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Liquid Fertility Management Bait Uptake by Urban Rats within New York City Subway Refuse Rooms

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ABSTRACT: Traditional rodent management tools, such as traps and lethal rodenticides, are acute measures to reduce commensal rodent populations. Given the growing concerns regarding effectiveness, environmental safety, and animal welfare related to these tools, it has become imperative to find new methods. Fertility control is an emerging potential alternative to these tools as a safe, humane and effective method of long-term population management. SenesTech, Inc. has developed a liquid fertility management bait that causes follicle depletion in the ovaries of female Sprague Dawley rats and compromises sperm production in male Sprague Dawley rats in laboratory settings. These studies have shown significant decreases in litter sizes following bait consumption, but acceptance of this bait by wild rodents needed to be confirmed. Bait acceptance was tested within refuse rooms of the New York City subway system. Bait was provided *ad libitum* for 90 days. Uptake was evaluated by examining, via fluorescence microscopy, the presence of the bait marker rhodamine B, which manifests as fluorescent bands in whiskers. Presence of these bands indicated that 51% of the captured population had consumed the bait, and of these, 58% had taken the bait more than once. These results demonstrate that wild rats will consume a liquid fertility management bait, even in the presence of highly palatable and abundant food within the refuse rooms. This study establishes the successful acceptance of a bait by wild rats. Further investigation is needed to evaluate the effectiveness of this product for the management of urban Norway rat populations.

KEY WORDS: bait acceptance, fertility control, New York City, Norway rat, *Rattus norvegicus*, refuse rooms, rhodamine B, rodent control, subways

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INTRODUCTION

Several species of rodent have been commensal with humans for millennia. Their success is due to these elusive animals being nocturnal, omnivorous, able to migrate with food availability, and reproduce at high rates (Singleton et al. 2003). Commensal rodents are sources of numerous zoonotic diseases (Meerburg et al. 2009), cause considerable loss in agricultural settings (Singleton 2003), prey on eggs or young of native wildlife species (Tobin and Fall 2004), and can be a source of building or electronic destruction (Tobin and Fall 2004). Despite aggressive culling campaigns to reduce rodent populations, there are an estimated 175 million Norway rats (*Rattus norvegicus*) in the U.S. that cost an estimated \$19 billion in damage annually (Pimentel et al. 2005). Until now, the common approach to reduce overabundant rodent populations has been the use of lethal rodenticides.

Lethal rodenticides are classified into two general groups: non-anticoagulants and anticoagulants. Non-anticoagulants attack the central nervous system, cause heart failure, or disrupt kidney function, while anticoagulants cause death by internal hemorrhaging (Corrigan 2001). Anticoagulants are favored because the delay in the onset of symptoms prevents rodents from associating toxicosis with bait consumption, thus conditioned bait avoidance or "shyness" is unknown (Buckle 1994). Anticoagulants are further categorized into first-generation and second-generation. First-generation anticoagulants required multiple daily deliveries to produce a lethal dose. They were the main method of rodent control worldwide in the 1950s and 1960s until cross-resistance to all first-generation

anticoagulants was discovered (Hadler and Buckle 1992). Second-generation rodenticides were created in response to the developed resistance in rodents. Second-generation anticoagulants are up to 100 times more potent, and a fatal dose can be ingested in a single delivery (Hadler and Buckle 1992).

While anticoagulant rodenticides are currently the main method of rodent control around the world, there are major concerns associated with such use. Negative environmental impacts can arise from exposure of non-target species through consumption of either baits or residue in prey and carrion (Eason et al. 2002, Singleton et al. 2007). Symptoms of anticoagulant toxicity, such as weakness, lameness, and respiratory compromise are often detectable for several days prior to death (PSD 1997, Mason and Littin 2003). Anticoagulant use has thus been classed as a markedly inhumane method of lethal control (PSD 1997, Mason and Littin 2003, Meerburg et al. 2008). Public awareness of these concerns have increased consumer requests for alternative pest management techniques (Corrigan 2009). Non-rodenticide rodent control options exist, but they often take longer and can be less effective (Corrigan 2009).

