Applied Animal Behaviour Science 163 (2015) 167–174

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Conditioned taste aversion in the grey fox (*Pseudalopex griseus*), in Southern Argentine Patagonia

Sigrid Nielsen[a,b,*], Alejandro Travaini[a,b], Aldo Iván Vassallo[c,b], Diego Procopio[a], Sonia Cristina Zapata[a]

[a] *Centro de Investigaciones Puerto Deseado, Universidad Nacional de la Patagonia Austral, Avenida Prefectura Naval S/N, 9050 Puerto Deseado, Santa Cruz, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, C1033AAJ Buenos Aires, Argentina*
[c] *Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, B7602AYJ Mar del Plata, Argentina*

ARTICLE INFO

Article history:
Accepted 11 December 2014
Available online 23 December 2014

Keywords:
Behaviour
Conditioned taste aversion
Levamisole
Masking agent
Patagonia
Pseudalopex

ABSTRACT

Attempts to control cougar (*Puma concolor*) and culpeo fox (*Pseudalopex culpaeus*) populations in Patagonia through poisoning, negatively affects grey foxes (*Pseudalopex griseus*) and many other non-target species. It is therefore advisable to develop selective predator control methods. Among a wide range of potential methods, are those based on manipulating the behaviour of non-target species. Conditioned taste aversion (CTA) develops when an animal associates taste with subsequent illness, and, after recovery, avoids the referent taste. Food aversions can be potentially useful in efforts to reduce losses of non-target species when lethal baits are distributed to control other predators. We evaluated two ways to add the aversion agent Levamisole hydrochloride to a bait to generate CTA in grey foxes. We established one control (without Levamisole) and two treatments: plain Levamisole and an ion-exchange resin complex (resinate: Amberlite® IRP-64) aiming to mask the taste of Levamisole. Foxes that ate plain Levamisole baits, ate fewer baits than those in control (26.7% plain Levamisole baits vs. 83.3% untreated baits, $P = 0.002$) even after plain Levamisole was no longer present in the baits (40.0% vs. 86.6% untreated baits, treatment and control respectively, $P = 0.007$). Foxes that ate Levamisole resinate baits, also ate fewer baits than those in control (50.0% Levamisole resinate baits vs. 83.3% untreated baits, $P = 0.043$) but then resumed consumption when baits no longer contained the Levamisole resinate (73.3% vs. 86.6% untreated baits, treatment and control respectively, $P = 0.329$). Thus, foxes that failed to detect the plain Levamisole in baits acquired CTA to the bait. Contrary to our expectations, foxes detected the Levamisole resinate, associated illness with it and so resumed eating baits as soon as the resinate was withdrawn. Additionally, in the control, where foxes were familiar with untreated “safe” baits, we repeated the treatment with Levamisole resinate baits to test latent inhibition (decrement in conditioning to a stimulus as a result of its prior exposure). We found that treated bait consumption did not decrease as before, during a similar number of days (76.6% Levamisole resinate baits vs. 83.3% untreated baits, $P = 0.631$), suggesting that latent inhibition prevented development of CTA within our experimental time period. CTA induced among grey foxes that consume plain Levamisole baits might spare them from being poisoned in attempts to control other species of predators.

* Corresponding author at: Avenida Prefectura Naval S/N, 9050 Puerto Deseado, Santa Cruz, Argentina. Tel.: +54 297 4870511; fax: +54 297 4870511.
E-mail address: sigridnielsen@yahoo.com.ar (S. Nielsen).

http://dx.doi.org/10.1016/j.applanim.2014.12.006
0168-1591/© 2015 Published by Elsevier B.V.

1. Introduction

Learned aversion to bait, historically referred to as 'bait shyness', occurs when an animal ingests a sub-lethal dose of a toxin added to bait and thereafter refuses to consume the same type of bait (e.g. Gustavson et al., 1974; Morgan et al., 1996; Massei et al., 2003a; Cagnacci et al., 2005). When animals do not detect the illness-causing agent, they form a conditioned taste aversion (CTA) to the bait itself and so avoid the baits even when they no longer contain the aversion agent (García et al., 1974; Cagnacci et al., 2005). On the other hand, when they detect the toxin, they associate it with illness and tend to avoid only baits containing the toxin (García et al., 1974; Cagnacci et al., 2005). CTA may be weaker if the flavour associated with illness is familiar rather than novel. This may be due to a process known as latent inhibition (LI). LI therefore refers to a decrement in conditioning to a stimulus as a result of its prior exposure (Roman et al., 2008; Lubow, 2009).

The aversion agent used to generate CTA should produce severe short-term illness, the effective dose 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 et al., 1989). Many substances are known to induce severe, short-term illness in a wide range of animals (e.g. Reynolds, 1999; Cowan et al., 2000). However, only few of them can be successfully hidden in baits while retaining the taste and smell of target food. Levamisole hydrochloride (Levamisole) is used as an anthelmintic agent and causes nausea, emesis and gastro-intestinal discomfort in many mammals and birds (Remington, 1975; Budavari, 1996). It is a safe and stable chemical that induces a robust and long-lasting CTA in laboratory rats (*Rattus norvegicus*), when its taste is 'masked' by oral gavage (Massei and Cowan, 2002), and in red foxes (*Vulpes vulpes*) by embedding a treated core of meat within plain minced meat (Massei et al., 2003a). Levamisole is soluble in water and is said to have a bitter taste which could be detectable (Cotterill et al., 2006). We expected that to be more successful as a CTA agent in the field, its taste should be masked through the formation of a resinate using an acidic ion-exchange Amberlite® IRP-64 resin or by microencapsulation (Cotterill et al., 2006). We chose the resin because it is easier to prepare, more economic and stable in the mouth. Microencapsulation though an effective method, is an expensive and complex process (Cotterill et al., 2006) that has been poorly tested in animals (Burns, 1983).

