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Statistical design for health monitoring in laboratory animal facilities using sentinel animals

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Abstract

Regular health monitoring is crucial in laboratory animal facilities to determine the presence or absence of specific pathogens. One common approach to monitoring involves the use of sentinel animals, which are periodically exposed to biological material from the cages being monitored. At a certain point, some of these sentinel animals are tested for pathogens. This article discusses designing an effective sampling scheme to meet desired quality standards. It addresses questions such as the number of sentinel animals required, the frequency of sampling biological material, the selection of cages based on facility set-up, and the optimal frequency and quantity of sentinel animal tests. While existing design formulas are available for simple random sampling, no quantitative recommendation exists for using sentinel animals to the best of our knowledge. We propose a Monte Carlo simulation-based approach in this article to address this. Our algorithm has been implemented in a publicly accessible web page at http://nolan.cnb.csic.es/sentinelcagesmanager.

Keywords

Animal facilities, quality assurance/control, sample size, statistics

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Introduction

Health monitoring is essential to animal facility management across various settings, including research laboratories, veterinary clinics, zoos and agricultural facilities.[1–3] Its primary objective is to ensure the well-being and welfare of animals through systematic observation and assessment of their health. By actively monitoring the animals, health monitoring aims to detect and prevent disease spread, identify potential health issues or the presence of agents that interfere with the results of scientific experiments, and enable appropriate interventions.

The ultimate goal of health monitoring programmes is to maintain animals' physical and behavioural health. Robust monitoring efforts allow facility managers and veterinarians to establish effective disease prevention and control measures, improve animal care practices and safeguard the health of both animals and humans.

Health monitoring in animal facilities involves a combination of strategies, including regular physical examinations, behavioural observation and various diagnostic tests. These tests encompass blood and urine analysis, microbiological cultures, serological testing, molecular diagnostics and imaging techniques such as X-rays or ultrasounds. The selection of specific tests depends on the species involved, the facility's purpose and the potential risks associated with the animals.

Animal facilities establish comprehensive health monitoring protocols to meet specific needs and requirements. These protocols encompass routine health checks, disease surveillance and targeted testing for known pathogens.[4] Biosecurity measures, such as stringent hygiene practices, quarantine periods for new

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animals and restricted access to certain areas, are often implemented to prevent the introduction or spread of pathogens.[5–7] The standardization of the procedures and tests is also of primary importance.[8]

Effective health monitoring relies on regular communication and collaboration among facility staff, veterinarians and researchers. Sharing observations, test results and relevant information enables a coordinated approach to animal health management, early detection of potential health issues and the implementation of appropriate interventions.

Using sentinel animals is a valuable approach within health monitoring programmes.[9,10] These selected animals are placed close to others in the facility and act as early warning systems for potential health concerns. By exposing sentinel animals to the same conditions and pathogens as the monitored animals, facility managers and veterinarians can closely monitor their health status, gaining insights into the animal population's overall health and detecting pathogens early. Sentinel animal programmes involve introducing these animals into monitored cages or areas and collecting samples of biological material, such as bedding. After a specific period, some sentinel animals are thoroughly tested to identify pathogens they may have contracted. This approach enables the detection of asymptomatic or subtly symptomatic diseases and helps assess the effectiveness of the facility's disease prevention and control measures.

Designing a sentinel animal programme requires careful consideration, including determining the optimal number of sentinel animals, the frequency of sampling, the selection of cages or areas for sampling based on the facility's layout, and the appropriate frequency and quantity of sentinel animal tests. Finding a balance between obtaining accurate results and minimizing the impact on the animal population is crucial.

Several questions arise when considering the utilization of sentinel animals for facility monitoring. These include determining the number of animals required to serve as sentinels, establishing the frequency of sampling biological material and testing the sentinels, and exploring the existence of an optimal sampling plan. Addressing these enquiries helps ensure that the facility effectively implements sentinel animal-based monitoring protocols. However, these questions are seldom addressed in scientific papers or monitoring recommendations.

