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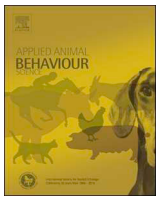

Using conditioned taste aversion to reduce human-nonhuman primate conflict: A comparison of four potentially illness-inducing drugs

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ABSTRACT

Human-wildlife conflict in the form of crop- and livestock depredation is escalating worldwide and many species of nonhuman primates are considered serious crop pests throughout areas within their ranges that humans inhabit. Animals become habituated to many non-lethal mitigation strategies, which then become ineffective at reducing crop-foraging intensities by nonhuman primates, so people have turned to culling to reduce crop losses. An example of this problem is primate crop depredation in northern India, where rhesus macaques (*Macaca mulatta*) have been declared vermin. Conditioned Taste Aversion (CTA) develops when humans and nonhuman animals associate the taste and odor of food with post-consumption illness and results in subsequent refusal to consume the food associated with illness. The length of time that the food is avoided indicates the aversion's strength. CTA can be induced deliberately when food is paired with a drug that causes nausea. Thus, exploiting CTA could be a non-lethal and effective method to control crop damage caused by vertebrate pests. We tested four drugs on 88 rhesus macaques to assess their ability to induce a CTA and determine safe and effective doses. Our results suggest that fenbendazole, an anthelminthic drug with a high margin of safety, is ineffective. A similar drug, levamisole also was ineffective, as the monkeys detected it during the acquisition phase. However, we were able to create aversions using thiabendazole, another anthelminthic, and 17 alpha-ethynyl estradiol (EE). Once a dose appropriate to induce a CTA was determined, EE demonstrated a success rate of 86 %, and thiabendazole 46 %. Both drugs have strengths and weaknesses. Only a small dose of EE (25 mg/kg of body weight) was required to induce a CTA, which can be concealed in a small amount of food. However, it is a synthetic hormone, so access to the drug should be limited, and its distribution in the environment controlled. Thiabendazole required a considerably higher dose (160 mg/kg of body weight) to establish a CTA and may be a greater challenge to conceal. Nonetheless, both drugs appeared to go undetected in these tests and could be used with mild baits, e.g., wheat and corn/maize. We urge continued conditioned taste aversion studies across species to reduce crop damage.

1. Introduction

Conditioned taste aversion (CTA) is an evolved defense mechanism found in all vertebrates that prevents an organism from being poisoned fatally (Bernstein, 1999; Cohn and MacPhail, 1996; Conover, 1995; Sinclair and Bird, 1984). An animal can acquire a CTA when it associates the taste of a food with nausea and post-consumption illness and subsequently refuses to eat that food (Garcia et al., 1974, 1955; Gustavson et al., 1974). CTA can also be induced deliberately by adding an undetectable, illness-inducing compound to food and animals potentially can acquire an aversion in a single trial (Gustavson, 1977; Nachman and Ashe, 1973; Nicolaus et al., 1989a, 1989b). When this type of learning occurs, animals do not associate the illness with a particular place or time, but only with the *taste* and *odor* of the offending food item. Five key neurobiological properties are associated with strong aversions: 1) the interval between consumption and illness (typically 30–60 min: (Garcia and Koelling, 1966); 2) the taste of the food (particularly those that are unfamiliar or novel: Garcia et al., 1974); 3) the illness symptoms (the more severe, the stronger the aversion: Revusky, 1968); 4) potentiation by other food characteristics (odor, color, and texture: Brett et al., 1976), and 5) negligible difference in taste and odor between treated and untreated food items (Garcia and Kimeldorf, 1957).

The strength of the aversion acquired is a crucial element in inducing and maintaining a CTA successfully. The crux of creating a strong aversion is determining and administering a safe and effective illness-

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inducing drug dose. Forthman-Quick (1986, p. 265) suggested that an aversion's strength be assessed by the animal's behavior. In a weak aversion, animals eat a preferred food but show appetitive disgust behavior (sniffing, manipulating, rubbing, staring at, eating with retracted lips). Those with a moderate aversion take more than 15 min to eat a preferred food, while those with a strong aversion refuse to eat a preferred food and may even show some uneasiness while near the food.

CTA has been used effectively in wildlife conservation and management to reduce coyote and wolf predation on sheep (Ellins et al., 1977; Gustavson et al., 1976), raven and raccoon predation on eggs (Avery et al., 1995; Nicolaus and Nellis, 1987), and bear consumption of pre-prepared meals (MREs) that they sought out and consumed previously (Polson, 1983; Ternent and Garshelis, 1999). In these studies, a food item that the animals actively targeted was treated with an aversive compound, and after the animals consumed the treated food and became ill, they avoided the food item.