Fertility control is an alternative strategy to rodenticides, with potential to manage overabundant commensal rodent populations. Fertility control reduces fecundity without increasing mortality rates (Jacob and Singleton 2008). Currently, fertility control agents being researched fall into two broad categories: immunocontraceptive vaccines, and chemical contraception (Fagerstone et al. 2010). Immunocontraceptive vaccines generate antibodies to key components of reproductive systems and can be

effective for 1 to 4 years after a single dose (Fagerstone et al. 2010). The limitation to these vaccines is they can only be delivered by injection. This is not a suitable approach for small mammals, such as rodents, where the fertility of a relatively high proportion of the target population must be reduced (Cowan et al. 2006). Chemical contraception interferes with ovulation, fertilization, or implantation and development of the egg in females, or by impairing spermatogenesis in males (Fagerstone et al. 2002). Chemical contraceptives can potentially be delivered orally via fertility management baits, and thus they are suited to small mammal targets such as rodents. Fertility management baits must be highly palatable to attract rodents away from normal food sources and to ensure consumption of an effective dose (Cowan and Townsend 1994). However to date, no such formulation has been developed.

In an attempt to establish more humane approaches and improve the quality of human life, SenesTech, Inc. (Flagstaff, AZ) has developed and tested a liquid fertility management bait (FMB) on Sprague Dawley laboratory rats. The active ingredients of FMB, 4-vinylcyclohexene diepoxide and triptolide, target the reproductive systems of males and females, rendering both sexes sub-fertile (Dyer and Mayer 2014). In female rats, all follicular stages of the ovarian cycle are targeted, degenerated, and reabsorbed; therefore, eggs do not mature and ovulate (Lue et al. 1998, Flaws et al. 1994, Huynh et al. 2000, Mayer et al. 2002). In males, FMB targets the number of epididymal sperm and sperm mobility (Liu et al. 2010, Xu and Zhao 2010). Laboratory studies have confirmed that FMB is highly palatable; both sexes will consume approximately 10% of their body weight while in the presence of ad libitum rodent chow and water (Dyer and Mayer 2014). Studies have also demonstrated a reduction in litter sizes in the first breeding round with baited females and unbaited males (Dyer and Mayer 2014). A more recent study showed males and females consumed approximately 4% and 10% of their body weight, respectively, and no females delivered litters in the first breeding round (Dyer and Mayer 2014). These results confirm FMB is palatable to Sprague Dawley rats and effective in reducing litter size; however, these results can only suggest how wild rats might respond to FMB.

Commensal Norway rats are prevalent in major cities worldwide, as they easily coexist within human habitats (Corrigan 2006). New York City, New York (NYC), in particular, is a favorable environment for this species due to readily available and abundant food resources in close proximity to extensive structural and subterranean harborages (Corrigan 2006). For these reasons, the NYC subway system was chosen as a suitable location for the first field study of FMB. The main objective of this study was to determine preference and acceptance of FMB by wild Norway rats within 3 refuse rooms in the NYC subway system.

METHODS

Experimental Phases

The study had two phases. Phase 1 was designed to determine liquid versus semi-solid bait preference and took place in 4 locations within the subway system:

Grand Central Station, 2nd Avenue, 86th Street, and 34th Street. Phase 2 was designed to examine long-term bait acceptance. Phase 2 continued in Grand Central, 2nd Avenue, and 86th Street Stations. All work conducted in this study was approved by the SenesTech Animal Care and Use Committee (ACUC, protocol 03-11). The United States Environmental Protection Agency (US EPA) approved all work conducted in this study and waived the need for an experimental use permit.

