

Environmental Health Monitoring: A Holistic Approach for All Housing Types



Megan R. LaFollette | The 3Rs Collaborative

AALAS 2023-10-25
12:30-2pm

Today's Schedule

- Introduction, Systematic Review, & Survey Updates – Megan LaFollette
- Program Overviews & Research from Brianna Hible, Wendy Williams, and Marnie Metzler
- 3RsC Resources by Caroline Winn

Time for 1-2 questions after each presentation

Additional time for questions at the end

Traditionally, soiled bedding sentinel rodents are used to ensure colony health status.

The background of the image is a blue-toned ECG (heart rate) monitor display. It features a grid pattern and several black lines representing the heart's electrical activity. The lines are slightly blurred, giving a sense of motion or a focus on the text in the foreground.

**With the advent of PCR
technology, we now can
replace sentinels.**

**But just because a replacement exists,
doesn't mean that change is easy or immediate.**



The 3Rs Collaborative was created by professionals who understand the challenge of 3Rs change firsthand.



**The 3RsC's mission is to advance
better science – for both people & animals**



**Refine.
Reduce.
Replace.**

The 3RsC's Strategy has 3 Goals:



1. Promote the 3Rs Broadly by creating a research landscape that is knowledgeable & supportive of the 3Rs



2. Promote Specific 3Rs Strategies by advancing implementation of high-impact, evidence-based, & practical 3Rs techniques.



3. Promote Our Organization by building collaborations & awareness of resources and programs from The 3Rs Collaborative.

Core Terminology & Types

- **Soiled Bedding Sentinels (SBS)** = traditional rodent health monitoring that involves transferring soiled bedding to a cage with live rodents which are periodically sampled/euthanized to determine colony health status. (Sometimes referred to as “sentinels”)
- **Environmental Health Monitoring (EHM)** = any type of health monitoring that does not require use of live animal sentinels

There are two major types of EHM:

- **Exhaust Dust Testing (EDT)** = EHM via swabbing plenums or using in-line media for cages that filter at the rack level.
 - This is vendor-independent terminology, but you may have heard this called, Exhaust Air Dust (EAD®), Environmental Diagnostics (Edx), EnviroRax, Sentinel™ EAD® or Sentinel2™, or Interceptor EAD®.
- **Sentinel-Free Soiled Bedding (SFSB)** = EHM via transferring soiled bedding into a cage or bin and exposing media or swabs before sending to the laboratory.
 - This is vendor-independent terminology, but you may have heard this called, Shake & Bake, PathogenBinder™, REPLACE, or Sentinel Swab.

Supplemental EHM can include:

- **Direct Colony Sampling (DCS)** = EHM via testing the research colony directly such as via feces, cage swabs, etc.
 - This is a common supplemental strategy
- **Room & Equipment Monitoring (REM)** = EHM via testing bedding dump stations, IVC rack pre-filters, cage change stations, BSC, floors, etc.
 - This is uncommon as a main strategy

**Taking a step back,
what **evidence** is there
for soiled bedding
sentinels?**

In 2016, there was a systematic review that evaluated the efficacy of soiled bedding sentinels.



RESEARCH ARTICLE

Efficacy of Soiled Bedding Transfer for Transmission of Mouse and Rat Infections to Sentinels: A Systematic Review

W. C. C. de Bruin^{1*}, E. M. E. van de Ven¹, C. R. Hooijmans²

1 QM Diagnostics BV, Nijmegen, The Netherlands, **2** Radboud University Medical Centre, SYRCLE at Central Animal Laboratory, Nijmegen, The Netherlands


* Wieke.debruin@qmdiagnosics.org

As of 2016, **only 15** articles,
conference presentations, or
posters had been published on the
efficacy of soiled bedding
sentinels.

Sufficient data to conclude soiled bedding sentinels are effective for **ONLY 5 pathogens.**

MHV, MPV, TMEV
Helicobacter spp.
Fur Mites*

Sufficient data to conclude that soiled bedding sentinels are **INEFFECTIVE** for Sendai Virus

A photograph of a snow-capped mountain peak against a clear blue sky. The mountain is covered in snow and has a sharp, jagged peak. The sky is a clear, light blue. The text is overlaid on the right side of the image.

**Try not to hold
new 3Rs
changes to
higher standards
than the
status quo**

Use equal/equivalent standards for the 3Rs.



Our goal was to establish **the evidence base** behind environmental health monitoring.



**Our review has high rigor & objectivity,
avoiding bias and cherry-picking.**

PRISMA Guidelines

**Preferred Reporting Items for
Systematic Reviews and Meta-Analyses**

SYRCLE Guidelines

**Systematic Review Center for
Laboratory Animal Experimentation**

We searched 3 databases to identify articles



*Conference abstracts were NOT included

- Peer-reviewed
- In English
- Rats or Mice
- Biological Monitoring OR Environmental/Health/Hygenic/Microbiological/Routine Sampling/Monitoring/Surveillance OR Exhaust Air/dust/debris

20% of extraction was replicated by a second reviewer

For each article we coded:

- **Sampling type: SBS, EDT, SFSB, etc.**
- **Pathogen Evaluated:**
 - **Type (Bacterial or Fungal = Parasites = Virus)**
 - **Importance (3Month/Annual Panel = Supplemental Panels)**
- **Did the sampling type detect the pathogen? (Yes/No)**

This allows us to use statistical models to determine the detection rate of each sampling method.

Data were analyzed via descriptive statistics & mixed linear logistic regression.

Dependent Variable

Detection (Yes/No)