Conditioned taste aversion can be applied in diverse situations to reduce predatory attacks upon livestock and in conservation of endangered species. Captive and field research has indicated that large predators such as wolves (*Canis lupus*) and coyotes (*Canis latrans*) that form CTA by eating lithium-treated meat baits subsequently avoid not only baits whether they contain lithium or not, but also live prey with the same taste and scent as the baits (Gustavson et al., 1974; Nicolaus et al., 1983). In the last few years, CTA has been also applied successfully to protect many species. For example, CTA was used to protect eggs of the threatened hooded plover (*Thnornis rubricollis*) from red foxes (Maguire et al., 2010). Also it was used to prevent northern quolls (*Dasyurus hallucatus*, O'Donnell et al., 2010) and blue-tongued skinks (*Tiliqua scincoides intermedia*, Price-Rees et al., 2013) from consuming a toxic invader, the cane toad (*Rhinella marina*).

In Patagonia, culpeo fox (*Pseudalopex culpaeus*) control started over a century ago (García Brea et al., 2010), but using non-selective methods, affecting many non-target species. More recently, bait-delivered control agents were tested in wild foxes from Santa Cruz Province (Travaini et al., 2001), and accepted as an effective method by patagonian sheep ranchers (Travaini et al., 2000).

There are two closely related fox species (Wayne et al., 1989) distributed from Ecuador to Tierra del Fuego Province, Argentina (Redford and Eisenberg, 1992): the larger culpeo fox (Novaro, 1997) and the smaller grey fox *Pseudalopex griseus* (Duran et al., 1985). Both species are sympatric in Santa Cruz Province (Johnson et al., 1996). In this work we focused on the grey fox, which is killed when it consumes poisoned baits intended to control culpeo foxes and cougars (*Puma concolor*) (García Brea et al., 2010). Since the grey fox is an omnivorous generalist canid with a home range between 103.5 ha and 279.9 ha (Silva-Rodríguez et al., 2010) and so is usually at higher densities than culpeo fox and cougar, it seemed an ideal subject for CTA research.

The aims of the present study were to (1) induce CTA in grey foxes using Levamisole as the aversion agent, (2) compare the efficacy of two methods for administering Levamisole: plain Levamisole and an ion-exchange resin complex (resinate) to mask the taste of Levamisole, and (3) evaluate the existence of LI through an observable delay in conditioning. Our main hypothesis was that foxes that detect the plain Levamisole should discriminate between baits lacking Levamisole and those containing it, by returning to eat baits once they find that the illness-inducing Levamisole is no longer present in the baits. If resin effectively masked the flavour of the Levamisole, then foxes could only associate illness with the flavour of the bait itself and so should continue to avoid baits even when they no longer contain the masked aversion agent.

2. Material and methods

2.1. Study area

We conducted the study at "Monte León National Park", in Santa Cruz Province, southern Argentina (50.35° S, 69.20° W). It comprised 62,700 ha of grasslands and shrub lands, included in the Patagonian phytogeographic Province (León et al., 1998).

2.2. Materials

Levamisole hydrochloride was obtained from Parafarm® (Nanchang, Jlangxi, China), and Amberlite® IRP-64 ion-exchange resin was obtained from Sigma–Aldrich Chemical (Gillingham, Dorset, UK).

2.3. Experimental design

We performed the bait-aversion study for 26 consecutive days during the early autumn 2010. We based the experimental design on the work of Cagnacci et al. (2005), with one control and two treatments. We established 15 transects with six pairs of bait stations spaced 2–3 m apart each, divided into three groups of five transects each: control, Treatment I (TI) and Treatment II (TII). We randomly assigned each group of transects to one of the three treatments. Control consisted of "safe" baits without the aversion agent, to determine baseline consumption, Treatment I consisted of plain Levamisole treated baits and in Treatment II the Levamisole was masked with resin. Additionally, at the same transects as control, we repeated Treatment II placing baits also with Levamisole resinate to test LI and we identified this as Treatment III (TIII).

Bait stations consisted of a circle of 1 m diameter of smooth earth or sand, with the bait placed at the centre, buried 5 cm deep. Transects consisted of six pairs of bait stations 500 m apart, and they were at least 1000 m apart from the next transect. We situated each pair of bait stations 10 m beside a secondary road. Within each pair, bait stations were 2–3 m apart. Each transect was considered as an independent sample unit since we assume that, based on grey foxes home range (Silva-Rodríguez et al., 2010), an individual fox could visit more than a pair of bait stations from the same transect, but would rarely encounter more than one transect during a single night.

