Research Article

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Sanitary measures considerably improve the management of resistant Norway rats on livestock farms

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Abstract

BACKGROUND: Norway rats (*Rattus norvegicus*) need to be controlled to prevent transmission of pathogens and damages to stored products and material, leading to considerable economic risks and losses. Given increasing resistance in Norway rats, the most persistent, bio-accumulative and toxic anticoagulant rodenticides are widely used for management, which presents hazards to the environment especially for non-target species. We investigated how sanitary measures improved management of Norway rats on 12 paired livestock farms in a region of Germany with a high population of resistant rats for reducing application of rodenticides. We recorded food intake, and tracked activity and resistance frequency during the pre-treatment, treatment and post-treatment periods.

RESULTS: In the post-treatment period, farms using sanitary measures had a higher control success with > 13% more bait boxes without feeding than farms not using sanitary measures. In addition, the reoccurrence of rats was delayed by 85 days. With increasing accessibility to buildings and more precise positioning of the boxes, control success improved, especially when rats could not spread from water-bearing ditches through the sewer system, and when rat-hunting animals were present. Resistant animals were more common indoors than outdoors, and there were more resistant rats recorded before and during treatment than in the post-treatment period.

CONCLUSION: The control success was substantially higher and reoccurrence was delayed using sanitary measures on farms. Sanitary measures can reduce resistance indirectly due to delayed re-colonization and establishment of resistant populations inside buildings. Hence, sanitary measures help to reduce economic losses, rodenticides required for rat management and environmental risk especially in the resistance area.

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

Keywords: rodent pest management; infestation; rodenticides; integrated pest management; Y139C; VKORC1

1 INTRODUCTION

Norway rats (*Rattus norvegicus*, Berkenhout, 1769) are commonly found throughout livestock farms because conditions are made favourable by numerous feeding and nesting opportunities. These rats need to be managed to avoid serious damage to infrastructure and stored feed through gnawing, eating and contamination.[1] Management also reduces the risk of disease transmission as rats can be reservoirs and vectors for several zoonotic pathogens such as cowpox virus and Leptospira.[2]

Anticoagulant rodenticides (ARs) are usually used for management on farms. After consumption, they prevent the coagulation process by the inhibition of the vitamin K epoxide reductase enzyme complex 1 (VKORC1). This enzyme catalyses the reduction of vitamin K epoxide into vitamin K quinone facilitating vitamin K recycling, the inhibition of which leads to bleeding and usually death.[3,4]

In response to frequent use since the 1960s, resistance emerged quickly and became established in rodents targeted with the first-generation ARs (i.e. warfarin, chlorophacinone, diphacinone, coumatetralyl).[5] Although metabolic resistance has been discussed,[6] resistance to ARs is mainly due to VKORC1 polymorphisms.[7,8] Resistance-imparting VKORC1 polymorphisms such as Y139C,

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which also affect humans using therapeutic anticoagulants,[7,9] can be found worldwide in Norway rats (*R. norvegicus*).[10]

AR can influence frequency of resistance on farms and resistance-breaking AR can control both resistant and non-resistant individuals alike.[11–14] Resistance-breaking ARs are the most toxic, bio-accumulative and persistent anticoagulant substances and carry a risk of poisoning non-target species, which has been shown in residue monitoring across the world (Mustelidea: Denmark[15]; Foxes: Germany,[16] Norway[17]; avian predators: Finland,[18] Germany[19]). Non-target species such as voles, mice and songbirds can consume rodenticide baits directly.[20,21] There is also a potential risk of secondary poisoning of predators such as barn owls if they feed on contaminated prey. Favourable conditions for rats on farms support rapid reoccupation and population establishment after control measures through immigration from infestations nearby, such that repeated use of rodenticides is necessary.[22]

Sanitary measures are suggested as a way of eliminating favourable conditions for the establishment of Norway rats on farms, and are often referred to in the literature as preventative measures.[23–25] Such measures could both increase the control success and delay reoccurrence, so their potential should be evaluated especially in the case of resistance when attempting to reduce the use of the most toxic AR.

Leirs *et al*.[23] found, using an extensive questionnaire survey in Denmark, that the correlation between farm characteristics and the occurrence of rodents seems to play an important role in rodent pest management. Rats are found particularly in straw stacks and animals feed near the pigsties and when automatic feeders and open drinking basins are used. For preventative rodent management, they recommended keeping feed in rodent-proof containers, avoiding spillage of fodder and general cleanliness. Accessible feed might support high reproduction rates of both individuals who have survived rodenticide treatment or individuals who immigrate. Measures such as sealing holes, joints and door gaps in silos and buildings, as well as rodent wire in front of fans, ventilation slots and drains, could prevent infestation by immigrants. Piles of bulk material, wood and other material outside of buildings encourage renewed rat infestation after control measures have been used.[24] Sanitary measures that remove feeding and nesting sites might make farms unattractive to Norway rats, may delay rat infestation and reduce the use of the most toxic anticoagulants.

In this study, we investigated how sanitary measures affected rat infestations after AR application on livestock farms within an area of Germany with a high population of resistant rats ('German resistance area'). In this area, rats with the VKORC1 polymorphism Y139C are found on farms[7] and the resistance-breaking AR Brodifacoum is commonly used for successful management.[20] We examined, in detail, farm characteristics such as types of buildings and types of livestock, and compared the control success and reoccurrence events on farms with and without sanitary measures. Our study found new factors influencing control success, creating the possibility of reducing the frequency of resistance and the use of the most toxic, bio-accumulative and persistent ARs in order to reduce the risk to the environment.