Independent Variables

Sampling Type

Pathogen Type

Sampling Type*Pathogen

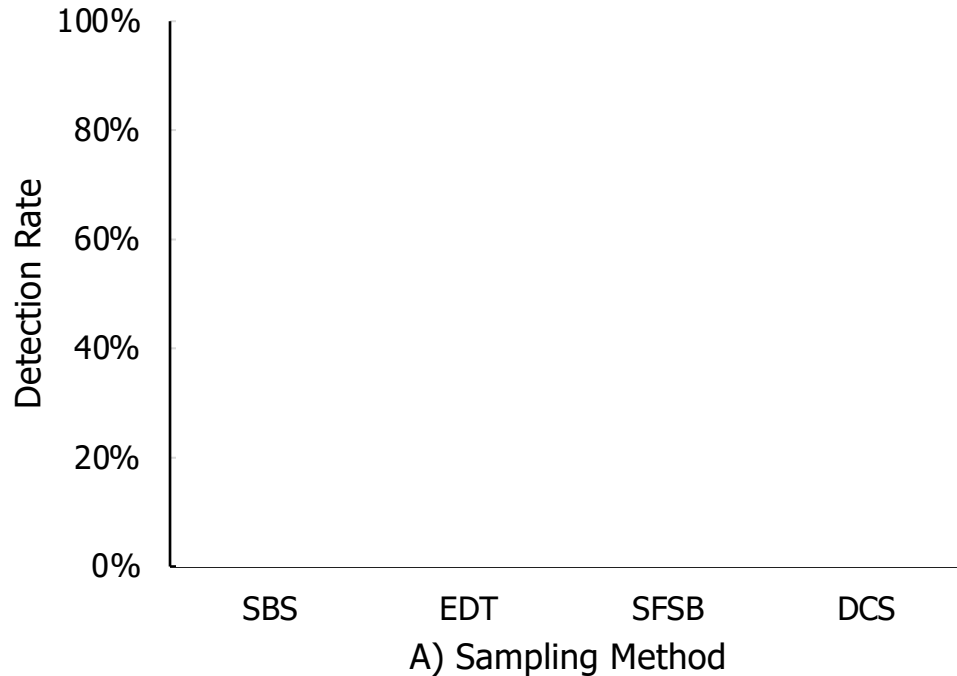
Thank you to

**- Caroline Starla Clement for
data collection**

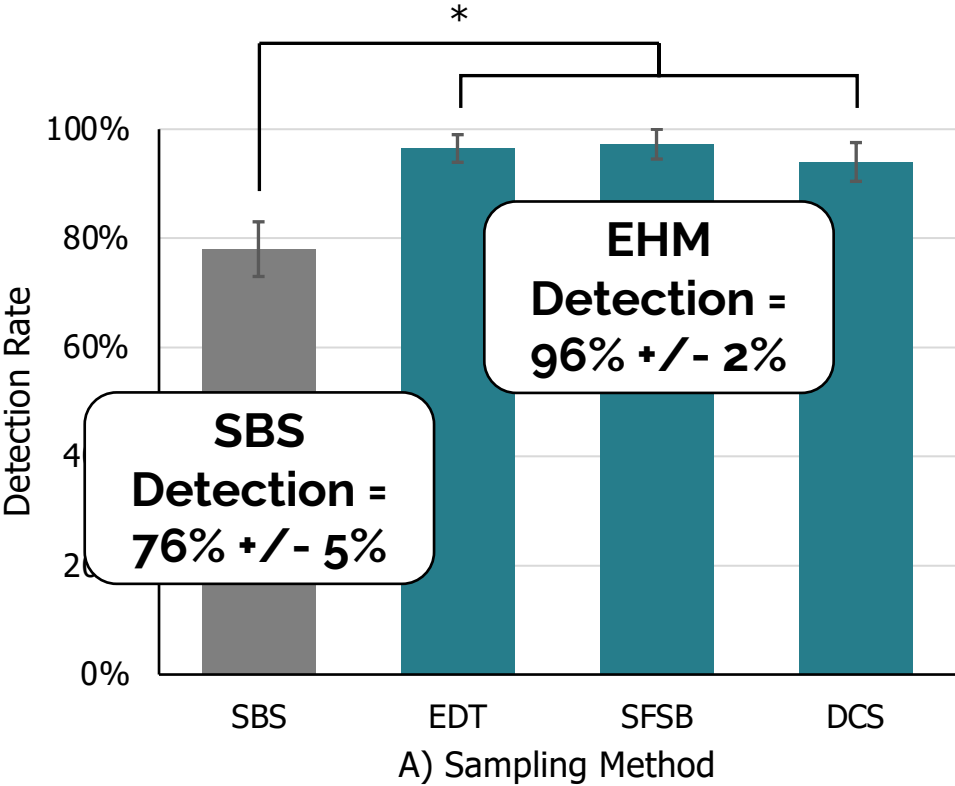
**- Joe Garner for statistics &
supervision**

**EHM is superior to SBS
based on
logistic regression**

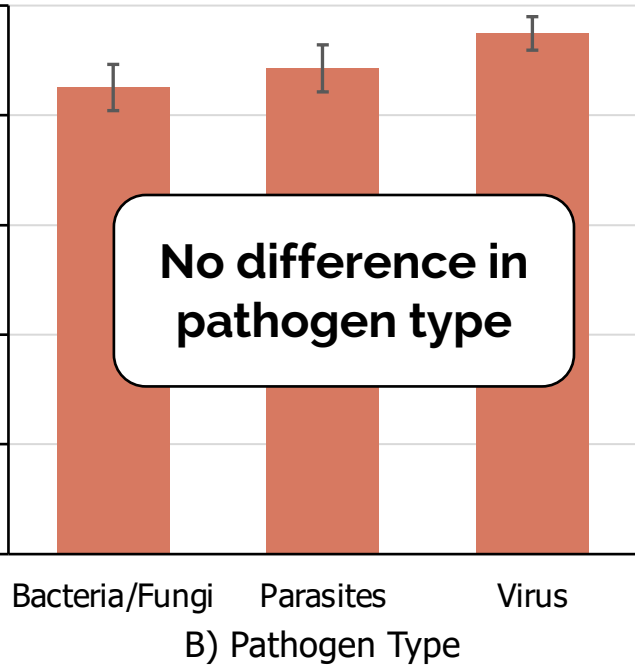
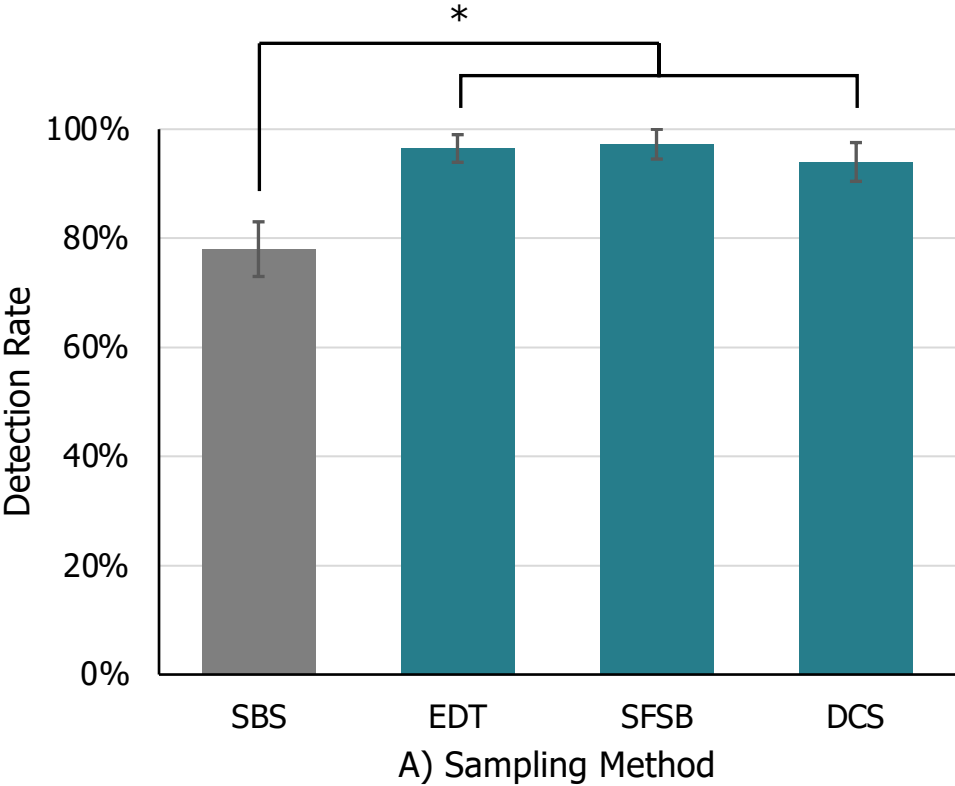
Results: Environmental health monitoring detects pathogens more often than soiled bedding sentinels, regardless of sampling method or pathogen type or importance.