Fosgate 2009[11] provides existing guidance on the number of animals needed to calculate the prevalence of a disease within a colony. However, this guidance is based on a random population sampling, which does not accurately reflect the complex conditions of real animal facilities, especially the use of sentinel animals. While random sampling enables closed-form formulas to calculate the sample size, this is not the procedure normally followed in many animal facilities, in which the number of tests is minimized through the use of sentinel animals due to the high cost of the testing procedures (around €300 per animal) and because the rest of the animals are participating in scientific experiments and cannot be removed for health monitoring.

In this study, we propose that a Monte Carlo simulation-based approach is better suited for determining the appropriate number of sentinels, sampling frequency and other relevant factors. To make estimates, Monte Carlo simulation typically selects multiple values for uncertain variables from historical data. Then, it averages the results to provide multiple possible outcomes and the probability of each. Using simulations can account for the intricacies and variability in real animal facilities, leading to more accurate and reliable results.

To facilitate the use of our approach, we have developed a web page publicly accessible at http://nolan.cnb.csic.es/sentinelcagesmanager. This web page provides facility managers and researchers with a user-friendly platform to input specific parameters and obtain customized recommendations regarding sentinel animal numbers and sampling frequencies. By employing this simulation-based tool, animal facilities can make informed decisions tailored to their unique circumstances, enhancing the effectiveness of their health monitoring programmes.

Previous work

Initially, we provide a summary of the classical approach proposed by Fosgate.[11] This classical approach is based on random population sampling without using sentinel animals. Subsequently, in the remaining sections of the article, we present our innovative approach, which aims to address and overcome the limitations associated with the simplistic assumptions of the classical approach.

Let us assume there are D animals with the disease and N animals in total. We may model the sampling with a binomial or hypergeometric distribution (binomial if N is large with respect to the sample size or hypergeometric distribution if N is not so large). The hypergeometric distribution is more accurate for random population sampling because it assumes sampling without replacement. Whilst the binomial distribution assumes sampling with replacement, the formulae are simpler and can be used to approximate the true hypergeometric distribution in larger populations. Let us denote as X the number of diseased animals observed from a random sample of size N. The goal is to design a sample size such that

$$\Pr\{X = 0 \leq \alpha \tag{1}$$

That is, we will take n animals at random from the facility, and if we do not observe any infected animal in our sample, then we will declare our facility infection-free. In the previous equation, α is the probability that the pathogen is present in the facility, but we have not observed any infected animal in our random sample.

For the binomial distribution, this results in a very simple formula for the sample size design. Let us define the prevalence of the disease in the animal warehouse as $p_0 = D/N$, then the sample size, n, can be solved through the equation

$$\Pr\left\{X = 0 = \binom{n}{0} p_0^0 (1-p_0)^n \Rightarrow n \geq \frac{\log\alpha}{\log(1-p_0)}\right. \quad (2)$$

That is, given a prevalence p_0 of disease in the facility, the formula above gives us the size of a random sample that will allow us to detect it with probability 1–α.

The case of hypergeometric sampling is much more difficult because the resulting equation cannot be easily solved for n

$$\Pr\left\{X = 0 = \frac{\binom{D}{0}\binom{N-D}{n}}{\binom{N}{n}} = \frac{(N-D)!(N-n)!}{N!(N-D-n)!} \leq \alpha\right. \quad (3)$$

An approximated solution is given by Gedanken[7]

$$n \geq (1-\alpha^{1/D})\left(N - \frac{D-1}{2}\right) \quad (4)$$

This solution has been used in animal facilities,[11] detection of defects[12] and environmental protection.[13]

Methods

However, a different approach is often followed in animal facilities. Cage bed material from randomly chosen cages is combined into a single cage. Some animals, typically from two to four, are then housed in that cage and later tested for the presence of the disease. These animals are called sentinels. The rationale is that we can monitor many animals in the original cages with relatively few sentinels. Every three months, the oldest two sentinels from the cage are analysed for the presence of pathogens and replaced by two new sentinel animals. This analysis is performed on microbiological units of the animal facility, that is, those animals that share some common area and, therefore, have the same probability of acquiring a disease. This common area can be a ventilated rack (25–100 cages), a room (about 200 cages) or the whole facility (up to 3000 cages), depending on the isolation measures in place.[14] Typical microbiological sizes can go from 100 to 1000 cages.