2 MATERIAL AND METHODS

2.1 Study area

This study was conducted on 12 livestock farms located within a radius of 11 km around the city of Warendorf, in the northwest of North-Rhine Westphalia in Germany, which is a well-known rodenticide resistance area.[26,27] We selected six livestock farm pairs, each matching in attributes that may influence rat infestations, e.g. number of buildings, type of livestock and food storage (Supporting Information Table S1). One farm from each pair was randomly selected as the 'control' where farmers carried out their usual rat management routine and focused on rodenticide application (method: without sanitary measures). On the other farm of each pair, additional sanitary measures were used in addition to the farmer's rat management routine (method: with sanitary measures). Each farm pair was monitored at the same time (Table S1). Additional factors were recorded which could be important for successful rat control, such as the building accessibility (i.e. bait box attribute related to building accessibility), the precise positioning of the boxes (i.e. position near infestation), the presence of water-bearing ditches that give rats access to the sewer system of the farm, and the presence of dogs and cats, which were proven to hunt rats (Table S2).

2.2 Standard protocol

The fieldwork was conducted from September 2018 until September 2019 (Table S1) during the farmer's usual pest management routines, which commonly included AR applications carried out according to Best Practice Codes and bait label instructions.[28,29]

We ensured a standard protocol, covering AR, control procedure, rat activity census, placing of bait boxes, sanitary measures and trapping, was followed to enable comparability. AR cereal baits, with the active ingredient brodifacoum in the concentration of 50 mg kg^{-1}, were used, following the bait label instructions (Fertigköder b').[30] Brodifacoum is known for its resistance-breaking properties in the case of Norway rats with the VKORC1 polymorphism Y139C.[13]

The control procedure consisted of three consecutive periods, the pre-treatment, the treatment and the post-treatment periods. The pre-treatment period lasted 7 days, the treatment period finished when no rat activity was determined in bait boxes after a minimum of one visit and the post-treatment lasted 138 days.

A rat activity census was carried out in all three periods following a standard procedure for rodenticide efficacy evaluation (TNsG[31]) which measured food intake (g/visit) and tracking activity index. During the pre-treatment period, one census was carried out. During the post-treatment period, eight census were carried out, one in each of the weeks 1, 2, 3, 4, 8, 12, 16 and 20 after the treatment period. For each census, two consecutive feeds of non-toxic rolled oats were measured, which were offered to the rodents for 24 h in the bait boxes, in quantities (100–400 g including bait tray of about 30 g) that exceeded the maximum daily intake. During the treatment period, rat activity was measured twice per week. A maximum of 200 g ± 5 g (including bait tray) of the brodifacoum product was placed in each bait box and refilled when necessary. The tracking index was measured at the same time as the feeding index using sand plates near the bait boxes. The percentage of plate covered with rat tracks was categorized as 0 (0%), 1 (1–5%), 2 (6–33%) 3 (34–66%), and 4 (> 66%) coverage.

The bait boxes ('Rattenköderstation A' from Detia Garda GmbH®) were placed using a standardized scheme for the placement of rodenticide baits for rat eradication on confinement livestock farms, and allocated to specific structural elements such as stacks, straw and hey, feed silos and stored old material.[32,33] Structural elements were provided with one bait station and two neighbouring bait stations were reduced to one if the distance between them was < 5 m. We recorded individual box data as potential co-variables, describing the location (indoor/outdoor), building accessibility, feeding and nesting options around the box, and the potential for optimal positioning (i.e. position near infestation) of the bait box when monitoring (Table S2). Geographic coordinates for each bait box were recorded from a global positioning system (GPS) device (Garmin GPSMAP 64st).

Sanitary measures were carried out in a participatory approach with the farmers by removing open feeding sites during the treatment period, and cleaning up the farm by removing nesting sites etc. during post-treatment (Table S3).

Live trapping and snap trapping were routinely carried out by farmers. Any dogs and cats on the farm behaved as normal. Farmers used e.g. Fox Metallrattenfalle by DeuFa® as snap traps, which were baited with oat flakes or a mixture of peanut butter and oat flakes. To prevent trapping of non-target animals, such as feral cats (*Felis silvestris catus*) and birds (e.g. *Passer domesticus*), traps were covered, e.g. with a tin plate.

2.3 Animal sampling

Trap removal and carcass collection were carried out during all three periods, with the following information being recorded: animal identifier (ID), farm number, date, sex (if determined visually) and monitoring period (Table S4). Position of individuals was recorded to differentiate the spatial organization of colonies into indoor *versus* outdoor.[34] A tail tip was collected from each animal and stored in 2 mL Eppendorf tubes filled with 80% undenatured ethanol.