To keep track of the consumption throughout the experiment and given that if aversion is generated it will be to a specific food, we used two types of baits, designed as "A" and "B". We placed baits "A" always in the nearest bait station from the road and baits "B" in the other bait station of the pair. Not interchanging baits between stations was done to prevent their contamination because of odour or particles of the bait left in it. All baits were of equal size, weighed 15 g and designed to be attractive only to foxes (Travaini et al., 2001). Previous experiments showed that there was no preference between the two baits. Bait "A" was based on minced meat (92.7%), animal fat (4.6%), maize starch (2.2%) and a commercial trap lure called Cat Passion (O'Gorman Enterprises Inc., Broadus, MT, USA) (0.5%). Bait "B" consisted of chicken based dry pet food for domestic animals (39.7%), raisins (39.7%) and hydrogenated vegetable oil (20.6%). The two baits had no common ingredients to avoid aversion to one type of bait affecting consumption of the other. We used bait "B" alone or with the aversion agent, which could be added directly to the bait (15 g bait, containing 324 mg of Levamisole, "BL") or masked through the formation of a Levamisole resinate complex using an ion-exchange resin (Amberlite® IRP-64, Cotterill et al., 2006) (15 g bait treated with Levamisole resinate, 4.5 g, containing 324 mg of Levamisole, "BLR"). We used a 70 mg/kg dose of Levamisole, as has been previously used for red foxes (Gentle et al., 2004).

The experiment consisted of four phases (see design in Fig. 1):

Pre-Conditioning: In the nearest bait station from the road of each pair, bait "A" was placed in control and in each of the treatments, while the other station remained without any bait. This phase lasted for 3–6 days in order to attract foxes, allow them to become familiar with bait stations and establish a baseline consumption.

Conditioning: Following Pre-Conditioning, bait "B" was placed in the farthest bait station from the road of each pair (bait "B" in control, "BL" in TI, "BLR" in TII and TIII) for 5–10 days, leaving the other station without bait.

Hypothesis (a). After initial high consumption, foxes would then consume fewer treated baits "BL" and "BLR" as compared with control baits "B".

Post-Conditioning 1: For 3–7 days both types of untreated baits ("A" and "B") were placed on the same site where they had been placed in previous phases, baits "A" on the nearest bait station from the road and "B" on the other one.

Hypothesis (b). If Levamisole induces CTA, foxes should consume fewer untreated bait "B" compared with the control sample but should continue to consume bait "A".

Post-Conditioning 2: For 1 to 2 days, in control transects we placed baits "A" and "B" and in treatment transects we placed baits "A" and the corresponding treated bait "BL" or "BLR".

Hypothesis (c). If foxes in treatments showed a decrease in consumption of treated baits in Conditioning (bait "BL" or "BLR") and untreated baits in Post-Conditioning 1 (bait "B") it would indicate that they acquired CTA and at Post-Conditioning 2 would be expected to follow a similar pattern to Conditioning.

Hypothesis (d). If foxes detected the Levamisole, in Post-Conditioning 2 they would consume fewer treated baits ("BL" or "BLR") than baits "A" and fewer treated baits ("BL" or "BLR") than baits "B" in control.

We checked bait stations every morning, tracking which species has visited the station and consumed the bait. We assumed that the bait was consumed by the species whose tracks were inside the bait station. Culpeo and grey foxes live in sympatry in the study area but their tracks were easily differentiated by size, culpeo having bigger tracks than grey fox (Travaini et al., 2001). It is possible that foxes removed the bait and cache it rather than consuming it, but in our experience of 16 years of activating similar bait stations in Patagonia, foxes mostly eat the bait at the bait station or near it. Finally, we erased the tracks and add more sand when necessary, before replacing the bait following the experimental protocol previously described.

2.4. Statistical analyses

To examine if Levamisole produced CTA or not, we used a generalized linear model (GLM) to analyze the effect of treatment and phases on the proportion of baits eaten during the final Pre-Conditioning and Conditioning day, and the first Post-Conditioning 1 and 2 day, because during these days behavioural responses were strong in each experimental phase. The model included treatments, experimental phases and bait type as explanatory variables (three factors with four, four and two levels, respectively), and

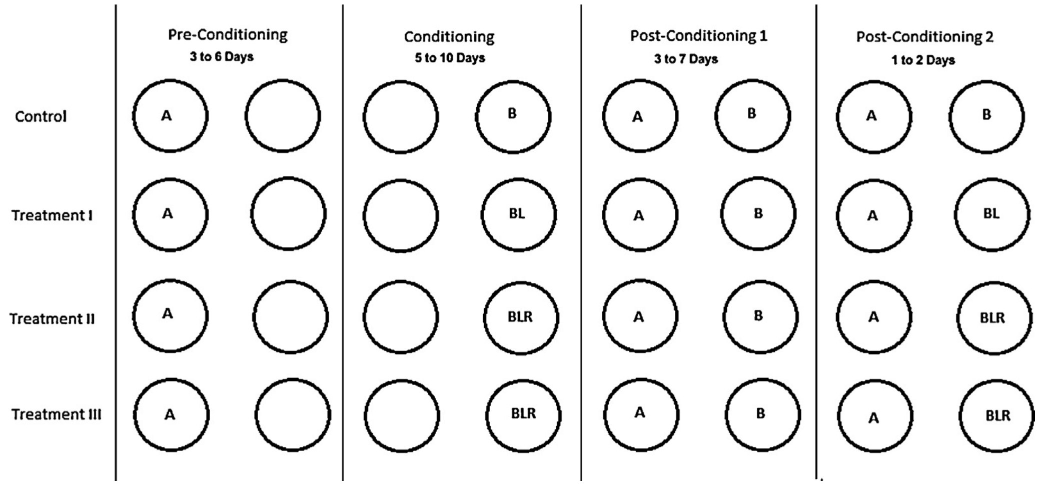

Fig. 1. The experimental design of the bait-aversion study at Monte León National Park. Baits "A" were meat baits, baits "B" were untreated dry baits, baits "BL" were baits with plain Levamisole and baits "BLR" were baits with the Levamisole masked with Amberlite® IRP-64.