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**EHM is superior to SBS
based on
descriptive statistics**

For viral agents, EHM has some superior detection.

Viral Agents	SBS	EDT	SFSB	DCS	EHM Detected Where SBS Failed?
LDEV		1/1			
LCMV		1/1			
Murine Hepatitis Virus	2/2	2/2	1/1	1/1	
Murine Norovirus	8/8	5/6	4/4	4/4	
Murine Parvoviruses	4/4	3/3	2/2	2/2	
Murine Astrovirus	3/3	2/2	1/1	2/2	
Murine Rotavirus	0/1	0/1			
Sendai Virus	0/1	1/1			Yes (1)
TMEV	1/1		1/1		

articles pathogen detected/# articles pathogen evaluated

For bacteria, EHM has clear superior detection.

Bacterial Agents	SBS	EDT	SFSB	DCS	EHM > SBS
Chlamydia muridarum	1/1			1/1	
Citrobacter rodentium	1/1			1/1	
Corynebacterium bovis		2/2			
Helicobacter spp.	7/8	8/8	4/4	6/6	Yes (1)
Klebsiella spp.	2/2	2/3	1/1	2/3	
Mycoplasma spp.	1/1		1/1	1/1	
Proteus mirabilis	2/4	4/4	2/2	2/3	Yes (2)
Pseudomonas aeruginosa	1/1	1/2		1/1	
Rodentibacter spp.	5/8	9/9	2/2	7/7	Yes (3)
Staphylococcus spp.	5/6	5/6	2/2	6/6	Yes (1)
Streptococcus spp.	2/2	0/1	0/1	2/3	

articles pathogen detected/# articles pathogen evaluated

For fungi & parasites, EHM has clear superior detection.

Fungal Agents	SBS	EDT	SFSB	DCS	EHM > SBS
Pneumocystis spp.	0/2	2/2			Yes (2)
Parasites	SBS	EDT	SFSB	DCS	EHM > SBS
Ectoparasites (Myobia musculi, Mycoptes musculus, Radfordia affinis)					Yes (4)
Entamoeba spp.	4/5	5/5	2/2	6/6	Yes (1)
Pinworms (Aspicularis spp. Syphacia obvelata)	4/5	4/4	2/2	4/4	Yes (1)
Spirotrichomonas spp.	2/3	1/1	2/2	3/3	Yes (1)
Tritrichomonas spp.	3/4	4/4	1/1	4/4	Yes (1)

articles pathogen detected/# articles pathogen evaluated

**In 10 different articles, Exhaust
Dust Testing or Sentinel-Free
Soiled Bedding detected
11 different pathogens,
where SBS failed.**

Murine Rotavirus, Sendai Virus, Helicobacter spp., Proteus mirabilis,
Rodentibacter spp., Staphylococcus spp., Pneumocystis spp.,
Ectoparasites, Entamoeba, Pinworms, Spironucleus spp.,
Tritichomonas spp.

Environmental Health Monitoring helps replace rodents & save costs

- **Across articles, 6876 rodents from 4 institutions are being replaced annually with environmental health monitoring.**
- **9 Articles mentioned that cost-savings were identified from switching to environmental health monitoring**

Limitations & Cautions from the Current Research

- **Environmental Health Monitoring is so effective at detection that false positives are possible.**
 - Any unexpected positives should be discussed with the diagnostic lab
 - Proper cage washing is important to remove residual nucleic acid (see our resource page)
- **More data would be beneficial for some pathogens, although consider prevalence & exclusion lists**
- **EHM may not be perfect, but it is better than sentinels**

Benchmarking EHM: How common is environmental health monitoring (EHM)?



Baseline results from our year 1 survey are published in JAALAS.

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Month 2023
Pages 1-10

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

Kerith R Luchins,^{1,4*} Kate V Gates,^{2,4} Caroline B Winn,³ Christopher A Manuel,⁴ Christina Pettan-Brewer,⁵ Patricia L Foley,⁶ Norman C Peterson,⁷ Joseph P Garner,⁸ Wai Hanson,⁹ and Megan R LaFollette¹⁰

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

Data were analyzed via descriptive statistics, mixed linear models, & thematic analysis.



Aleeza Stephens

*****Caution: Today's analysis is preliminary with only basic statistics**

Although >120 participants responded each year, **only 29** Participants replied in Year 1, 2, & 3.

Country

79% USA

21% Other

Institution

82% Academic

18% Other

(Industry, CRO,
Government, etc.)

Roles of Primary Response

63% Vets

13% Managers

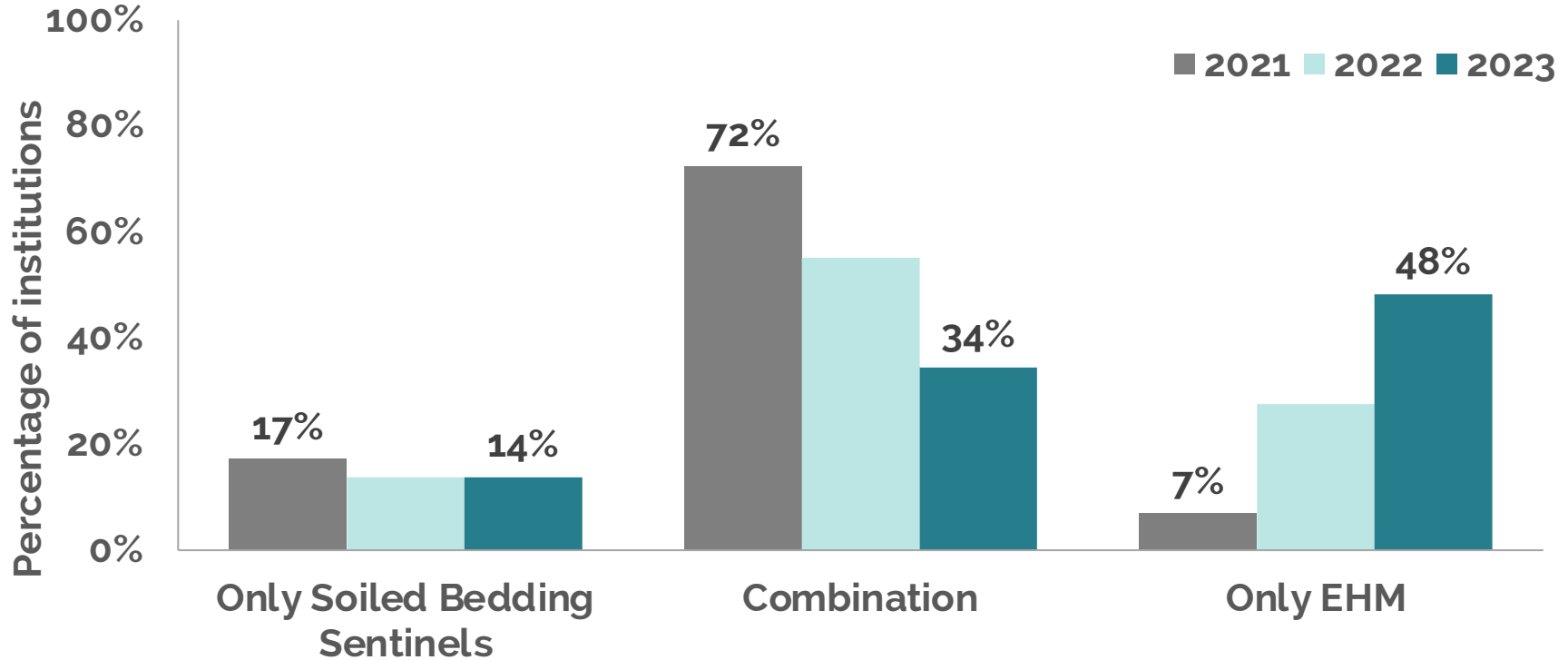
**24% Other (techs,
researchers, etc.)**

