Report 19 - 2019

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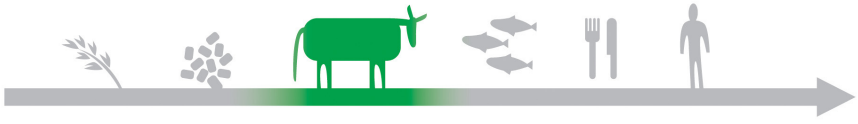
- Results from an experimental study



Veterinærinstituttet
Norwegian Veterinary Institute

The use of electricity to protect buildings against rodents

Results from an experimental study

Content

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Commissioned by TxGuardian

Photo front page: Kristian Ellingsen-Dalskau

ISSN 1890-3290

Summary

There is a need for efficient rodent control to prevent damage of property, spoilage of food and spread of disease. However, the humaneness of the methods and the environmental side effects have received little attention. Traps and poisoned baits are commonly used methods. The second generation anticoagulants have a long half-life and accumulates in tissues and are found in a proportion of dogs and various species of wildlife. Further, many of the rodent species which may invade our houses in winter, are a part of an ecosystem and thus, the goal should not be eradication, but exclusion from buildings. The concept of TxGuardian is to install an electric barrier around the building. Rodents about to enter receive an electric shock which should be aversive enough to demotivate them from proceeding, but without causing death, physical damage or lasting pain. The prototype equipment had previously been tested in an urban environment and found to be effective. By testing the equipment in a controlled setting, a laboratory, the aim was to document any harmful effects on the individual animals regarding physical injury and pain behaviour, and to test aspects of learning/memory.

In the laboratory room, we installed a test house with openings at all four sides, 13 cm above the floor, which could be accessed by rodents climbing up metal pipes. A metal floor list separated a small part of the room. Both the metal pipes and the floor list were connected to the electric power source. Standard food, water, wood shavings, plastic shelters, and nesting material were available inside the test house, on the floor outside the test house and on other side of the floor list. Inside the house and on the other side of the floor list, we also placed very attractive food items (hazelnut kernels and sunflower seeds). The animals were habituated to the test room for one week, with the current switched off, until we knew that all animals made use of the house and crossed the list readily.

After the current was switched on, the animals were released in the room, one by one. We used level 4 giving up to 2600 V. The shock resulted in an instant behavioural reaction, lasting for a moment, only. All parts of the room, including inside the test house, were videotaped until the next morning. The animals were then put into standard cages for 14 days, before they were tested again in the room, with the current switched on overnight. Video recording of the mice (N=6) from 00 to 08 h the first night showed 406 approaches to the house whereof 15 crossings (3.7%), and 929 approaches to the floor list whereof 13 crossings (1.4%). After the pause of 2 w, there were 291 approaches to the house whereof 9 crossings (3.1%), and 1814 approaches to the floor list whereof 11 crossings (0.6%). Among the rats (N=13, videos from 02-06 h) no rat crossed into the test house nor over the floor list of a total of 2569 approaches the first night and 1982 approaches two weeks later. On this basis, we find that the equipment is very efficient against mice and even more so against rats, and that the animals learn and remember. Results from one of the mouse groups (A) also suggest that exposure to electrified barriers over time is more important for learning than the strength of the current.

Clinical examination of the animals after exposure to electricity and autopsy after euthanasia did not reveal any severe injuries or damage caused by the shock. However, at autopsy, some redness (hyperaemia) were found inside the front paws of many of the mice, a finding that was not noticed on live animals, but which may be connected to the current.

During the pilot trials we found that both mice and rats would discover any route to cross the barriers without being shocked, if there were any. Thus, care should be taken to protect all parts of a building. The laboratory breeds used are known to be active and exploratory, and were chosen because we expected their behaviour to be comparable to wild rodents. It is likely that wild mice and rats would have been more cautious, especially at the first encounter, but at the same time their motivation to seek food and shelter is probably higher.

Sammendrag på norsk

Mus og rotter bekjempes fordi de kan gjøre betydelig skade på eiendom og varer og spre smitte. Bekjempelsesmetodenes innvirkning på miljøet og dyras velferd har imidlertid vært lite påaktet. Ulike typer feller og forgiftet åte er de mest benyttede metodene. De mest brukte giftstoffene, annengenerasjons antikoagulanter (vitamin K-hemmere), har lang halveringstid og akkumulerer i næringskjeden. De påvises eksempelvis hos husdyr som hund og i ulike viltlevende predatorer. Flere av smågnagerartene som ofte invaderer hus vinterstid, er en del av naturen, og det bør ikke være et primært mål å utrydde dem, men heller hindre dem adgang til bygninger. Tx-Guardians utstyr er en elektrisk barriere som monteres rundt bygninger, og som gir smågnagere et elektrisk støt hvis de prøver å forsere. Støtet skal være avskrekkende nok til at de trekker seg unna, også når motivasjonen for å gå inn er høy, men ikke så sterkt at de dør eller påføres fysisk skade eller langvarig smerte. Utstyret har tidligere vært testet i felt og vist god effekt. Formålet med å teste utstyret under kontrollerte former i et laboratorium, var å sjekke eventuelle skader av strøm på dyra og å undersøke læringseffekten. Det ble benyttet laboratoriemus og –rotter som var kjent for å være aktive og eksplorative.

Testrommet inneholdt en kasse der dyra hadde tilgang gjennom en slisse på alle fire sider, 13 cm over gulvnivå. På vei inn måtte de forsere metallrør som kunne kobles til strøm. En gulvlist av metall, som også kunne elektrifiseres, skilte av en del av rommet. Inne i kassen, på gulvet utenfor kassen og på andre siden av gulvlisten ble det plassert vann, standard fôr, skjul, strø og redemateriale, mens inne i kassen og på andre siden av listen var det i tillegg spesielt attraktiv mat (hasselnøttkjerner og solsikkefrø). Dyra ble tilvent rommet over ca. en uke før strømmen ble tilkoblet.

