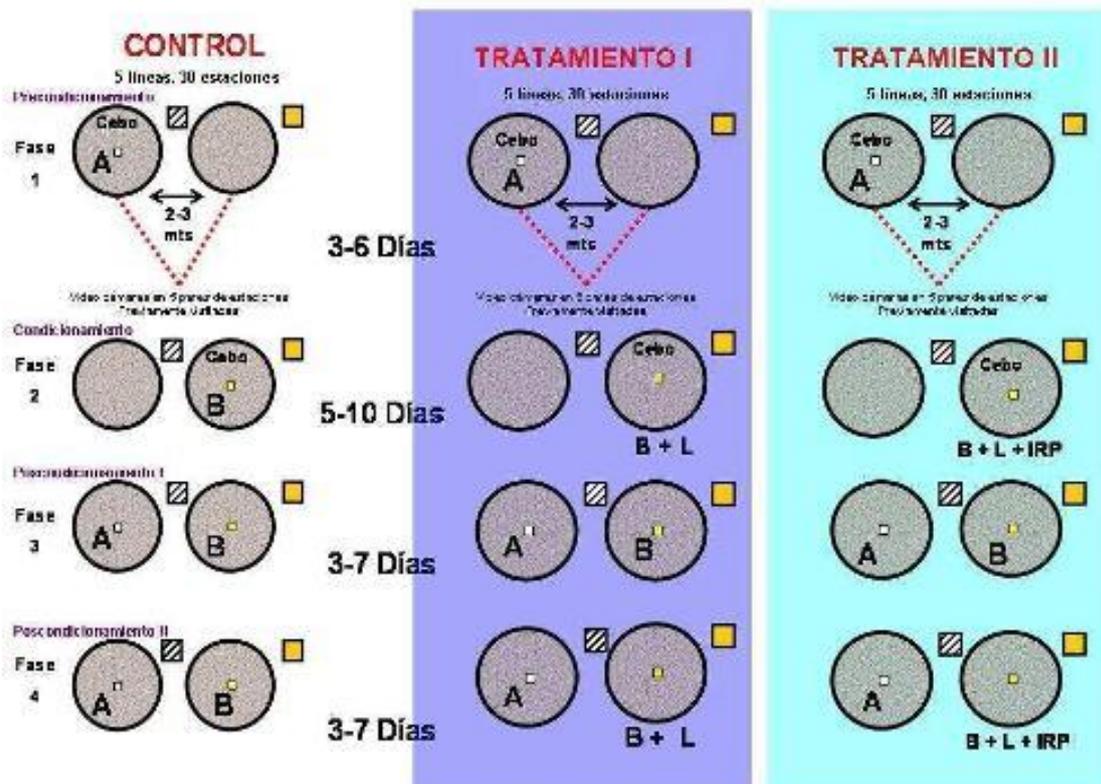
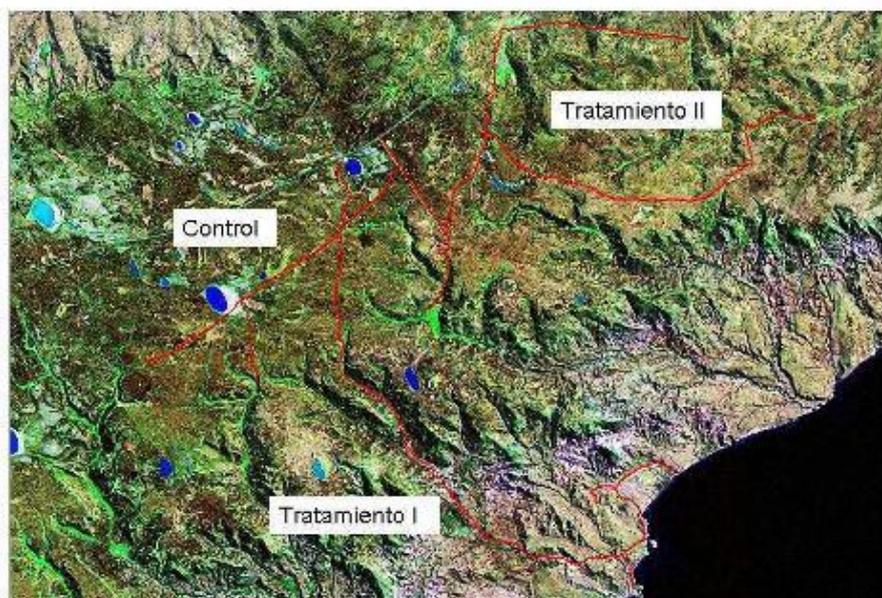