2.4 Analysis of resistance and sex

DNA from tail tissue was extracted using the Gentra Puregen Mouse Tail kit (Qiagen®, Hilden, Germany) following the manufacturer instructions. All samples were analysed for the VKORC1 polymorphism tyrosine139cysteine (Y139C) using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) test.[35] Outer and inner primer sequences were used, as described by Pelz *et al.*[26] PCR was carried out using Carl Roth® Taq polymerase, and products were analysed using gel electrophoresis with 3% agarose gel (high-molecular grade, Sigma®), adding ethidium bromide for identification of the mutation under ultraviolet (UV) light. If the sex of the animals could not be determined visually, it was identified by PCR using two primer sets to amplify parts of the non-pairing sex-determining region on the 'Y' chromosome in an optimized protocol.[36]

2.5 Statistical analyses

2.5.1 Control success

Bait intake data were heavily zero-inflated (Fig. 1). Hence, to assess the effectiveness of sanitary measures on rat control success, the proportion of observations of inactive boxes per period (i.e. number of observations of boxes where no feeding took place relative to total number of observations of boxes) was analysed. To compare the control success between farms with and without sanitary measures and related farm characteristics such as types of buildings and types of livestock we used generalized mixed effect models. Fixed effects were method (with *versus* without sanitary measures) and period (pre-treatment *versus* treatment *versus* post-treatment) and the following co-variables at the farm scale: presence of hunting dogs/cats, presence of ditches and distance to the next farm. The co-variables at box scale were bait box attributes, feeding/nesting attributes, optimal position of bait box and noticeably good nest site (Tables S1 and S2). The modelling procedure involved four steps, each based on comparing models with period and method as fixed effects using the second-order Akaike information criterion (AICc). We first selected beta binomial family as the optimal distribution in order to model proportional response, since it gave a better fit than either binomial family or binomial family with observation-level random effects (due to overdispersion, Table S5). Subsequently, we choose farm ID as the random effect in combination with an exponential spatial correlation function at farm level, since this random effect structure revealed the best fit compared to other random effect terms, with and without the autocorrelation function (Table S5). In the third step, we selected the fixed effects for a global model. Given that a model with all measured co-variables would be overparameterized, we selected co-variables by fitting models with two-way and three-way interactions (when possible) between method, period and one co-variable. The terms of those models with a lower AICc, when compared to the model with method and period and its two-way interaction, were used to fit a global model (Table S5). In the fourth step, we used multimodel inference to fit candidate models, which are subsets of the global model, and compared them using the AICc.[37] We restricted the complexity of candidate models to a maximum of eight terms. The models with delta AICc < 4 were considered to have substantial empirical support and were used for interpretation. Full and conditional model averaging of coefficients was applied across candidate models with delta AICc < 4. Akaike weight and sum of Akaike weight were calculated across all candidate models to describe the empirical support of each model and the variable importance, respectively. *Post hoc* Tukey tests were used with $\alpha = 0.05$.

Associations between the observed frequencies of tracking index on farms with and without sanitary measures were tested for each period using Pearson's Chi-squared (χ^2) test (or Fishers Exact test) and visualized with mosaic plots. In mosaic plots, the area of the tiles is proportional to the observed cell frequencies and the colour represents Pearson residuals, which show departures from independence. The size of residuals determines the shading of a tile[38]: dark grey stand for large residuals (> 4, corresponding to individual $\alpha = 0.0001$), grey for medium-sized residuals (< 4 and > 2, corresponding to $\alpha < 0.05$), and light grey tiles for small residuals (< 2).

2.5.2 Reoccurrence

A time-to-event analysis using non-parametric Kaplan–Meier curves was carried out to assess the probability of reoccurrence of Norway rats on farms with and without sanitary measures. We determined, for each box, the duration in days between the last visit in the treatment period and the first food intake in the post-treatment period (i.e. the first date with food intake per visit > 0 during post-treatment). These measurements were taken in relation to sanitary measures and factors that were important for successful rat management (i.e. presence of ditches and hunting dogs/cats). Based on Kaplan–Meier curves the reoccurrence was described by the time until 20% of all boxes were active again. Since we installed on average 22 boxes per farm this translates to approximately 4.4 boxes per farm. In addition, we determined the overall reoccurrence after 138 days in the post-treatment period. Observations were censored when no feeding was observed during post-treatment. *Post hoc* log rank tests were used to test for differences between groups.