Etter at strømmen ble tilkoblet, ble de sluppet en og en på gulvet. Utstyret, som var innstilt på nivå 4 (opptil 2600 V) ga støt som førte til en tydelig atferdsreaksjon, men som kun varte et øyeblikk. Alle deler av rommet, inkludert inni kassen, ble deretter videoovervåket over natt. Neste dag ble dyra plassert i bur for de neste 14 dagene, før de igjen ble testet i rommet over natt, med strømmen slått på. Gjennomgang av videoene av musene (N=6) tatt mellom kl. 00-08, viste at det var 15 kryssinger inn til kassen av 406 forsøk (3,7 %) og 13 kryssinger av gulvlisten av 929 forsøk (1,4 %) første natten. Ved gjentaket etter 14 dagers fravær var tilsvarende tall 9 kryssinger til kassen av 291 forsøk (3,1 %) og 11 kryssinger av listen av 1814 forsøk (0,6 %). Blant rottene (N=13, registrering av videoopptak kl. 02-06) var det ingen som krysset verken inn til kassen eller over gulvlisten, verken første natten (av totalt 2569 ganger de forsøkte seg) eller etter 14 dagers fravær (av totalt 1982 ganger). På dette grunnlaget vurderes utstyret å være meget effektivt overfor mus og særdeles effektivt overfor rotter. De husker ubehaget og lærer å holde seg unna. Resultater fra en av musegruppene (A) kan tyde på at det er viktigere for læringseffekten at strømmen er tilkoblet over tid, enn at strømstyrken er høy.

Ved kliniske kontroller underveis og ved obduksjon i ettertid ble det ikke avdekket alvorlige skader av strømeksponeringen. Under den patologiske undersøkelsen ble det funnet noe rødme under tær på framlabbene hos mange av musene, noe som ikke var observert på de levende dyra, men som kan ha sammenheng med strømeksponeringen.

I pilotforsøket erfarte vi at både mus og rotter fant enhver mulighet til krysse barrierene uten å få støt, hvis slike muligheter fantes. Lærdommen er derfor å sikre alle deler av en bygning. Musene og rottene som ble brukt i forsøket var laboratoriestammer som er aktive og eksplorative, fordi vi ønsket at de skulle være mest mulig sammenlignbare med ville smågnagere. Det er likevel sannsynlig at ville smågnagere ville vært mer forsiktige, samtidig som de også kan ha en sterkere motivasjon for å finne mat og varme.

Introduction

Rodent control

Mice and rats represent a threat to human health, food supply and property, and rodent control measures are considered necessary in most human societies. Rodents may make nests inside houses, gnaw through walls and cabinets, damage insulation, water pipes and electric wiring. They spoil food meant for human or farm animal consumption by eating or contaminate it by urine and faeces, and they may spread harmful pathogens.

A widely used control measure is poison; rodenticides. The most common rodenticide in Norway is second generation anti-coagulant poisons. These poisons work by hampering the coagulation mechanisms in the blood, so that the animals will become bleeders and eventually die from blood loss from internal or external traumas. In contrast to the first generation anticoagulants, the target animals only have to eat from the bait once, or a small amount a few times, as the half-life is very long. This property is also the main drawback of the second generation anticoagulant compounds, as they are poisonous for a large range of species and may thus cause poisoning of non-target animals, including birds, mammals and reptiles (e.g. Sánchez-Barbudo et al., 2012). Further, rodents which have eaten the poisons and become sick, are easy prey for predators and scavengers. Thus, high levels of rodenticides are found in wild predatory birds, e.g. in Denmark (Christensen et al., 2012), Finland (Koivisto et al., 2018) and Norway (Bernhoft et al., 2018). In Norwegian Eagle owls (*Bubo Bubo*), 1-4 different rodenticides were found in the liver of 72% of 100 dead owls sampled in the period 1994-2014 (Bernhoft et al., 2018). For some of the birds, poisoning was likely the cause of death. In his review, Vyas (2017) reported that rodenticides were found in 190 different non-raptor bird species. Anticoagulant rodenticides were found in the faeces of 54% of 139 wild Norwegian red foxes shot in 2016 (Seljetun et al. 2019), implying relatively recent exposure. Further, these substances were found in the liver of 67% of at total 254 Norwegian mammal predators (wolf, red fox, polar fox, lynx, wolverine and American mink, sampled 1997-2017) (Madslien et al., 2019). Poisoning of dogs occur from time to time (e.g. Valheim and Wisløff, 2013) and in a survey among dogs hospitalized for other reasons at the NMBU Small animal veterinary clinic, low levels of rodenticides was found in 21% of the dogs. Exposure to even low levels of anticoagulant rodenticides are expected to have negative effects on non-target animals, such as reduced fertility (poor sperm quality and increased embryonic death), neonatal mortality, increased parasite load and chronic weakening (reported in Seljetun et al. 2019).Thus, there are strong environmental and animal welfare reasons to reduce the use of rodenticides. However, animal welfare considerations should also include the target animals themselves, with the goal to use more humane methods.

Among alternatives to poison are lethal spring traps and other traps which aim to either kill the target rodents immediately or to catch them alive. Traps are not always feasible in practice, because they must be controlled on a regular basis and reset. Not only rodenticides, but even traps may cause severe suffering to the pest animals (Mason and Littin, 2003; Baker et al., 2012), for example due to mishits or too low impact power (Engeland et al., 2010).

Another important aspect is that many rodent species which invade barns and houses to seek food, shelter, and warmth, are wild species which fill an ecological niche in nature. Most of these mice species enter buildings in autumn and leave in spring, and do not breed indoors. The goal in rodent control should not necessarily be to kill all pest animals, but rather to prevent individuals from entering buildings in their search for resources. Methods to frighten the rodents away, e.g. the use of aversive ultra-sound has been tried, but the effect vanishes over time due to habituation. TxGuardian follows a similar but still different line; using an electric barrier (“fence”) around the building which delivers a painful electric shock to the animal if it tries to cross. The goal is that the shock is perceived as unpleasant, but it should not be lethal or physically harmful beyond the immediate and short lasting pain. Successful avoidance learning means that the rodents will lose their motivation to pass the electric “fence” barrier.

Effects of electric current on the animal body

Electric signals are part of all animals' normal physiology, e.g. in the heart and throughout the nervous system. The body consists of approximately 70% water with electrolytes, which also leads electric current well. Other tissues may lead electric current poorly, such as adipose tissue, and fur/wool will protect the skin. When exposed to an external source of electricity, the current will mainly follow nerves and blood vessels. In rodents, the nose and the foot pads are not covered by fur, and thus, electric current from an external source will easily pass the body. Pain is experienced in the body part which is in touch with the electric source and not at the "exit", i.e. the body part in contact with earth.