This methodology significantly reduces the number of animals needed solely for observation. However, as far as we know, no systematic procedure exists for determining the number of sentinel creatures needed to maintain the same level of certainty as with binomial or hypergeometric random sampling. This is likely because there is no precise formula for calculating this sample size. We have relocated all technical details to the Supplementary Material online to simplify reading the main body of text. We present the methods in a manner that is easy to understand for everyone and direct only those interested in the mathematical and algorithmic specifics to the Supplementary Material.

Below, in Monitoring a single microbiological unit, we provide a Monte Carlo simulation method that allows calculating the sample size of a single microbiological unit. In Spatial sampling of a single microbiological unit, we provide an algorithm that proposes a sampling plan over time and space to maximize the probability of identifying a locally spreading disease. Finally, in Monitoring multiple microbiological units, we extend this methodology to sampling multiple microbiological units.

Monitoring a single microbiological unit

Monte Carlo simulation is a method that allows us to mimic the actions taken at the facilities many times over. In each simulation, we check whether we would find the disease. By changing certain elements in the simulation, particularly the number of sentinel cages, the sampling period and the number of weeks between controls, we can verify whether there is a chance that we would miss the disease and make sure that this chance is less than α. The following paragraphs introduce the different effects we consider for our simulated experiments.

Since we are taking samples from animal cages, we consider each cage an experiment unit. Let us say the chance of a cage having infected animals is p_0. We will refer to this as the disease prevalence in the testing area. The total number of cages in the microbiological unit we are dealing with is B_{cage}. It is important to highlight that many of these diseases manifest subclinically, meaning animals do not display symptoms. Therefore, the term infected is more appropriate than sick.

We collect bedding from M_{cage} cages each week and put it in the sentinel cages. Even though we can choose any period to do this, we will keep referring to it as a weekly event to make things easier. We call p_1 the

chance that the cage bedding can spread disease if any infected animals are in the cage. And we call p_2 the chance that a sentinel animal gets infected in a week if its bedding can spread the disease.

Every week, we randomly pick cages for sampling (the exact sampling mechanisms are given i, Spatial sampling of a single microbiological unit). At the end of N_{weeks}, we check M_{health} sentinel animals to see whether the disease we are looking for is present. We assume that once a sentinel animal gets infected, we will be able to find out in the next checkup (either through testing for antibodies or some other way).

We can keep the M_{health} animals in one cage or two separate cages, where the bedding has come from the same cages. We call this second scenario *splitting*. This method helps us avoid losing all sentinel animals immediately if something bad happens to their cage.

We repeat this pretend experiment many times and count how often the disease is detected and not detected. This way, we do not have to assume any specific pattern for the chance of missing the disease among the sentinels (like we had to do with the random sampling of animals).

All these factors together (prevalence of the disease in the microbiological unit and its size, the probability of the bedding being infectious and the sentinel animals getting infected, the number of sampling weeks, and the number of cages from the microbiological unit used to produce the bedding of each sentinel cage) determine the chance of detecting the disease. Using the Monte Carlo simulation, we can devise different sampling plans by adjusting factors under our control, such as those related to the sampling scheme.

All the algorithmic details of the simulation can be seen in the Supplementary Material Section 1.1. Here, it suffices to keep the idea that we can alter the sampling plan until we make the probability of missing the disease smaller than α.

Spatial sampling of a single microbiological unit

In the previous section, we suggested a method to design the number of facility cages that we need to sample each week to have a given detection probability. Groups of M_{cage} cages are used to make the bed of a single sentinel cage. Now we wonder how to choose these cages from our microbiological unit (see, for instance, Figure 1). The reason is that the infections typically originate in one of the cages, and neighbouring cages are more susceptible to infection, leading to localized disease spread. Therefore, it is crucial to design a sampling plan that effectively covers the entire microbiological unit, maximizing the weekly coverage. Several sampling possibilities may seem sensible:

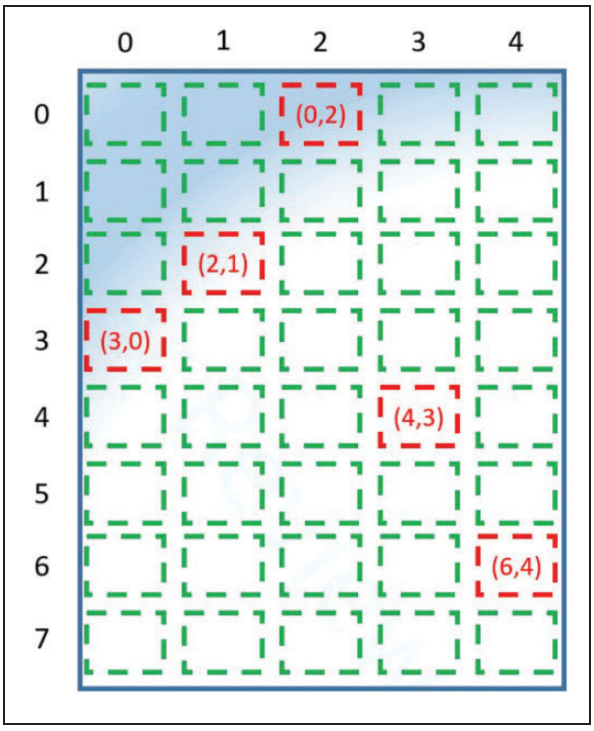

Figure 1. Illustration of a microbiological unit. Rows and columns are numbered. The cages marked in red are selected to make the bed of a single sentinel cage. The numbers in red indicate the row and column of each cage. The shadow in the top left corner indicates the origin of an infection within the rack and its spatial spread.

1. *Sequential sampling*. We enumerate the cages and sample them in an ascending, sorted way: cage 1, 2, 3, 4, . . . Although the simplest to apply, this approach has the disadvantage of poor spatial coverage.
2. *Random sampling*. We may enumerate the cages and sample them randomly every week by employing a computer random generator. This approach is better than the previous one, but it may suffer from random clustering and insufficient spatial coverage in specific weeks or missing a specific cage between consecutive tests. Nevertheless, this should not be a severe problem in the long run.
3. *Optimal sampling*. We may look for a sampling plan that covers all cages and maximizes the spatial coverage over all weeks between testing weeks.

In Supplementary Material Section 1.2, we present an algorithm for achieving optimal sampling to ensure comprehensive coverage of a single microbiological unit. The algorithm presumes all cages are organized in a rectangular array (for instance, 15 cages $\times$ 10 cages). With this configuration, we want to produce a

spatial sampling plan to maximize the distance between weekly sampled cages over all weeks until we fully cover the microbiological unit. The optimization is performed with a genetic algorithm whose details are given in the Supplementary Material.

Monitoring multiple microbiological units

Very often, a shelf of the animal facility contains multiple microbiological units. There are several reasons for this: 1) the facility staff change gloves and take special hygienic measures when they change from one line to another; 2) a set of cages contains animals coming from a single litter; ... Whatever the reason is, the whole set of cages in the shelf can be regarded as multiple microbiological units whose sizes are different. Let us refer to them as N_1, N_2, ..., N_B. Let us assume that only one of the units is infected. The prevalence of the disease in this line is p_0 (the expected number of infected cages will be $\mathrm{d}p_0N_b\mathrm{e}$, where N_b is the size of the infected unit, and $\mathrm{d}x\mathrm{e}$ represents the rounding up of x.

$N_{sentinelCages}$ can still monitor the multiple microbiological units. If we take M_{cage} samples for N_{weeks}, we have in total $M_{cage}N_{sentinel}C_{ages}N_{weeks}$ samples for the whole testing period. We design our sampling plan by taking a random sample from $\{1,2,\ldots, B\}$ with a weight proportional to the expected number of infected cages in each line. Within each line, we randomly sample without replacement of the cages, but otherwise, we may still use the algorithm devised in Monitoring a single microbiological unit to calculate the probability that we miss the disease.

Results

We now illustrate the use of our tools in some scenarios to evaluate the impact of the different parameters on the number of sentinel cages needed.

