

Comparison of baiting strategies in common vole management

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Abstract

BACKGROUND: Worldwide, pest rodents can cause extensive damage to agriculture, forestry, food storage, and infrastructure and pose a risk to public health and livestock due to the spread of zoonotic pathogens. In Europe, the most common pest rodent species is the common vole (*Microtus arvalis*). Management during periodic outbreaks largely relies on rodenticidal bait with zinc phosphide. Efficient baiting with rodenticides or possibly anti-fertility products in the future require baiting methods that allow a sufficient proportion of the population to consume an effective dose of bait. We used a bait with the quantitative marker ethyl-iophenoxic acid (Et-IPA) to evaluate baiting strategies in enclosure experiments. This wheat-based bait with Et-IPA was placed in bait boxes or directly into the tunnel system entrances in different seasons and common vole abundances. Voles were live-trapped, individually marked and blood samples were collected to relate Et-IPA blood residues to bait uptake.

RESULTS: The percentage of animals consuming bait was not heavily affected by the baiting strategy but voles had higher Et-IPA blood residues if tunnel baiting was used in autumn and if bait boxes were used in winter. Non-reproductive as well as lighter animals tended to have higher Et-IPA blood residues than reproductive individuals, whereas sex had no effect. Population density had a negative effect on the probability of residues present as well as on Et-IPA blood concentration.

CONCLUSION: The results of this study might help to improve baiting techniques to manage overabundant rodent pest species regardless of the compounds to be delivered.

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Keywords: baiting strategies; ethyl-iophenoxic acid (IPA); *Microtus arvalis*; bait marker

1 INTRODUCTION

Pest rodents have a significantly negative impact on economic (damage) and environmental (invasive species) aspects and pose health risk in developed as well as in developing countries. In Europe, rodent damage is most pronounced in rural areas during multi-annual outbreaks.^{1–6}

One of the most common methods to manage overabundant rodent pest species is the use of rodenticides, but orally delivered contraceptives gain more and more interest.^{5–7} In the past decades, ecologically based rodent management emphasized the importance of a comprehensive understanding of the ecology and population dynamics of pest species to effectively apply rodenticides and other management methods.^{8,9} Combining both lethal methods such as the use of rodenticides and non-lethal methods like fertility control are considered to be most cost effective.^{7,10–12}

Contraceptive techniques that require individual capture (e.g., sterilization or insertion of hormonal implants) are impractical for overabundant, small rodent species.¹³ All management strategies that are based on delivering compounds rely on effective baiting methods to achieve effects at population level. This is the case for rodenticide use and for oral delivery of anti-fertility

compounds for managing short-lived animals with clearly defined breeding seasons.⁵ About two-thirds of house mice (*Mus musculus domesticus*, Linnaeus, 1758) and ricefield rats (*Rattus argentiventer*, Robinson & Kloss, 1916) need to be rendered infertile to reduce the reproductive output within a breeding season.^{14–16} When

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rodenticides are registered, the percentage of reduction in rodent numbers is usually required to be close to 100%.¹⁷

Compounds used for rodent management can pose exposure risk to non-target animals. Therefore, regulation for application are in place to minimize such risk including the use of bait boxes, indoor use only or placing the bait directly into the tunnel system of the target animals.¹⁸ In addition, bait bases and baiting strategies need to be used that increase the probability of bait consumption only by target species. However, it is necessary to find a balance between the efficacy needed to reach a certain proportion of the target species, workload and the aim to exclude non-targets, which also might affect baiting success for targets.

Common voles (*Microtus arvalis*, Pallas, 1778) are one of the major rodent pest species in Europe.¹⁹ During outbreaks, they cause severe damage to crops due to their rapid reproduction and high food intake rates.^{19,20} We used the common vole as a model species in a series of enclosure trials to test bait uptake for different baiting strategies. Ethyl-iophenoxic acid (Et-IPA) was applied as a bait marker to identify the effects of extrinsic factors (season) and intrinsic factors (population size, sex) and baiting strategy (bait stations *versus* tunnel baiting) on bait uptake. In a prior laboratory trial, wheat bait containing Et-IPA was developed and a dose-residue relation established that allows estimating bait uptake quantitatively.²¹ In the enclosure trials, the proportion of common voles ingesting bait and the amount of bait eaten were considered.

