Journal of the American Association for Laboratory Animal Science
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Vol 62, No 1
January 2023
Pages 64–73

A Cross-sectional Survey on Rodent Environmental Health Monitoring Practices: Benchmarking, Associations, and Barriers

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Tens of thousands of rodents are used each year in Rodent Health Monitoring programs. However, Environment Health Monitoring (EHM) could replace sentinel rodent use while maintaining or even improving diagnostic quality. Despite its advantages, widespread implementation of EHM appears to be relatively low. To better understand EHM's prevalence and factors influencing its use, we surveyed research animal professionals. Our hypotheses were (1) EHM prevalence would be low and (2) EHM use would be associated with beliefs and knowledge about EHM. Participants were recruited via online promotion. A total of 158 individuals completed a mixed-methods survey about current practices, beliefs, and knowledge about EHM. Qualitative data were coded using thematic analysis and analyzed using generalized linear models. Results showed that current EHM implementation was low; only 11% of institutions used EHM exclusively. Across the 111 institutions surveyed, over 20,000 soiled bedding sentinels were used each year. However, most participants believed EHM to be advantageous in replacing sentinel animals (78% of participants). Some participants believed EHM could save time (31%), cost less (27%), and be highly accurate (15%). Conversely, some participants believed EHM would be difficult to use due to their current caging type (40%), higher costs (21%), lower accuracy (16%), and personnel attitudes/expertise (14%). Overall, respondents with higher planned EHM use also had more positive attitudes, norms, and control of EHM. We also identified several factors that could promote the implementation of EHM. Communication efforts should emphasize that EHM is compatible with various types of caging, can provide cost savings, has high accuracy, and is consistent with the 3Rs as a replacement. Efforts should also focus on improving attitudes, encouraging peers, and providing resources to facilitate implementation. Implementation in just the surveyed institutions could eliminate the need for well over 20,000 rodents each year, consistent with 3Rs goals.

Abbreviations and Acronyms: EHM, environmental health monitoring

DOI: 10.30802/AALAS-JAALAS-22-000086

Introduction

Rodent vivaria have traditionally used soiled-bedding sentinel health-monitoring programs to detect and exclude pathogens in rodent colonies. However, in 2016, a systematic literature review concluded that relatively little literature supported this commonly accepted health monitoring practice.[5] In addition, the authors could make no comprehensive recommendations for an effective sentinel health monitoring program as many questions remained about soiled-bedding transfer dose/frequency and sentinel strains.[5]

Environmental Health Monitoring (EHM) involves the surveillance of rodent colonies without the use of sentinel animals. EHM has been proposed as a replacement for soiled bedding sentinels for several reasons; in particular, soiled bedding sentinels have limitations for the detection of certain pathogens.[5,23,34,38,42] EHM can also potentially decrease labor and cost.[24] In addition, EHM is consistent with the 3Rs principles of animal research by replacing the use of live sentinel animals with a scientifically appropriate health monitoring alternative. EHM could also contribute to refinement in that housing of rodents on pooled dirty bedding could be stressful as shown in a recent study in which sentinel mice had a lower net weight gain over the study period as compared with control mice.[31] Most importantly, EHM has been shown across a number of studies from different authors to be effective at detecting multiple pathogens when used as an adjunct or as a complete replacement to traditional health monitoring programs.[2,4,7,8,11,14,15,17-19,26-29,33,35,37,39,40,44,45]

Different methods can be used to perform EHM depending on cage type and rack design. Regardless of the specific method, an environmental sample is ultimately collected and submitted for PCR testing. For IVC racks with unfiltered exhaust at the cage level (for example, Allentown, Tecniplast), Exhaust Dust Testing (EDT) involves collecting dust samples using plenum swabbing or collar mounted media.[17,18,25-28,34,35,39,40,44,45]

Submitted: 07 Sep 2022. Revision requested: 30 Sep 2022. Accepted: 12 Dec 2022.
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This article contains supplemental materials online.

For all types of caging, including static cages or IVC rack systems with filtered exhaust at the cage level (for example, Animal Care Systems, Innovive, Thoren, Lab Products), a technique we term Sentinel-Free Soiled Bedding (SFSB) testing can be used. With this technique, pooled dirty bedding is transferred to an empty cage or container that does not contain sentinel animals. This bedding is sampled via filter paper, swabs, or other media.[11,14,15,37,43] Finally, noninvasive samples can be taken directly from colony animals, including sick animals and those found dead, regardless of the housing system used.[7,10,16,42,43] Although the scientific literature supporting EHM is still evolving, more publications in 2022 support its application as compared with the number supporting traditional soiled bedding sentinels in 2016.[5]

Despite the increasing amount of published literature and number of real-world examples showing the benefits of EHM, many institutions still use soiled bedding sentinels. Previous surveys on rodent health monitoring programs at research institutions revealed that in 2006, 100% of institutions used soiled bedding sentinels for surveillance.[6] In 2017, the survey was repeated because the "use of PCR analysis of colony animals or exhaust manifolds was early in its widespread use as a primary method of disease detection during the past 10 years."[30] At this point, 95% of institutions used soiled bedding sentinels for surveillance.[30] Low adoption of EHM may be related to lack of knowledge about the recent evidence supporting EHM or to operational concerns such as cost or time. However, benchmarking data or evaluation of constraints to EHM implementation that could guide its adoption has not been published to date.

The theory of planned behavior can be used to understand, predict, and design interventions to change human behavior.[1] This theory states that people are more likely to perform behaviors when they plan to do them. These plans are influenced by 3 main factors: beliefs about the consequences of doing a behavior (attitudes), beliefs about social and professional pressure to do a behavior (subjective norms), and beliefs about their personal control over doing a behavior (perceived behavioral control). This theory is the basis for over 832 published studies, is highly predictive, and can be used to develop interventions to promote behavior change.[13] Recently, this theory has been used to successfully change the behavior of animal research personnel.[20,21]

Our objective in this study was to characterize the current level of EHM use and identify factors that prevent or enable its implementation. Our specific aims were to gain insight into adoption of EHM, personnel knowledge and attitudes toward the method, and, in particular, barriers to its implementation. Based on previous research, we hypothesized that current implementation of EHM is low and this limited adoption is in part due to individual beliefs. We hope this study will identify promising areas for intervention that will help institutions adopt EHM to promote the 3Rs.

Materials and Methods

All procedures and informed consent protocols were approved by University of Washington's Human Research Protection Program Institutional Review Board (IRB), protocol #00012787. No interactions occurred between the researchers and animals during the study. Therefore, we did not seek IACUC approval.

Participants and procedures. Participants were recruited between April 7th and 26th, 2021 by a widespread online convenience sampling designed to maximize sample size. Online contacts were made using 4 modalities: direct emails to known relevant personnel, listservs (for example, LAREF), email lists (for example, the 3Rs Collaborative's), and social media (LinkedIn, Facebook, Twitter). All modalities were used up to 3 times using the same flyer but with different text, as is a recommended survey procedure.[9] Participants were informed that they would be entered into a drawing for a $25 gift card for completing the survey. Participants were also informed that they would be contacted during the following 2 y to gather additional longitudinal data. The current manuscript only contains data from the first year of data collection. After voluntary informed consent, participants completed a 10-min online survey. Participants were over the age of 18, currently worked at an institution that uses mice or rats, and were at least somewhat familiar with their institution's rodent health monitoring program.

Measures. This survey was created by members of the 3Rs Collaborative's (3RC) Rodent Health Monitoring Initiative via a thorough review of the literature, the theory of planned behavior,[1] and consultation with experts in the field of survey methodology and EHM. When possible, we used validated survey instruments such as the theory of planned behavior survey. However, when validated instruments were not available, additional items were created, reviewed by our expert team, piloted, and revised as necessary. All survey question text and scoring scales are shown in Supplemental Table S1.