Electric current is used to control the behaviour of animals in various situations, e.g. shock collars for dogs used for aversion training to prevent chasing of sheep (VKM 2017). In farm animal management, electric fences are widely used. These usually consist of one or two heights of steel wire or plastic ribbons with a steel thread woven into the mesh. The voltage is usually very high, up to several thousand volts for a large enclosure, but there is a fixed limitation for maximum current to be delivered so it is not dangerous for humans or animals. The electricity is sent in pulses (e.g. every 2-10 seconds). A simple electric fence may allow a farmer to control cattle, horses or pigs that otherwise would require very solid fences. In general, animals quickly learn to avoid contact with the electric fence since the association between the perceived pain and the happening "contact with the fence" is rapidly made. Nevertheless, it is also known that some individuals may learn to pass an electric fence without being shocked, usually by running quickly under the fence between two pulses, more rarely to jump over. Physiological factors of the animal (e.g. insulation properties of the fur) may affect the strength of the current the animal is exposed to, and psychological factors (e.g. motivational state) will affect pain perception and thus the reaction to electric shock. For instance, an individual may find that the reward on the other side of the fence is so tempting that the animal is willing to "pay" for it with a shock. Further, the properties of the environment where the electric fence is placed is important. A dry ground will lead current less well than a moist ground due to the higher resistance, meaning that the current passing from the fence through an animal to the ground will be larger in the last example.

Exposure to high voltage/amperage is dangerous as it may cause tonic immobility of muscles, cardiac arrest if passing the heart, and unconsciousness and epileptic seizures if passing the brain. A stroke of lightening is usually fatal. In contrast, electric stimulation with low voltage / low amperage equipment is not dangerous but still aversive. Depending on the stimulus strength, the experience may vary from unpleasant to very painful. Behavioural signs of pain will depend somewhat on species and the individual, but withdrawal/retreat, twitches and vocalizations are common signs. Limping of the exposed limb indicate pain, but also licking or rubbing the exposed body part may be signs of pain. Increased auto-grooming, a conflict behaviour which can be seen in animals which are not sure what to do, may be a sign of stress.

Electric current - principles

Ohm's law describes the relationship between current (Ampere), voltage (Volt) and resistance (Ohm).

Current (A) = Voltage (V)/Resistance (Ω)

If the voltage is kept constant, the current strength will depend on the resistance of the conductor. The resistance describes how easily or difficult current is lead through the material. For instance, current is easily led through water and metal but not rubber and wood. The equation is not directly valid for live tissues but the principle is the same: The current which passes through the animal tissue (i.e. the conductor) between two points (e.g. the nose touching the electric fence and the foot on the ground) will depend on the voltage across the two points (the fence and the ground) and the resistance in the system (how well the conductor, here the animal tissue, leads current). The animal body becomes a part of the electric circuit. Hence, standing on a moist ground will reduce resistance (and increase current) whereas wearing rubber boots will increase resistance (and reduce current).

For electrical fences, we usually talk about effect (Joule), instead of amperage. Ampere and Joule are related but different units. One Ampere is defined as the current needed to transport one Coulomb of electric charge per second. Joule is defined as the work needed to move one Ampere through one Ohm of resistance for one second. The production of one Joule per second is one Watt (W).

TxGuardian equipment

In short, the equipment is designed to prevent rodents from entering buildings, by delivering an electric shock if they touch the electric "fence". The installation consists of two horizontal metal pipes (looking like water pipes) which are mounted on the wall just below the outside wall covering, all around the walls of the building to be protected. The pipes are electrified. Further, for the doors and gates into the building for deliveries of goods, a metal list is placed on the ground. This is also electrified.

The equipment has been developed based on published work on electric parameters including current strength in shocks delivered to mice and rats. Because electric shocks given to rodents have been widely used in science to study learning processes and stress behaviour, and to induce depression as models for human disorders, there are many published papers in this matter. The aim for TxGuardian is to use electric parameters which are shown to be aversive but not physically harmful for the animals. For mice, the scientific literature indicated that 0.18 mA is the lowest effective level. For rats, the literature is less abundant but 0.4 mA was suggested to be effective. Development of the TxGuardian equipment was based on this scientific knowledge.

TxGuardian has conducted field studies with prototypes of the equipment. The company placed small test houses in town areas and attracted rodents by providing feed inside the house. After approximately four months, the electric power was turned on. Rodent activity was assessed using video cameras both inside and outside the house, both before and after the current was turned on. The equipment was modified according to the gained field experiences to the version which we have tested in the laboratory.

The voltage of the equipment can be adjusted (level 1-10), but only by the company and not by the customer. According to TxGuardian, the calculated theoretical maximum amperage is 0.448 mA. Maximum effect is 1.5 Joule and the theoretical level is < 1.0 Joule at the highest level (i.e. level 10). With few exceptions, described later, we used level 4, which provides 2600 V. The number of pulses per second (Hz) was equal to the frequency used in the field experiment.

Test facilities

Approval of experiments and humane end points

The experiments were approved by the Norwegian Research Animal Authority with FOTS id. No. 19062. Animals were weighed regularly during the experimental period. A scoring scheme was developed to follow the animals' health and welfare. Scoring was done before and after exposure to electric current, in addition to the daily care. The scheme included the animals activity score (1=normal, explorative behaviour; 2= reduced activity, sitting in a corner; 3=inactive, lying flat); appearance score (1=normal, shiny hair coat, normal body posture; 2=dull hair coat, abnormal movements or posture; 3=hunchback, no self-grooming, reduced appetite, squinted eyes), and damage score (1=no injuries or damage visible; 2=curly/burned whiskers, burns any place on body, sore feet). If the animal was scored as 2, the animal must be attended to by a veterinarian who will consider the need for analgesic treatment. If the animal got a score of 3, or a weight reduction of at least 15%, or received more than 5 shocks during direct observation, the animal should be euthanised.

Pain behaviour assessment and counts of shocks was done by the researchers during the first minutes after the first exposure with electric current, and the scoring scheme was used on the morning before expose and repeated at the end of the day and on the following day, by experienced lab animal personnel.

If any animal showed pain behaviour like limping lasting more than five minutes after exposure, or showed lasting signs of discomfort, it was to be euthanized. If any animal showed physical injury (e.g. burns on feet), the voltage was to be adjusted (down regulated). Animals always had access to water, food and shelter without being at risk for receiving electric shocks.