What method does your institution use for routine colony health surveillance?

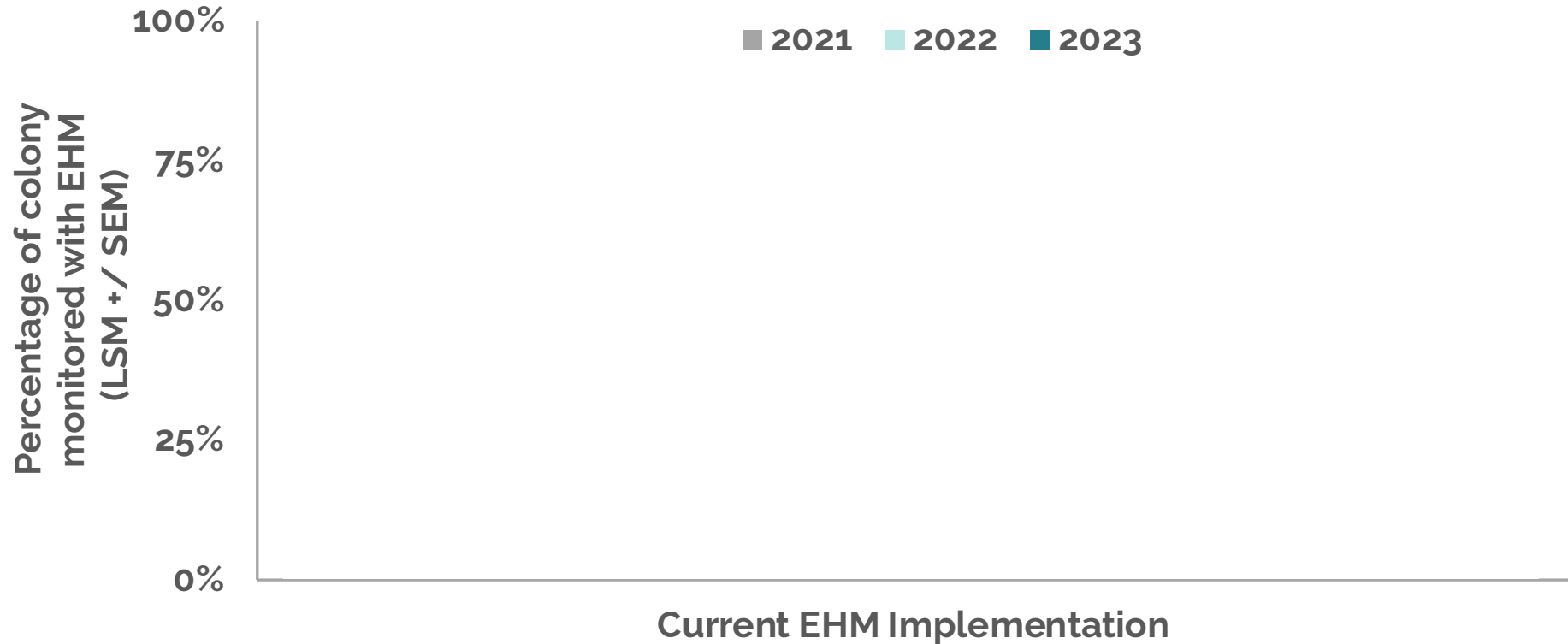


(n = 29)

Takeaway: Environmental health monitoring implementation is **increasing over time.**

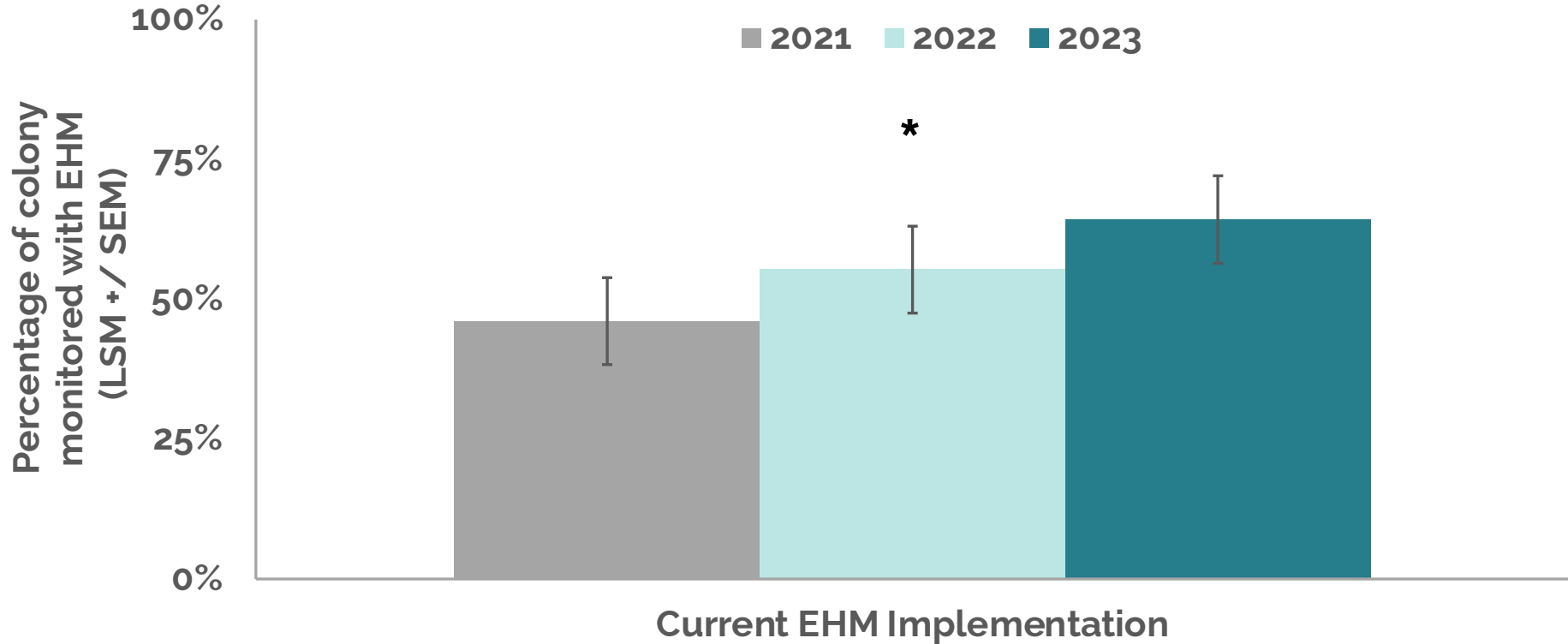


What % of your institution's routine colony health monitoring of mice & rats relies on primarily environmental health monitoring?