Several compounds have been tested to induce CTA in a variety of birds and mammals, including apomorphine (Chapman et al., 1998; Revusky and Bedarf, 1967; Wittlin and Brookshire, 1968); carbachol (Bogliani and Bellinato, 1998; Cox et al., 2004), cinnamamide (Gill et al., 1998), cyclophosphamide (Matsuzawa and Hasegawa, 1983), ethynyl estradiol (Forthman et al., 2005; Nicolaus et al., 1989a, 1989b; Semel and Nicolaus, 1992); levamisole (Massei et al., 2003a; Nielsen et al., 2015); lithium chloride (Burns, 1980; Ellins et al., 1977; Ellins and Catalano, 1980; Gustavson et al., 1976), and thiabendazole (Gustavson et al., 1983; Indigo et al., 2017; O'Donnell et al., 2010).

Human-wildlife conflict comes in many forms, one of the most continuous and long-standing of which is wildlife crop foraging. Many primate species across Africa and Asia are labeled as conflict animals, or even vermin, because of their proclivity to forage for crops (Anand et al., 2018; Lee and Priston, 2005). Humans continue to use strategies to reduce crop foraging that require human presence or use inanimate visual repellents and stationary noise-makers to which animals become habituated, even though these strategies are ineffective (Hockings, 2016). However, CTA is a strategy to which animals cannot habituate because associations between the taste and odor of a specific food and illness are thought to occur in the brain stem where learning takes place unconsciously (Bernstein et al., 1986; Borison and Wang, 1953). Hence, we proposed to test CTA as a conflict mitigation strategy for crop-foraging primates. We conducted our study on rhesus macaques (*Macaca mulatta*), a species that has been categorized as vermin in India because of its high level of crop depredation. The goals of our study were to: 1) identify compounds that can be used to induce a CTA in rhesus macaques; 2) determine a safe and effective dose of these compounds to induce a CTA in different age-classes of rhesus, and 3) improve our understanding of which food items can be used to establish a CTA (Fig. 1).

We tested the efficacy of three anthelmintics and one synthetic steroid on wild rhesus macaques. One drug, fenbendazole, had not been used previously to establish a CTA in *any* species and another, levamisole, had been trialed in other species but had never been tested on nonhuman primates. Ethynyl estradiol and thiabendazole had been used on other nonhuman primates previously, but not on rhesus macaques, so no effective dose to induce a CTA had been recommended for this species.

Based on published research, we predicted that establishing a CTA would be more straightforward in 1) strong-tasting foods, 2) juveniles and 3) compounds that required a smaller dose that could be concealed easily.

2. Materials and methods

2.1. Study subjects

We conducted our experiments on 101 rhesus macaques (88 experimental, 13 control) at the Baul Monkey Sterilization Center (BMSC), Una Division, Himachal Pradesh, India. The experimental subjects included 51 juveniles (27 males, 24 females), 27 sub-adults (18 males, 9 females), and 10 adults (8 males, 2 females). These monkeys were trapped in the wild and brought in for sterilization by the Himachal Pradesh Forestry Department. To minimize stress, the center's goal is for the monkeys to spend approximately one week at the sterilization center. Typically, they are observed for three days, sterilized, allowed to recover for three days, and then returned to the same location where they were captured. The monkeys initially were housed with troop members captured in the same location. When lactating females were trapped, they were housed with their infant and used as controls.

2.2. Experimental protocol

2.2.1. Processing

We conducted experiments only on healthy male and female juveniles, male and female sub-adults, and adult male and non-lactating adult female monkeys (Fig. 1). The Himachal Pradesh Forestry Department trapped the monkeys in the wild and brought them to the BMSC for sterilization. After they were brought in, staff members who routinely work with the monkeys estimated their weight and assessed their health. Before the experiments began, adults and juveniles chosen for the CTA study were housed singly to ensure that we could monitor food consumption and each monkey's behavior. We photographed all monkeys, documented any distinguishing characteristics, and recorded where each monkey had been captured.

The CTA experiments began before sterilization and we used one type of food bait per experiment (*e.g.*, wheat or corn/maize balls, bananas) (Fig. 2). We selected a control subject(s) for each experiment and this individual received an untreated food bait while test subjects received treated baits. After sedation for sterilization surgery, each monkey was weighed. This information was used to determine the actual drug dose given.

2.2.2. CTA acquisition-first day

We tested five different techniques with five different food items to administer potentially illness-inducing compounds to test subjects (captive and field setting). The technique we used depended on the amount of drug we needed to conceal, the food item's characteristics, and whether a previous trial suggested that the monkeys could detect it. We added the potentially aversive compounds to 1) approximately 100 g of corn/maize kernels or wheat grains that were held together with tasteless gelatin; 2) porridge made with ground wheat or corn/maize; 3) whole grains of wheat; 4) the surface of cut fruit and/or vegetables, and 5) a small amount of fruit that was removed from the whole fruit, and then reinserted (Fig. 2). The initial dose was based on the monkey's weight as estimated by the sterilization center. We also considered what dose was previously successful if it was used in other studies.