Figure 3. Experimental design of the CTA study at "Monte León National Park".

The bait-aversion study was conducted for 26 consecutive days in autumn 2010, from 2 to 27 March. The experiment consisted of one Control and two Treatments, each one with 5 transects of 6 double bait stations. A bait station consisted of a 1-m-diameter circle of smoothed earth with the bait placed at the centre, buried at depths of about 5 cm (Travaini et al. 2001). Each station was spaced 0.5 km apart and transects were at least 1 km apart (Figure 4).



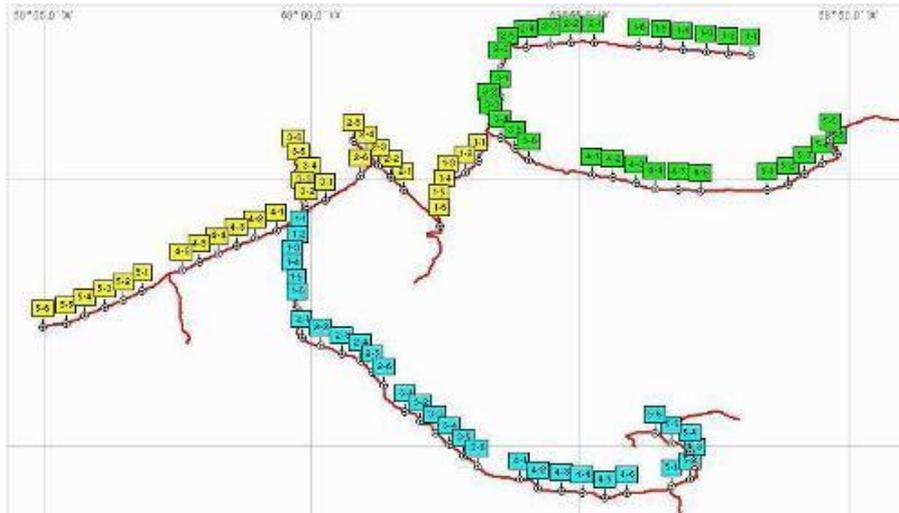


Figure 4. Landsat 7 view of the entire study area with internal road in red and schematic view of Control and Treatment Units along the same roads.

The second station was placed 2-3 meters besides the one previously described (Figure 5). Each transect was regarded as an independent sampling unit, and we assumed that individual foxes might visit more than one station within transect but would rarely encounter more than one transect/night. Stations were reviewed every morning, registering the species who visited, the pattern of visit based on footprints left and if also consumed the bait. We also recorded all identifiable behaviour based on marks or signs (scratch, urine and faeces) and footprints abundance were registered as Low (less than 15 footprints inside the station), Medium (between 15 and 30 footprints inside the station) or High (more than 30 footprints inside the station) (Figure 6). Any bait that disappeared was replaced. Finally, we conditioned the surface and the bait was replaced as indicated by the experimental protocol in each moment.



Figure 5. A pair of bait-stations separated by 2-3 mts. Six of these pairs conformed a transect, our sampling unit. We systematically used the station closer to the road to deliver bait A, the meat bait.



Figure 6. Bait-station with footprints of grey fox and signs of scratch.

Bait elaboration

Both baits, A and B weight about 30 grs. One was based on minced meat, animal fat, maize starch and a commercial trap lure called CAT PASSION (O'Gorman Enterprises, Inc.), called bait "A" (Figure 3,7). The other consists of dry pet food for domestic animals, raisins, and hydrogenated vegetable oil (Figure 3,8). Both baits contains no common ingredients to avoid that aversion to one type of bait affect the consumption of the other type.