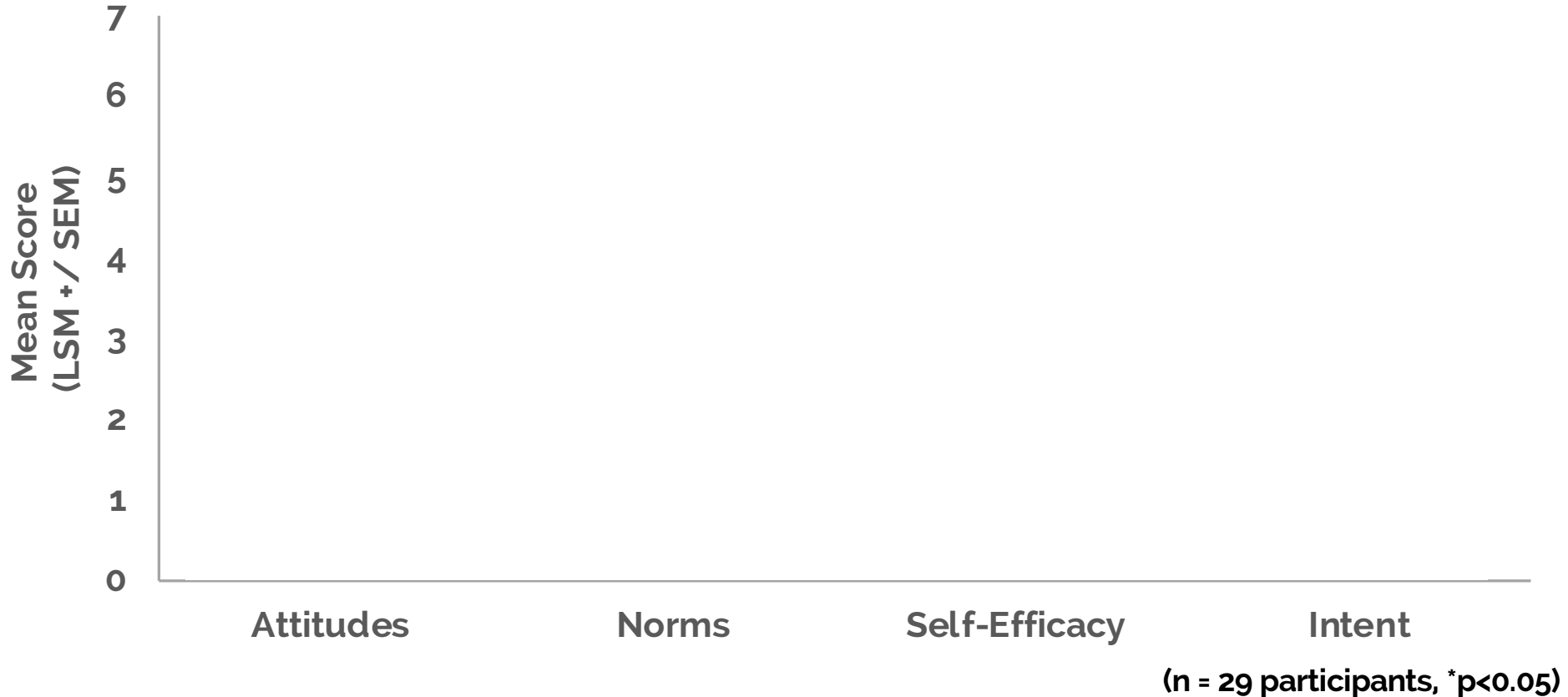
(n = 29 participants, *p<0.05)

Takeaway: Environmental health monitoring implementation is **increasing over time.**

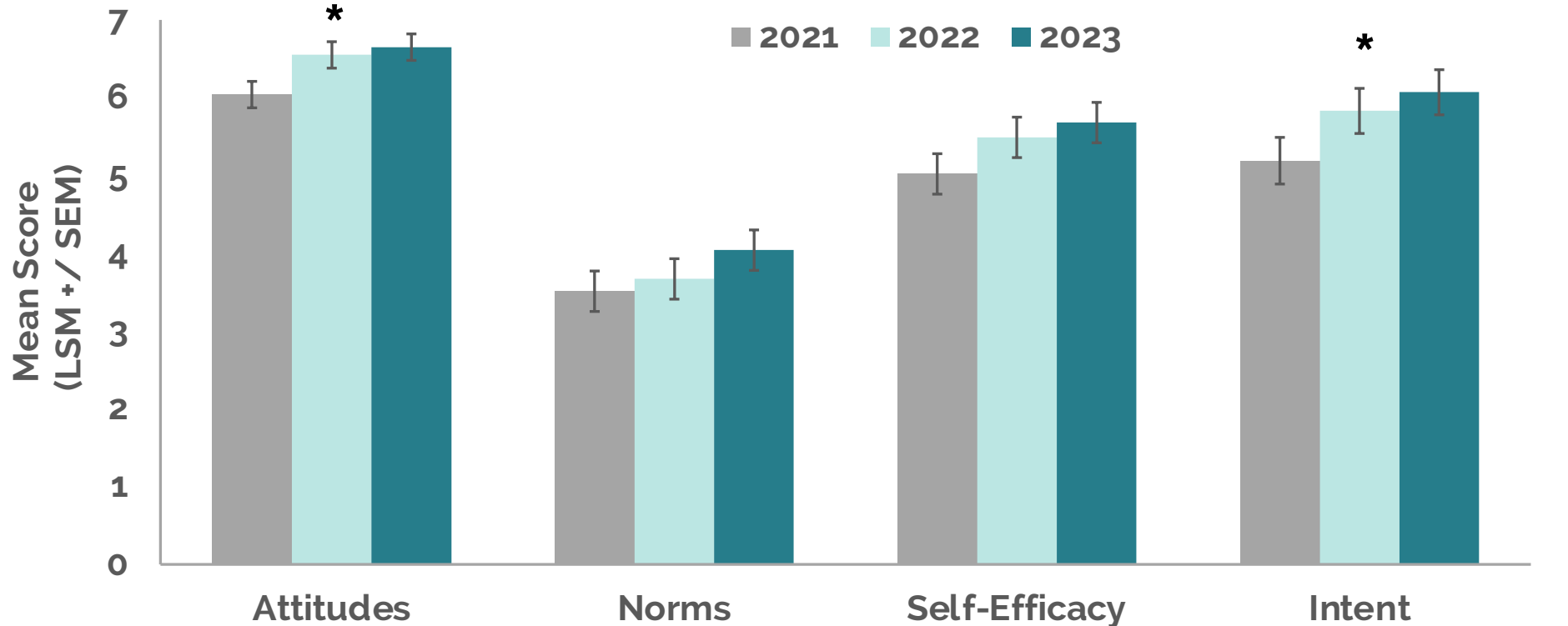


(n = 29 participants, *p<0.05)

How positive or negative are your attitudes, norms, self-efficacy, and intent towards EHM?