baits consumed as response variables (converted to proportions with respect to the total bait stations throughout the study). A quasibinomial error structure and logit link function was used for the response variable (Crawley, 2007).

Model fitting was visually assessed by inspecting plots of standardized deviance residuals for each model. We assessed goodness of fit for all models and estimated the variance inflation factor ($\hat{c}$) as residual deviance divided by degrees of freedom (Burnham and Anderson, 1998). The alpha value was set at 0.05. All analysis was performed using R 2.11.0 (R Development Core Team, 2010).

3. Results

Grey fox visitation rate was 76% for the 20 experimental units, with a total of 2230 operative night stations during the whole experiment. By their tracks, we also identified the presence of other species to our stations, such as cougar, skunk (*Conepatus humboldtii*), european hare (*Lepus europaeus*), guanaco (*Lama guanicoe*), dwarf armadillo (*Zaedyus pichiy*) and lesser rhea (*Pterocnemia pennata*). However, none of them ever consumed any bait, since it was intact in the stations with tracks that did not belong to foxes. We found 25 uneaten baits up to 5.2 m away from the bait station, using a constant searching effort. Twenty four were Levamisole treated baits (10 plain and 14 masked Levamisole baits) and only one was an untreated dry pet food bait but belonging to Post-Conditioning 1 from Treatment I.

We saw no differences in meat bait "A" uptake between control and treatments transects in any of the three phases where it was placed (GLM, $n=5$ for each phase, per treatment. Pre-Conditioning: control vs. TI: $t=0.215$ [$P=0.830$], vs. TII: $t=-1.002$ [$P=0.319$] and vs. TIII: $t=0.012$ [$P=0.991$]. Post-Conditioning 1: control vs. TI: $t=-1.044$ [$P=0.299$], vs. TII: $t=-0.597$ [$P=0.552$], and vs. TIII: $t=-0.020$ [$P=0.984$]. Post-Conditioning 2: control vs. TI: $t=-0.534$ [$P=0.595$], vs. TII: $t=0.281$ [$P=0.779$], and vs. TIII: $t=0.998$ [$P=0.321$]). Also, bait uptake was high, indicating that there were no differences in base consumption between places throughout the entire experiment (Fig. 2).

3.1. Hypothesis (a) and (b)

During Conditioning, in Treatment I, consumption of baits "BL" was fewer than baits "B" in control (GLM, $t=-3.176$, $P=0.002$, $n=5$ per treatment, Fig. 3). In this phase there was a reduction of percentage of baits "BL" consumed from 30.0% (day 1) to 13.3% (day 6) (Fig. 4), lower than the average consumption of baits "A" during Pre-Conditioning (67.7%, Fig. 2). During Post-Conditioning 1, in Treatment I, when foxes were offered baits "A" and "B", they consumed fewer baits "B" than baits "B" in control (GLM, $t=-2.712$, $P=0.007$, $n=5$ per treatment Fig. 3) and there was no difference in consumption of baits "A" (Fig. 2). These results indicated that foxes acquired an aversion to untreated dry pet food baits "B".

In Treatment II, during Conditioning, consumption of baits "BLR" was lower than baits "B" in control (GLM, $t=-2.045$, $P=0.043$, $n=5$ per treatment, Fig. 3). During this phase, the consumption of baits "BLR" has a maximum at day 5 and then low again reaching 30% of baits consumed at day 6 (Fig. 4). In Post-Conditioning 1, there was no difference with control for both types of baits (GLM; $t=-0.597$, $P=0.552$ for baits "A" (Fig. 2) and $t=-0.980$, $P=0.329$ for baits "B", $n=5$ (Fig. 3)). These results indicated that foxes had not acquired an aversion to untreated dry pet food baits "B".

In Treatment III, during Conditioning, the consumption of baits "BLR" was fewer than of baits "B" in control but with no statistical difference (GLM, $t=-0.481$, $P=0.631$, $n=5$ per treatment) contrary to the results found in Treatment II. In Post-Conditioning 1, there was no difference in bait consumption (GLM; $t=-0.020$, $P=0.984$ for baits "A" and $t=1.011$, $P=0.314$ for baits "B", $n=5$) and it was higher than the other phases of the treatment. These results indicated that foxes had not acquired an aversion to untreated dry pet food baits "B".