Figure 7. Hand preparation of "A" baits



Figure 8. Elaboration of "B" baits

The compound used to generate aversion, levamisole hydrochloride, is an antiparasitic of wide use in veterinary, which produced emesis in domestic canids with a 54 mg/kg dose (Thienpont et al. 1966). We used a dose of 70 mg/kg, the same that caused learned repellence in wild red foxes (*Vulpes vulpes*) (Massei et al. 2003, Gentle et al. 2004). Total aversive incorporated into each bait must covers the dosage to provoke emesis in a fox weight of 9-10 kg, corresponding to an adult Red Fox. We decided to use half baits in cases which we have no doubt that the station was visited only by a Grey Fox, 3-4 kg for an adult (Travaini et al. 2001).

Statistical analysis

We performed generalized linear models (GLMs) to analyze (1) the effect of treatment and phases on the proportion of baits eaten (the contrast were made inside each phase and between Treatment and Control by bait type), (2) abundance of footprints among control and both treatments by bait type to determine if there were differences in the use of the bait-stations, and (3) urine marks left inside the bait station.

Were excluded from the analysis data from culpeo foxes because they only visit 0.1 % of the bait-stations. Destroyed stations by wind or rain, and one line from Treatment I and one from Treatment II were also excluded because they were close to the control and have the same pattern of behaviour opposite to the rest of the treatment lines.

All analysis was performed using R 2.11.0 (R Development Core Team 2010).

Preliminary Results

Visitation rates were 0.1% and 76%, for the Culpeo and Grey foxes, respectively, of a total of 2230 operative stations. Grey foxes visited the station without consuming the bait in only 8.3% of the occasions opposite to culpeo foxes with 69.2% stations visited without consuming the bait.

Conditioned taste aversion

No differences in meat bait "A" uptake were observed between treatments and control groups in any of the three phases where it was placed (Figure 9).

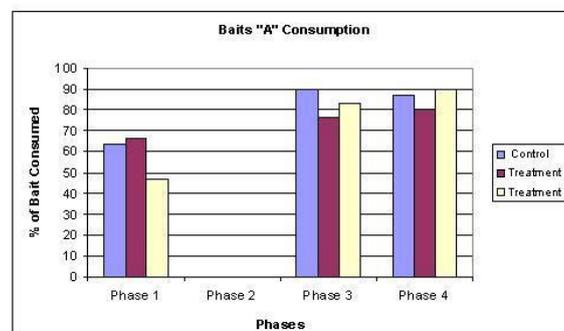


Figure 9. Percentage of meat baits "A" consumed in the experiment phases by treatment I (TI) and II (TII) and control (averages across transects, n = 5 for both treatments and control). Phase 1, pre-conditioning; Phase 2, conditioning; Phase 3, post-conditioning-part one; Phase 4, post-conditioning part two. Levamisole was added to meat baits during Phase 2 and Phase 4.

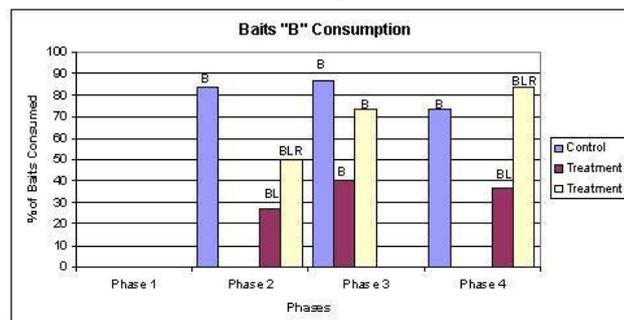


Figure 10. Percentage of bait "B" consumption in each experimental phase by treatment I (TI), II (TII) and control (averages across transects, n = 5 for both treatments and control). Phase 1, pre-conditioning; Phase 2, conditioning; Phase 3, post-conditioning-part one; Phase 4, post-

conditioning part two. Levamisole was added to meat baits during Phase 2 and Phase 4. Baits "B" were pet food untreated baits, baits "BL" were pet food baits with levamisole and baits "BLR" were pet food baits with the levamisole masked into a resin.