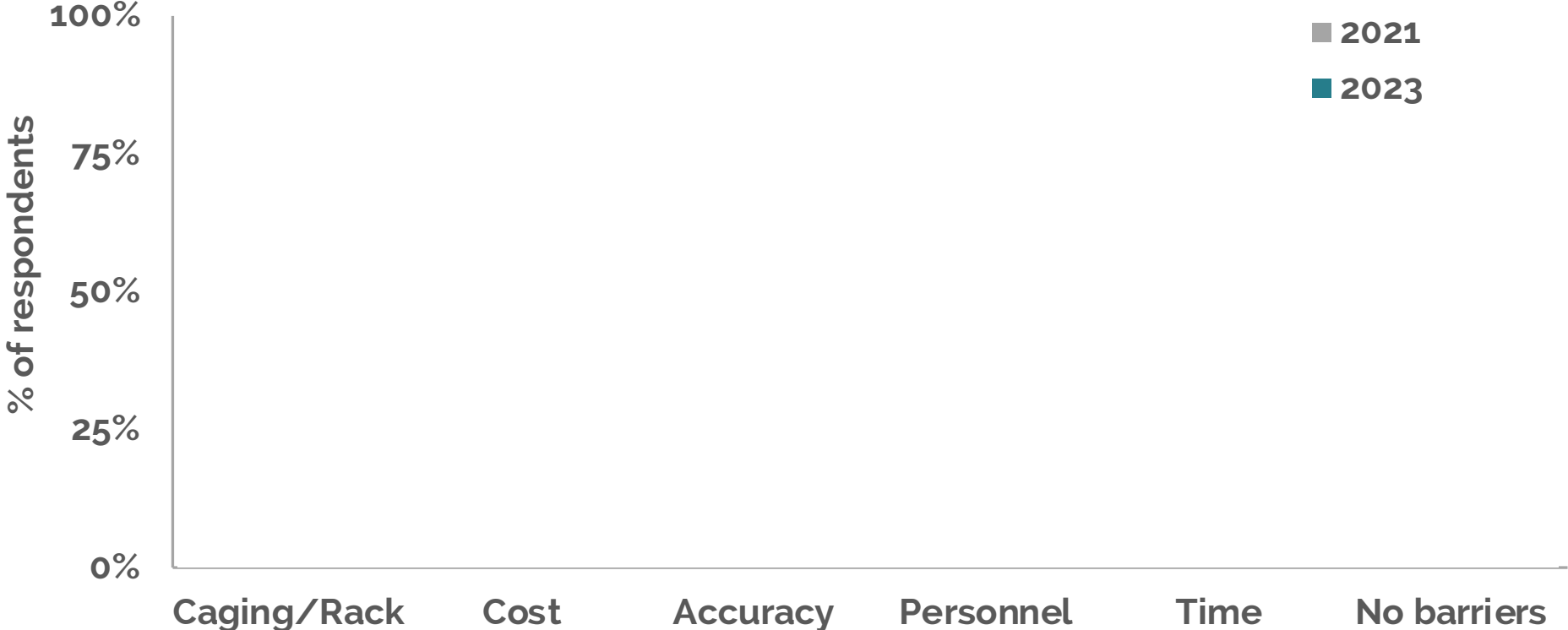


Takeaway: Attitudes & intent have increased in a statistically significant manner. (norms/efficacy numerically)



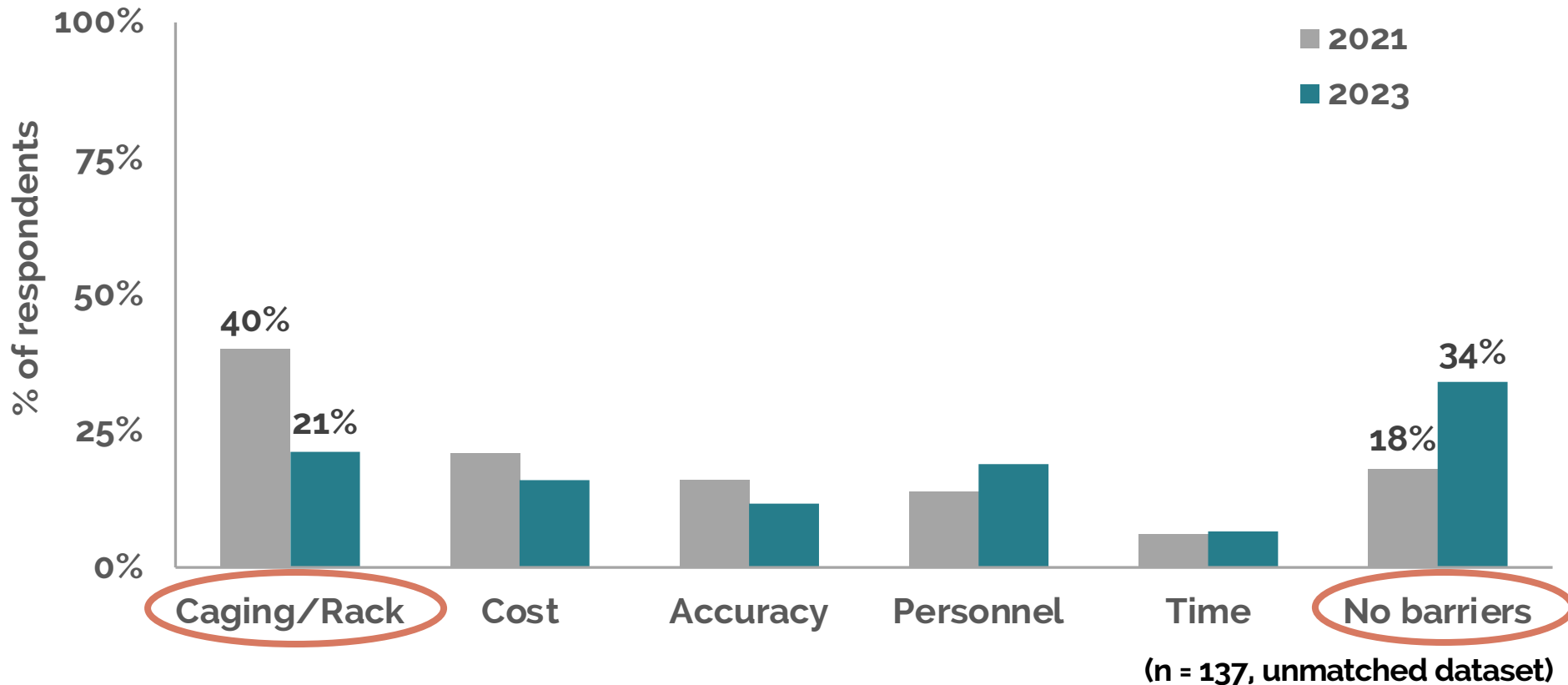
(n = 29 participants, *p<0.05)

What makes it difficult or impossible for your institution to use EHM?