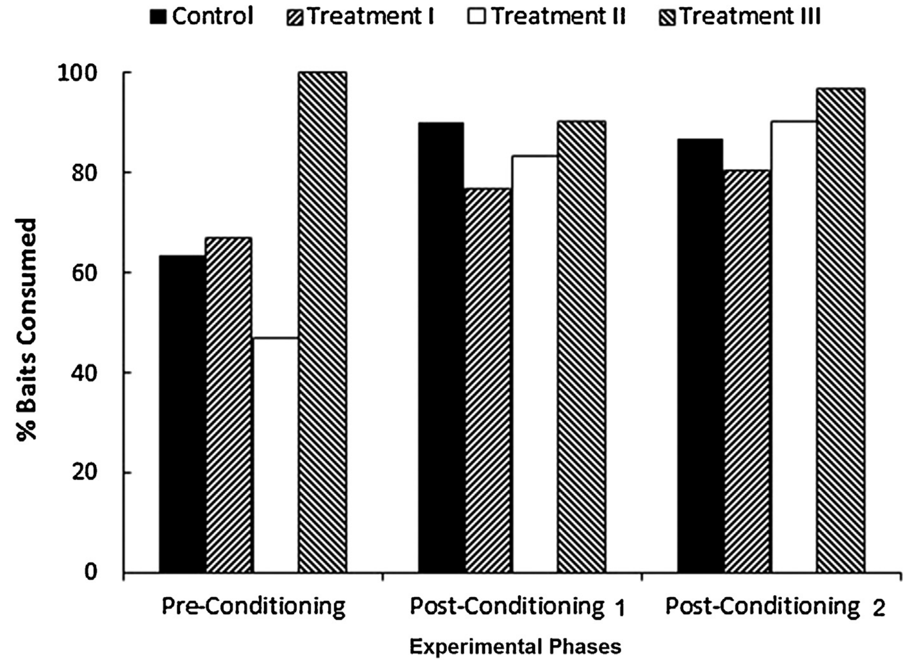

Fig. 2. Consumption of untreated meat baits "A" during control (with paired dry pet food bait "B", without Levamisole) and treatments (with appropriate paired dry pet food bait "B": Treatment I with plain Levamisole, Treatment II with Levamisole masked with resin and Treatment III with Levamisole masked with resin using a familiar bait). Only during Pre-Conditioning meat baits "A" were placed alone. Averages across experimental units, sample size $n = 5$ for control and treatments. There were no differences in bait "A" consumption between control and treatments throughout the entire experiment.

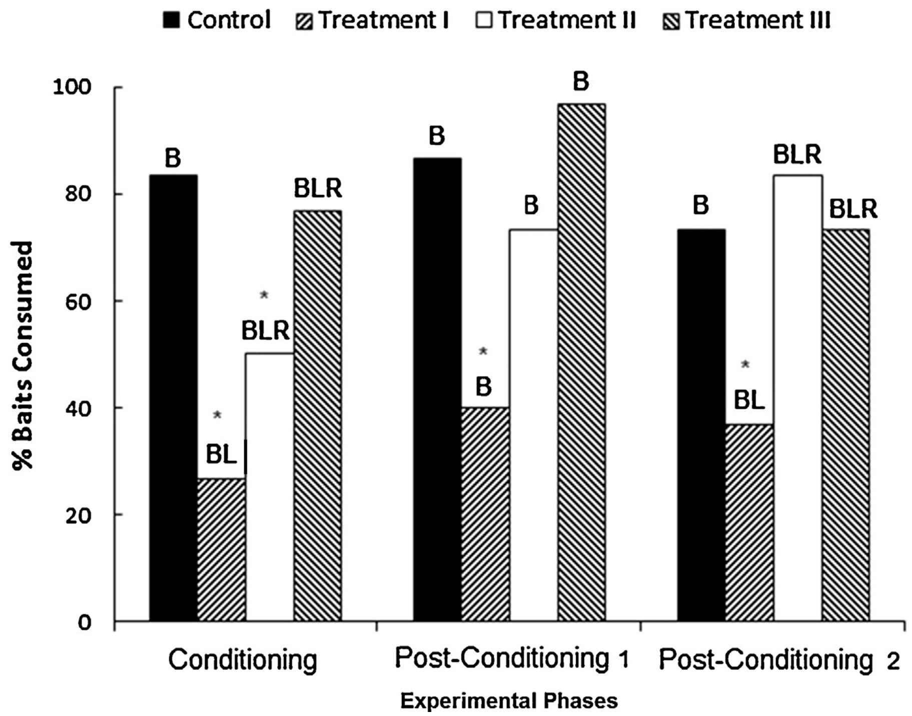

Fig. 3. Consumption of dry pet food bait "B" during control (without Levamisole), Treatment I (plain Levamisole), II (Levamisole masked with resin), and III (Levamisole masked with resin using a familiar bait). Averages across experimental units, sample size $n = 5$ for control and treatments. Baits "B" were untreated baits, baits "BL" were baits with plain Levamisole and baits "BLR" were baits with the Levamisole masked with resin. $^{*}P < 0.05$.