During Phase 2, in both Treatment I and Treatment II consumption of baits "B" were less than Control (Fig. 10, $z=-3.105$, $p<0.01$ for Treatment I and $z=-1.564$, $p>0.01$ for Treatment II). The lack of significance in Treatment II could be because small sample size. In Phase 3, when foxes were offered baits "A" and "B", the results were different between treatment I and II. Foxes in treatment I consumed less baits "B" than in control ($z=-2.363$, $p<0.05$) but there were no difference in consumption of baits "A" ($z=-0.100$, $p>0.05$). In treatment II there was no difference with the control for both types of baits (Fig. 9 and 10, $z=-0.326$, $p>0.05$ for baits "A" and $z=-0.577$, $p>0.05$ for baits "B"). These results indicated that foxes in Treatment I acquired an aversion to untreated baits but not the ones in Treatment II.

In Phase 4, when levamisole-treated baits "BL" were re-offered to foxes in Treatment I, they eat less baits "B" than control ($z=-2.337$, $p<0.05$). Baits with the levamisole masked by the resin "BLR" were eaten even a little more than control and there were no statistical difference ($z=0.437$, $p>0.05$). In the same phase, the consumption of meat baits "A" did not differ between treatment and control groups ($z=0.003$, $p>0.1$) (Fig.9 and 10).

Use of the bait station

During control there were more abundance of footprints than during any of both treatments ($z=-6.2$, $d.f.=14$, $p<0.01$) (Figure 11). This could indicate that they spent more time in the bait-station or visit it more than one/night.

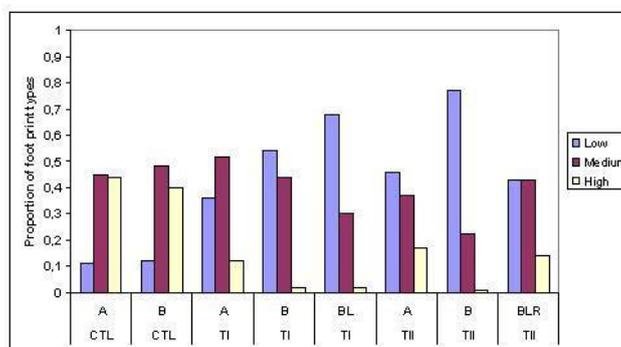


Figure 11. Abundance of footprints by treatments and bait type.

Urine marks

The urine marked of bait stations also showed differences between control and treatments ($t=2.37$, $p<0.05$ for Treatment I and $t=2.87$, $p<0.05$ for Treatment II) and this difference was mainly during phase 4 ($t=3.72$, $p<0.01$). In this phase, during treatment I, there were significant differences between stations with baits "A" and stations with treated baits "BL" ($t=-2.293$, $p<0.05$), with less "BL" bait stations urined (12 urine marks in "A" bait stations and 1 in "BL" bait stations). This difference was not found in treatment II ($t=-0.743$, $p>0.05$), where the urine marked of the bait stations were similar and high compared with "BL" bait stations (23 urine marks in "A" bait stations and 20 in "BLR" bait stations).

Discussion

The results indicated that grey fox can acquired conditioned taste aversion to baits with unmasked levamisole in pet food baits (Figure 8) as was found by Massei et al. (2003) with captive foxes *Vulpes vulpes*. After a single portion of treated meat bait, strong longlasting CTA was induced. Previous studies found the opposite, in ferrets (*Mustela putorius*) and free-living foxes and badgers (*Meles meles*). Massei 2003, Gentle 2004 and Cagnacci 2005 viewed that animals could detect the levamisole and therefore acquire repellence to the chemical rather than aversion to the food itself. More experimentation in canids is needed to reach a conclusion.