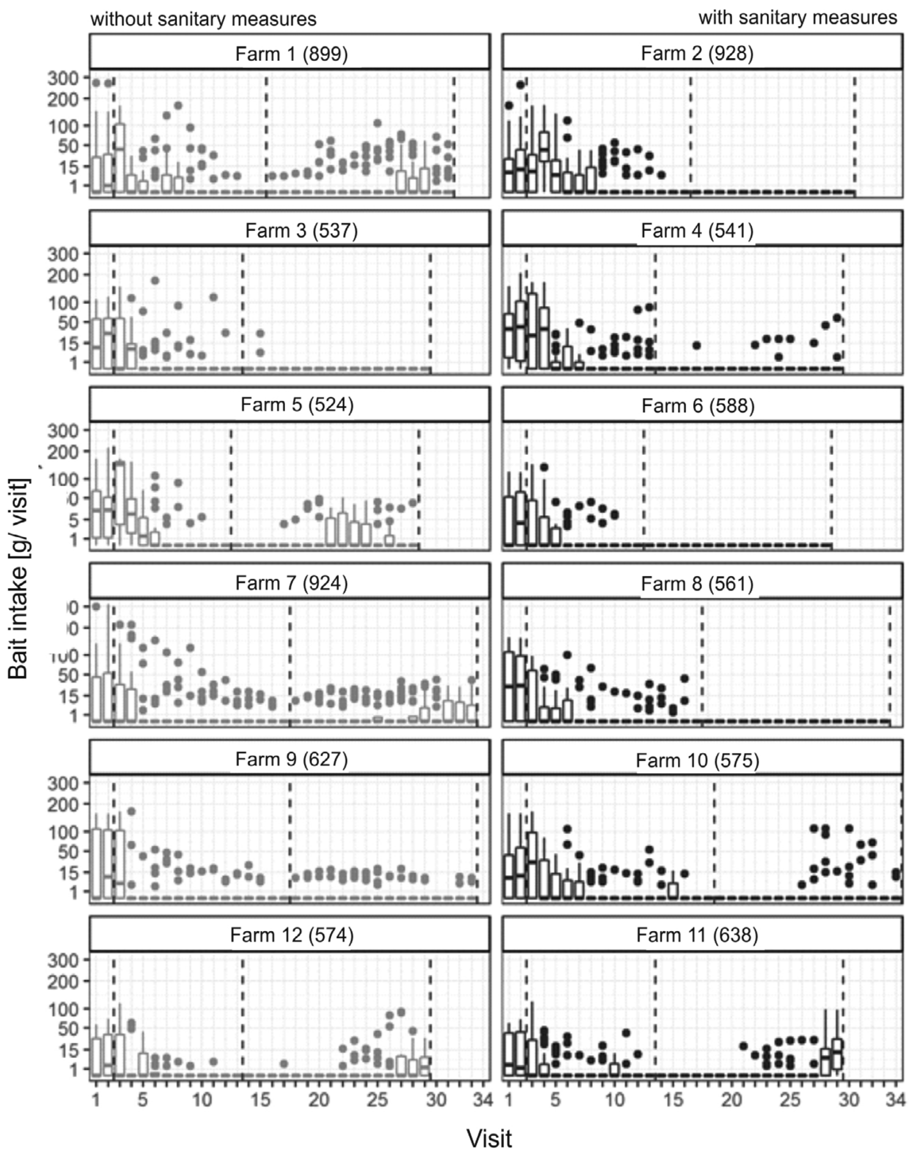

Figure 1. Bait intake (g/visit) for each farm and farm pairs with (dark grey) and without sanitary measures (light grey). Box plots visualize median, lower and higher quartiles, minimum and maximum, and observations as points, which are outside the 1.5 interquartile range. Vertical lines show the three periods: pre-treatment, treatment and post-treatment. The *Y*-axis is on a square root scale. Number of observations per farm in brackets. SaM means sanitary measures.

2.5.3 Resistance

To describe frequency of resistance the relative frequency of the polymorphism Y139C was calculated and expressed as a proportion of rats collected for each period. Associations between the observed zygosity frequencies in Y139C (heterozygote, homozygote, not present) on farms were tested in relation to period, sanitary measures, location and sex using Pearson's χ^2 test and visualized with mosaic plots.

All statistical analyses were carried out using R-project 3.6.3 with the packages vcd, glmmTMB, MuMIn, effects, emmeans and ggplot2.[39–45]

3 RESULTS

3.1 Rat infestation

Intake was on average 773.6 g per farm and day (range: 304.5–1040 g per farm and day) in the pre-treatment census and 6.5 g per farm and day (0–39 g per farm and day) in the first post-treatment census resulting in a 99.2% reduced food intake (Table S6). While mean food intake per farm and day was similar between farms without and with sanitary measures in the pre-treatment (761.4 *versus* 785.8 g per farm and day), it differed in the post-treatment period (12.9 *versus* 0 g per farm and day).

Intake of bait (Fig. 1) and the tracking index (Supporting Information Fig. S1(a)) showed very similar patterns, with both decreasing over time during the treatment period. The treatment period lasted between 35 days (Farms 5 and 6) and 56 days (Farm 10) until the rat infestation reached the stop criteria and mean bait intake was 2149.8 g per farm (range: 753–4342 g per farm) (Table S1). On some farms (e.g. Farms 1, 4, 5, 10, 12 and 11), an increasing food intake (= infestation) was observed during the post-treatment while, on other farms (e.g. Farms 2, 3, 6 and 8), hardly any food intake (= infestation) occurred within the post-treatment period.