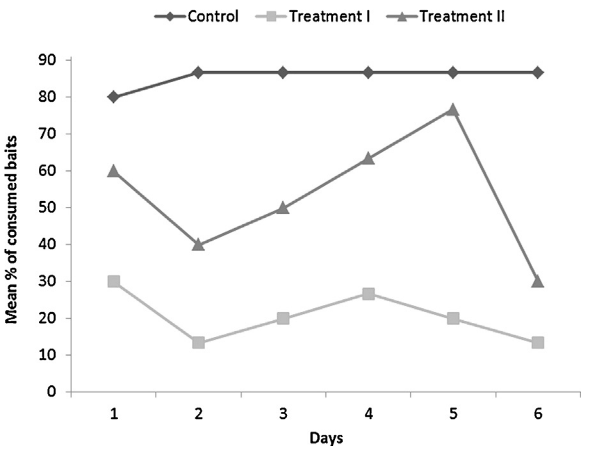

Fig. 4. Consumption of dry pet food bait "B" during the first 6 days of Conditioning, during control (without Levamisole), Treatment I (plain Levamisole) and II (Levamisole masked with resin). Averages across experimental units, sample size $n = 5$ for control and treatments.

3.2. Hypothesis (c) and (d)

During Post-Conditioning 2, in Treatment I, when baits "A" and "BL" were re-offered, foxes ate fewer baits "BL" than baits "B" in control (GLM, $t = -2.163$, $P = 0.030$, $n = 5$ per treatment, Fig. 3), but the consumption of baits "A" did not differ between treatment and control transects (Fig. 2). In Treatment II, baits "BLR" were eaten even a little more than baits "B" in control but there were no statistical differences (GLM, $t = 0.726$, $P = 0.469$, $n = 5$ per treatment). The results of this phase confirm that foxes in Treatment I acquired a CTA to untreated dry pet food baits but not in Treatment II. In Treatment III, consumption of bait "BLR" was similar to bait "B" in control transects (GLM, $t = -7.42\text{e-}16$, $P = 0.973$, $n = 5$ per treatment). This result join with the results of the other phases indicated that foxes in Treatment III acquired neither aversion to the bait itself nor to the Levamisole resinate in the period of time we evaluated.

4. Discussion

Grey foxes acquired conditioned taste aversion to untreated dry pet food baits ("B") based on baits treated with plain Levamisole ("BL"). This conclusion is supported by the fact that during Conditioning there was a reduction in plain Levamisole treated bait consumption and this lower consumption, compared to control, was maintained for the next 5 days even when plain Levamisole was not present in the bait. The observed decrement in the consumption of plain Levamisole treated dry pet food baits in the present study cannot be attributed to a 'bait-site avoidance' (Dimmick and Nicolaus, 1990; Ogilvie et al., 2000), as throughout all phases foxes visited the bait stations (there were foxes' tracks inside them) with treated baits regardless of whether they consumed the bait or not. Massei et al. (2003a) found that after a single portion of Levamisole treated meat bait (beef-flavoured minced turkey) captive red foxes acquired strong long-lasting CTA. On the other hand, ferrets (*Mustela furo*) in a study with Levamisole treated meat baits (roast-chicken flavoured minced turkey) (Massei et al., 2003b), free-living red foxes with Levamisole treated meat baits (day-old chickens) (Gentle et al., 2004), and Eurasian badgers (*Meles meles* L.) with Levamisole treated meat baits (minced beef mixed with porcine skin gelatine) (Cagnacci et al., 2005), could detect plain Levamisole and therefore acquired aversion to the chemical rather than aversion to the food itself. One possible explanation for these diverse results is the type of food used to make the bait and the absolute amount of Levamisole relative to the mass of the bait material.

With the masked Levamisole, we found that grey foxes detected the Levamisole resinate and did not acquired CTA to dry pet food baits, contrary to what we expected and differing to what Cotterill et al. (2006) found. They showed that the taste of Levamisole could be masked in a biscuit bait using the same resin as used by us and that rats could acquire CTA to untreated baits. The effects we found were demonstrated during Conditioning, when treated bait ("BLR") consumption was lower than control, and in the other two phases the consumption rose again, being higher than control. We suspect that the proportion of resinate in Levamisole treated baits (0.3 bait weight) was high, so foxes could differentiate the baits by their scent and/or palatability, and avoided consuming masked Levamisole treated baits. The Levamisole resinate is a voluminous white powder that in the mix with dry pet food resulted in a whitish bait that could be visually differentiated from untreated baits ("B"). Those differences not only in colour, but perhaps in taste and scent of the bait were because we used a relatively small bait of 15 g to ensure that it would be entirely consumed in a single visit. A smaller proportion of Levamisole resinate such as that used by Cagnacci et al. (2005), 0.01 bait weight, may be undetectable and generate CTA in grey foxes.

In Treatment III, our results were consistent with LI, since the foxes had become familiar with untreated "safe" baits "B" before we offered the Levamisole resinate treated ones. There was a high rate of consumption from the beginning of baits "A" and baits "B", with no differences in any phases between treatment and control transects showing that they did not acquire aversion to the bait itself or to the Levamisole resinate. One reason for the failure to acquire an aversion may have been because the bait was familiar to the foxes. Since it takes longer to acquire an aversion to a familiar food we could try extending the conditioning phase (with treated baits) until we saw a significant decline in consumption before further testing for CTA. De la Casa and Lubow (2000) examined conditioned taste aversion with rats showing LI as a function of time interval between conditioning and test phases. They found that even after 21 days of Conditioning phase, the pre-exposure group had a high consumption compared to the non-pre-exposure group. We only had approximately 7 days of Conditioning phase and there was no indication of a decline in consumption so we might have to extend this phase many days or a few weeks in order to fully test whether a CTA develops.