The results can be used to evaluate the efficacy of baiting strategies for common voles and potentially other small rodent species that are relevant for the protection of crops, infrastructure, health and for conservation.

2 MATERIAL AND METHODS

2.1 Animals

Common voles were trapped in the field near Münster (51°58'29.6'' N 7°34'02.2'' E), North Rhine Westphalia, Germany and held in the animal holding facilities in Münster. Five females and three males were released as founder population in each of the six semi-natural enclosures in Remderoda, Jena (50°56'19.4'' N 11°31'42.9'' E), Thuringia, Germany in July 2020 and in June 2021. Care was taken to allow voles to acclimatize to enclosure conditions. The shortest period from releasing voles to sampling (see later) was about 3 weeks. This period covered a reproductive cycle of common voles,⁴ which seemed appropriate for acclimatization.

Each of the quadratic rodent proof enclosures measured 50 m × 50 m, and were separated by a sheet metal fence buried 40 cm deep into the soil. Vegetation inside the enclosures consisted of a grass mixture and was mown once a year in autumn. Along the fence, a 2.5 m stripe was mown regularly to keep the vegetation low. Plots were surrounded by a fence that excluded terrestrial predators but voles were accessible for avian predators. Before the first trial, there was a period of 4 months to allow acclimatization of voles and reproduce to build up self-sustaining enclosure populations. Population size was checked by live trapping 1–3 weeks before the start of each trial and further animals were released when necessary. In case of heavily biased densities or population structure among the enclosures, differences were balanced by transferring individuals.

2.2 Bait

The Et-IPA bait was developed and tested in a previous laboratory study.²¹ It consisted of broken wheat that was coated with 1280 µg/g Et-IPA dissolved in sunflower oil. Dose-dependent

residues can be derived from blood samples enabling generation of a quantitative estimate of consumption based on Et-IPA blood residues.

2.3 Enclosure trials

In a series of five trials considering different seasons and population densities, Et-IPA bait was either inserted directly into tunnel entrances or placed in a bait box for mice (Tomcat RTU, Bell Laboratories, Inc., Madison, WI, USA). Three enclosures were randomly chosen for each baiting strategy. The assignment of baiting strategy to the enclosures was switched after each trial to avoid habituation. Per trial, 50 g Et-IPA bait in total was placed in each enclosure, 5 g in each of ten active tunnel entrances (marked with a bamboo stick) or 6.5 g in each of eight bait boxes that were placed in the corners and in the centre of each enclosure wall. The two methods reflected practical use of burrow baiting with an applicator and perimeter baiting with bait boxes.

Seven days after placing the bait, bait boxes and remaining bait from tunnel baiting was removed. The voles were live trapped for 3 days with Oos-traps with wooden boxes using wheat, rodent pellets (Altromin 1324; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) and apple slices as bait, and paper as bedding, if necessary, in winter.²² All traps were checked about every 12 h. Body weight was measured with a spring scale (Pesola Medio-Line; Pesola-Werke, Switzerland) to the nearest gram. A blood sample of each individual was collected by puncture of the retrobulbar sinus with a capillary pipette (Hirschmann ring-caps 50 µL #9600150). Whole blood samples were stored at –80°C until further analysis. For identification (and to prevent multiple blood sampling) all captured animals were individually marked by injecting a subcutaneous passive integrated transponder (PIT) tag (ID-100B Microtransponder, TROVAN, supplier: Anitech; Edewecht, Germany) into the scruff of the neck. Furthermore, sex, reproductive status (lactating, pregnant, testis scrotal), enclosure and trap number were recorded. Afterwards, the animals were released from where they had been caught. Animals that were trapped more than once within one trial were released without processing. Trapping was terminated when new captures were < 5% (except for summer). The estimate of population density per enclosure was based on the number of individuals caught. Trials were conducted in November 2020 (174 samples; 114 animals/ha), June 2021 (56 samples; 27 animals/ha), September 2021 (236 samples; 157 animals/ha), November 2021 (151 samples; 102 animals/ha) and March 2022 (150 samples; 100 animals/ha).