In treatment II, with the resinate, against our expectations, we found that grey fox have repellence to resinate and not acquired CTA to untreated pet food baits like found in rats Cotterill et al. (2006) when the taste of levamisole was masked in a biscuit bait using the IRP- 64 resinate. As was showed in the results, in phase 2 bait consumption was less than control but in phase 3 the consumption rise again. In phase 4 the consumption was higher than control. This last result could be because the foxes stole the baits and leave them near the station. During the last part of the experiment we observed that and registered it in five occasions during Treatment II and sixteen occasions during Treatment I, but there's no doubt that there were more stolen baits than we registered. We suspect that one of the problems in Treatment II was that the proportion of resinate in treated baits was high and affected its palatability. May be also that foxes avoid consumed masked levamisole treated-baits based on visual cues. All foxes in the first day of revision of the second phase of Treatment II consumed baits and 60% more than the half of baits available and we found no significant differences in consumption between Treatment II and Control in that day ($z=-0.8$, $p>0.05$).

One possible explanation in the differences found in bait station use, is that in control all stations had "good food" and foxes explore more the stations searching for extra food. Instead, in treatment stations, they not always found "good food" so they reduce searching for more.

About urine marked results we speculate that they were used to indicate "good food", because during phase 4 the number of urine marks were higher in bait "A" stations compared to bait "B" stations in treatment I. In treatment II there were no differences between stations. Also as the urine mark increased during phases in both treatment and control, also indicated a place were find food daily.

Conclusion

We generated conditioned taste aversion toward baits treated with levamisole and repellence with the levamisole masked within the resin. There was high variability between individuals behaviour, for example, many foxes took the bait but not consumed it and left it near the bait station (in some occasions we found it even 10 meters from the station). Camera traps would be useful to identify bait consumption at the bait station from those just taken away. In future experiments we have to put more distance between Treatments and Control, because the two treatment lines nearer control seemed affected by control protocol.

Acknowledgements

We want to thank APN for their permission to perform field trials inside the protected area. We also want to thank all the staff from Monte León National Park for their interest and permanent help. Many thanks to Marcela Pinedo, from the "Instituto de Investigaciones Biológicas", FCEyN, UNMdP, for her assistance with the resine.

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Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

We were unable to prepare the resin for ourselves so that took more time than initially considered. Now we have the elements to prepare it for ourselves.

Nevertheless, we must also get some help from the lab at the Universidad de Mar del Plata for the chemical quantification of the amount of Levamisol effectively trapped by the resin. This is a variable parameter that must be evaluated in order to properly dose the baits.

Culpeo foxes were at very low densities at Monte León National Park, so we must search for another area with higher densities of this species. We already have an additional study area, the Monumento Natural Bosques Petrificados, where we can try for this species.

Levamisol masked within the resin is quite bulky, so to deliver emetic doses we should prepare quite big baits. We are now trying to prepare microcapsules of Levamisol. This could be a good option if economically affordable.

Briefly describe the three most important outcomes of your project.

Levamisol produced aversion in grey foxes so it could be used to protect the species from poisoning, as was our initial objective.

Aversion should be considered in future experiment to reduce sheep predation by foxes, as was demonstrated for Coyotes in California.

Individual variability should be considered in future experiments and bigger samples sizes should be incorporated in the experimental design.

Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant). Are there any plans to continue this work?

This is only an initial trial that should continue with new experiments, including the generation of aversion to sheep. Some neighbour ranchers were interested in our work.

How do you plan to share the results of your work with others?

A copy of the poster with this results presented at a national mammalogist meeting in Argentina will be available for download. We are also preparing a manuscript for publication with the results presented in this preliminary report.

Timescale: Over what period was the RSG used? How does this compare to the anticipated or actual length of the project?

Our trials were done accordingly to the timescale originally presented to RSG. Nevertheless we have found that one of the two fox species were almost absent from our study area. We plan to repeat the trials in another protected area, with Culpeo foxes, as well as make the first trials with microencapsulated Levamisol, accordingly to remnant funds.