A mixed methods approach of both qualitative and quantitative questions (that is, open and close ended questions) was used to both allow participants to freely share their opinions and enable quantitative statistical analysis.

Demographics and work factors. Participants were asked questions about their demographics and current work. Demographic questions included age, country, and highest education level. Current work questions included type of institution (for example, academic or industry), institution name, role (for example, veterinarian, manager, caretaker, or researcher), and an estimate of the number of cages of mice and rats at their institution.

Beliefs: Theory of planned behavior. The theory of planned behavior was used to assess EHM intentions and beliefs, including behavioral attitudes, subjective norms, and perceived behavioral control. Surveys constructed using this theory typically have excellent reliability and validity.[13] To avoid confusion, the following statement was given before participants answered questions about EHM: "*In this survey, environmental monitoring (otherwise known as EHM) is defined as a variety of sampling methods that are used to indirectly perform rodent colony health surveillance without the use of live 'sentinel' animals.*"

First, participants were asked 2 qualitative, open-ended questions about the barriers to and advantages of EHM. These questions allowed participants to answer freely without direct prompting as to what the answers should be. Second, participants were asked quantitative close-ended questions. These included questions about attitudes (for example, whether they think their institution using EHM is bad compared with good), control beliefs (for example, their confidence in their institution using EHM), and norms (for example, whether there is any professional/social pressure to use EHM). Finally, participants were asked about their general intentions for using EHM and for the percentage of cages expected to be monitored via EHM in the next year.

Benchmarking. To benchmark knowledge about EHM, participants were asked a series of questions with right or wrong answers about EHM. These questions were developed by research animal veterinarians and experts in rodent health monitoring, and were based upon a thorough review of the literature. Responses to these questions were then tallied to determine how knowledgeable the respondent was about EHM. Participants were also asked which resources they used to learn about rodent health monitoring.

To benchmark and understand factors that could affect EHM implementation, participants were asked to describe the current rodent health monitoring programs at their institutions. Participants were asked what type(s) of methods were used for routine health monitoring (that is, only live animal sentinels, only EHM, both, or none). If EHM was used at all, participants were asked what percentage of their colony was currently tested using primarily EHM. Participants were asked about quarantine monitoring practices and what percentage of quarantine animals were monitored with EHM. Participants were asked about the caging and rack types and how many times a year racks were taken out of service for sanitation. If live animal sentinels were used, participants were asked how many were used in 1 year, what testing methods were used (for example, PCR, serology, parasitology, or microbiology), and test frequency.

At the end of the survey, participants were also asked if their institution would accept rodents from other institutions that use only EHM. Finally, participants were asked what the single most important factor was that allowed their site to adopt EHM.

Data analysis.

Quantitative analysis. Data analysis was conducted in JMP (version 16.2.0; JMP Statistical Discovery, Cary, NC) using descriptive statistics and general linear models. Where appropriate, descriptive statistics are presented as the Mean ± SD. Prior to testing, all appropriate assumptions were visually confirmed including independence of residuals, homogeneity of variance, and normality of residuals. For all summary scales, an average of individual items was calculated; respondents missing over 50% of the data per scale were excluded. For example, a summary score for attitudes was only calculated for participants that answered at least 2 of the 3 questions about their attitudes towards EHM. Responses to categorical questions were collapsed into larger categories for use in linear models when fewer than 20 respondents met a particular category. For example, the education levels of Associate Degree and High School Diploma, GED, or equivalent were collapsed into one category "Associate Degree or Lower."

For benchmarking of institutional adoption of EHM, we analyzed information from only one individual for each institution. If multiple individuals responded to the survey from the same institution, we only used the response from the individual thought to be the most knowledgeable participant, rather than trying to average potentially contradictory information. To select the response used for institutional benchmarking analysis, we first considered the individual(s) who reported being the most familiar with their institutional rodent health monitoring program. If more than one person reported the same high-level familiarity, we then chose a veterinarian or manager. Finally, if multiple individuals remained, we chose the most complete response. Benchmarking data are reported using descriptive statistics.

To determine the association between planned use of EHM and potential influential factors (for example, individual beliefs about EHM), we ran a general linear model for all participant responses. The dependent variable for analysis was the planned level of EHM. Relevant independent variables included the theory of planned behavior beliefs, familiarity, knowledge, and institutional/demographic factors.

The initial model used was:

$$\begin{aligned}\text{Intention to Implement EHM} &= \text{Attitudes} + \text{Social norms}\\ &+ \text{Control beliefs} + \text{Knowledge}\\ &+ \text{Exhaust Dust Compatible Racks} + \text{Country} + \text{Role}\\ &+ \text{Education} + \text{Number of Cages}\end{aligned}$$

Qualitative analysis. An inductive conventional content analysis was used to code qualitative data related to EHM adoption.[12] Two of us (KL and KG) read all responses for a particular question multiple times, noting potential themes next to each response. We attempted to create themes that were primarily driven by the participant responses. However, we recognize that our knowledge and internal biases also influence theme creation. After completion of initial coding, KL and KG looked for patterns to that could suggest broader themes and subsequently we drafted a coding manual. This initial coding manual was discussed by 3 authors to determine potential code names, definitions, key phrases, and representative quotes. Subsequently, data were reread and formally coded based on this manual. During coding, the manual was further refined in response to any questions that arose based on discussion between the 3 authors. Ultimately, a formal coding manual was created that described each code, key words, definitions, and example quotes (Supplemental Table S2). Interrater reliability was assessed by having a second individual code a random 20% of the qualitative data using the same coding manuals. The following formula was used: Reliability = # of agreements/(# of agreements + # of disagreements).[32] Ultimately, any subthemes that did not meet 80% reliability were dropped and incorporated into the main theme.

Each participant's response to a particular question was broken down into its unique clauses for coding, with no limit to the number of codes per response. For example, the response "*Reduction in the number of live animals used (and the consequent impact on staff and resources)*" was broken into 2 clauses. The first clause, "*Reduction in the number of live animals used*" was coded as Replacement. The second clause "*(and the consequent impact on staff)*" was coded as Personnel. Responses that that we could not understand were coded as ambiguous. Ultimately, we calculated the prevalence of each theme and subtheme by taking the total number of participants mentioning the category divided by the total number of respondents that gave an understandable answer in that category. For example, X individuals referenced Y out of X total respondents that gave a codable response to that question. Therefore, blank responses were not included in the total number of respondents.

Results

Demographics. A total of 158 participants were included in the study. Detailed demographic information is displayed for all participants in Table 1. Overall, participants were primarily veterinarians (52%) with graduate or veterinary degrees (66%). Participants worked mostly at universities (73%) and in the United States of America (80%).

Benchmarking institutional practices. Analyzing only one response per institution produced the following results across 111 institutions. Most institutions had a combination of IVCs and static cages (69%). In terms of specific rack types, 36% of institutions had only IVC racks with unfiltered exhaust at the cage level. The number of rodent cages per institution ranged from 80 to 50,000 with an average of 9,878 ± 11,581.

Table 1. Demographic and Work Information for Animal Research Facility Personnel Participants (n = 158).