2.4 Analysis for Et-IPA residues

The preparation of the blood samples was based on Berentsen *et al.* and is described in detail in Jacoblinnert *et al.*^{21,23} In brief, whole blood samples were spiked with the surrogate propyl-IPA (PR EuroChem Ltd, Zug, Switzerland) as internal standard. Et-IPA and propyl-IPA were extracted and liquid chromatography-electrospray tandem mass spectrometry was used to measure concentrations. All samples were measured twice, concentration of Et-IPA calculated with peak areas using Analyst 1.7.1 without surrogate-correction. The limit of Et-IPA detection was 0.05–0.1 pg/µL whole blood.

2.5 Statistical analyses

In general, two different dependent variables were evaluated using generalized linear mixed models (GLMMs). First, we evaluated the probability of detecting residues in each individual. For

this model the dependent variable followed a binomial error distribution, where individuals were either showing residues regardless of quantity or no residues. Second, we evaluated individual residue levels in a GLMM with a gamma error distribution. In both cases, enclosure was used as random factor to account for the spatial design of the study. Seasonal models were constructed to evaluate the impact of baiting strategy in conjunction with individual (sex, reproductive activity, weight) and population level (density) parameters. Single factor effects in each model were explored using the *ggeffects* package (estimated marginal means) and displayed using the *ggplot* package in program R.

3 RESULTS

According to Jacoblinnert *et al.*, Et-IPA residue values after the consumption of 1 g bait coated with 1280 µg/g Et-IPA are 119 µg/g after 1 day and 85 µg/g after 7 days.²¹ Based on the mean of these values (102 µg/g) and mean Et-IPA residues in the enclosures of 1404.3 ng/g/body weight (multiplied by 20 g mean vole body weight = 28 086.5 ng/g), average bait consumption was assumed to be about 0.28 g. Overall and independent of season and other intrinsic and extrinsic factors, the mean probability of an animal consuming Et-IPA bait was 0.73 (box) or 0.82 (tunnel).

The probability of an animal consuming Et-IPA did not differ statistically significantly between sexes, seasons or baiting strategies (Fig. 1(a)). The mean Et-IPA blood concentration per body weight was 1456.95 ng/g/body weight (box) and 1354.95 ng/g/body weight (tunnel). There was a trend that the Et-IPA blood concentration was up to 33.3% higher if the bait was directly inserted into tunnel entrances in autumn ($P = 0.073$) (Fig. 1(b)). In contrast, Et-

IPA blood concentration tended to be up to 44% higher if bait boxes were used in winter ($P = 0.053$) (Fig. 1(b)). The statistical analysis data is provided in Supporting Information, Table S1.

No significant differences have been observed for the probability of Et-IPA residues in common vole blood within reproductive and non-reproductive animals in all seasons, neither for box nor for tunnel baiting (Fig. 2(a)).

Non-reproductive voles had up to 61% higher Et-IPA-blood residues per body weight in autumn than reproductive animals ($P < 0.01$) (Fig. 2(b)). In addition, in winter, Et-IPA-blood residues were up to 67% higher, if the animals were non-reproductive ($P < 0.01$) than reproductive and twice as high if bait boxes were used ($P < 0.01$) instead of tunnel baiting (Fig. 2(b)). The statistical analysis data is provided in Table S2.

There was a trend in spring, that voles of lower body weight were more likely to have Et-IPA-blood residues than heavier animals ($P = 0.051$) (Fig. 3(a)). In autumn, this pattern tended to be reversed ($P = 0.060$) (Fig. 3(a)).

In autumn and winter, there was a clear negative effect of body weight on Et-IPA blood residues per body weight (autumn: $P < 0.01$; winter: $P < 0.01$) (Fig. 3(b)). Furthermore, residues were up to 31% higher when bait boxes instead of tunnel baiting was used in winter ($P = 0.01$) (Fig. 3(b)). The statistical analysis data is provided in Table S3.

There was a negative effect of population density on the probability of IPA residues in autumn ($P < 0.01$) (Fig. 4(a)) and the concentration of Et-IPA blood residues per body weight in autumn and winter (autumn: $P < 0.01$, winter: $P < 0.01$) (Fig. 4(b)). In autumn, Et-IPA residues were up to 37% higher if the bait was placed directly into the tunnel entrances compared to using bait boxes ($P = 0.02$) (Fig. 4(b)). However, in winter, Et-IPA blood concentration was up to 37% higher if bait boxes were used compared to baiting tunnel entrances ($P = 0.01$) (Fig. 4(b)). The statistical analysis data is provided in Table S4.