(n = 137, unmatched dataset)

Takeaway: Barriers to EHM are decreasing especially “caging/rack”.



Acknowledgments

- **Aurore Dodelet-Devillers, McGill U.**
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- **Ken Henderson, Charles River Laboratories**
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- **Massimo Foa, IDEXX**
- **Megan LaFollette, 3RsC**
- **Norman Peterson, Seagen**
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- **Patricia Foley, Georgetown U.**
- **Robert (Bob) Livingston, IDEXX**
- **Theresa Faughnan, Long Island U.**
- **Wai Hanson, Emory U.**

Thank you to our current members

charles river

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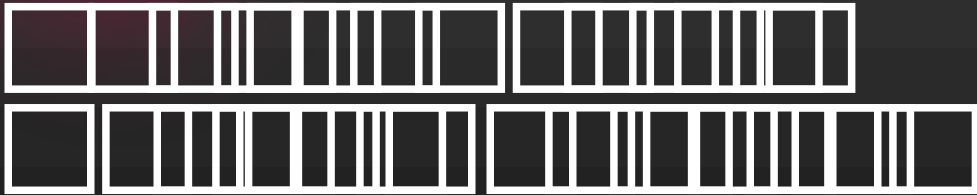
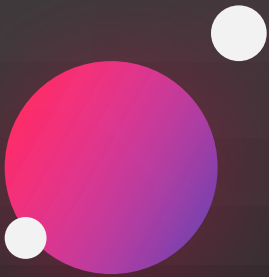
https://bit.ly/3RC_Newsletter

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A

B

C

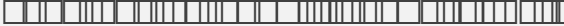
















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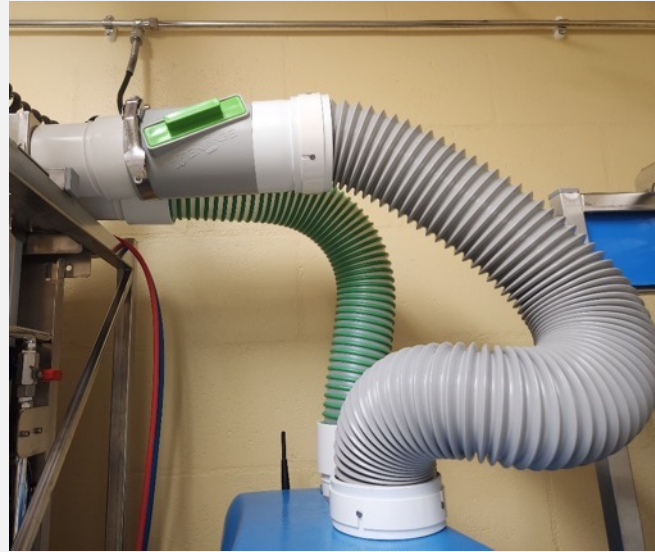
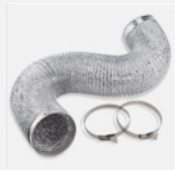
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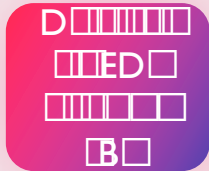
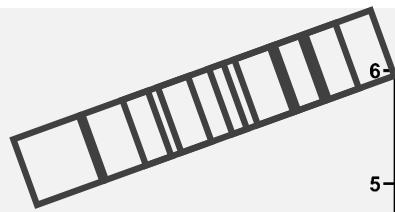
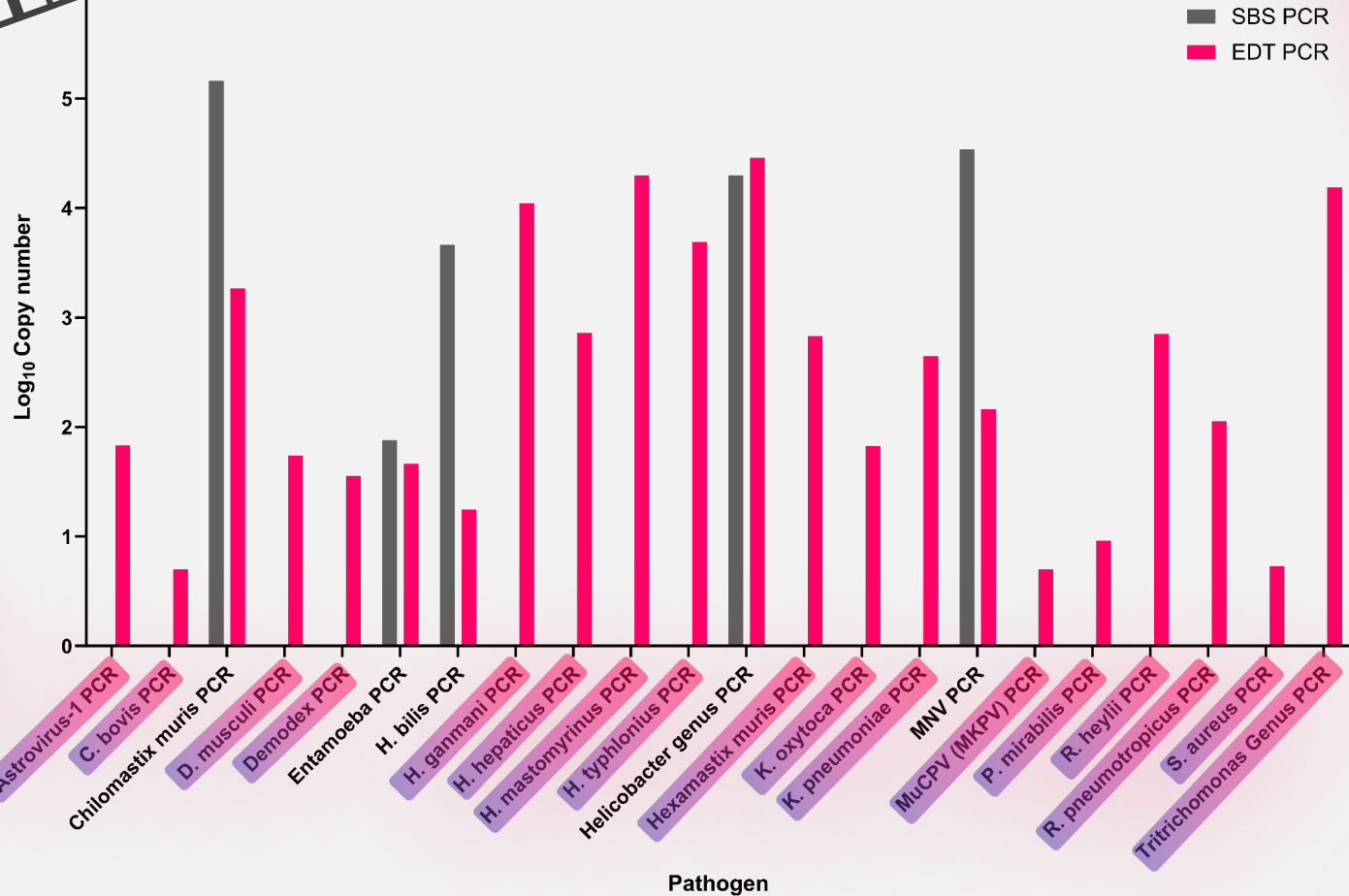
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- F     