Monitoring a single microbiological unit

The following results have been obtained with 50,000 simulations. We have fixed

$$N_{weeks} = 12,\ M_{cages} = 5$$

These are typical values in an animal facility. Then, we explored the effect of the size of the microbiological unit, the prevalence,

$$M_{health},\ p_1,\ \text{and}\ p_2$$

(the two latter in the range 0.1 to 1.0).

Tables 1 and 2 show the results for a prevalence $p_0 = 0.3$ with a non-split and split experiment. The number in parentheses is the number of sentinel cages, while the number outside is the probability of detection. As expected, it is more efficient to perform sanitary controls with two sentinel animals in two separate sentinel cages (splitting is better than non-splitting). For this reason, from this point on, all experiments will be performed by splitting. The Supplementary Material shows similar tables for p_0 equal to 0.2, 0.1 and 0.05. We also show the corresponding table for $p_0 = 0.3$ with only one tested animal, $M_{health} = 1$.

An interesting finding is that the sentinel strategy can effectively detect pathogens in most practical scenarios with only one or two sentinel cages. This holds as long as the probabilities p_1 and p_2 are sufficiently high, typically above 0.3 (refer to the tables for precise values). Notably, the detection capability remains remarkably effective even in extremely low disease prevalences, reaching as low as 5%. By employing a sampling strategy of just two sentinel animals and conducting weekly sampling over three months, we can successfully detect pathogens with prevalences as low as 5%. This stands in stark comparison with the nine

Table 1. Probability of detection and number of sentinel cages for $B_{cage} \geq 50$, $p_0 = 0.3$, $M_{health} = 2$, $N_{weeks} = 12$

$B_{cage} \geq 50$, $p_0 = 0.3$, $M_{health} = 2$, $N_{weeks} = 12$										
p_2										
p_1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.1	0.96 (10)	0.96 (5)	0.97 (4)	0.97 (3)	0.98 (3)	0.96 (2)	0.97 (2)	0.97 (2)	0.98 (2)	0.98 (2)
0.2	0.96 (5)	0.98 (3)	0.97 (2)	0.99 (2)	1.00 (2)	0.96 (1)	0.97 (1)	0.98 (1)	0.98 (1)	0.98 (1)
0.3	0.97 (4)	0.97 (2)	1.00 (2)	0.97 (1)	0.98 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.4	0.97 (3)	0.99 (2)	0.97 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.5	0.98 (3)	1.00 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.6	0.96 (2)	0.96 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.7	0.97 (2)	0.97 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.8	0.98 (2)	0.98 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.9	0.98 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
1.0	0.99 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)

Table 2. Probability of detection and number of sentinel cages for $B_{cage} \geq 50$, $p_0 = 0.3$, $M_{health} = 2$, split, $N_{weeks} = 12$.

p_2										
p_1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0.1	0.96 (9)	0.97 (5)	0.96 (3)	0.99 (3)	0.97 (2)	0.99 (2)	0.99 (2)	1.00 (2)	0.96 (1)	0.98 (1)
0.2	0.96 (5)	0.98 (3)	0.98 (2)	1.00 (2)	0.97 (1)	0.99 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.3	0.98 (4)	0.98 (2)	1.00 (2)	0.98 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.4	0.97 (3)	0.99 (2)	0.98 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.5	0.98 (3)	1.00 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.6	0.96 (2)	0.96 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.7	0.97 (2)	0.97 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.8	0.98 (2)	0.98 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
0.9	0.98 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
1	0.99 (2)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)

animals required for detecting a prevalence of 30% using random sampling (as indicated in equation (2)), or the 39 animals needed for a prevalence of 5% (as indicated in equation (4)).