Role	N	% of Total
Veterinarian	82	52%
Manager	25	16%
Animal Caretaker or Laboratory Technician	22	14%
Other	29	18%
Education		
Graduate or Veterinary Degree	104	66%
Bachelor's degree	33	21%
High School Diploma or Associate Degree	21	13%
Institution Type		
Academic	115	73%
Industry	18	11%
Other	25	16%
Location		
USA	125	80%
Canada	14	9%
United Kingdom	7	4%
Europe	10	6%
Africa	1	1%

Currently, only 11% (n = 12) of institutions used EHM alone for routine colony health monitoring of mice and rats (Figure 1). Most institutions used soiled bedding sentinels only (41%, n = 45) or a combination of methods (46%, n = 51). Of the institutions that used a combination of methods, an average of 44% ± 34% of their colony used primarily EHM although that ranged from 0% to 100%. For quarantine, most institutions typically used only EHM (63%); however, some used a combination of methods (14%), only soiled bedding sentinels (15%), or no testing (8%).

The institutions that used sentinel animals reported using between 1 and 2,100 per year (380 ± 488) with a total of 20,917 sentinel animals used yearly from all institutions. Of the 98 institutions that reported the methods used to test their sentinels, PCR was most used (94% of institutions), followed by serology (93%), parasitology (71%), and microbiology (44%). Of the 88 institutions that reported testing frequency, sentinels were generally tested quarterly (69%), although this ranged from 1 to 12 times a year.

In terms of the specific type of EHM method used, exhaust air dust swabbing was used most often (68%, n = 41), followed by exhaust air dust media (41%, n = 24), running filter paper over or swabbing specific surfaces (32%, n = 19), filter paper in static soiled bedding cages that did not contain live rodents (22%, n = 13), and other (15%, n = 9). EHM was generally conducted 3 or 4 times a year (67%, n = 30). Racks were generally removed from service for sanitation 1 or 2 times a year (81% of institutions, n = 38).

Although few institutions currently used EHM, 40% of participants expected to use EHM next year in over half of their colonies. Finally, most participants (76%, n = 78) indicated that their institution would accept rodents from other institutions that use only EHM, although some may require additional testing.

Benchmarking familiarity, knowledge, and beliefs. Overall, most participants were moderately (27%) or very (56%) familiar with EHM, with very few who were not at all (1%), slightly (6%), or somewhat (10%) familiar with EHM. Generally, participants had some inaccuracy in their knowledge of EHM as indicated by an average knowledge quiz score of 65%. Specific question text and responses are shown in Figure 2. Most participants were aware that EHM can replace soiled bedding sentinels (99% correct), require less time (95%), and that for most pathogens, is equal to or more sensitive than soiled bedding sentinels (85%). However, most participants were unaware that over 20 peer-reviewed publications support EHM (32%) and that most institutions will accept rodents from institutions that use only EHM (22%).

Participants used a variety of resources to learn about rodent health monitoring. Most commonly, their resources were from diagnostic companies (74%), conferences/webpages/workshops (71%), peer-reviewed manuscripts (69%), and colleagues (61%). Less commonly used were technical articles (36%) and the 3RC's newsletter/webpage/webinars (36%).

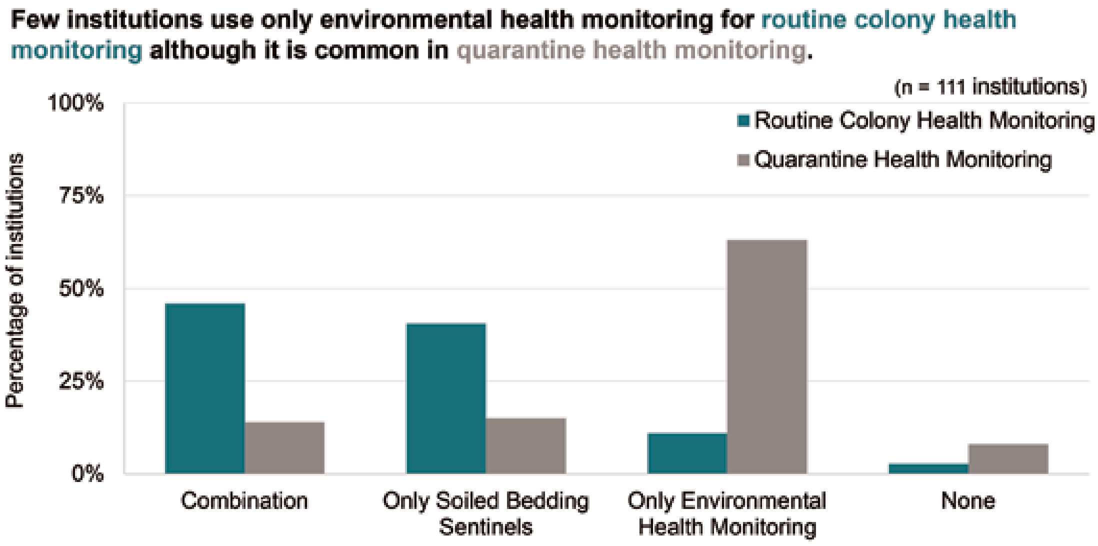

Figure 1. Health Monitoring Program Benchmarking. The percentage of surveyed institutions (n = 111) that use only environmental health monitoring, only soiled bedding sentinel programs, a combination, or no programs in their routine colony health monitoring or quarantine health monitoring programs.

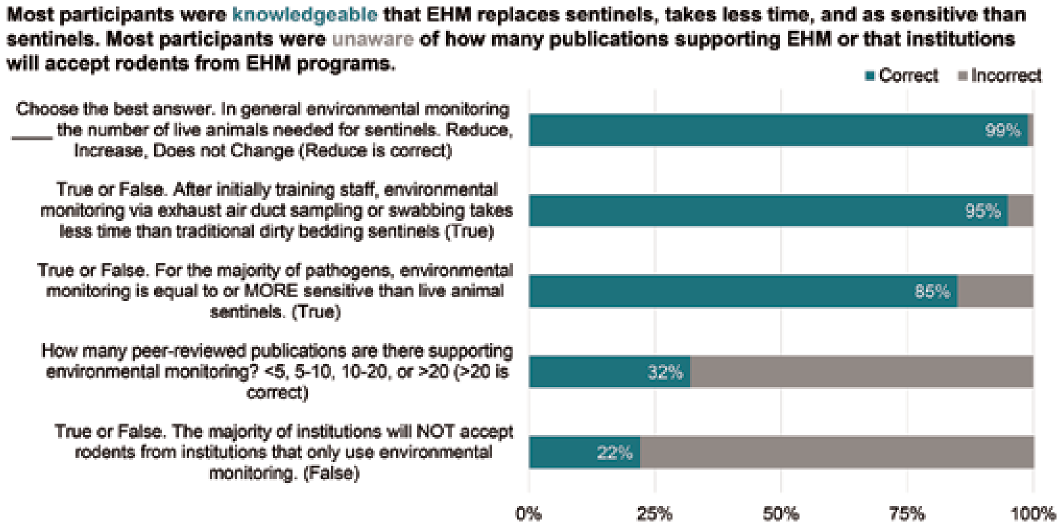

Figure 2. Participant Knowledge of Environmental Health Monitoring. The figure shows percentage of participants that gave the correct answer in a knowledge quiz about environmental health monitoring. The correct answer, as determined by scientific research and expert consensus, is shown in parentheses.

On a scale from 1 to 7, where 1 indicates negative and 7 indicates positive, individuals had relatively positive attitudes about EHM (5.8 ± 1.5; that is, on average they think EHM is good), had neutral to negative subjective norms (3.4 ± 1.5; that is, on average they did not feel any professional pressure to use EHM or even felt professional pressure to not use EHM), and had neutral perceived control beliefs (4.8 ± 1.7; that is, on average they were not confident they could implement EHM).