4 DISCUSSION

In this replicated series of enclosure trials, Et-IPA residues were present in 77% of the voles. This seems a suitable fraction of the population to deliver compounds either for fertility control or for rodenticidal treatment to achieve the desired population-level effects. This value might be even higher if bait was to be offered for longer than the 7 days in this study or at higher bait density. Eight bait stations at the perimeter of the enclosure or ten baited tunnels in the 0.25 ha area of an enclosure were sufficient to target a large proportion on voles present. This is not surprising because common vole home ranges of 125 m² allow most animals access to bait during movement in or occasionally venturing outside their home ranges.²⁴

Baiting strategy, sex, season, and reproductive status did not generally affect the proportion of common voles accessing bait. However, there was an effect of body weight. While body weight was negatively correlated to the percentage of voles consuming bait in spring, it was positively correlated to bait uptake in autumn. This indicates season effects that can alter diet choice and/or dominance structure.^{25,26} In spring, there are large overwintered individuals present that reproduce early and may require more food to boost reproduction. In autumn, many juveniles are present when the reproductive season comes to an end. Young (and therefore lighter) animals have a higher food intake rate than heavier conspecifics, which may explain the change of pattern in autumn.²⁰ This may explain the negative effect of body weight on Et-IPA residues in autumn and winter

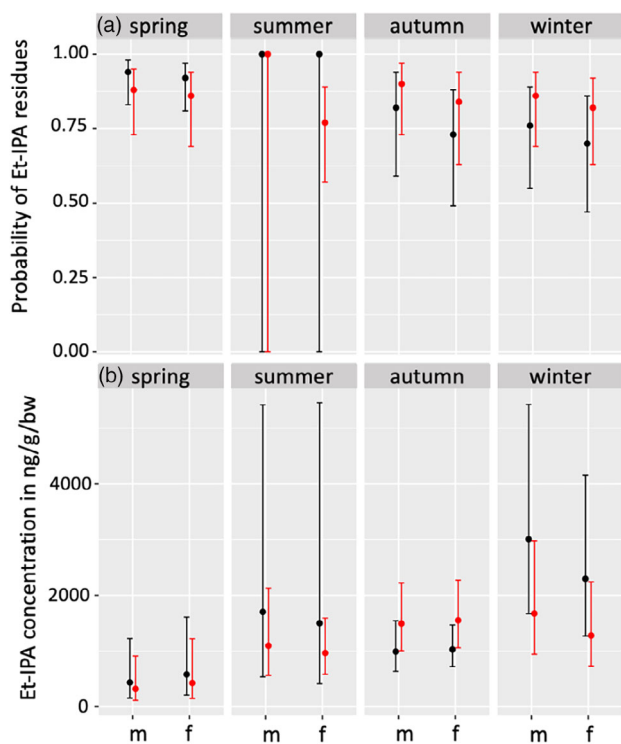


Figure 1. (a) Probability of Et-IPA residues in common vole blood and (b) Et-IPA blood concentration (in ng/g/body weight) for male (m) and female (f) voles with blood residues considering the different baiting strategies (bait box – black symbols, tunnel baiting – red symbols) and seasons. Measure of variance is \pm standard error.