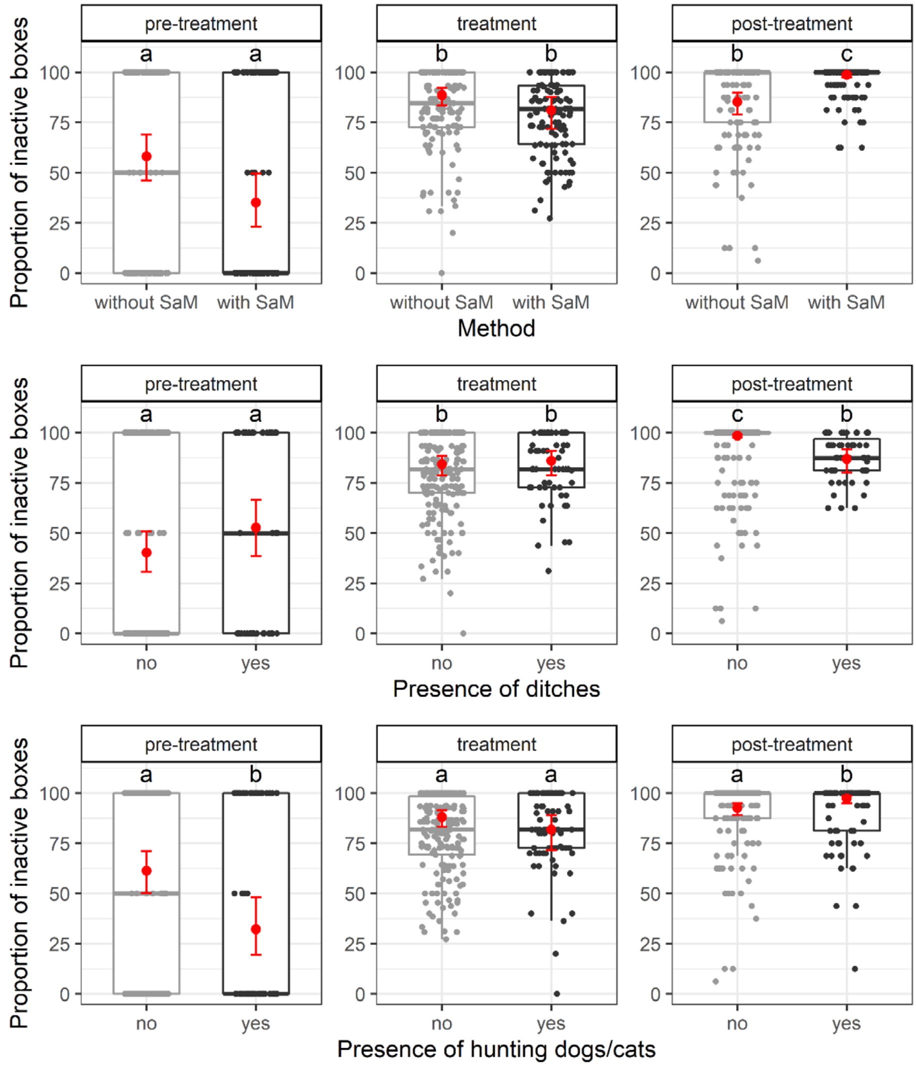

Figure 2. Proportion of inactive boxes (i.e. boxes where there was no feeding by rats) for the three periods and farms without/with sanitary measures, with ditches (yes/no) and with hunting dogs/cats (yes/no). Boxplots are with observed values overlaid as jittered points. Estimated marginal means with 95% confidence interval of the best model are displayed in red. Groups sharing a letter do not differ according to Tukey's *post hoc* test at α of 0.05. SaM means sanitary measures.

3.2 Control success

The proportion of inactive boxes was affected by sanitary measures, periods, presence of ditches, bait box attributes relating to building accessibility, presence of hunting dogs/cats and positioning of the bait box (see Table S7 for sum of Akaike weights, and model averaged odds ratio in Fig. S2). Other explanatory variables, such as distance to the next farm, bait box location (e.g. indoor *versus* outdoor), feeding/nesting attributes or noticeably good nest site, were of minor importance (see Table S5).

Modelling resulted in two candidate models within a range of delta AICc < 4 (Table S8). Both top candidate models contained the interactions between method and period, presence of ditches × period and the main effect of building accessibility. The best model further included an interaction between presence of hunting dogs/cats and period, while the second-best model contained the interaction between optimal positioning of bait box and period.

The proportion of inactive boxes (i.e. boxes without rats feeding) was low during the pre-treatment period at all farms without and with sanitary measures (58.1% *versus* 35.2%) but increased in the treatment period (88.7% *versus* 81.0%) on all farms (Fig. 2). In the post-treatment period, farms without sanitary measures had a lower proportion of observations of inactive boxes than those with sanitary measures (85.3% *versus* 98.8%). Similar effects were observed for the presence of ditches and dogs/cats: during pre-treatment and treatment. The proportion of inactive boxes was at a comparable level, while in the post-treatment period, a higher proportion of inactive boxes was seen at farms without ditches (98.6% *versus* 87.0%), and with dogs (97.3% *versus* 92.6%) (Fig. 2). The proportion of inactive boxes was higher in the post-treatment period, when optimal positioning of the bait box was possible (96.1%) or indeterminant (97.6%) in contrast to when optimal positioning was not possible (91.8%; Fig. S3). Independent of method and period, the proportion of inactive boxes was highest when building accessibility was high (93%), which differed from outdoor boxes (82%) and from boxes in buildings with medium and low accessibility 75.5% and 73% respectively (Fig. S3). Consequently, a farm with sanitary measures, no ditches and with hunting dogs/cats present might result in 99.8% inactive boxes during post-treatment (best case) while a farm without