On the first day of the experiments, morning rations were withheld and we gave all experimental monkeys (except for the control) a treated food bait. Once the experiment began, all monkeys were monitored for signs of gastrointestinal distress (*e.g.*, vomiting, gagging, retching, restlessness: Andrews, 1992; Stadtländer et al., 1998). Water was available *ad lib.*, but no other food was given until the afternoon. Approximately five hours after the experiment began, monkeys were given food rations that did not include the item used in the experiment (e.g., garbanzo beans, fresh fruits, and vegetables).

2.2.3. CTA acquisition-second day

On the second day of the experiment, cages were cleaned and any remaining food given the previous afternoon was removed. To determine if the dose previously given created a food aversion, we offered test subjects the same food baits containing an increased drug dose. If the monkeys *refused* to eat the treated food bait, this demonstrated that they had potentially acquired some level of food aversion. They went on to the extinction phase, which tested whether food refusal was due to a conditioned taste aversion or if they detected the drug.

If the monkey *ate* the treated bait, this demonstrated that the previous dose was too low, so no taste aversion had been acquired. The following

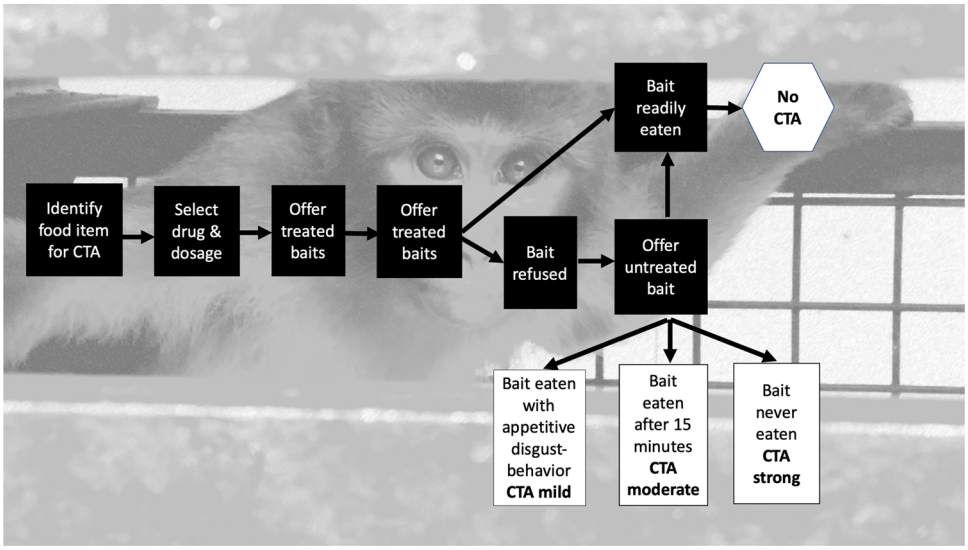

Fig. 1. A general conditioned taste aversion (CTA) flowchart describing the steps of a CTA experiment.

day, we offered these individuals the same food bait containing an increased dose. We monitored these individuals for illness symptoms and, if required, gave them a higher dose on the following day.

Monkeys were usually observed by the BMSC for three days prior to surgery, however, there were three occasions when monkeys were held longer than three days. If the first drug had failed to create a CTA, we trialed a second drug on 36/88 (41 %) monkeys. The second drug trial did not commence for at least 48 h after the first drug was given, which ensured that it was completely metabolized and excreted from the body.

2.2.4. Extinction trials

After acquisition trials, we tested subjects that had refused the *treated* food bait with an *untreated* food bait. This process determines how resistant the aversion is to "extinction" (consumption of the untreated food). Interaction with the food was documented to assess the strength of the aversion (Fig. 1). If the untreated food was quickly eaten, this suggested that the monkeys could detect the difference between treated vs. untreated.

Fig. 2. Potentially illness-inducing food baits: A) bananas; B) wheat balls; C) corn balls, and D) scattered wheat.

2.3. Potentially illness-inducing drugs

We tested four compounds that have been used previously to induce CTA in nonhuman primates (Forthman et al., 2005) and/or had been recommended for use in nonhuman primates (Mukaratirwa et al., 2008; Reichard et al., 2008). These drugs were relatively tasteless and nearly odorless compounds. We used pharmaceutical grade compounds obtained from Sigma-Aldrich/Merck Group (Bangalore, India). When given at high enough doses, all four drugs cause nausea; all doses given were below the LD50. Signs of nausea can be subtle or overt. We monitored the monkeys for overt symptoms of nausea, that included vomiting, gagging, retching, and restlessness (Andrews, 1992; Stadtländer et al., 1998).

We used the same criteria as Forthman-Quick (1986) to define an aversion's strength. In weak aversions, food eaten previously is still eaten, but animals exhibit some disgust behavior. In a moderate aversion, the bait is eaten after 15 min, and in the strongest aversion, the bait is never eaten. If an animal refuses to eat the treated food bait but eats the untreated food bait, this suggests that the monkey is able to detect the compound.