Participant's intentions to use primarily EHM in the next year was strongly associated with participant's beliefs about EHM (Table 2). A more positive intention to use primarily EHM in the next year was positively associated with more positive attitudes, social norms, and control beliefs. No demographic or institutional factors (for example, country, role, education, number of cages, or cage type) were correlated with planned EHM use.

Qualitative analysis. Most participants responded to open-ended questions meant to reveal the key barriers to (n = 136, or 86% of participants) and advantages of using EHM (n = 142, or 90% of participants). However, fewer than half of the participants (n = 65, or 41% of participants) were both eligible for and chose to respond to the question meant to reveal the key enabling factors for institutional use of EHM. Overall, their responses were captured with 8 key themes: replacement, caging/rack type, cost, time, accuracy, personnel, rack sanitation, and none. These themes and representative quotes are summarized below in order of frequency across questions and in Figure 3 and Supplemental Table S2.

Table 2. Intention to Implement Environmental Health Monitoring. The table presents associations from a general linear model of self-report data from research animal personnel. Participants were asked about their beliefs, knowledge and familiarity with EHM and about institutional and work factors. We then determined the association of these responses with their intent to implement EHM in the next year. (+) indicates a significant positive association between the independent and dependent variables, $P < 0.05$.

Independent Variable	Intent to Implement EHM (n = 125)
Attitudes	**(+) $F_{1,111}$ = 21.45, P < 0.0001**
Subjective Norms	**(+) $F_{1,111}$ = 17.14, P < 0.0001**
Control Beliefs	**(+) $F_{1,111}$ = 14.91, P = 0.0002**
Knowledge	$F_{1,111}$ = 1.74, P = 0.1903
Exhaust Dust Compatible Racks	$F_{1,111}$ = 1.25, P = 0.2654
Familiarity	$F_{1,111}$ = 0.01, P = 0.9312
Education	$F_{2,111}$ = 1.20, P = 0.3065
Role	$F_{3,111}$ = 0.38, P = 0.7710
Institution	$F_{2,111}$ = 2.02, P = 0.1370
Country	$F_{1,111}$ = 0.36, P = 0.5474

Participant responses were segmented based on which open-ended question they were answering. "Barriers" were responses that participants believed make EHM difficult or impossible to implement. "Advantages" were factors that participants believed were positive aspects of EHM. Finally, "enablers" were factors that participants believed were most important in enabling their institution to switch to EHM; this question was only shown to participants that had either switched or planned to switch their facility to over 50% EHM.

Theme 1: Replacement of animals. The most frequently cited advantage of EHM was *Replacement of Animals*, which was stated by 78% of participants. Participants described EHM as allowing their institution to avoid the use of, reduce the total numbers of, or replace live sentinel animals in health monitoring programs. In particular, some participants reported that EHM is "helping fulfill the 3 R's" and that by using EHM they can "reduce animal use."

Replacement of animals was the 2nd most common enabler reported by 20% of participants. For example, one participant cited "a desire to reduce the number of live animal sentinels" as being the most important factor that enabled their institution to switch to EHM.

Theme 2: Caging and rack type: Easy compatibility and consistency. The most common perceived barrier to implementing EHM was *Caging and Rack Type* which was reported by 40% of participants. These individuals believed that implementing EHM was difficult due to the specific type of caging or IVC rack design used at their institution. Some participants just generally described "cage/rack design" as a reason. Others mentioned particular types of cages or racks that they believed made EHM difficult such as stating that "we have some animals on static rack" or "some of our ventilated racks do not lend

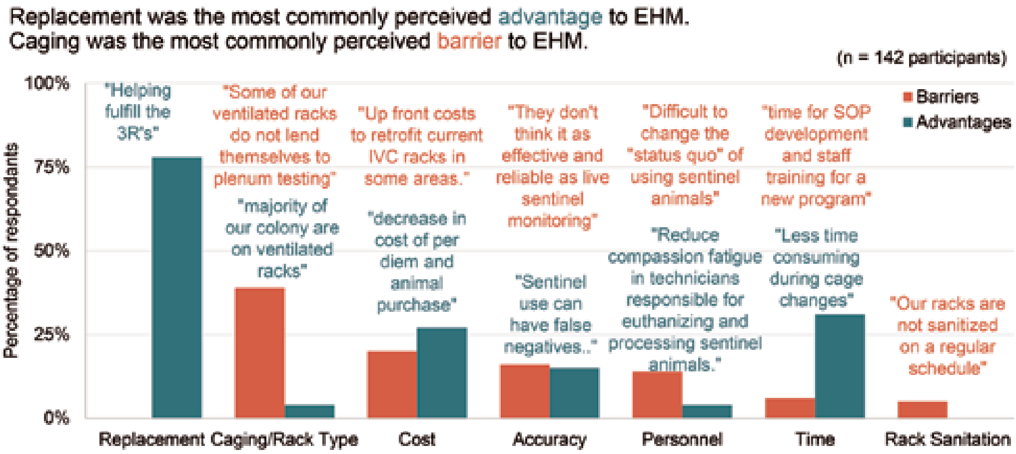

Figure 3. Barriers and Advantages to Environmental Health Monitoring. The most common themes related to the benefits/advantages and barriers to implementing environmental health monitoring as identified by 142 participants familiar with their institutions' rodent health monitoring program. Graphic includes representative quotes. The complete coding manual including theme definitions, sub theme results, coding manual, and representative quotes are presented in Supplemental Table S2.

themselves to plenum testing." Several others reported that simply having multiple caging types would make EHM difficult due to perceived incompatibilities or inconsistencies. One participant stated that they had "multiple caging types, some with single-cage filtration as opposed to rack-based filtration" while another indicated "different types of racks used, [soiled bedding sentinels allow] uniformity of testing across the institution (so different methods don't need to be used)."

Conversely, a few participants (4%) stated that their *Caging and Rack Type* was advantageous for using EHM. Those participants mentioned having racks that are easily compatible with EHM (for example, IVC racks with unfiltered exhaust at the cage level) or generally having consistent rack types at their institution. For example, one participant stated, "the majority of our colony are on ventilated racks" while another stated, "we use exclusively Lab Products caging (both IVC and static), so we're able to employ a consistent SOP across campus."

Similarly, 8% of respondents considered *Caging and Rack Type* to be one of the most important factors that enabled EHM use. Generally, these responses referenced uniformity and racks that were compatible with Exhaust Dust Testing. For example, one respondent stated, "We have almost all Allentown racks which made the conversion pretty easy over the last 2 years" while another indicated "Ventilated rack design supports EAD testing."

Theme 3: Time. The second most cited advantage of EHM was *Time* which was reported by 31% of participants. Participants reported multiple possibilities for saving time by switching to EHM. One area of time savings was related to the time required to manage and collect samples from sentinel animals. For example, one participant stated, "By eliminating the sentinel animal, you eliminate the time needed to care (housing, cage changes, veterinary needs, etc.) for the animals." Another participant cited "reduced time in packing sentinels for shipment" and another stated, "Collection of EAD filters is also a much less laborious task than collecting blood from sentinel animals and ectoparasite tests from colony animals." Other participants specifically referenced time saved from soiled bedding transfers. For example, "Less time-consuming during cage changes."

Despite its frequent mention as a key advantage of EHM, *Time* was cited by only 3% of participants as one of the most important factors enabling facility wide change. One participant noted "fast turnaround time and easy collection" while another specifically noted "less workload."

Similarly, only 6% of participants cited *Time* as a barrier to EHM. In those cases, participants were concerned both about the time needed to establish and maintain an EHM program. Specifically, one respondent mentioned "time for SOP establishment and re-training of staff."