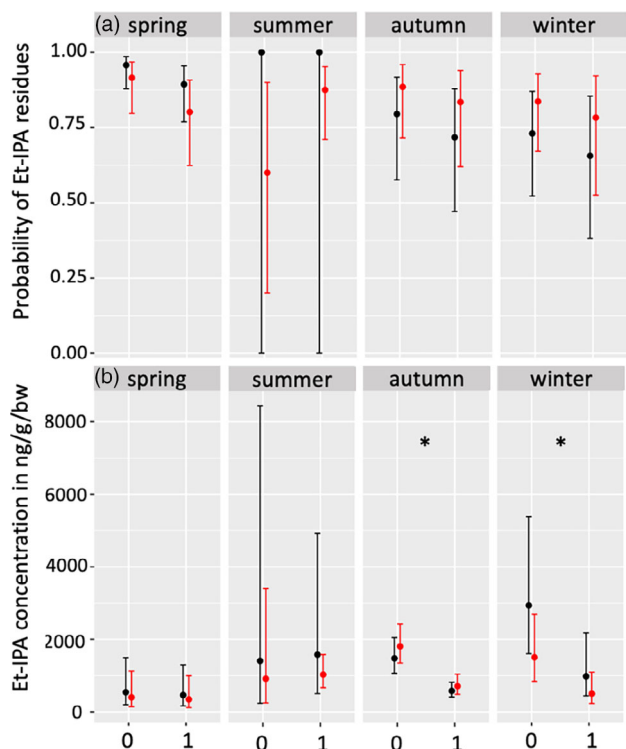


Figure 2. (a) Probability of Et-IPA residues in common vole blood and (b) Et-IPA blood concentration (in ng/g/body weight) for reproductive (1) and non-reproductive (0) common voles with blood residues considering the different baiting strategies (bait box – black symbols, tunnel baiting – red symbols) and seasons. Measure of variance is \pm standard error.

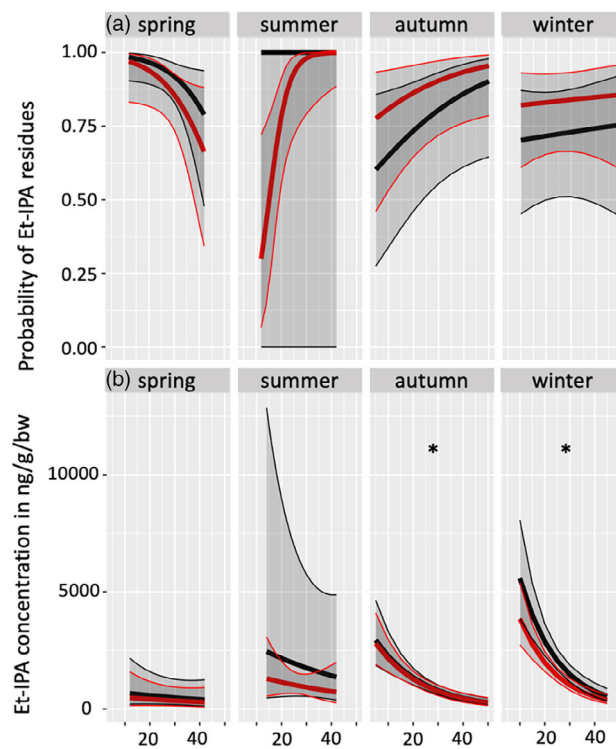


Figure 3. Effect of body weight (in grams) on (a) the probability of Et-IPA blood residues and (b) Et-IPA blood concentration (in ng/g/body weight) in common voles with blood residues considering the different baiting strategies (bait box – black symbols, tunnel baiting – red symbols) and seasons (95% confidence intervals in grey).

as less food competition, which gives access to bait not only to dominant but also subordinate individuals, seems unlikely at these seasons.²⁵

There were several intrinsic and extrinsic effects on Et-IPA concentration, which reflects the amount of bait consumed. Neither baiting strategy performed consistently better in all conditions considered and some of the effects (body weight) were not consistent across models. In particular seasons, one or the other baiting strategy tended to be superior but differences in residues may not be highly relevant as long as an effective dose is delivered to reach the management aim.

The findings for body weight effects on the probability of bait uptake were confirmed by Et-IPA concentration that was lower in heavier animals in autumn/winter and higher densities were also correlated to lower residues. During summer, few voles were present and if so, most of them in the same enclosure despite of similar procedures of balancing uneven densities. As a result, data from summer is of high variance and may lack robustness.

It is somewhat surprising that higher Et-IPA blood residues occurred in non-reproductive animals, because lactating Brandt's voles (*Lasiopodomys brandtii*, Radde, 1861) have higher energy demands than non-lactating females.²⁷ This is similar in ricefield rats and house mice.^{25,26} The contrasting finding of higher Et-IPA residues in non-reproductive animals was restricted to autumn at the end of the reproductive period. It seems unlikely that bait boxes are more attractive or more accessible to non-reproducing voles so other unknown mechanisms may matter.

When managing rodent pest species, effective baiting strategies are essential for both fertility control and the use of rodenticides.