Our results may have been conservative, since we found that foxes stole baits and left them away from the bait station. We found some of these, but certainly there may have been others and it is possible that some stolen baits might have been left where we did not find them since they have similar colour to the environment or because of caching (Saunders et al., 1999). This may have led us to overestimate the number of dry pet food baits eaten by foxes. However, that would only make the levels of aversion and repellence found much stronger.

Future field experiments testing CTA as a management tool for wild non-target populations are justified. CTA could be used to deal with two different concerns related particularly to livestock farms in Patagonia. First, it could be used in resident foxes in national parks to prevent them from being poisoned by generating CTA to the baits used by neighbouring ranchers (for example, chicken eggs) to poison culpeo foxes and cougars in their fields. Second, it could be used to generate CTA to livestock using beef hides retaining the typical taste and scent of domestic livestock prey on the outer surfaces wrapped securely around ground meat. This was indicated by Ellins et al. (1977) in coyotes, inhibiting predation between two sheep herds using sheep carcasses laced with lithium chloride, adjacent to the herds. Since Levamisole may be safer and easier to work with than lithium chloride and our results indicate that it produced reliable aversion among grey foxes, it might be a worthy addition as a management tool for wild populations. In the implementation, LI would need to be considered if the bait material used is familiar to the target animal.

5. Conclusion

Free-ranging grey foxes that consumed baits containing plain Levamisole formed aversion to the bait material, refusing to consume the baits further even after they no longer contained the aversion agent. However, foxes that consumed baits containing Levamisole resinate avoided the baits as long as the Levamisole resinate was present but resumed consumption as soon as it was no longer present. The result of this study encourages future research on CTA as a promising tool to mitigate wildlife-livestock conflicts and to improve selectivity in predator control programmes.

Conflict of interest

I declare that any author of this manuscript have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

Acknowledgments

Funds were provided by The Rufford Small Grant Fundation, the Universidad Nacional de la Patagonia Austral (PI 29B/119) and CONICET (PIP 0646). Also Sigrid Nielsen has a PhD scholarship (CONICET). We are very grateful to Administración de Parques Nacionales for their permission and support. We thank Pablo Rosso, Lorena Martínez, Marina Martínez, Leonardo Martínez, Cristian Vellido, Rubén Sosa and Cecilia Ernst for their field support within the protected area. We also thank Lowell K. Nicolaus for his valuable comments on the manuscript.

References

Budavari, S.E., 1996. The Merck Index: An Encyclopaedia of Chemicals, Drugs, and Biologicals, 12th ed. Merck & Co, Whitehouse Station.

Burnham, K.P., Anderson, D.R., 1998. Model Selection and Inference: A Practical Information-Theoretic Approach. Springer-Verlag, New York, New York, USA.

Burns, R.J., 1983. Coyote predation aversion with lithium chloride: management implications and comments. Wildl. Soc. Bull. 11, 128–133.

Cagnacci, F., Massei, G., Cowan, D.P., Delahay, R.J., 2005. Can learned aversion bee used to control bait uptake by Eurasian badgers? Appl. Anim. Behav. Sci. 92, 159–168.

Cotterill, J., Massei, G., Cowan, D.P., 2006. Masking the taste of the conditioned taste aversion agent Levamisole using an ion-exchange resin, for practical application in wildlife management. Pest Manage. Sci. 62 (2), 120–125.

Cowan, D.P., Reynolds, J.C., Gill, E.L., 2000. Reducing predation through conditioned taste aversion. In: Cosling, L.M., Sutherland, W.J. (Eds.), Conservation Biology Series 2: Behaviour and Conservation. Cambridge University Press, New York, pp. 281–299.

Crawley, M.J., 2007. The R Book. J. Wiley, Chichester, pp. 950.

De la Casa, L.G., Lubow, R.E., 2000. Super-latent inhibition with delayed conditioned taste aversion testing. Anim. Learn. Behav. 28 (4), 389–399.

Dimmick, C.R., Nicolaus, L.K., 1990. Efficiency of conditioned aversion on reducing predation by crows. J. Appl. Ecol. 27, 200–209.

Duran, J.C., Cattan, P.E., Yañez, J.L., 1985. The grey fox *Canis griseus* in Chilean Patagonia (Southern Chile). Biol. Conserv. 34, 141–148.

Ellins, S.R., Catalano, S.M., Schechinger, S.A., 1977. Conditioned taste aversion: a field application to coyote predation on sheep. Behav. Biol. 20 (1), 91–95.

García Brea, A., Zapata, S.C., Procopio, D.E., Martínez Peck, R., Travaini, A., 2010. Evaluación del interés de productores ganaderos en el control selectivo y eficiente de predadores en la Patagonia austral. Acta Zool. Mex. (n.s.) 26 (2), 303–321 (in Spanish).

García, J., Hankins, W.G., Rusiniak, K., 1974. Behavioural regulation of the milieu interne in man and rat. Science 185, 824–831.

Gentle, M., Massei, G., Saunders, G., 2004. Levamisole can reduce bait monopolization in wild foxes *Vulpes vulpes*. Mammal Rev. 34, 325–330.

Gustavson, C.R., Garcia, J., Hankins, W.G., Rusiniak, K.W., 1974. Coyote predation control by aversive conditioning. Science (Washington) 184, 581–583.