Test room and equipment

The trials were conducted at the facilities at Norwegian University of Life Sciences (NMBU), the Veterinary Faculty, Section for Experimental Biomedicine. We used one room with a pen floor area of 275x250 cm available to the animals. A test house was placed in the middle of the room. It measured 58x58 cm and the hight was 76 cm. The roof could be opened. The animals had access to the house via openings (3cm wide) at all four sides of the house, 13cm above the floor. In the setup for mice, three metal pipes were placed on the walls between the floor and the openings, so that the animals could climb up. In the setup for rats, the top pipe was removed to make it easier for them to enter the house. The metal pipes were connected to electric power and could thus be electrified.

Along one side of the room, a floor list, i.e. a metal skirting board, was installed. It was a model of the barrier designed to be used in door and gate openings of the building to be protected. It consisted of three 3 cm wide metal bands which were connected to the current source, and one wider (5 cm) which was not connected. The bands were separated by rubber.

Figure 1. The photos show the experimental room with the test house (left photo), the floor list (right photo) and some of the cameras. The low wall seen on the photo to the right is a barrier separating the entrance from the experimental pen. Photos: Kristian Ellingsen-Dalskau.

To secure that current was delivered from the lower metal pipe and the first metal band, we placed a carpet under the house and under the floor list, and kept it moist. This was necessary because the floor of the room was too well insulated, and thus different from the normal outdoor situation. At the chosen level 4 of the equipment (giving 2600 V), the measured voltage was as follows:

- House: From carpet to lower pipe 1.5 V; from carpet to second pipe 1.2 V; between 2nd and 3rd pipe 2.6 V
- Floor metal list: From carpet to first metal 1.2V; from carpet to 2nd metal list 1.5 V; from 2nd to 3rd metal list 2.6V.

Voltage was checked at all four sides of the house and the floor metal list before each testing.
For the animals' well-being, enrichment items and other resources were provided three places; inside the test house, outside the test house and on the other side of the floor metal list. The enrichment items were red plastic shelters (mouse size for mice and rat size for rats) and red plastic pipes placed on wood shavings, and nesting material (paper cloths and hamp fiber). Standard mouse/rat food pellets and water were available at all three places. In addition, sun flower seeds and hazel nut kernels were offered inside the test house and by the shelter on the other side of the floor metal list, to make these places more attractive to the rodents, and motivating them to pass the barriers.

Eleven cameras were distributed so that every part of the floor, the test house walls, and also the inside area of the test house were covered.

Figure 2. The left photo shows mice inside the test house with food, kernels, water, red plastic shelters and nesting material. On each side there is a slot for entrance/exit. The metal pipes which can be electrified (only visible to the left) are used by the animals to climb on to get inside. The photo to the right shows the test area with the carpet underneath the test house and floor list (installed to improve conduction) and placement of enrichments (here in rat size) and feed/water. Photo: Cecilie Mejdell.

Material and methods - mice

Animals

The mice were of the black laboratory breed C57BL/6JRj and were all females. This breed was chosen because it is known to be active and explorative and it is therefore widely used in behavioural studies. The mice were 8 weeks old when delivered from France via NAISER – Norwegian Animal Import Services, Gladengveien 3B, 0661 Oslo, Norway. After arrival, the animals were kept in groups of 3 (4 mice in group C) in standard, enriched cages for 2-4 days before each group was gradually habituated to being loose in the test room, over 1 week. For identification, the mice were tagged by holes in the ear, according to a standard system used in the lab.

We first used 3 mice in a pilot study to check out suitable marking techniques so that each individual could be identified also during the night, by the infrared cameras. We ended up by using white non-toxic paint (Kelco fine line marker spray) on the tail (not painted; tail painted white on distal part; tail painted white on proximal part). We observed how the animals behaved when tested one by one compared to as a group, to decide upon the experimental set-up. We also evaluated avoidance and pain behaviour after the current was turned on and adjusted the settings accordingly.

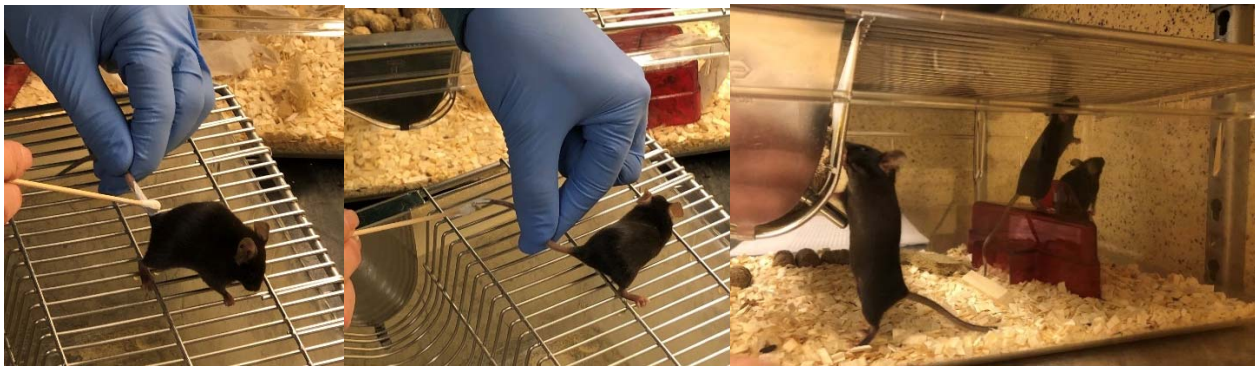

Figure 3. Painting the tails for individual recognition (left and middle). Standard mouse cage in which the mice were kept between the test days. Photos: Kristian Ellingsen-Dalskau and Cecilie Mejdell.

In the main study, we used 9 mice, in 3 groups of 3. There was a 10th mouse which was not included in the study.

Test procedure

After the acclimation period and when we were sure that all mice in the group made use of the test house and crossed the metal list readily, testing started. The first test was done without the electricity. The mice were first taken out of the test pen and placed in a cage. One by one, each mouse was put in a box inside the pen and then released by lifting the box, using a stick operated from outside the pen. The time in seconds until the mouse entered the house or crossed the floor list was recorded. After 5 minutes, the next mouse was released.