Study Areas

The study took place within 4 below-grade subway system refuse rooms in New York, NY from February to August 2013. The study areas were chosen based on room size, reported rat populations, and ease of accessibility. Potential rat harborages were determined by observing rat movements. Study areas were spread throughout the city to ensure that migration between study areas was not possible. The average size of below-grade refuse rooms is 11.8 m² (127 ft²); the study areas chosen represent below average, average, and above average size. During daily operations, city workers added refuse in black plastic bags to metal uncovered dumpsters to these study areas 3 times per day, and it was removed a minimum of one time per week. The lights in these rooms were on 24 hours a day, 7 days a week. During Phase 1, all study areas had 2 tamper-resistant bait boxes (JT Eaton 903TP Tamper Resistant Rat Fortress Bait Station), while during Phase 2 both 86th Street and 2nd Avenue had 1 bait station and Grand Central Station had 5 bait stations.

34th Street

The 34th Street Station refuse room was average size at 13.24 m² (142.5 ft²), had an abundant trash supply, and a nearby rat haborage area. The temperature ranged from 18°C to 22°C with an average of 20°C. Humidity varied from 37% to 62% with an average of 45%. The experiment was discontinued at this site after Phase 1, due to inconsistent rodent activity, excessive trash accumulations preventing bait box access, and safety concerns.

86th Street

The 86th Street Station refuse room was below average size at 4.46 m² (48 ft²) and had a limited trash capacity. The temperature ranged from 17°C to 33°C with an average of 24°C. Humidity varied from 35% to 67% with an average of 52%.

2nd Avenue

The 2nd Avenue room was average size at 13.94 m² (150 ft²), had an abundant trash supply, and a nearby rat haborage area. The temperature ranged from 16°C to 33°C with an average of 23°C. Humidity varied from 33% to 73% with an average of 55%.

Grand Central Station

The room at Grand Central Station was above average size at 67.17 m² (723 ft²), had abundant trash supply, and multiple nearby rat haborage areas. The temperature ranged from 18°C to 33°C with an average of 25°C.

Humidity varied from 31% to 65% with an average of 52%.

Fertility Management Bait

Both semi-solid and liquid FMB (SenesTech, Inc., Flagstaff, AZ) were placed within tier 1 tamper-proof bait boxes along active rat runs within the refuse rooms. Liquid FMB was dispensed from Helland Rodent Liquid Poison Auto Dispensers (J. T. Eaton & Co. Inc., Twinsburg, OH). Rhodamine B (RB) (Acros CAS# 81-88-9; Acros Organics, Thermo Fisher Scientific, Waltham, MA), a commonly used fluorescent biomarker (Fisher 1999), was incorporated into both bait formulations at a concentration of 0.03% to assess FMB acceptance by individual rats.

During Phase 1, all boxes contained 50 g of control, no active ingredients, semi-solid, and one dispenser containing 50 mL of control, no active ingredients, liquid FMB. To prevent mold growth, the semi-solid FMB was coated in a thin cheese wax layer (New England Cheesemaking Supply Co., Inc., South Deerfield, MA). FMB consumption was measured biweekly to determine palatability, rates of consumption, and bait form preference. Consumption was analyzed by paired t-test.

Throughout Phase 2, two liquid dispensers were placed in each box providing 400 mL of bait weekly. FMB was monitored weekly to determine rates of consumption. A linear model was used to test effects of bait station location, room, and day of study on liquid consumption.

Trapping

Data collectors had a uniformed subway system escort during most of the study for safety and because of public awareness campaign “see something, say something,” promoted for public safety. Trapping occurred only at Grand Central Station because data collectors were able to obtain a badge to enter this room without an escort, allowing them to follow the strict schedule of checking traps set forth by IACUC protocol. The study site at 86th Street Station was too small to allow data collectors to comfortably work out of the public eye. The study site at 2nd Avenue Station was large enough to allow data collectors to work out of the public eye, but accessing the room was inconsistent due to logistical problems.