Spatial sampling of a single microbiological unit

We assume we have a single biological unit arranged as a rack with 10×5 rows and columns. We will make the bed of the sentinel cages by taking the bed from $M_{cage} = 5$ cages from the biological unit. Using the genetic algorithm proposed in Spatial sampling of a single microbiological unit, we get an average distance within each sampling group of 2.99. The sampling plan is:

- Batch 0: (6, 1)(9, 2)(0, 3)(5, 4)(2, 0)
- Batch 1: (7, 3)(3, 2)(4, 4)(7, 0)(0, 1)
- Batch 2: (7, 1)(1, 4)(5, 3)(8, 0)(2, 2)
- Batch 3: (2, 3)(4, 2)(6, 4)(8, 1)(0, 0)
- Batch 4: (8, 2)(4, 1)(7, 4)(1, 3)(3, 0)
- Batch 5: (2, 1)(0, 4)(6, 3)(5, 2)(9, 0)
- Batch 6: (9, 4)(7, 2)(1, 0)(4, 3)(3, 1)
- Batch 7: (5, 1)(8, 3)(6, 0)(1, 2)(2, 4)
- Batch 8: (6, 2)(9, 3)(3, 4)(1, 1)(5, 0)
- Batch 9: (0, 2)(9, 1)(4, 0)(3, 3)(8, 4)

This sampling is depicted in Figure 2. If we have two sentinel cages, in the first week, we would use batches 0 and 1; the second week, we would use batches 2 and 3; et cetera. Once we run out of batches, we may start from the beginning of the list again, or we may use any of its randomizations (e.g. batches 4, 7, 6, 1, 9, 5, 3, 0, 2, 8).

The sampling plan for a 10×10 rack is shown in the Supplementary Material.

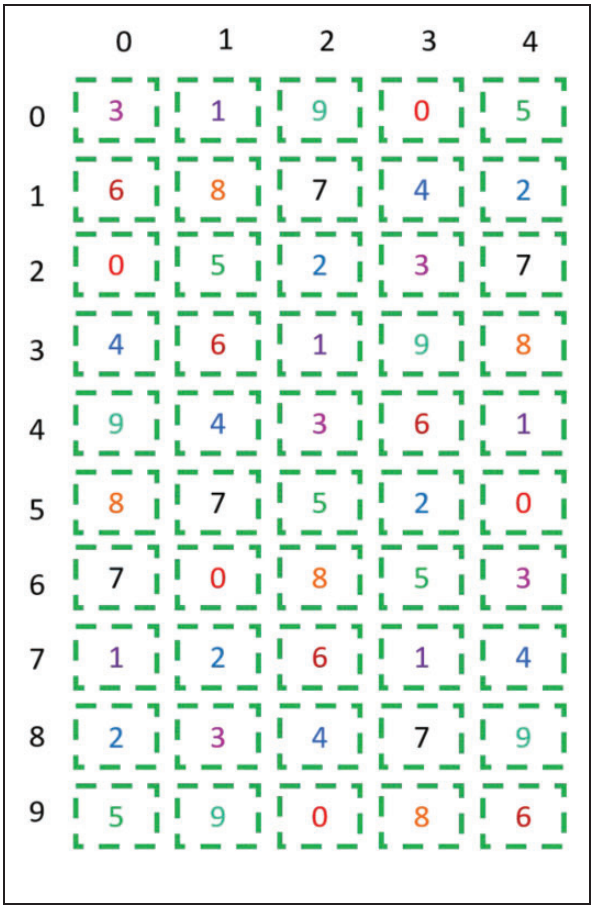

Figure 2. Illustration of the spatial sampling plan. Cages with the same number belong to the same sampling batch, and their beds are used to construct the bed of the same sentinel cage.

Monitoring multiple microbiological units

Let us consider an example where we have a rack containing 50 cages. These 50 cages are divided into five separate microbiological units, each containing 10 cages. The goal is to determine the number of sentinel cages required to detect a disease with a 95% probability when the disease prevalence is $p_0 = 0.3$ in only one of the microbiological units. Table 3 illustrates this

Table 3. Probability of detection and number of sentinel cages for $B_{cage} = [10, 10, 10, 10, 10]$, $p_0 = 0.3$, $M_{health} = 2$, split, $N_{weeks} = 12$.