The next day, the mice were placed in the cage and scored according to the scoring scheme by the animal caretaker. The current was turned on, and the voltage was checked. The mice were released one by one as described above, and time to entering the house and/or crossing the floor list was recorded, together with the number of electric shocks received and the animals' behavioural reaction (vocalization, withdrawal, limping, licking paw). Each mouse was observed for 5 min before the next was released. The group of mice was then left in the pen until the next morning, only interrupted by a pain score check in the afternoon. The pen and animals were monitored by the cameras. The next morning, a new scoring was done of each animal. Thereafter, the mice were put in a standard cage. They were retested in the pen after 2 weeks in the cage, with current turned on, and stayed overnight in the pen, as described above.

After the second test, the mice were euthanised using isofluaran gas followed by injection of mebumal ip. The same day, they underwent autopsy at the Norwegian Veterinary Institute, section for pathology.

Analyses

Two observers were present when animals were released in the test room. Average time to enter the house or to cross the floor list after release, was calculated for the two situations, before and after the electricity was turned on, and thereafter with electricity turned on after 2 weeks in the cage. Number of crossings, and whether or not the animals received a shock, was registered.

Regarding the video recordings, emphasis was put on analysing the videotapes taken during the dark hours, when rodents are most active. Behaviour was recorded for 8 h in mice (from 00-08h). The period was longer in mice than in rats because of practical reasons: only the date, not the time, was visible after the mouse videos were saved on the hard disc. Therefore, we had to start right after midnight (new date), and we continued until the light was turned on at 08 in the morning. Altogether, we went through 32h of video recordings comprising around 1800 video clips.

We recorded the number of approaches to the barriers (i.e. metal pipes around house, and the floor list separately); whether the animal crossed or turned away; whether a shock was received or not; deviations from expected reactions to the shock, and, if possible, registration of the individual animal. Video tape analyses were performed during two nights; the first night with the electricity turned on (first exposure), and finally after the animals had been kept away from the test room in 14 days (second exposure).

Material and methods - rats

Animals

The rats were of a hooded laboratory strain named RjOrl:LE and all were males. This breed was chosen because it is known to be less docile and more explorative than most other laboratory strains. The rats were 8 weeks old when delivered from France via NAISER – Norwegian Animal Import Services, Gladengveien 3B, 0661 Oslo, Norway. After arrival, the animals were kept groups of 3 (4 in group C) in standard, enriched rat cages for 2-4 days before the animals (groupwise) were gradually habituated to being loose in the test room, over 1 week. For identification, each rat was marked with colour: no, red, green or blue non-toxic marker pen on the white haircoat (see figure 4).

For the rats, the top metal pipe on the wall of the test house was removed to widen the slot where the rats could enter the test house. We first used 3 rats in a pilot study. In the mouse pilot, we had already found the marking method which was visible in infrared light (white painting on parts of the tail). Further, the testing procedure (i.e. one by one) worked fine with the rats, and we stuck to voltage level 4, which seemed to be effective without causing strong pain behaviour. Therefore, the results from the pilot rat group could be included in the results.

In the main study, we used 10 rats, in 2 groups of 3 and one group of 4.

Figure 4. Left photo shows two rats outside the test house, the closest marked with a red pen on the haircoat and the tip of tail painted white. Right photo shows a rat about to enter the house, climbing up the metal pipes. Photos from video tapes, by Kristian Ellingsen-Dalskau.

Test procedure and analyses

After the acclimation period, and when we were certain that all individual rats in the group made use of the test house and crossed the metal floor list readily, testing started. The procedures used were the same as those described for mice. For the video analyses, we followed the rats during 4 h in the dark period (02-06 h). We analysed the videos online and therefore had all information on both date and time available to us, in contrast to the situation for the mouse videos. Since we could use recordings from all 13 rats, we are confident that analyses of 4 h per night gives sufficient information. Altogether, we went through 32h of recordings comprising around 5000 video clips.

After the tests were completed, the rats were euthanized by inhalation of gradually increasing concentrations of CO_2 gas in air. The same day, they underwent autopsy at the Norwegian Veterinary Institute, section for pathology.

Results - mice

At all behaviour and health scorings (general appearance and pain scale) all mice had normal scores. The mice reduced their weight slightly during the days when they were kept loose in the test room, probably due to the abundant space and many enrichments causing a high level of activity in the large room.

For the mice in the pilot study, the equipment was first set to the lowest level of current (level 1). This had hardly any aversive effect. The three pilot mice show mostly some behavioural reaction to the shock but it did not slow them down or prevent them from passing the barrier. The voltage was increased to level 4, which was the level we had meant to use based on experiences from the field studies. Now, the pilot mice showed more evident aversive behaviours, but they still passed the barriers. Further, they adopted means to pass without being shocked. This could be climbing on plastic covered cables to pass the floor metal list or jump directly to the upper metal pipe. During this pilot trial, we decided to increase earth contact by installing a moist carpet under the test house and the floor list. Further, the fact that the pilot mice readily passed the electrified barriers led us to believe that level 4 was not aversive enough. When we tested group A (n=3) for the first time, we therefore set the equipment to level 5 (~5000 V). However, this was evidently painful. The mice reacting by loud vocalizations, rolling and jumping away, or limping the first seconds after exposure. We quickly decided not to leave the mice in the pen overnight (as was the plan according to the test protocol). A thorough check of the animals, using the scheme and pain score, was performed one hour later, and then all the mice were judged to be fine. Therefore, they were not euthanized at that time but left in a standard cage to be checked twice a day and eventually be retested two weeks later. Mice in groups B and C (n=6) were tested according to the test procedure, using level 4.

The pilot study gave us valuable information: if there is a way to evade the electric shock, mice will find the way to do so. They learned to jump directly to the top metal pipe, to climb on plastic covered cables, to jump via cameras, and to find any non-electrified part of the system and enter there. The take home message is to be very accurate when installing the equipment, to close all alternative routes. This is also the case with rats.

There were some missing information from the video-cameras. Some of the cameras stopped recording, and we did not always discover this in time to replace them. The cameras were supposed to film only when there were animals moving, but they stopped recording after five minutes even when animals were present, and thus, the longest video clip is five minutes. At some places, especially by the floor list, light reflections made it difficult to distinguish between individuals. As explained under the Materials and Methods section, information on exact time of the day when each of the clips was taken was lost when we downloaded the video clips. Due to this, and a very high number of video clips in total, making it far too time consuming to watch all, we chose to analyse the video clips from midnight (00:00 h) until the light in the research animal facility was turned on in the morning, which happened around 08.