Theme 4: Cost of implementation. More participants considered *Cost Savings* to be an advantage to using EHM (27%) compared with 21% who believed that *Increased Cost* made it difficult to use EHM. As an advantage, participants reported cost savings due to a decrease in the cost of maintenance, purchase, and diagnostic testing of sentinel animals. For example, one respondent mentioned that EHM can result in a "decrease in cost of per diem and animal purchase." Similarly, *Cost Savings* were cited as the third most common enabler by 12% of participants. For example, one participant stated "reduced costs associated with dedicated sentinel animal care and housing charges" was one of the most important factors that convinced their institution switch to EHM.

When cost was cited as a barrier, some participants noted the cost of the equipment (8%) and/or the cost of testing (7%). Concern with testing costs were typically related to the increased cost of PCR as compared with serology. For example, one participant indicated "Our racks require an attachment to use a commercially available filter media. This custom attachment is expensive, especially when multiplied by 400 racks." Another participant stated, "Also switching from a predominantly serology-based system to a PCR based system increases cost significantly. Even with pooling of media, PCR costs for panels are 3× more than serology panel costs."

Theme 5: No barriers or No advantages. A total of 18% of participants reported no barriers to using EHM. Some of these individuals worked at institutions that had already switched to EHM as stated by one participant, "We use Exhaust Air Dust throughout all of our facilities." Others simply did not see any barriers to switching to EHM by responding "none" or "no factors make it difficult or impossible." Conversely, only 1% of participants saw no advantages to EHM stating that "this method would not be beneficial for our site."

Theme 6: Accuracy of EHM. *Accuracy* was considered an advantage (15%) almost as often as a barrier (16%). Those who viewed *Accuracy* as an advantage thought that EHM could reduce false negatives, improve sensitivity, and ultimately provide more consistent results. Overall, participants emphasized EHM's superiority in disease screening to maintain appropriate biosecurity for a pathogen-free environment. This view was conveyed by one respondent who wrote that EHM can "maintain the biosecurity of the facility and ensure the health of the animals (and therefore the quality of the science)." Another stated "Better sensitivity (we always pick up any new unexpected finding first by Env. PCR vs colony animal sampling, or bacteria swabs in isolators)."

In fact, the superior *Accuracy of EHM* was the most cited factor (reported by 23% of participants) that enabled institutions to switch. One participant stated that "Evidence that environmental monitoring is as good or better than sentinels for assessing colony health." Another specifically stated that there are "numerous high quality publications on its efficacy."

Despite these positive views of the accuracy of EHM, other participants were still concerned about the accuracy, reliability, and lack of published data on EHM. For example, one participant was "Still skeptical of the reliability of results from environmental monitoring" and another stated there is a "lack of data." Some respondents use a hybrid program (EHM and soiled bedding sentinels) stating that they "do environmental monitoring in the breeding rooms but not all the experimental rooms. The vets were unsure when environmental testing became available, so we use it in a small area but have not eliminated other health monitoring methods."

Theme 7: Personnel. *Personnel* were more often considered a barrier (14%) than an advantage (4%). Participants who viewed *Personnel* as a barrier stated that soiled bedding sentinel health monitoring programs represent the '*status quo*' and institutional culture may make change difficult. One respondent explained, "My institution is used to doing things a certain way. We could use environmental monitoring, but the preference is to use live sentinels." Alternately, some respondents believed their institution did not have the personnel expertise or knowledge to convert to EHM.

Conversely, respondents who reported *Personnel* as an advantage thought that EHM could improve personnel mental and physical health. For example, one participant stated that EHM can "reduce compassion fatigue in technicians responsible for euthanizing and processing sentinel animals" while another stated, "We strive for a dust free environment. For occupational health concerns…"

A total of 8% of respondents considered *Personnel* to be one of the most important factors that helped their institution change. These participants indicated that openness to change or commitment from staff was important. Specifically, respondents cited "openness from our AV" and "researcher buy-in".

Theme 8: Rack sanitation. The least common perceived barrier was *Rack Sanitation*, which was reported by 5% of participants. Participants indicated difficulties with washing racks at all or difficulties on a regular basis. Respondents specifically noted that they "cannot wash and autoclave the racks" and that "rack sanitation schedules and timing drift make scheduling and regular results reporting difficult."

Theme 9: Testing advancements and ease. A unique response to what was the most important factor enabling an institution to switch was related to advancements and ease in testing. For example, one participant simply stated, "the development of PCR techniques to detect pathogens." Another specifically cited "CRL-LTM program for analysis of samples and providing the supplies for sample collection."

Discussion

Through our survey and subsequent analysis, we have benchmarked the rodent health monitoring practices and potential factors influencing EHM implementation in the animal research environment. We successfully surveyed 158 research animal personnel from 111 institutions who were familiar with their institution's rodent health monitoring programs. Our results indicate that most animal research personnel are at least moderately familiar with EHM, but that it is not yet used exclusively at most institutions.

Overall, our analysis indicates that although personnel generally believe EHM is good, they do not feel professional pressure to make the switch to EHM and are not confident in making the switch. However, more positive intent to use EHM in the next year is associated with positive attitudes, norms, and control beliefs. When asked about EHM, participants generally indicated that replacement of animals was the largest advantage to EHM while caging and rack type was the most common perceived barrier. From these data, we now know that well over 20,000 animal lives could be replaced yearly in just these surveyed institutions through use of EHM.

Current practices. In April of 2021, surveyed institutions reported moderate to high familiarity with and low exclusive implementation of EHM. Although peer-reviewed articles about EHM appeared as early as 2004,[8] soiled bedding sentinels have remained the most commonly used method of rodent health monitoring likely due to historical precedent.[30] Techniques to apply EHM to static cages or cages with filtered exhaust at the cage level were first described over a decade later.[11,15,43,44] In addition, the evidence base has not yet been synthesized into a formal review article, although the 3RC has a systematic literature review in progress as of the publication of this article. Furthermore, prior to launch of the 3RC's Resource Hub in late 2020 (www.na3rsc.org/health-monitoring), no central, vendor-independent online resource had been available for professionals to learn about EHM.

Furthermore, despite reporting relatively high familiarity with EHM, the average knowledge score quiz result for participants was 65%. Furthermore, fewer than a third of participants were aware that over 20 peer-reviewed articles supported EHM and that the majority of institutions will accept rodents from EHM only institutions. An outdated understanding of the evidence behind and acceptance of EHM could contribute to hesitancy to implement EHM.

Overall beliefs and associations of EHM. Most research animal personnel have positive attitudes about EHM; on average, they believe that EHM is good and beneficial. Furthermore, participants who had more positive beliefs about EHM were also more likely to indicate an intention to use EHM in the future. In particular, EHM was qualitatively considered to be beneficial for the 3Rs technique of Replacement. Factors such as cost, time, and accuracy were commonly listed as both benefits and barriers.

Overall, participants had very low subjective norms about EHM; on average, they generally felt either no professional pressure to use EHM or no professional pressure to *not* use EHM. However, participants that did have higher subjective norms about EHM were more likely to indicate higher levels of planned EHM implementation. Typically, research animal veterinarians and managers play key roles in designing rodent health monitoring programs. As soiled bedding sentinels have been in use for 50 y, a key cited barrier in our qualitative data was convincing these individuals to change the status quo. In addition, concerns about the accuracy of EHM likely influence subjective norms about EHM. A change in these norms will likely result in greater adoption of EHM.

The control that participants perceived over providing EHM was overall reported to be neutral; on average, participants were not sure if they could implement EHM. Furthermore, participants who had more positive control beliefs about EHM were also more likely to report higher levels of planned use of EHM. If institutions wish to adopt EHM, providing training and resources may be beneficial.