Snap-trapping of rats was done to detect FMB uptake, indicated by the presence of RB fluorescent bands in whiskers collected from trapped individuals (Fisher 1999). Traditional rat traps (Victor Easy Set Rat Traps, Woodstream Corp., Lititz, PA and Jawz Reusable Mouse and Rat Traps, J.T. Eaton & Co., Inc.) were placed every 4.57 m (15 ft) along the rat pathways within the Grand Central Station refuse room in clusters of 1-3 traps for a total of 15 traps. In order to improve trap success, unset traps were baited with peanut butter, french fries, or bacon grease for 1 week prior to set trapping and during non-trapping weeks. Two trapping periods occurred every 24 hours: daytime (0700-1700) and overnight (1700-0700). Any animals that were found alive in the traps were humanely euthanized with an overdose of isoflurane (MWI Veterinary Supply Co., Boise, ID; NDCA code 13985-030-60), and death was assured by

cervical dislocation or bilateral pneumothorax. Animals were classified as juveniles or adults by examining external genitalia (Aplin et al. 2003). Males were classified as juveniles if the testes were undescended and classified as adults if the testes were descended. Females were classified as juveniles if the vagina was sealed off by the hymen and were classified as adults when the vagina was perforated.

Whisker Analysis

After euthanasia, whiskers were plucked from one side of the snout and sent to SenesTech for further analysis. Whiskers of 2 animals were unable to be collected due to destroyed tissues. Whiskers were mounted on glass microscope slides and imaged using an Olympus BX51 microscope (Olympus Corp. of the Americas, Center Valley, PA) at 2× in Surveyor software (Objective Imaging, Cambridge, UK). The fluorescence was visualized using a TRITC fluorescence filter and an Xcite Series Q mercury burner. Images were analyzed for presence or absence of RB using the bright reddish-orange glow of RB under fluorescence. Band number was determined by looking for bright areas of RB with diffuse, faint glow in between.

RESULTS

Phase 1

For all study sites, more liquid than semi-solid was consumed. Semi-solid bait consumed per day ranged from 0 to 0.33 g, while liquid consumed per day ranged from 0 to 22.5 mL. A paired t-test (bait station by date) of liquid consumed per day versus solid consumed per day showed there was significant preference for liquid over the solid bait ($P < 0.0001$). Therefore, in Phase 2 we used liquid FMB to determine consumption in each field site.

Phase 2

Consumption of FMB

FMB was readily consumed in the presence of abundant natural food sources during Phase 2 (Figure 1). A linear regression model was created to test for effects of room, day of study, and the interaction between room and day. The results show that room and day of study both have significant effects on amount consumed per day ($P = 0.0004$ and $P < 0.0001$ respectively), but there was no evidence of an interaction between room and day of study ($P = 0.53$).

The model showed that more liquid was consumed per day, per bait station in the 2nd Avenue room compared to the other two rooms ($P = 0.0029$). There was no difference in liquid consumption per day per bait station between Grand Central Station and 86th Street ($P = 0.65$).

A decrease in consumption was seen throughout the experiment in Grand Central Station and at 2nd Avenue. The above model suggests that consumption decreased by about 0.4 mL per bait station per day throughout the experiment ($P < 0.0001$).

FMB Acceptance

Analysis of RB bands under fluorescence microscopy showed 43 of 84 (51%) animals trapped in Grand Central Station refuse room consumed the bait. Further analysis

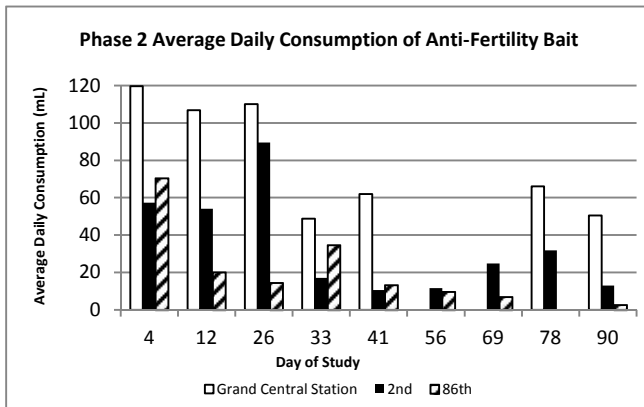


Figure 1. Average consumption of active FMB over time during Phase 2. Construction prevented data collection on days 56 and 69 at Grand Central Station, therefore no data are shown. Trash accumulations prevented data collection on Day 78 at 86th Avenue, therefore no data are shown.