Results from the visual observation period (5 minutes per animal) is shown in Table 1, and results from video recordings in Tables 2 and 3. Table 1 shows time to cross a barrier (enter test house or cross floor list) after habituation, and at first exposure and after two weeks. Number of animals not crossing increases, as does the time before crossing in those animals which do cross.

Table 1. Results from visual observations of mice in test room, groups B and C.

	Without electricity	**First exposure, electricity turned on**		**Exposure after 2 weeks, electricity turned on**	
	Time to enter house or cross list (sec)	**Time to enter/cross (sec)**	**No. of shocks**	**Time to enter/cross (sec)**	**No. of shocks**
Groups B & C N=6	$\overline{X}$ =5.33 (N=6) (range 3-7)	1 did not enter $\overline{X}$ =56 (N=5) (range 2-264)	$\overline{X}$ =3.33 (N=6) (range 0-5)	4 did not enter $\overline{X}$ =44 (N=2) (range 33-55)	$\overline{X}$ =0.33 (N=6) (range 0-1)

Table 2 shows the number of approaches to a barrier resulting in turning or crossing, and whether the mice receive a shock before turning or at crossing, at second exposure. Some mice manage to cross without being shocked.

Table 2. Results from video recordings of mice from group B and C (N=6) in test room, overnight, at second exposure (i.e. 2 weeks after first exposure overnight, and in the meantime kept in cages). Percentage with/without shock may not sum up to 100, because it sometimes was uncertain whether the animal received a shock or not when crossing, partly due to failure of cameras.

	Test house			Floor list			Total (house and floor list)		
	Number	% with shock	% without shock	Number	% with shock	% without shock	Number	% with shock	% without shock
Approach and turn	282	0	100	1803	0	100	2085	0	100
Approach and cross	9	0	100	11	27	36	20	15	65
Total approaches	291	0	100	1814	0.2	99	2105	0.1	99.7

The ability of the equipment to prevent mice from passing a barrier is given in Table 3, showing results from video recordings the first and second exposure and combined. The electric barrier effectively prevented the mice from passing and that there was a learning effect.

Table 3. Comparison of the number of approach and turn and approach and cross by mice from group B and C (N=6) in the test room at night (8h from 00-08) in the 1st versus 2nd exposure (i.e. after a pause of 2 weeks). Efficiency measured as percentage of crosses is shown.

	Test house		Floor list		Total (house and floor list)		Total for 1st and 2nd exposure
	No 1st	No 2nd	No 1st	No 2nd	No 1st	No 2nd	
Approach and turn	391	282	916	1803	1307	2085	3392
Approach and cross	15	9	13	11	28	20	48
Total approaches	406	291	929	1814	1335	2105	3440
Efficiency: % crossings	3.7%	3.1%	1.4%	0.6%	2.1%	1.0%	1.4%

The number of approaches at the test house was reduced by 28 % from the first to second exposure (Table 3). This indicates that the mice had learned to associate entering the test house with a negative happening and preferred to stay outside. However, at the floor list, the number of approaches was almost doubled at the night of the second exposure compared to the first. We believe is caused by some individual mice which actually had crossed the list: they seemed to be greatly distressed for being “trapped” behind the list at the same time as they were very reluctant to cross back, getting another shock. Thus, they approached and turned again and again. Likely, the mice were trying to find a way to cross back over the floor list without getting shocked.

Group A mice were exposed to more aversive current (level 5 with 5000V compared to level 4 with 2600V) than the other groups, as judged by their display of pain behaviours. However, group A mice were exposed to barriers with this high voltage over a shorter span of time (3 hours versus overnight). When testing them two weeks later (data not shown), all 3 mice crossed the electrified barrier, on average 62 seconds after release (range 4-119s) and they got on average 4 shocks (3-6) before entering the test house or crossing the floor list. These results suggest that exposure to electrified barriers over time is more important than the strength of the current for the learning outcome.

After euthanasia, all 13 mice underwent autopsy with external and internal gross examination (results presented in Table 4). One of these, C4 was not included in the analyses but had been exposed to electricity once. Five mice had partial keratitis/cataract in both eyes, and we do not know whether they had this on arrival or developed during the housing period at the laboratory, and if so, whether it has any connection with exposure to electric shocks or other environmental factors. Ten mice were found to have hyperaemia (redness) of some toes of at least one front paw. The rodents will normally touch the electrified barrier with the front paws, and the hyperaemia is probably caused by physical contact with the electrified barriers. Except for Group A mice, which were exposed to the higher voltage, the hyperaemia was categorized as weak. The three pilot mice did not show hyperaemia. A reason for this might be that they learned to avoid the shocks already at the lowest voltage (level 1).

Table 4. Results from the autopsy of mice. N means normal (i.e. no pathological findings).

	Pilot mice			Group A			Group B			Group C			
Organ	P1	P2	P3	A1	A2	A3	B1	B2	B3	C1	C2	C3	C4
Whiskers	N	N	N	N	N	N	N	N	N	N	N	N	N
Nose	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes (keratitis/cataract)	Yes	N	N	N	N	N	N	N	N	Yes	Yes	Yes	Yes
Front paws, no. toes w. hyperaemia	0	0	0	5	4	5	5	4	3	8	4	8	7
Hind paws, no. toes with hyperaemia	0	0	0	0	0	0	0	0	0	0	0	0	0
Internal findings, including heart	N	N	N	N	N	N	N	N	N	N	N	N	N

Results - rats

We did not need to adjust any procedure after testing the set-up on pilot rats, and the same setting of the electric apparatus as for mice (group B and C) was used (level 4). Results from all 13 rats are showed in the tables below.

At all behaviour and health scorings (general appearance and pain scale) in live rats, all had normal scores. They reduced their weight slightly during the days when they were kept loose in the test room, probably due to the abundant space and many enrichments causing a high level of activity compared to the cage.

Similar to the situation in the mouse trial, there were some missing information from the video-cameras in the rat trial. Rats often climbed onto the cameras, and this could change the camera angle or even make them fall down. Some cameras stopped recording, and this was not always discovered in time to replace them. Further, cameras did not record continuously for more than 5 minutes even if there was a rat present, resulting in some missing data. To reduce the number of video clips to be analysed, we only analysed clips taken in the dark hours between 02 and 06.