EHM for static cages and cage-level filtration. By far, participants most often cited caging and rack design as a barrier to EHM. This result is likely due to early publications and presentations that focused on Exhaust Dust Testing, which is not compatible with all IVC rack designs and static caging. However, between 2018 and 2022, 6 publications have described Sentinel-free Soiled Bedding Testing (a method of EHM compatible with all cage types) as equal to or superior to the use of soiled bedding sentinels.[11,14,15,37,43,44] This information complements anecdotal reports to the 3Rs Collaborative of increased numbers of institutions switching to Sentinel-free Soiled Bedding Testing. However, as with traditional soiled bedding sentinels, research is still being conducted to determine the best testing protocols for EHM.[5] Greater dissemination of specific peer-reviewed publications and real-world experiences of using EHM for static cages and cage-level filtration may help address this perceived barrier.

Conflicting barriers and advantages to EHM. Of the 7 coded themes for barriers and advantages to EHM, 5 appeared in both categories. For example, some participants listed cost as a barrier to using EHM while others listed it as an advantage. These conflicts may be in part due to differences between institutions, but also may be influenced by misconceptions.

In our survey, cost was more often considered an advantage to EHM (27%) rather than a barrier (20%). This finding is consistent with a study showing that the EHM program was 26% less expensive than soiled bedding sentinels.[24] From a practical standpoint, budget must be considered when modifying an animal care and use program. Some participants were concerned that EHM would be more expensive due to the cost of PCR assays as compared with serology. These participants may not be considering that these costs can be offset by eliminating the need to purchase and maintain sentinel animals.[24] In addition, participants cited up-front costs for collar mounted media, which we note were not included in the only published cost analysis to date.[24] Therefore, this initial investment may be a valid concern depending on when the rack was purchased. In addition, the published cost analysis focused on an institution whose racks were mostly amenable to Exhaust Dust Testing. An additional cost related benefit for institutions with racks that have unfiltered exhaust at the cage level is that a sentinel cage is not required, allowing a potential increase in revenue in per diems by the use of this cage space for research purposes. This potential revenue was also omitted from the published cost analysis.[24] Publications from additional institutions that use other methods of EHM, particularly sentinel-free soiled bedding techniques or hybrid methods, would allow more thorough cost comparisons. In addition, greater dissemination of the results of such cost analyses might mitigate this barrier.

Time was much more often cited as an advantage (31%) rather than as a barrier (6%) to using EHM. This finding is consistent with a previous study reporting that EHM decreased the amount of time spent by technicians on performing health-monitoring activities.[24] The activities involved ear punching mice for identification, daily care, necropsies, and sample collection (blood, fecal, fur swab, and oral swab) for the soiled bedding sentinel program. These activities were more time intensive than the EHM program, which involved collecting the exposed collection medium and placing a fresh one. These time savings amounted to approximately 1.5 h each week for every 10,000 rodent cage[24] and should be viewed as mitigating cost. However, as stated by some participants, the process of making the change itself requires additional time for conversion and retraining. To help mitigate this concern, the 3RC developed editable SOPs for various EHM sampling methods and a downloadable and editable introductory presentation slide set; both can be found on the 3RC's website (https://www.na3rsc.org/health-monitoring/).

Accuracy was considered to be a barrier (16%) as often as an advantage (15%). These conflicting views may result from a relatively recent increase in the number of publications reporting that EHM is equal to or more sensitive to soiled bedding sentinels.[2,4,7,8,11,14,15,17-19,26-29,33,35,37,39,40,44,45] These divergent views may also be related to a status quo bias and lack of knowledge that soiled bedding sentinels have relatively limited support in published literature.[5] In addition, both positive and negative views on accuracy were commonly conditional on the type of rack at the institution. Respondents with racks amenable to Exhaust Dust Testing, which is supported by a larger evidence base, typically had greater confidence in the accuracy of EHM as compared with participants whose racks or caging required Sentinel-free Soiled Bedding Testing, for which published data is more recent and techniques are less standardized. We are in the process of conducting a systematic literature review to provide a comprehensive and unbiased understanding of the scientific literature supporting EHM to address this barrier.

Related to accuracy, some participants (5%) were concerned about their ability to sanitize racks. These sanitation concerns are typically related to the possibility of finding false positive results due to legacy nucleic acids, which may be present even after an agent has been excluded from a particular population of rodents. The stability of these legacy nucleic acids can confound the ability of PCR diagnostics to detect the reintroduction or continued presence of an agent.[2] Sanitization practices may require customization depending on the legacy nucleic acids of concern. For example, typical cage wash practices (use of temperatures of 180°F and detergents) at one institution were sufficient to remove nucleic acids for *Helicobacter* spp., *Rodentibacter* spp., and MNV from IVC racks and ensure negative rack plenum swab PCR results.[25] However, manual scrubbing of the plenums with a detergent before cage wash was required to remove *Ornithonyssus bacoti* adhered eggs to eliminate legacy nucleic acids.[7] Other strategies are the use of dedicated IVC racks for higher barrier rooms or 2 cage wash cycles to avoid legacy nucleic acids for *Helicobacter* spp. and MNV.[39] For agents that shed large amounts of genetic material into a rack exhaust system, a single cycle through a 180°F rack washer, using detergent, was not adequate to remove legacy nucleic acids. However, autoclaving racks effectively removes legacy nucleic acids for *C. bovis*,[28] *Rodentibacter* spp.,[34] and *Helicobacter hepaticus*.[35] If whole rack autoclaving is not available, vaporized hydrogen peroxide sanitation of IVC racks and rack blowers is effective in eliminating legacy nucleic acids, so that none are detected by PCR.[40] Finally, while not specific to EHM, a recent publication demonstrates the effectiveness of standard cage wash practices for the removal of common rodent pathogens.[8] However, additional research on this particular topic could support the switch of more institutions to EHM.

Personnel were more often cited as barriers to EHM (14%) rather than as recipients of the benefits of EHM (4%). Generally, participants referred to poor personnel attitude toward EHM (10%) or the lack of personnel expertise (5%). Participants discussed the difficulty in changing the status quo and concerns about moving away from traditional methods that have historical precedence. These factors are commonly cited issues for any type of behavior change. Due to its ubiquity, personnel discomfort stemming from both attitudes and expertise can be alleviated with targeted education focused on human behavior change. The 3RC's Rodent Health Monitoring Initiative has created an extensive Resource Hub (https://www.na3rsc.org/health-monitoring/) and panel presentations that are designed to help with this. This Hub includes educational information and resources to promote conversion to EHM. Finally, an effort is being made to change subjective norms by directly citing examples of institutions that have successfully switched. Furthermore, through conducting this survey, we can document that most institutions already accept rodent imports from exporters that use only EHM.

Participants in this study indicated that EHM can be beneficial to personnel in terms of mental health because it reduces the need to perform euthanasia, which could mitigate compassion fatigue. Compassion fatigue is a common concern for those who work with research animals.[22] This emotional exhaustion and impaired empathy can significantly reduce the quality of life of those who are chronically placed in morally stressing situations.[36,41] This can include the euthanasia of large numbers of nonexperimental, sentinel rodents that likely appear healthy independent of their diagnostic results. Between these direct benefits to personnel and the potential use of EHM to reduce zoonotic pathogen transfer, EHM can contribute to the goal of One Health and One Welfare by benefiting humans and animals simultaneously.[3]

Limitations. This project had several limitations due to the use of convenience sampling, cross-sectional, and self-report characteristics. The convenience sampling may have been influenced by sampling bias and may not truly be representative of the full research animal personnel population, especially for countries with limited responses. While we received responses from over one hundred institutions, this is ultimately only a fraction of all AAALACi-accredited facilities. Participants may have been skewed to individuals who felt particularly passionate either for or against EHM. In addition, although previous work indicates that the theory of planned behavior is highly predictive,[1] our cross-sectional design is not suitable for determining the cause-and-effect relationship of any of the associations we identified. Finally, because personnel were asked to report current and planned implementation of EHM based on their own knowledge, they may have over or under-estimated these levels. However, despite these limitations, this study still provides valuable insight into current rodent health monitoring practices and beliefs that provide direction for interventions and future studies.