2.3.1. Ethynyl estradiol

17 alpha-ethynyl estradiol (EE) is a common synthetic hormone used in oral contraceptives that causes low acute and chronic toxicity. This drug is neither teratogenic nor does it have clastogenic properties. Published studies have demonstrated that long-term exposure to EE poses very few health risks to primates (Maier and Herman, 2001). EE has been used previously to induce CTAs in baboons (Forthman et al., 2005), raccoons (Semel and Nicolaus, 1992), and free-ranging predators (e.g., raccoons, skunks, opossums, red fox, badgers, and coyotes: L.K. Nicolaus et al., 1989a, 1989b). We conducted eight EE experiments on 43 captive monkeys using techniques 2, 3, and 5. We began with a dose of approximately 10 mg/kg body weight, and the largest dose given was 30 mg/kg body weight (Table 1).

2.3.2. Fenbendazole

Fenbendazole is a benzimidazole anthelmintic compound that is slightly soluble in water and active against roundworms (adults, larvae, and eggs) and tapeworms. This drug is prescribed for nonhuman primates and has a wide margin of safety (Plumb, 2002). It has not been used previously to establish a CTA in wild animals, but we trialed it because of a veterinarian's recommendation, cost, and availability. We conducted three fenbendazole experiments on 14 captive monkeys using technique 1. We began with a dose of approximately 125 mg/kg body weight, and the highest dose given was approximately 375 mg/kg body weight (Table 1).

2.3.3. Levamisole

Levamisole is an imidazothiazole that has anthelmintic and immunostimulating properties (Jacobs and Taylor, 2001). This water-soluble, relatively tasteless, and nearly odorless compound has been used to control badgers' consumption of oral baits (Cagnacci et al., 2005) and foxes' consumption of meat (Massei et al., 2003a; Nielsen et al., 2015), but has not been used with nonhuman primates. We conducted eight levamisole experiments on 30 captive monkeys using techniques 1, 2, 4, and 5. Because levamisole had not been used to establish a taste aversion in nonhuman primates, we began with a dose of 20 mg/kg body weight and increased it to 190 mg/kg body weight (Table 1).

2.3.4. Thiabendazole

Thiabendazole is a systemic benzimidazole anthelmintic compound that also possesses antifungal activity (Plumb, 2002). It is water-insoluble, odorless, and nearly tasteless, and has been used to establish a CTA in olive baboons (Forthman et al., 2005), black bears (Ternent and Garshelis, 1999), dingoes and wild dogs (Gustavson et al., 1983), laboratory rats (Massei and Cowan, 2002), wolves (Ziegler et al., 1983), and quolls (Indigo et al., 2017; O'Donnell et al., 2010). We conducted three thiabendazole experiments on 37 captive monkeys using techniques 3, 4, and 5. We began with a dose of 130 mg/kg body weight and increased it to 225 mg/kg body weight (Table 1).

Table 1
Captive rhesus macaque conditioned taste aversion (CTA) experiments using four drugs: fenbendazole (FBZ), levamisole (LV), ethynyl estradiol (EE), and thiabendazole (TBZ). Five techniques were used to add a "dose" of these potentially aversive compounds to crops damaged by monkeys: 1 = corn/maize kernels or wheat grains held together with gelatin; 2 = wheat grains held together with ground wheat porridge; 3 = whole wheat grains; 4 = surface of cut fruit and/or vegetables, and 5 = small amount of fruit removed, mixed and then reinserted.

Drug	Food item treated	Dose mg/kg BW	Technique	# Individuals tested	# Individuals acquiring a CTA (%)
FBZ	Wheat	125-187	1	8	0
	Wheat	245-336	1	8	0
	Wheat	346-377	1	7	0
LV	Wheat	21-23	2	4	0
	Wheat	28-38	2	7	1 (14)
	Wheat	54-68	2	4	0
	Wheat	* 83-90	2	4	0
	Wheat	* 63-77	1	12	0
	Wheat	* 63-77	2	12	1 (8)
	Banana	71-145	4	21	2 (9.5)
	Banana	88-174	5	21	2 (9.5)
EE	Wheat	12-23	2	4	1 (25)
	Wheat	23-27	2	4	2 (50)
	Wheat	27-32	2	4	2 (50)
	Wheat	* 21-26	2	2	0
	Wheat	17-25	3	4	4 (100)
	Eggplant	19-21	5	13	6 (46)
	Eggplant	19-30	5	13	11(85)
	Banana	23-25	5	7	7 (100)
TBZ	Wheat	130-152	3	5	1 (20)
	Banana	136-181	4	13	3 (23)
	Banana	133-225	5	7	7(100)
	Banana	177-184	5	13	12(92)

* Weight-underestimated.