Results from the visual observation period (5 minutes per animal) are shown in Table 5, and results from analyses of video recordings in Tables 6 and 7.

Table 5. Results from visual observations for of rats in test room, pilot, A, B, and C groups. Rats were observed for 5 minutes each after release.

	No electricity	**First exposure with electricity turned on**		**Exposure after 2 weeks, electricity on**	
	Time to enter house or cross list (sec)	**Time to enter or cross (sec)**	**No. of shocks**	**Time to enter or cross (sec)**	**No. of shocks**
4 groups N=13	1 did not enter or cross $\overline{X}$ =19 (N=12) (range 6-53)	1 did not enter or cross $\overline{X}$ =28 (N=12) (range 5-139)	$\overline{X}$ =1 (N=13) (range 0-2)	12 did not enter or cross $\overline{X}$ =7 (N=1)	$\overline{X}$ =0.5 within 5 minutes (range 0-1); $\overline{X}$ =1.2 within 30 minutes (range 0-3)

Table 5 shows the number of rats crossing the barrier and the latency to cross after release at three times: after the habituation period, after electricity was switched on, and after being in the cage for 14 days. The rats remembered well, and 12 of the 13 rats did not cross during the visual observation period at the second exposure.

Table 6 shows the number of approaches to an electrified barrier resulting in turning or crossing, and whether the rats received a shock before turning or at crossing, at second exposure. There were many approaches, but none of the rats crossed a barrier, neither into the house nor over the floor list. Only 1% of the approaches resulted in a shock, indicating that the rats remembered well from two weeks before.

Table 6. Results from video recording of rats in test room, pilot, A, B, C (N=13) over night, at second exposure (i.e. 2 weeks after first exposure overnight and in the meantime kept in cages).

	Test house			**Floor list**			**Total (house and floor list)**		
	Number	**% with shock**	**% without shock**	**Number**	**% with shock**	**% without shock**	**Number**	**% with shock**	**% without shock**
Approach and turn	1483	1	99	499	1	99	1982	1	99
Approach and cross	0	0	0	0	0	0	0	0	0
Total approaches	1483	1	99	499	1	99	1982	1	99

To check whether rats actually can sense the voltage, i.e. know whether the electricity is on or off without being in direct contact with the electrified barriers, we put the pilot rats back in the test room two days after the second exposure, now electricity switched off. None of the three rats crossed any barrier within 30 minutes observation time. However, video recordings showed that all of them entered shortly after we had left the room, indicating that current should be turned on at all times to keep rats away.

Table 7 shows the ability of the equipment to prevent rats from passing a barrier. Data from video recordings from the first and second exposure is shown separately and combined. There were many approaches but no crossings within the chosen observation period. However, it should be noted that two rats in group B had entered the test house before the video recording started in both trials, and did not leave it again. In addition, in 4 of the 8 trials, there were rats which entered the test house and did not leave it again within the observation period. Nevertheless, there is no doubt that the electric barrier very effectively prevented the rats from crossing.

Table 7. Comparison of the number of approach and turn and approach and cross by rats from group pilot, A, B and C (N=13) in test room, overnight, in the 1st versus 2nd exposure (02-06 h).

	Test house		Floor list		Total (house and floor list)		Total for 1st and 2nd exposure
	No 1st	**No 2nd**	**No 1st**	**No 2nd**	**No 1st**	**No 2nd**	
Approach and turn	2183	1483	386	499	2569	1982	4551
Approach and cross	0	0	0	0	0	0	0
Total approaches	2183	1483	386	499	2569	1982	4551
Efficiency: % of crossings	0%	0%	0%	0%	0%	0%	0%

There was a 32% reduction in the number of approaches to the test house at the second exposure compared to the first. A similar reduction was also observed in the mice trial, and, as with the mice, this can probably be explained by the animals associating the house with the negative experience of receiving a shock. The much higher number seen for approach and turn at the test house compared to the floor list can probably be attributed to the fact that there were rats remaining in the test house for the full duration of the test

periods, motivating the other rats to join. The rats which remained outside in the test area, had frequent nose-to-nose contact with the rat in the test house, resulting in a high number of approach and turn. At the floor list, an increase in the number of approaches was observed from the 1st to 2nd exposure also for the rats. A possible reason for this could be that during the first exposure, the rats could climb up on a device close to the floor list and jump across the list without receiving a shock. This possibility was later removed.

The rats seemed to be in less distress by being "trapped" behind the floor list compared to the mice. Whereas the mice would desperately seek for a way to cross back without receiving a shock, the rats which had crossed remained calm behind the list.

All 13 rats underwent autopsy with external and internal gross examination. Results are shown in Table 8. Only minor findings were reported. One rat had a haemorrhage in the subcutis of the nose which can be connected to electricity or to other trauma. One rat had red spots on two toe pads of a front paw (without reactions in subcutis). The method of euthanasia (using CO_2 gas) caused some bleeding in the airways of most animals. Group B rats had some indications of beginning lung infection, although this was not notice during the daily checks of live rats.

Table 8. Results from the autopsy of the rats. One broken whisker in one rat (*). One rat had a swollen nose with subcutaneous oedema (**). Group B rats had indication of a respiratory disease (***).

	Pilot			Group A			Group B			Group C			
Organ	P1	P2	P3	A1	A2	A3	B1	B2	B3	C1	C2	C3	C4
Whiskers	N	N	N	*	N	N	N	N	N	N	N	N	N
Nose	N	N	N	N	N	N	**	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N
Front paws, no. of red toes	N	N	N	N	N	N	N	N	2	N	N	N	N
Hind paws, no. of red toes	N	N	N	N	N	N	N	N	N	N	N	N	N
Internal findings, including heart	N	N	N	N	N	N	***	***	***	N	N	N	N

Discussion

In the laboratory set up, the equipment from TxGuardian turned out to be very effective, as measured at the second exposure two week after the first exposure. However, there are some important differences to the natural situation to keep in mind. First of all, we tested the equipment on laboratory rodents, not wild individuals. Laboratory strains may deviate from wild rodents in some behaviours and motivational levels. In general, the domestication history has made them less fearful and less cautious. Laboratory rodents live in a protected environment with easy access to basic needs (food, water, company) and nowadays the cages are furnished with enrichment items like shelter and nesting material in addition to wood shavings. To counteract the likely differences in behaviours between wild and domesticated rodents, we picked strains known be active and explorative, which they turned out to be. While laboratory rats are variants of the brown rat (*Rattus norwegicus*) which lives in the wild, laboratory mice are variants of the house mouse species (*Mus musculus*), which actually is quite uncommon in Norway. Most of the mice which enter our buildings belong to other mouse species, which generally have much larger home ranges.