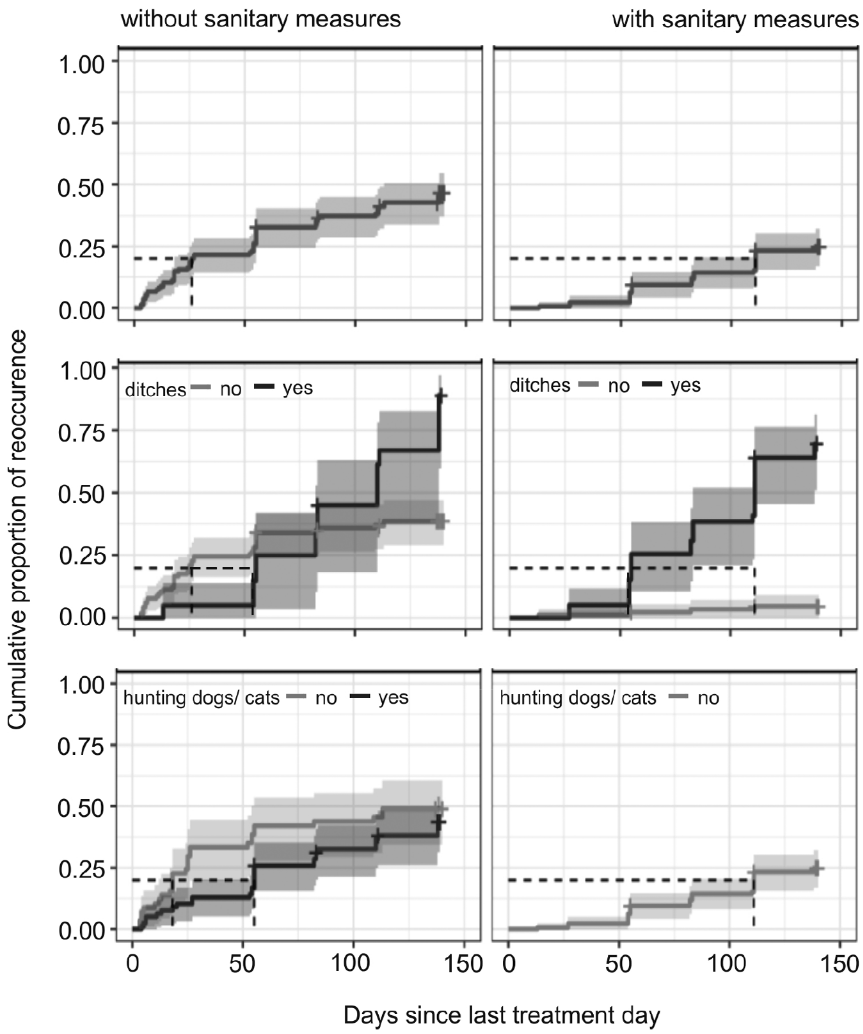

Figure 3. Kaplan–Meier plots showing the cumulative proportion of reoccurrence (and 95% confidence interval) derived from time since last treatment to first feeding reoccurrence in days on farms without/with sanitary measures combined with ditches (yes/no) and combined with hunting dogs/cats (yes/no). Dashed lines mark reoccurrence event by rats as indicated by first feeding from 20% of bait boxes (see also Supporting Information Table S11 for summary of risk table).

sanitary measures, with ditches and no dogs and cats might result in only 51.4% inactive boxes during post-treatment (worst case).

Likewise, for the tracking index, we observed a similar pattern. There was no departure from independence in the frequency of tracking index between farms without or with sanitary measures during the pre-treatment period (P-value = 0.21, df = 4, $\chi^2 = 5.8$, $n = 367$; Fig. S1(b)) and treatment periods (P-value = 0. 58, df = 4, $\chi^2 = 2.9$, $n = 2144$; Fig. S1(b)). In the post-treatment period, tracking indexes 1 and 2 were more often observed at farms without sanitary measures (Fisher's Exact Test P-value < 0.00001, $n = 2623$), indicating lower infestation at farms with sanitary measures (see Table S9 for detailed frequencies).

3.3 Reoccurrence

Kaplan–Meier plots for the duration in days between last treatment visit and first food intake in the post-treatment period show different reoccurrences of Norway rats in relation to method (without/with sanitary measures) ($P = 0.0001$, $\chi^2 = 16.3$, df = 1, Fig. 3).

The reoccurrence of rats, as indicated by first feeding from 20% of all boxes, was 85 days earlier on farms without compared to farms with sanitary measures (26 days *versus* 111 days; dotted line in Fig. 3). Farms without sanitary measures had a steeper increase over the first few days and a higher overall reoccurrence after 138 days, than farms with sanitary measures (46.7% *versus* 25%; Fig. 3). The presence of ditches increased reoccurrence rates even more ($P = 0.0000$, $\chi^2 = 65.8$, df = 3; Fig. 3 and Table S10). In fact, 89% reoccurrence was observed at farms with ditches and without sanitary measures, while 70% reoccurrence was observed at farms with sanitary measures. Overall, the lowest cumulative proportion of reoccurrence was observed at farms with sanitary measures and without ditches, with just 5% reoccurrence after 138 days (Fig. 3). Farms without ditches and without sanitary measures had a steep increase in reoccurrence over the first few days, i.e. 20% of boxes were re-visited by rats after 25 days (dotted lines in Fig. 3), but this slowed down leading to an overall reoccurrence of 39% after 138 days. The Kaplan–Meier plot for the presence of hunting dogs/cats and sanitary measures show different rates ($P = 0.00008$, $\chi^2 = 19.0$, df = 2), but the effect of hunting dogs/cat was much weaker compared to the sanitary measures (Fig. 3). At farms without sanitary measures (Fig. 3, left panel), 20% reoccurrence was observed after 55 days with, and after only 18 days without, hunting dogs/cats. The overall reoccurrence was 44% with and 49% without hunting dogs/cats, but those data did not differ according to the log rank *post hoc* test (Table S10).