2.4. Field trials

We tested EE, levamisole, and thiabendazole opportunistically in two rural villages in northern India. Treated baits were placed where adult monkeys traveled frequently: near a temple, on the roof of a house, on the steps of a water tower, and along a roadside. In particular, we targeted adult male bachelor groups. We offered five different food baits (corn/maize, wheat, pumpkin, and apple). We based the drug doses on average weights that we observed at the Baul Monkey Sterilization Center: juveniles, n = 59, 1.56–3.4 kgs, mean 2.25 kgs; sub-adults, n = 27, 2.66–5.5 kgs, mean 3.95 kgs; adult females, n = 6, 4.3–7.6 kgs, mean 5.7 kgs; adult males, n = 9, 5.46–11.28 kgs, mean 8.5 kgs. We used a dose that had created an aversion with trapped monkeys at the BMSC: 25 mg/kg body weight for 13 EE trials (wheat and corn, technique 1 and 4; apple, technique 4), 160 mg/kg body weight for two thiabendazole trials (wheat and corn, technique 1), and 190 mg/kg body weight for three levamisole trials (wheat, technique 1; pumpkin, technique 4).

2.5. Influence of Age/Sex class on CTA

The effects of age-class, sex, drug used, and CTA acquisition were investigated. We used Chi Square ($X2$) because our data was not normally distributed, and set alpha to 0.01. All statistical tests were conducted using Stata 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp, LLC).

2.6. Ethical clearance

Before beginning these experiments, we received ethical clearance from the National Institute of Advanced Studies in Bangalore and research permits from the Himachal Pradesh Forest Department. The veterinarian associated with the Sterilization Center assisted us in our experiments.

3. Results

We applied four aversive compounds to crops monkeys in India eat frequently. In these experiments, we tested 88 monkeys to determine if we could induce a conditioned taste aversion in a captive setting. We also trialed three aversive compounds in the field to determine if and how monkeys would interact with treated crops.

3.1. Signs of illness

During the CTA experiments, we monitored the monkeys for signs of illness. In total, we observed restlessness, dry retching, and vomiting five times (Table 2). The majority of those symptoms (4/5) occurred with levamisole.

3.2. Potentially illness-inducing compounds

3.2.1. Ethynyl estradiol (EE)

A juvenile male acquired the first aversion at a dose of 12 mg/kg body weight using technique 2. Most monkeys ate the entire treated bait (Fig. 3). Overall, we had a success rate of 21/43 (49 %: Table 1). Once we determined the correct dose, we had a success rate of 6/7 (86 %: Table 1).

3.2.2. Fenbendazole

We conducted three fenbendazole experiments with 14 different monkeys and were unable to induce any aversions (Table 1).

3.2.3. Levamisole

A juvenile male acquired the first aversion with Levamisole at a dose of 70 mg/kg body weight. As the dose increased, more monkeys ate only some or none of the treated food bait (Fig. 4). We achieved the best results with treatment 5 at a dose of 125 mg/kg body weight. Overall, we had a success rate of 3/30 (10 %: Table 1).

3.2.4. Thiabendazole

An adult female acquired the first aversion with Thiabendazole at a dose of 155 mg/kg body weight. Varying amounts of the baits were eaten according to the food and dose (Fig. 5). Overall, we had a success rate of 9/37 (24 %). Once we determined the correct dose, we had a success rate of 6/13 (46 %: Table 1).

3.2.5. Two drugs given

We offered 36 monkeys two different drugs. Of these, the recommended dose was consumed by 17/36 monkeys and 13/17 monkeys also consumed a second treated food bait suggesting that a CTA was not acquired. Of these monkeys, 5/13 received thiabendazole (164 – 205 mg/kg of body weight). For the additional 8/13 monkeys, their weight was under-estimated and the dose offered was less than the recommended dose.

Two monkeys received three increasing doses of EE (25 – 32 mg/kg of body weight). Even though these additional doses were equal or greater than recommended doses, the monkeys failed to acquire a CTA. Levamisole was given subsequently, it also failed to induce a CTA.

Table 2
Signs of illness observed during the conditioned taste aversion experiments.

Drug	Dose (mg/kg BW)	Symptom	Age class	Sex
Levamisole	20	restlessness	Juvenile	M
	60	restlessness	Juvenile	M
	83	restlessness	Juvenile	M
	69	dry retching	Juvenile	M
EE	25	vomiting	Juvenile	M

3.3. Field trials

We conducted field trials using EE, levamisole, and thiabendazole. We trialed EE eleven times in the field. Five adult monkeys readily ate baits treated with EE (technique 1 – wheat and cornball baits); however, two adult females and two adult males, picked up, smelled, and then refused similar baits. One sub-adult male ate treated cut fruit (technique 4) readily. Three adult males ate a portion of corn/maize on the cob (technique 5).