Conclusions

In conclusion, as of April 2021, EHM appears to be relatively rarely implemented in animal research institutions, but the field is moving toward higher implementation. Although most research animal personnel believe EHM to be beneficial for replacing sentinel rodents and reducing time, they also believe that implementation is limited by barriers that include caging and rack design, cost, accuracy, and personnel. Furthermore, higher current and planned EHM implementation is related to factors such as attitudes, social/professional norms, and control beliefs. The results of this survey can be used in the context of a growing societal desire to work toward the replacement of animal use in research when scientifically appropriate. Overall, our results suggest that interventions to promote EHM should target improving attitudes to EHM (for example, addressing misconceptions about limitations related to caging and rack type, cost, and accuracy), establishing a professional norm of using EHM methods, and helping institutions to implement EHM. These interventions may ultimately increase the use of EHM thereby replacing over 20,000 sentinel rodents used each year in just the 111 surveyed institutions and many more around the globe.

Supplemental Materials

Table S1. Survey data and dictionary. Survey question text and scoring scales for the 3RC's Rodent Health Monitoring Initiative survey performed in 2021.

Table S2. Survey qualitative results & manual. This table shows the formal coding manual, the percentage of responses for each theme, a description, key phrases, and example quotes.

Acknowledgments

We gratefully acknowledge the laboratory animal personnel who took the time to participate in and promote this study. We also thank the 3Rs Collaborative staff, volunteers, and sponsors for making this research possible. We also appreciate all laboratory animal personnel who have been integral in helping their institutions switch away from using soiled bedding sentinels to using EHM. Finally, we thank all of the sentinel rodents that have been and are currently used to ensure optimal colony health—we hope our efforts ultimately replace their need to be used.

References

1. **Ajzen I**. 1991. The theory of planned behavior. Organ Behav Hum Decis Process **50**:179–211. https://doi.org/10.1016/0749-5978(91)90020-T.
2. **Bauer BA, Besch-Williford C, Livingston RS, Crim MJ, Riley LK, Myles MH**. 2016. Influence of rack design and disease prevalence on detection of rodent pathogens in exhaust debris samples from individually ventilated caging systems. J Am Assoc Lab Anim Sci **55**:782–788.
3. **Bourque T**. 2017. One welfare. Can Vet J **58**:217–218.
4. **Brielmeier M, Mahabir E, Needham JRW, Lengger C, Wilhelm P, Schmidt J**. 2006. Microbiological monitoring of laboratory mice and biocontainment in individually ventilated cages: A field study. Lab Anim **40**:247–260. https://doi.org/10.1258/002367706777611497.
5. **de Bruin WCC, van de Ven EME, Hooijmans CR**. 2016. Efficacy of soiled bedding transfer for transmission of mouse and rat infections to sentinels: A systematic review. PLoS One **11**:e0158410. https://doi.org/10.1371/journal.pone.0158410.
6. **Carty AJ**. 2008. Opportunistic infections of mice and rats: Jacoby and lindsey revisited. ILAR J **49**:272–276. https://doi.org/10.1093/ilar.49.3.272.
7. **Clancy BM, Theriault BR, Schoenberger JM, Bowers CJ, Mitchell CM, Langan GP, Ostdiek AM, Luchins KR**. 2022. Identification and control of an *Ornithonyssus bacoti* infestation in a rodent vivarium by using molecular diagnostic techniques. Comp Med **72**:113–121. https://doi.org/10.30802/AALAS-CM-21-000105.
8. **Compton SR, Macy JD**. 2015. Effect of cage-wash temperature on the removal of infectious agents from caging and the detection of infectious agents on the filters of animal bedding-disposal cabinets by PCR analysis. J Am Assoc Lab Anim Sci **54**:745–755.
9. **Dillman DA**. 2011. Mail and internet surveys: the tailored design method. Hoboken (NJ): John Wiley & Sons.
10. **Dole VS, Zaias J, Kyricopoulos-Cleasby DM, Banu LA, Waterman LL, Sanders K, Henderson KS**. 2011. Comparison of traditional and PCR methods during screening for and confirmation of *Aspiculuris tetraptera* in a mouse facility. J Am Assoc Lab Anim Sci **50**:904–909.