Johnson, W.E., Fuller, T.K., Franklin, W.L., 1996. Sympatry in canids: a review and assessment. In: Gittleman, J.L. (Ed.), Carnivore Behaviour, Ecology and Evolution. Cornell University Press, Ithaca, New York, USA, pp. 189–218.

León, R.J.C., Bran, D., Collantes, M., Paruelo, J.M., Soriano, A., 1998. Grandes unidades de vegetación de la Patagonia extra andina. In: Oesterheld, M., Aguiar, M.R., Paruelo, J.M. (Eds.), Ecosistemas patagónicos. Ecol. Austral., vol. 8, pp. 75–308 (in Spanish).

Lubow, R.E., 2009. Conditioned taste aversion and latent inhibition: a review. In: Reilly, S., Schachtman, T. (Eds.), Conditioned Taste Aversion: Behavioural and Neural Processes. Oxford University Press, Oxford, pp. 37–57.

Maguire, G.S., Stojanovic, D., Weston, M.A., 2010. Conditioned taste aversion reduces fox depredation on model eggs on beaches. Wildl. Res. 36 (8), 702–708.

Massei, G., Cowan, D.P., 2002. Strength and persistence of conditioned taste aversion agents in rats: evaluation of eleven potential compounds. Appl. Anim. Behav. Sci. 75, 249–260.

Massei, G., Lyon, A.J., Cowan, D.P., 2003a. Levamisol can induce conditioned taste aversion in foxes. Wildl. Res. 30, 633–637.

Massei, G., Lyon, A.J., Cowan, D.P., 2003b. Potential compounds for inducing conditioned taste aversion in ferrets. New Zeal. J. Zool. 30, 95–100.

Morgan, D.R., Morriss, G., Hickling, G.J., 1996. Induced 1080 bait-shyness in captive brushtail possums and implication for management. Wildl. Res. 23, 207–211.

Nicolaus, L.K., Cassel, J.F., Carlson, R.B., Gustavson, C.R., 1983. Taste aversion conditioning of crows to control predation on eggs. Science 220, 212–214.

Nicolaus, L.K., Farmer, P.V., Gustavson, C.R., Gustavson, J.C., 1989. The potential of estrogen-based conditioned aversion in controlling depredation: a step closer toward the "magic bullet". Appl. Anim. Behav. Sci. 23, 1–14.

Novaro, A.J., 1997. *Pseudalopex culpaeus*. Mamm. Sp. 558, 1–8.
O'Donnell, S., Webb, J.K., Shine, R., 2010. Conditioned taste aversion enhances the survival of an endangered predator imperiled by a toxic invader. J. Appl. Ecol. 47, 558–565.
Ogilvie, S.C., Thomas, M.D., Morriss, G., Morgan, D.R., Eason, C.T., 2000. Investigation of sodium mono-fluoroacetate (1080) bait shyness in wild brushtail possum (*Trichosurus vulpecula*) populations. Int. J. Pest Manage. 46, 77–80.
Price-Rees, S.J., Webb, J.K., Shine, R., 2013. Reducing the impact of a toxic invader by inducing taste aversion in an imperiled native reptile predator. Anim. Conserv. 16, 386–394.
R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, http://www.R-project.org
Redford, K.H., Eisenberg, J.F., 1992. Mammals of the Neotropics, vol. 2. The Southern Cone, The University of Chicago Press, Chicago.
Remington, J., 1975. Remington's Pharmaceutical Sciences, 15th ed. PA Mack, Easton.
Reynolds, J.C., 1999. The potential for exploiting taste aversion (CTA) in wildlife management. In: Cowand, D.P., Feare, C.J. (Eds.), Advances in Vertebrate Pest Management. Filander Verlag, Fürth, pp. 267–282.
Roman, C., Lin, J.Y., Reilly, S., 2008. Conditioned taste aversion and latent inhibition following extensive taste preexposure in rats with insular cortex lesions. Brain Res. 1259, 68–73.
Saunders, G., Kay, B., McLeod, L., 1999. Caching of baits by foxes (*Vulpes vulpes*) on agricultural lands. Wildl. Res. 26, 335–340.
Silva-Rodríguez, E.A., Ortega-Solís, G.R., Jiménez, J.E., 2010. Conservation and ecological implications of the use of space by chilla foxes and free-ranging dogs in a human-dominated landscape in southern Chile. Austral Ecol. 35, 765–777.
Travaini, A., Zapata, S.C., Martínez-Peck, R., Delibes, M., 2000. Percepción y actitud humana hacia la predación de ganado ovino por el zorro Colorado (*Pseudalopex culpaeus*) en Santa Cruz, Patagonia Argentina. Mastozoología Neotropical 7, 117–129 [In Spanish.].
Travaini, A., Martínez-Peck, R., Zapata, S.C., 2001. Selection of odor attractants and meat delivery methods to control Culpeo foxes (*Pseudalopex culpaeus*) in Patagonia. Wildl. Soc. Bull. 29, 1089–1096.
Wayne, R.K., Kat, P.W., Fuller, T.K., Van Valkenburgh, B., O'Brien, S.J., 1989. Genetic and morphologic divergence among sympatric canids (Mammalia: Carnivora). J Heredity 80, 447–454.