Table 1. Percentage of trapped animals from Grand Central Station that consumed FMB as revealed by the presence of Rhodamine B in their whiskers.

Demographic Data	Total with Bands	Total Captured	Proportion
Male	17	39	44%
Female	26	45	58%
Juveniles	30	63	48%
Adult	8	21	38%

of the whisker bands showed 25 of 43 (58%) animals consumed FMB multiple times. Table 1 shows the percentages of trapped rats with RB detected in their whiskers.

DISCUSSION

For 3 months, we tested the acceptance and uptake of FMB by wild Norway rats in an urban field setting. Data from Phase 1 revealed that wild rats preferred liquid bait over semi-solid bait, which is consistent with observations made in laboratory rats (SenesTech Inc., unpubl.). The significance of using liquid bait in the presence of ample food may reflect the rodent digestive system. Lepkovsky et al. (1957) reported that food digestion requires an extensive amount of water to be transported to the digestive system. When water availability is limited, the body compensates by drawing water from other tissues in order to meet this demand, placing the animal in a state of dehydration. Given that increased food consumption in rodents leads to an increase in fluid uptake (de Castro 1989), it can be hypothesized that FMB liquid bait is readily accepted by urban rat populations because food supply is abundant but water availability is limited.

There was a decrease in bait consumption during Phase 2 at Grand Central Station and 2nd Avenue after 30 days, but it was still consumed consistently in the presence of highly palatable and abundant food within the refuse rooms. These apparent decreases could be due to several factors: however, these were not consistent

between the 2 sites. Firstly, monthly trapping operations at Grand Central Station could have reduced the population, which in turn caused a consumption decrease. However, this would not explain the reduction in consumption at 2nd Avenue, as no trapping was undertaken here. Secondly, rodenticide use could have caused population decreases. Rodenticide packs were found at Grand Central Station, however no packs were ever found at 2nd Avenue, and poisoned rodent carcasses were not found at either site. Bait consumption can potentially be used as an index of treatment effectiveness. However, bait consumption-independent methods of measuring effectiveness, for instance as described by Cowan and Townsend (1994), would be required to rigorously evaluate the population consequences of using FMB.

The aim of fertility control is to reduce rodent population density. Jacob et al. (2004) observed the population impacts of surgical sterilization of adult female ricefield rats in enclosures. Female rats were surgically sterilized via tubal ligation (0, 25, 50, or 75% of females) and then allowed to live undisturbed for approximately 100 days. Surgical sterilization of 50% and 75% of females decreased the initial population by 50%. The authors thus suggested that sterilizing a minimum of 50% of females would reduce population densities significantly (Jacob et al. 2004). Similar predictions have been made by population modeling for species with high population turnover rates (Cowan et al. 2006). Previously, we have shown that consumption of FMB by female Sprague Dawley rats for 15 days results in reduced litter sizes and increased days to parturition when compared to controls (Dyer and Mayer 2014).

In the current study, 51% of the trapped population consumed FMB and of these 58% did so multiple times. These data, in conjunction with Jacob et al. (2004), imply that if the amounts consumed per individual were sufficient to inhibit fertility, 51% of the population in the refuse rooms would have had reduced litter sizes therefore potentially causing population decreases. However, we do not know the amounts of bait consumed per individual in this study, nor the dose required to achieve equivalent levels of infertility to those of Jacob et al. (2004).

Our results confirm that urban rats will consume a liquid fertility management bait in the presence of abundant alternative food sources. These findings, coupled with data from laboratory experiments showing reduced fertility following bait consumption, warrant further studies on the effects of fertility management bait on urban rat populations.

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