p_2										
p_1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
0.1	0.95 (47)	0.96 (24)	0.96 (16)	0.95 (12)	0.96 (10)	0.96 (8)	0.96 (7)	0.96 (6)	0.97 (6)	0.97 (5)
0.2	0.95 (23)	0.96 (12)	0.96 (8)	0.96 (6)	0.97 (5)	0.96 (4)	0.98 (4)	0.96 (3)	0.98 (3)	0.98 (3)
0.3	0.96 (16)	0.96 (8)	0.95 (5)	0.96 (4)	0.95 (3)	0.97 (3)	0.99 (3)	0.97 (2)	0.98 (2)	0.99 (2)
0.4	0.96 (12)	0.96 (6)	0.96 (4)	0.96 (3)	0.98 (3)	0.97 (2)	0.98 (2)	0.99 (2)	0.99 (2)	0.95 (1)
0.5	0.95 (9)	0.97 (5)	0.95 (3)	0.98 (3)	0.97 (2)	0.99 (2)	0.99 (2)	0.95 (1)	0.97 (1)	0.98 (1)
0.6	0.96 (8)	0.96 (4)	0.97 (3)	0.97 (2)	0.98 (2)	0.99 (2)	0.96 (1)	0.98 (1)	0.98 (1)	0.99 (1)
0.7	0.96 (7)	0.98 (4)	0.99 (3)	0.98 (2)	0.99 (2)	0.96 (1)	0.98 (1)	0.99 (1)	0.99 (1)	0.99 (1)
0.8	0.96 (6)	0.96 (3)	0.97 (2)	0.99 (2)	0.95 (1)	0.97 (1)	0.99 (1)	0.99 (1)	1.00 (1)	1.00 (1)
0.9	0.96 (5)	0.97 (3)	0.98 (2)	0.99 (2)	0.97 (1)	0.98 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)
1	0.96 (5)	0.98 (3)	0.99 (2)	0.95 (1)	0.98 (1)	0.99 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.00 (1)

scenario, highlighting the increased difficulty in disease detection compared with Table 2.

In Table 2, there were 15 infected cages out of a total of 50 (30% prevalence). However, in the current situation with separate microbiological units, each consisting of 10 cages, only three cages (30% prevalence) are infected.

In the Supplementary Material, we show a similar table but with 10 microbiological units of five cages each and a prevalence of 30%. We also show a table for the same microbiological configuration where the whole unit is infected.

Conclusions

Implementing regular testing of sentinel animals to detect pathogens offers substantial benefits in terms of reducing the cost of colony health monitoring and minimizing the number of animals sacrificed. The disease detection capability is remarkably effective, provided that the biological material used for the tests exhibits a relatively high level of infectivity ($p_1 > 0.3$) and there is a reasonable probability for a sentinel animal to contract the disease upon exposure to the pathogen ($p_2 > 0.3$). These conditions are generally met by most pathogens of interest, making health monitoring with sentinel animals a widely adopted practice in research animal facilities.[15–18] If p_1 or p_2 are very low, then random sampling may still require fewer animals than the use of sentinels. Alternatively, exhaust dust surveillance and in-cage filter paper compare favourably to sentinel sampling for efficiency, sensitivity and animal welfare.[10,19]

There has been a lack of quantitative assessment regarding the detection capability of sentinel animal-based disease monitoring. The main challenge lies in the complexity of the infection and sampling process, which does not lend itself to straightforward mathematical formulas. Consequently, quantitative analysis using closed-form formulas has not been possible. However, Monte Carlo simulations have emerged as a valuable tool in this regard. By conducting thousands (in our example, 10,000) of simulations of the sampling process, we can effectively determine the probability of disease detection with a high degree of accuracy.

In addition to our previous work, we have also developed spatial sampling patterns specifically designed for individual microbiological units. These patterns are strategically designed to maximize the coverage of each unit, enabling us to detect and monitor localized infections that effectively spread within the unit.

Finally, we have expanded our methodology to address situations where multiple microbiological units are present, with the added complexity of only one infected unit. This scenario poses a greater challenge due to a significantly smaller number of infected cages within the system.

Data availability

This paper does not contain data obtained from animals. The algorithms described in the paper can be publicly accessed at http://nolan.cnb.csic.es/sentinelcagesmanager.

Declaration of conflicting interests

The authors have no conflicts of interest to declare.

Ethical approval

This paper does not contain data obtained from animals. It describes a mathematical procedure to design health monitoring plans using sentinels.