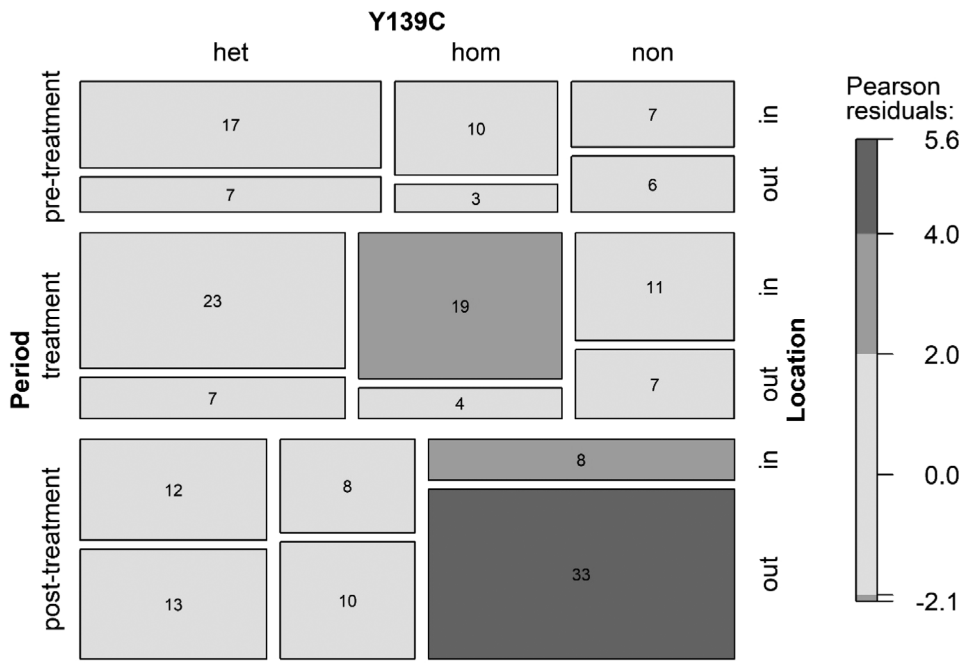

Figure 4. Mosaic plots derived from resistance analysis with observed frequencies of zygosity in Y139C: het(erozygote), hom(ozygote), non (wildtype) collected from all farms over all periods and locations (in = indoor, out = outdoor) using Pearson's χ^2 test. Sanitary measures had no effect. Tiles are arranged from left to right in the order of het, hom and non. Area (height and width) of the tiles is proportional to the observed cell frequencies and the colour represents Pearson residuals, which show departures from independence. The size of residuals determines the shading of a tile[38]: dark grey stand for large residuals (> 4, corresponding to individual $\alpha = 0.0001$), grey for medium-sized residuals (< 4 and > 2, corresponding to $\alpha < 0.05$), and light grey tiles for small residuals (< 2).

3.4 Resistance

Over the entire monitoring, a total of 230 dead rats were collected, of which 131 were males, 87 females and 12 were of unknown sex due to the degree of decay (Table S4). Seventy-five (33%) samples had no Y139C polymorphism. The Y139C polymorphism was found heterozygous in 79 (35%) and, in 58 (25%) samples, the Y139C polymorphism was found homozygous. Eighteen (8%) were with unknown resistance status because of insufficient quantity of the DNA. The overall Y139C frequency varied between the periods and was lowest, at 0.35, in the post-treatment period compared with 0.52 in the pre-treatment and 0.54 in the treatment periods.

The zygosity frequencies in Y139C differed between periods. There were more non-resistant animals (Y139C not present) compared to resistant animals (Y139C heterozygote and homozygote) in the post-treatment period (Fig. S4(a)). Therefore, non-resistant animals (Y139C not present) were more often collected outdoors compared to indoor locations. During the treatment period, homozygote animals were more often collected indoors than outdoors (Fig. 4). The frequency of zygosity in Y139C was independent of sex (Fig. S4(b)). Furthermore, in the post-treatment period, heterozygote animals were collected less often at farms without sanitary measures (Fig. S4(c)). The sex ratio was similar between resistant and non-resistant animals in all periods (Fig. S4(d)).

4 DISCUSSION

4.1 Sanitary measures

Our study demonstrated that sanitary measures considerably improve the management of Norway rats on livestock farms, in agreement with recommendations from other studies, for example, on pig farms.[23, 46] We identified that the beneficial effects of sanitary measures, such as reducing food and removing nesting sites, led to 99% of observations of inactive bait boxes (i.e. boxes without feeding by rats) occurring during the post-treatment period, in contrast to about 85% of observations on farms without sanitary measures. The reoccurrence of rats, as indicated by first feeding from 20% of all boxes, was delayed by 85 days on farms with, compared to farms without, sanitary measures in the post-treatment period. It is not possible to remove food and nesting sites completely in practice.[24,46] Nevertheless, basic sanitary measures have the potential to increase rat control success and delay reoccurrence noticeably. Farmers considering sanitary standards as suggested in the Danish rodenticide resistance strategy,[23,47] to avoid rat infestations, reduce rodenticide application, and mitigate the risk of infection and environmental damage.

Removal of food sources before treatment increases consumption of rodenticides. We have shown, that removal of nesting sites after treatment means that accessibility to buildings increases control success when using indoor bait boxes. Independent of method and period, the proportion of inactive boxes was highest when bait boxes were located in buildings with good accessibility.