We trialed levamisole in the field three times with pumpkin (technique 5) and wheat balls (technique 1). An adult male smelled the pumpkin but then refused it. An adult female took a small bite, then refused the bait. Another adult male ate the bait but did so very slowly.

We trialed thiabendazole in the field twice, once with wheat balls (technique 1) and once with apples (technique 5). Because the amount of compound needed was large, we created three wheat balls to conceal the dose required to create a CTA. One adult male ate one of three treated wheat ball baits (technique 1) and refused the remaining two. One sub-adult male took and appeared to eat treated cut fruit (technique 5).

3.4. Influence of Age/Sex class on CTA

We found no significant difference between age classes and the likelihood of acquiring a CTA ($X2(2) = 3.1860$, $P = 0.203$). We also found no significant difference between sex and the likelihood of acquiring a CTA ($X2(1) = 0.0357$, $P = 0.850$. However, we found a significant difference between the drug used and the likelihood of acquiring a CTA ($X2(3) = 25.5477$, $P = 0.000$).

4. Discussion

Our results demonstrated that thiabendazole and ethynyl estradiol are effective at inducing food aversions in rhesus macaques but fenbendazole and levamisole are *not* effective. In the case of fenbendazole, despite giving large doses, we observed no signs of illness and were unable to establish an aversion in any of the monkeys. However, these experiments were carried out in a captive setting so results for free-ranging monkeys could vary.

Regarding levamisole, at a higher dose, required to establish an aversion, few monkeys ate the food bait as they apparently could detect it (Fig. 4). Interestingly, 4/5 (80 %) observations of gastrointestinal distress (retching, vomiting) occurred when we used levamisole (20 – 83 mg/kg of body weight) as the aversive agent. Common side-effects are nausea and diarrhea and less common are anxiety, nervousness, and vomiting (https://www.mayoclinic.org/drugs-supplements/levamisole-oral-route/side-effects/drg-20064492). Only four monkeys out of 30 (13 %) refused to eat *untreated* bananas during the extinction phase. Similarly, free-ranging monkeys smelled and then refused food crops treated with this drug. We suggest that levamisole is not an effective drug for CTA as it was also detected by foxes (Nielsen et al., 2015), ferrets (Massei et al., 2003b), and badgers (Cagnacci et al., 2005).

We were able to establish food aversions in rhesus macaques using EE and thiabendazole. Thiabendazole is considerably cheaper than is EE, but a relatively large dose is required to induce an aversion in heavier sub-adults and adults. We found this to be the case for an adult male that weighed 10 kg, as approximately 1.5 g of thiabendazole was required to induce a CTA and it was not possible to conceal that much compound in one food bait (e.g., one banana or one wheat ball).

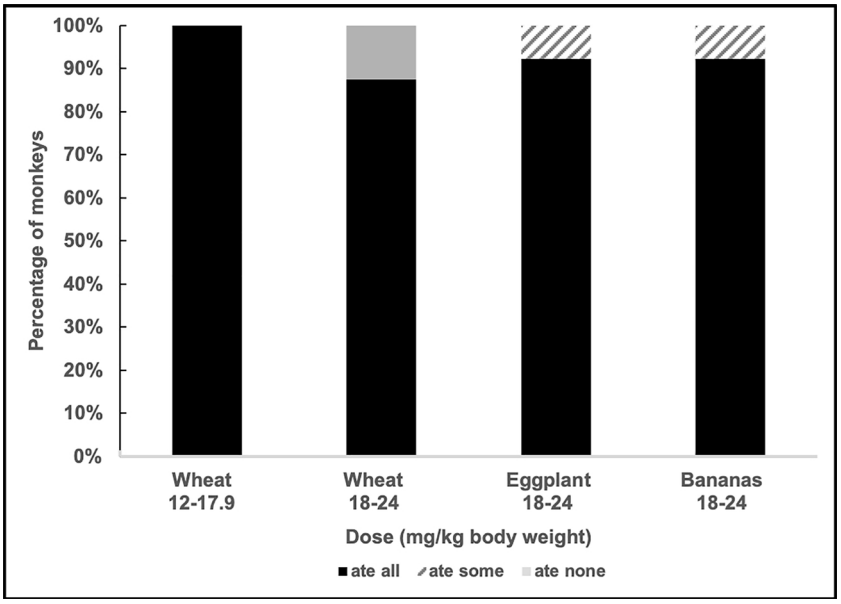

Fig. 3. The percentage of monkeys that ate all, some, or none of wheat, eggplant, and banana baits treated with ethynyl estradiol after the first acquisition day.

However, it was possible for juveniles that weighed 2 kg, as only 0.3 g was required and this amount of drug could be concealed in a single food bait.

Again, a single food bait containing the entire dose required to create a CTA is ideal. In a field setting, animals will typically "grab and go" leaving with one food bait. In our field trials, we observed that when multiple baits are required only one of the baits was consumed. In these instances, the individual would not have experienced gastrointestinal distress nor acquired a CTA for that food. This supports our prediction that compounds requiring a smaller dose are easier to conceal and beneficial in CTA acquisition.