For both methods, an appropriate percentage of the population has to consume the bait to achieve the desired effects at population level.^{5,28,29} Our results indicate that tunnel baiting and the use of bait boxes can deliver bait to a large percentage of individuals. Tunnel baiting is labour intensive because bait has to be placed manually in tunnel entrances unless (expensive) machinery is used. This technique is restricted to crop stages with low vegetation that allows finding of tunnel entrances. The use of bait boxes is rare. It requires manual filling of stations, distribution, checks and removal. Future research could consider developing biodegradable pre-filled bait stations that can be distributed with machinery. More bait was consumed from bait stations in winter and at tunnel baiting in autumn. Usually, bait application is conducted at the start of the breeding season in late spring. At this time, baiting strategy does not seem to matter. However, if baiting is conducted in winter or autumn, one or the other technique may yield better results but crop stage (vegetation height) may limit suitability.

Some rodent populations can recover rapidly from management action that lower population size. For example, midday gerbils (*Meriones meridianus*, Pallas, 1773) recover due to immigration within 4–8 months and common voles even within 10–15 days after population collapses.³⁰ Insufficient amount of bait, duration or inappropriate timing of baiting can lead to management failure and waste of resources.³¹ Zinc phosphide bait is the most common rodenticidal compound used in Europe to manage overabundant common vole populations.³² Assuming a wheat grain weight of about 45 mg, bait uptake of 0.28 g is equal to 6.2 wheat grains.³³ Assuming a zinc phosphide concentration of 0.8 g/kg (this is the lowest concentration of products available in

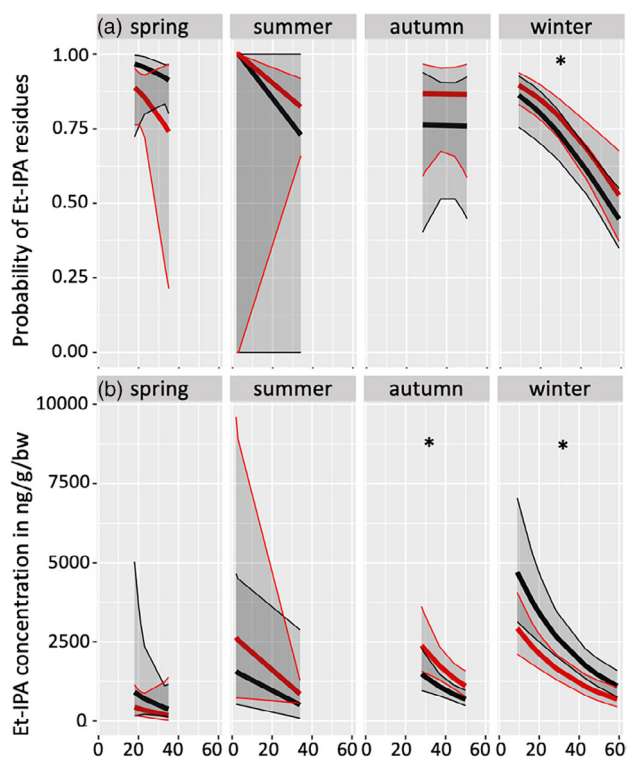


Figure 4. Effect of population density on (a) the probability of Et-IPA blood residues and (b) Et-IPA blood concentration (in ng/g/body weight) in common voles with blood residues considering the different baiting strategies (bait box – black symbols, tunnel baiting – red symbols) and seasons (95% confidence intervals in grey).

Germany), bait uptake of 0.28 delivers 2.24 mg zinc phosphide to a 20 g vole. This is almost three-fold the median lethal dose (LD50) of 39 mg/kg.³⁴ Similar calculations could be performed to estimate uptake of other active ingredients such as compounds relevant for future fertility control.

5 CONCLUSION

We tested two baiting strategies in enclosure trials with common voles. Both baiting strategies seem to be suitable to reach a large percentage of the population but extrinsic and intrinsic effects on bait uptake seem inconsistent. Overall, tunnel baiting results in 82% animals consuming bait, slightly more than the 73% when using bait boxes. Both percentages seem to be sufficient for fertility control. A higher percentage might be necessary for rodenticide use. However, the results of this enclosure study need validation in the field.

This study provides valuable information for field experiments and field application including the negative correlation of population density and bait consumption that points towards increasing bait density when abundance is high. Knowledge resulting from field trials should help farmers to select the most appropriate baiting strategy and application rate to manage common voles.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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