We found that the accessibility of buildings, has an effect on the control success, most likely because individuals living around the buildings have access to the bait boxes. Hence, we highly recommend that holes into buildings and other entrances for rat should actually only be closed after treatment, in order to ensure the best control success.

Lambert *et al.*[24] demonstrated that habitat modifications through removal of vegetation cover and items, such as bales and pallets, near farm buildings, reduced the survival rate and size of rat populations living near farm buildings. In collaboration with the farmers, we carried out such measures, for example, moving fire wood and tyres into neat stacks to avoid shelter for nesting sites. These measures were effective, but required a certain discipline and engagement on the part of the farmer. When such measures were neglected, a reoccurrence of rats quickly became visible.

4.2 Further factors influencing control success

Apart from sanitary measures and accessibility of buildings, sewer systems with rat barriers to ditches and rat-hunting dogs and cats increased control success and delayed the reoccurrence of rats. It is already known that ditches are preferred habitats of rats[48] and sewer systems are often used as refugium by rats, particularly in cities.[49] We have shown the effect of these habitat preferences on farms, whereby rats living in ditches seem to infest farms through sewer systems with no barriers. Such habitat structures represent a variety of ecological factors (protection of predators, for orientation and finding feed) that favour the presence of migratory rats and play a special role in the migratory processes of Norway rats between open fields and human settlement areas.[48,50] Unfortunately, it is not always possible to retrofit sewer barriers, but rat barriers should be considered when renewing sewer systems.

Although we monitored only four farms that had rat-hunting cats and dogs, the benefit of them seems evident. Studies have shown that dogs and cats are good at hunting rodents around farms and reduce their activity.[51,52] Cats could be able to prevent re-infestation if the rat population is very small,[53] in the case of immigration of single rats where avoidance of reoccupation becomes possible. Four out of five farms that had cats remained almost completely free from rats after treatment, while the farm that had no cats continued to suffer from recurring infestations.[54] Encouragement of the use of hunting dogs and cats would not only lead to lower rat infestation but also distract the animals from killing endangered species such as songbirds.[51]

4.3 Rodenticide application and resistance

Application of brodifacoum led to an eradication of rat infestations. Brodifacoum, along with flocoumafen and difethialone, is known for its resistance-breaking effects in rats with Y139C polymorphism on farms.[13] Other anticoagulant compounds such as warfarin, coumatetralyl, bromadiolone and difenacoum would result in lower control success because these are ineffective against resistant individuals. Calciferol could be an alternative to the resistance-breaking ARs. The active ingredient was recently approved and is available to farmers.

Analysis of resistance shows that we had more resistant rats in the pre-treatment period than in the post-treatment period and more indoors than outdoors. The lower frequency of resistant rats in the post-treatment period may be explained by both the control of the resistant indoor individuals and the immigration of individuals from outdoors, which were less often resistant. This may result from territorial behaviour with distinct separations of colonies in space[34] and fewer contacts of outdoor populations to ARs that are applied only in and around buildings. Using sanitary measures to provide effective rat control and prevent rat infestations could have the potential to reduce resistance indirectly because the establishment of indoor populations, which tend to have high resistance frequencies, is delayed.

5 CONCLUSION

Successfully of control resistant Norway rats, the most toxic, bioaccumulative and persistent ARs are required. We demonstrate in our study that sanitary measures combined with rodenticide application can considerably improve the management of resistant rats on livestock farms. Consequently, we recommend removing possible feeding and nesting sites indoor and outdoor to increase the control success and delay reoccurrence of rat infestations. The effect can be increased by timing the sanitary measures, like removing food sources before treatment to redirect rats to baits, and removing nesting sites and building accessibility after treatment to make sure that rats remain to consume bait during treatment. Rat-hunting dogs and cats, and sewer systems with rat barriers, are options that farmers can exercise to avoid reoccurrence. If it is not possible to retrofit barriers in the sewer system, then this should be considered when planning rat control measures. High control success and the definite delayed reoccurrence reduces the risk of zoonotic diseases for livestock and humans. Further, the application of rodenticides can be reduced which is especially important in the resistance area where the environmental risk is high because the most toxic anticoagulants are used for control of the resistant rat population. It should be proven if an effective management together with sanitary measures could reduce resistance occurrence in the resistance area of German.

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AUTHOR CONTRIBUTION

SCH: methodology, investigation, writing – original draft, writing – review and editing; DG: methodology, software, formal analysis, data curation, writing – review and editing, visualization; NK: conceptualization, methodology, validation, investigation, writing – review and editing, fieldwork management, funding acquisition; AE: conceptualization, methodology, validation, investigation, data curation, writing – original draft, writing – review and editing, visualization, supervision, project administration, funding acquisition.

CONFLICT OF INTERESTS

Not applicable.

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DATA AVAILABILITY STATEMENT

Study data can be requested from the authors. All data are available to the editor and reviewers during the review process, if requested. Code availability: Code can be requested from the authors.

ETHICAL APPROVAL

All procedures carried out in studies involving animals were in accordance with the ethical standards of the institution. Rodenticides applications were performed during regular pest control routines and recommendations from Federal Environment Agency[25] and bait label instructions (®DETIA).

SUPPORTING INFORMATION

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

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