Only a very small amount of EE was required to induce a food aversion. For example, an adult male that weighed 10 kg required approximately 250 mg of EE. In a captive setting, we observed that once an aversion was acquired with EE, the monkey left the item alone and would not eat it even if there was nothing else to eat (supplemental video). In one case, an *untreated* banana that remained in the cage from the previous day's trial went untouched for more than a day. Several monkeys refused even to touch the untreated banana, and others turned their backs on the untreated food item (supplemental video). In these instances, we used the correct dose in the *first* trial.

In comparison, some monkeys that had been treated with thiabendazole refused the untreated banana initially but ate it after 15 min, which suggests that they acquired a moderate aversion (Fig. 1). As mentioned, using the correct EE dose on the first trial appears critical. In four other trials, we used an EE dose that was too low but increased the dose in subsequent trials to a correct concentration and none of these monkeys acquired a CTA.

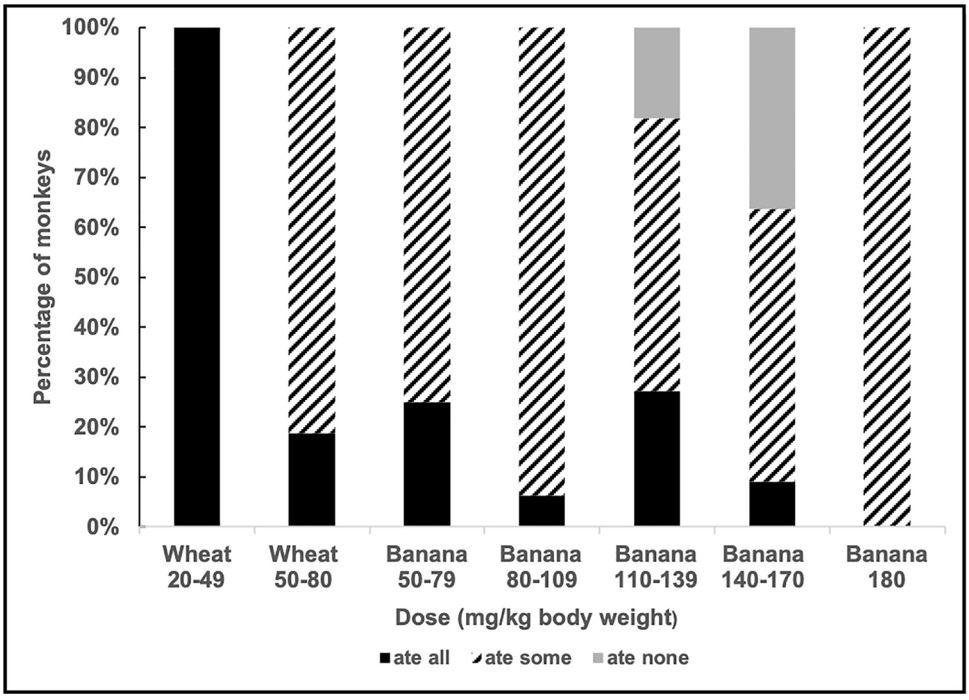

Fig. 4. The percentage of monkeys that ate all, some, or none of wheat and banana baits treated with levamisole after the first acquisition day.