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Conception statistique pour la surveillance sanitaire dans les installations d'animaux de laboratoire utilisant des animaux sentinelles

Résumé

Une surveillance sanitaire régulière est essentielle dans les installations d'animaux de laboratoire afin de déterminer la présence ou l'absence d'agents pathogènes spécifiques. Une approche commune de la surveillance implique l'utilisation d'animaux sentinelles, qui sont périodiquement exposés au matériel biologique provenant des cages surveillées. À un certain moment, certains de ces animaux sentinelles sont testés pour détecter des agents pathogènes. Cet article traite de la conception d'un système d'échantillonnage efficace pour répondre aux normes de qualité souhaitées. Il aborde des questions telles que le nombre d'animaux sentinelles requis, la fréquence d'échantillonnage du matériel biologique, la sélection des cages en fonction de la configuration de l'installation, ainsi que la fréquence et la quantité optimales de tests à pratiquer sur les animaux sentinelles. Bien qu'il existe déjà des formules de conception d'échantillonnage aléatoire simple, il n'y a, à notre connaissance, aucune recommandation quantitative quant à l'utilisation d'animaux sentinelles. Pour y remédier, nous proposons dans cet article une approche basée sur la simulation Monte Carlo. Notre algorithme a été implémenté dans une page Web accessible au public à l'adresse http://nolan.cnb.csic.es/sentinelcagesmanager.

Statistischer Rahmen für die Gesundheitsüberwachung in Labortiereinrichtungen unter Verwendung von Sentineltieren

Abstract

Laufende Gesundheitsüberwachung ist in Labortiereinrichtungen von entscheidender Bedeutung, um das Vorhandensein oder Nichtvorhandensein bestimmter Krankheitserreger festzustellen. Ein gängiges Konzept für die Überwachung ist die Verwendung von Sentineltieren, die in regelmäßigen Abständen biologischem Material aus den überwachten Käfigen ausgesetzt werden. Zu einem bestimmten Zeitpunkt werden einige dieser Sentineltiere auf Krankheitserreger getestet. In diesem Artikel wird erörtert, wie ein effektiver Probenahmeplan aussehen muss, um die gewünschten Qualitätsstandards zu erfüllen. Er befasst sich mit Fragen wie der Anzahl der erforderlichen Sentineltiere, der Häufigkeit der Probenahme von biologischem Material, der Auswahl der Käfige entsprechend der Einrichtungsstruktur und der optimalen Häufigkeit und Menge der Sentineltiertests. Während es für einfache Zufallsstichproben bereits Planungsformeln gibt, existiert unseres Wissens nach keine quantitative Empfehlung für den Einsatz von Sentineltieren. Wir schlagen in diesem Artikel einen auf Monte-Carlo-Simulationen basierenden Ansatz vor, um dieses Problem zu lösen. Unser Algorithmus wurde auf einer öffentlich zugänglichen Webseite vorgestellt unter http://nolan.cnb.csic.es/sentinelcagesmanager.

Diseño estadístico para el control sanitario en instalaciones de animales de laboratorio mediante animales centinela

Resumen

El control sanitario regular es crucial en las instalaciones de animales de laboratorio para determinar la presencia o ausencia de patógenos específicos. Un método habitual del control consiste en utilizar animales centinela, que se exponen periódicamente al material biológico de las jaulas vigiladas. En un momento dado, algunos de estos animales centinela se someten a pruebas de detección de agentes patógenos. Este artículo trata del diseño de un plan de muestreo eficaz para cumplir con las normas de calidad deseadas. Asimismo, el artículo aborda cuestiones como el número de animales centinela necesarios, la frecuencia de muestreo del material biológico, la selección de jaulas en función de la configuración de las instalaciones, y la frecuencia y cantidad óptimas de pruebas con animales centinela. A pesar de que existen fórmulas de diseño para el muestreo aleatorio simple, hasta donde sabemos no existe ninguna recomendación cuantitativa para utilizar animales centinela. En este artículo proponemos un enfoque basado en la simulación Monte Carlo para abordar esta cuestión. Nuestro algoritmo se ha implementado en una página web con acceso público en http://nolan.cnb.csic.es/sentinelcagesmanager.