The laboratory rodents rapidly made use of the extra resources in the room: the large space, the dark hiding place in the test house, the novel and attractive food items both in the test house and across the floor list. After the current was turned on, there were other differences between the test situation and the situation in "real life", which may have practical importance. If a wild rodent gets an electric shock when trying to enter a new building, it will probably flee back to the home nest or pass on and try to find food another place. In contrast to the natural situation, the laboratory animals could not return to a home nest elsewhere, but were stuck in the room. Their only "safe" place to be was the floor area (with necessary resources such as plain food, water and shelter. This lack of opportunity to leave the site, might have increased their

motivation to enter the attractive, “safe” test house, the most popular place for them to be before we turned on the current.

The electric barriers did not totally prevent the rodents from crossing, but reduced the frequency significantly. This was especially so for the rats. The mice tried to cross more often and got more shocks. The higher number of shocks received in mice compared to rats, might indicate that they were less good at learning, but can also be influenced by how the barriers were designed. Mice, being smaller and faster than rats, generally received the electric shock when more than half of the body had crossed the barrier. This combined with the fast forward-directed momentum, resulted in mice crossing the barrier despite receiving a shock. In contrast, rats received the electric shock earlier and were therefore more prone to turn around than to cross. On the other hand, because the mice were so explorative and seemed to never give up, they were also able to find any possible way to cross the barrier without being shocked. For instance, they jumped directly to the upper metal pipe or climbed on plastic covered cables over the floor list. We stopped as many of these routes as we could, but the rodents often found new opportunities. The take home message is to cover every inch of the building to be protected.

In the field test conducted prior to this experimental test, the equipment turned out to be very efficient. However, the question on how electric shocks influence on the welfare of the animal, and the risks for physical damage to them, could not be answered. This necessitated a controlled study allowing thorough supervision of the animals for signs of altered behaviour and suffering. The rodents underwent regular health checks and were pain scored after exposure to electric shocks, and they could be followed with the cameras. At the higher voltage (5000 V, group A mice only), the behavioural reaction was strong and the touch was obviously very painful. Although the pain reactions faded away over a few minutes time, we decided to alter the protocol and thus reduced the current to level 4 (2600 V). The level 4 voltage resulted in vocalisation and a withdrawal response, but no additional behavioural signs of reduced welfare.

We autopsied 26 animals which all had been exposed to at least one electric shock. The mice exposed to 5000 V (group A) also showed more and more evident redness (hyperaemia) inside their front paws compared to the other groups (mild hyperaemia in mouse group B and C, and normal paws in the pilot mice and in 12 of 13 rats. This finding possibly reflects the fact that the mice received more shocks than the rats did, but also their smaller body size. Our data indicate that the equipment used up to level 4, is aversive without causing any lasting or severe damage or harm to the rats, and only mild hyperaemia to the front paws of mice.

None of the methods used in society to control pest rodents have an efficiency of 100%. Pest rodents may learn to avoid a trap and not to eat from a poisonous bait, or they may develop resistance to the chemicals, as was the case with first-generation anticoagulant poisons, and partly also for some second generation chemicals. Likewise, rodents may figure out how to avoid electric shocks if there is a way to do so. On this background, the TxGuardian equipment showed to be very efficient. When the electricity was turned on, the mice crossed the electric barriers only 1.4% of the 3440 times they approached them. With rats the equipment was even more efficient. Despite a total of 4551 approaches to the barriers, they never crossed the electric barriers during the observation periods. When it comes to animal welfare, the equipment has advantages both to target and non-target animals. None of the rodents died or got severe bodily injuries. Actually, with a maximum of 2600 V (level 4), we did not reveal pain behaviours beyond the first second after exposure or severe bodily injury to the rodents. In comparison, poison will cause reduced welfare and suffering lasting for days before death and may potentially affect a large number of non-target animals. Spring traps may not always hit correctly and will then cause severe pain and suffering.

Conclusions

To avoid damage caused by rodents, a mechanical block of the animals’ access routes into buildings is the most important preventive measure. Some buildings are especially costly or difficult to protect mechanically, and the TxGuardian system provides a solution which should work well, even when doors or gates have to be left open. The overall goal of the TxGuardian equipment is to effectively prevent rodents

from entering buildings without killing or hurting them. The equipment, as tested in a laboratory setting, was very efficient in demotivating rodents from crossing the electric barriers. The electric shocks received by the mice and rats (level 4, up to 2600 V) did not cause pain behaviour beyond the immediate pain nor severe pathoanatomic lesions to the animals.

Acknowledgements

We thank the staff at the Section for Experimental Biomedicine at the Norwegian University of Life Sciences (NMBU) for sharing their profound knowledge, for excellent care for the research animals, for scoring the animals' health and welfare state, and for assistance during trials; Even Bostad and Christian Øhrn at TxGuardian for installation and maintenance of the equipment; Ane Julie Ellingsen for help with video analyses; and Arnulf Soleng at the Norwegian Institute of Public Health for his knowledge on rodent behaviour and valuable discussions on study design.

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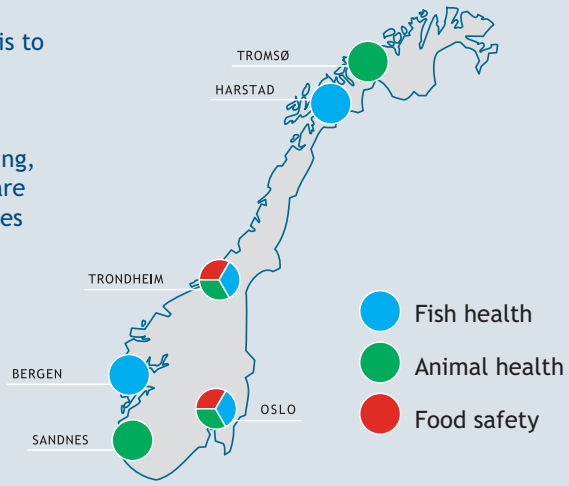

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