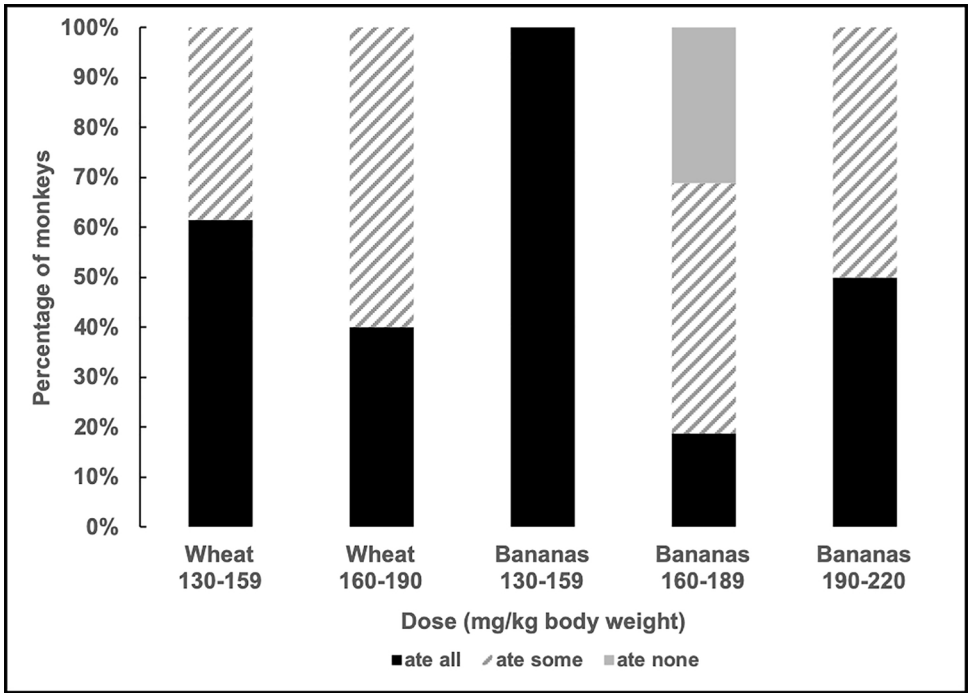

Fig. 5. The percentage of monkeys that ate all, some, or none of wheat and banana baits treated with several doses of thiabendazole after the first acquisition day.

Other disadvantages of EE are its cost and that it is a synthetic hormone that potentially could affect reproduction (Forthman et al., 2005). However, doses of EE required to create a taste aversion should produce only transient effects on reproduction. A previous CTA study conducted on baboons observed no disruption in mating behavior (consorting and copulating) among treated animals (Forthman et al., 2005). More research must be done to determine whether EE causes spontaneous abortions, disrupts lactation, and increases the probability of birth defects in pregnant females (Physician's Desk Reference Staff, 2000). To reduce non-target species' consumption of food baits, animals could be trapped, offered the treated food bait, marked if possible, and then released. In the field, food treated with EE was usually eaten, but because these monkeys were not routinely followed, we were unable to conduct extinction trials.

In addition to the drug itself, the food bait's nature may play a role in inducing a strong CTA. It has been suggested that a homogenous food bait is important in establishing a CTA (Gaynor, pers. comm.). We saw evidence of appetitive disgust behavior with two food baits (peeled eggplant and coarsely ground wheat porridge). In the acquisition phase, all monkeys ate the treated baits readily on the first day, but on the second day, they ate the eggplant flesh, but avoided the seeds, and in the case of wheat porridge, tried to pick out and eat only the more intact grains. Manipulating the food indicates a weak aversion (Fig. 1) (Forthman-Quick, 1986).

Bananas were a more effective bait than was wheat with respect to thiabendazole, but they were unable to mask the taste of levamisole at a higher dose. These data provided mixed support for our prediction that using a strong-tasting food bait is beneficial in CTA acquisition. Both food preference and the aversive compound's negligible taste and odor also influence efficacy.

Our results suggest that age and sex did not influence CTA acquisition. This finding does not support our prediction that it would be more straightforward in juveniles. Nonetheless, because CTA works best when a food item is novel, it should be most effective for reducing crop foraging with juveniles before they begin to eat cultivated food items. Therefore, we suggest that field trials begin with a dose appropriate for juveniles. Once all juveniles have been treated, the dose could be increased for larger animals. One way to ensure that the dose is appropriate for the animal consuming it is to trap animals, weight, and give the food bait inside the trap. Further, individuals who direct the troop's movement could be targeted.

Aside from inducing an aversion in the individual, studies have demonstrated that aversions can be passed down to offspring (Cremona et al., 2017). Treated mothers may deny their offspring the opportunity to consume foods for which they have an aversion (Semel and Nicolaus, 1992), and even subadults may try to prevent their siblings from eating a bait (Forthman, unpublished data).

Based on our experiments, we recommend using EE and thiabendazole to create food aversions in monkeys (*Macaca* in particular). Between these two compounds, EE was more effective (Table 1) and far less compound was required, which made it easier to conceal in food baits. Other studies have demonstrated that CTAs induced with EE were more resistant to extinction than those induced with cinnamamide and thiabendazole (Gill et al., 2000).

One limitation of this study was that 70 % of the subject monkeys were juveniles as monkeys brought into the sterilization center largely were juveniles and subadults. Because a long-term program of trapping and sterilizing macaques is currently in place in Himachal Pradesh, adult individuals are trap-shy and are therefore trapped rarely. Our data analysis suggested that there was no difference between age-class and sex of monkeys in acquiring CTA. Future studies should retest this with a normally distributed population.

Conditioned taste aversion shows much promise as a conflict mitigation strategy, especially since it can potentially be passed to offspring. However, more research and field trials are needed to test new illness-inducing compounds, determine the best way to introduce treated food baits in a wild setting, and measure CTA's efficacy with monkeys in urban and rural settings. EE and thiabendazole went undetected, but levamisole did not. Regardless of an appropriate dose, our field trials suggested that the willingness to consume treated baits will vary from one individual to another. Future studies will need to include testing individuals after they consume treated baits in their CTA protocol, as well as extinction trials to determine the duration of aversions. Inducing food aversions requires careful planning, but it is potentially a strategy to which animals cannot habituate. Thus, we urge researchers to conduct more food aversion studies across species to reduce crop damage.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2020.104948.

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