11. **Dubelko AR, Zuwannin M, McIntee SC, Livingston RS, Foley PL**. 2018. PCR testing of filter material from IVC lids for microbial monitoring of mouse colonies. J Am Assoc Lab Anim Sci **57**:477–482. https://doi.org/10.30802/AALAS-JAALAS-18-000008.
12. **Elo S, Kyngäs H**. 2008. The qualitative content analysis process. J Adv Nurs **62**:107–115. https://doi.org/10.1111/j.1365-2648.2007.04569.x.
13. **Francis J, Eccles MP, Johnston M, Walker AE, Grimshaw JM, Foy R, Kaner EFS, Smith L, Bonetti D**. 2004. [Internet]. Constructing questionnaires based on the theory of planned behavior: A manual for health services researchers. [Cited 01 January 2022]. Available at: https://openaccess.city.ac.uk/id/eprint/1735/.
14. **Gerwin PM, Arbona RJR, Riedel ER, Henderson KS, Lipman NS**. 2017. PCR testing of IVC filter tops as a method for detecting murine pinworms and fur mites. J Am Assoc Lab Anim Sci **56**:752–761.
15. **Hanson WH, Taylor K, Taylor DK**. 2021. PCR testing of media placed in soiled bedding as a method for mouse colony health surveillance. J Am Assoc Lab Anim Sci **60**:306–310. https://doi.org/10.30802/AALAS-JAALAS-20-000096.
16. **Henderson KS, Perkins CL, Havens RB, Kelly M-JE, Francis BC, Dole VS, Shek WR**. 2013. Efficacy of direct detection of pathogens in naturally infected mice by using a high-density PCR array. J Am Assoc Lab Anim Sci **52**:763–772.
17. **Jensen ES, Allen KP, Henderson KS, Szabo A, Thulin JD**. 2013. PCR testing of a ventilated caging system to detect murine fur mites. J Am Assoc Lab Anim Sci **52**:28–33.
18. **Kapoor P, Hayes YO, Jarrell LT, Bellinger DA, Thomas RD, Lawson GW, Arkema JD, Fletcher CA, Nielsen JN**. 2017. Evaluation of anthelmintic resistance and exhaust air dust PCR as a diagnostic tool in mice enzootically infected with *Aspiculuris tetraptera*. J Am Assoc Lab Anim Sci **56**:273–289.
19. **Körner C, Miller M, Brielmeier M**. 2019. Detection of murine astrovirus and *Myocoptes musculinus* in individually ventilated caging systems: Investigations to expose suitable detection methods for routine hygienic monitoring. PLoS One **14**:e0221118. https://doi.org/10.1371/journal.pone.0221118.
20. **LaFollette MR, Cloutier S, Brady C, Gaskill BN, O'Haire ME**. 2019. Laboratory animal welfare and human attitudes: A cross-sectional survey on heterospecific play or "rat tickling." PLoS One **14**:e0220580. https://doi.org/10.1371/journal.pone.0220580.
21. **LaFollette MR, Cloutier S, Brady CM, O'Haire ME, Gaskill BN**. 2020. Changing human behavior to improve animal welfare: A longitudinal investigation of training laboratory animal personnel about heterospecific play or "rat tickling." Animals (Basel) **10**:1435. https://doi.org/10.3390/ani10081435.
22. **LaFollette MR, Riley MC, Cloutier S, Brady CM, O'Haire ME, Gaskill BN**. 2020. Laboratory animal welfare meets human welfare: A cross-sectional study of professional quality of life, including compassion fatigue in laboratory animal personnel. Front Vet Sci **7**:114. https://doi.org/10.3389/fvets.2020.00114.
23. **Lindstrom KE, Carbone LG, Kellar DE, Mayorga MS, Wilkerson JD**. 2011. Soiled bedding sentinels for the detection of fur mites in mice. J Am Assoc Lab Anim Sci **50**:54–60.
24. **Luchins KR, Bowers CJ, Mailhiot D, Theriault BR, Langan GP**. 2020. Cost comparison of rodent soiled bedding sentinel and exhaust air dust health-monitoring programs. J Am Assoc Lab Anim Sci **59**:508–511. https://doi.org/10.30802/AALAS-JAALAS-20-000003.
25. **Luchins KR, Mailhiot D, Theriault BR, Langan GP**. 2020. Detection of lactate dehydrogenase elevating virus in a mouse vivarium using an exhaust air dust health monitoring program. J Am Assoc Lab Anim Sci **59**:328–333. https://doi.org/10.30802/AALAS-JAALAS-19-000107.
26. **Mahabir E, Durand S, Henderson KS, Hardy P**. 2019. Comparison of 2 prevalent individually ventilated caging systems for detection of murine infectious agents via exhaust air particles. Lab Anim **53**:84–88. https://doi.org/10.1177/0023677218785929.
27. **Mailhiot D, Ostdiek AM, Luchins KR, Bowers CJ, Theriault BR, Langan GP**. 2020. Comparing mouse health monitoring between soiled-bedding sentinel and exhaust air dust surveillance programs. J Am Assoc Lab Anim Sci **59**:58–66. https://doi.org/10.30802/AALAS-JAALAS-19-000061.
28. **Manuel CA, Pugazhenthi U, Leszczynski JK**. 2016. Surveillance of a ventilated rack system for *Corynebacterium bovis* by sampling exhaust-air manifolds. J Am Assoc Lab Anim Sci **55**:58–65.
29. **Manuel CA, Pugazhenthi U, Spiegel SP, Leszczynski JK**. 2017. Detection and elimination of *Corynebacterium bovis* from barrier rooms by using an environmental sampling surveillance program. J Am Assoc Lab Anim Sci **56**:202–209.
30. **Marx JO, Gaertner DJ, Smith AL**. 2017. Results of survey regarding prevalence of adventitial infections in mice and rats at biomedical research facilities. J Am Assoc Lab Anim Sci **56**:527–533.
31. **Merley AL, Hubbard JS, Rendahl AK, Duke Boynton FD, Collura Impelluso L**. 2022. Behavioral and physiologic effects of dirty bedding exposure in female ICR mice. J Am Assoc Lab Anim Sci **61**:42–51. https://doi.org/10.30802/AALAS-JAALAS-21-000060.
32. **Miles MB, Huberman AM**. 1994. Qualitative data analysis: An expanded sourcebook, 2nd ed. Thousand Oaks (CA): Sage Publications.
33. **Miller M, Brielmeier M**. 2018. Environmental samples make soiled bedding sentinels dispensable for hygienic monitoring of IVC-reared mouse colonies. Lab Anim **52**:233–239. https://doi.org/10.1177/0023677217739329.
34. **Miller M, Ritter B, Zorn J, Brielmeier M**. 2016. Exhaust air dust monitoring is superior to soiled bedding sentinels for the detection of *Pasteurella pneumotropica* in individually ventilated cage systems. J Am Assoc Lab Anim Sci **55**:775–781.
35. **Miller M, Ritter B, Zorn J, Brielmeier M**. 2016. Exhaust air particle PCR detects *Helicobacter hepaticus* infections at low prevalence. J Vet Sci Technol **7**:1000343. https://doi.org/10.4172/2157-7579.1000343.
36. **Newsome JT, Clemmons EA, Fitzhugh DC, Gluckman TL, Creamer-Hente MA, Tambrallo LJ, Wilder-Kofie T**. 2019. compassion fatigue, euthanasia stress, and their management in laboratory animal research. J Am Assoc Lab Anim Sci **58**:289–292. https://doi.org/10.30802/AALAS-JAALAS-18-000092.
37. **O'Connell KA, Tigyi GJ, Livingston RS, Johnson DL, Hamilton DJ**. 2021. Evaluation of in-cage filter paper as a replacement for sentinel mice in the detection of murine pathogens. J Am Assoc Lab Anim Sci **60**:160–167. https://doi.org/10.30802/AALAS-JAALAS-20-000086.
38. **Perdue KA, Copeland MK, Karjala Z, Cheng LI, Ward JM, Elkins WR**. 2008. Suboptimal ability of dirty-bedding sentinels to detect *Spironucleus muris* in a colony of mice with genetic manipulations of the adaptive immune system. J Am Assoc Lab Anim Sci **47**:10–17.
39. **Pettan-Brewer C, Trost RJ, Maggio-Price L, Seamons A, Dowling SC**. 2020. Adoption of exhaust air dust testing in SPF rodent facilities. J Am Assoc Lab Anim Sci **59**:156–162. https://doi.org/10.30802/AALAS-JAALAS-19-000079.
40. **Ragland NH, Miedel EL, Engelman RW**. 2019. PCR prevalence of murine opportunistic microbes and their mitigation by using vaporized hydrogen peroxide. J Am Assoc Lab Anim Sci **58**:208–215. https://doi.org/10.30802/AALAS-JAALAS-18-000112.
41. **Thurston SE, Chan G, Burlingame LA, Jones JA, Lester PA, Martin TL**. 2021. Compassion fatigue in laboratory animal personnel during the COVID-19 pandemic. J Am Assoc Lab Anim Sci **60**:646–654. https://doi.org/10.30802/AALAS-JAALAS-21-000030.
42. **Tierce RK, Winn AA, Albers TM, Poueymirou WT, Levee EM, Woods SE, Reddyjarugu B**. 2022. Detection and transmission of *Proteus mirabilis* in immunodeficient mice. J Am Assoc Lab Anim Sci **61**:256–269. https://doi.org/10.30802/AALAS-JAALAS-21-000104.
43. **Varela MMD, Bibay JIA, Ogden BE, Crim MJ, Htoon HM**. 2022. Using sterile flocked swabs as an alternative method for rodent health monitoring. J Am Assoc Lab Anim Sci **61**:370–380. https://doi.org/10.30802/AALAS-JAALAS-22-000024.
44. **Winn CB, Rogers RN, Keenan RA, Gerwin PM, Matthews KA, Ramirez JA, Bennet TE, Perkins CL, Henderson KS**. 2022. Using filter media and soiled bedding in disposable individually ventilated cages as a refinement to specific pathogen-free mouse health monitoring programs. J Am Assoc Lab Anim Sci **61**:361–369. https://doi.org/10.30802/AALAS-JAALAS-22-000013.
45. **Zorn J, Ritter B, Miller M, Kraus M, Northrup E, Brielmeier M**. 2017. Murine norovirus detection in the exhaust air of IVCs is more sensitive than serological analysis of soiled bedding sentinels. Lab Anim **51**:301–310. https://doi.org/10.1177/0023677216661586.