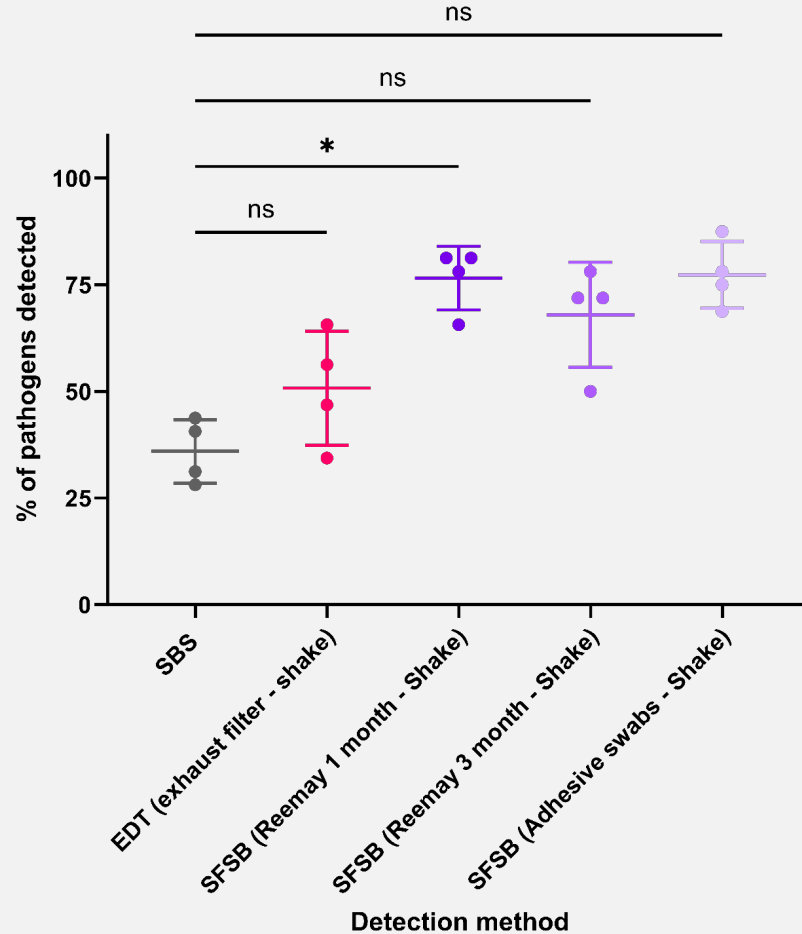
EDT detected 17 more pathogens than SBS



EDT and SFSB detected more pathogens than SBS



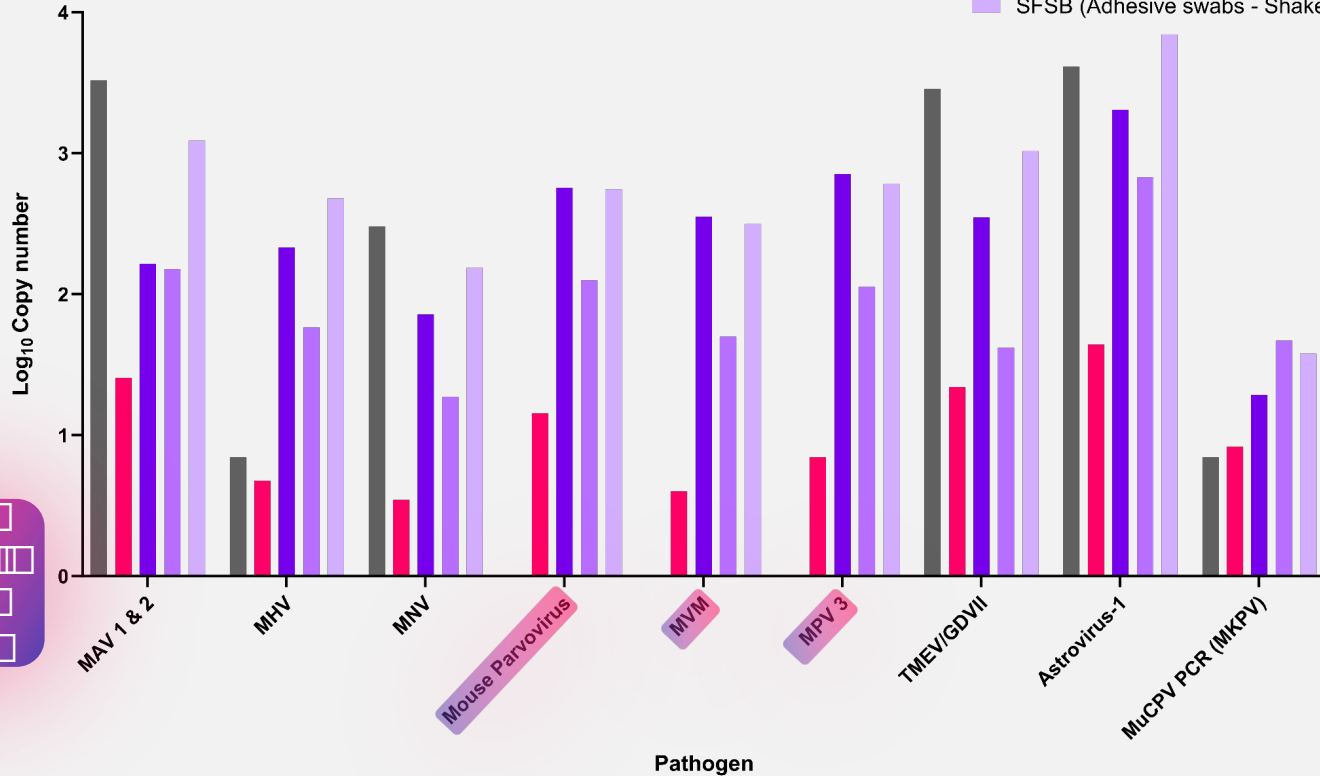
- $\bar{x} = 0.075$
 - $\bar{x} = 0.056$
- ED**
 - D
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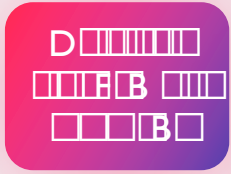
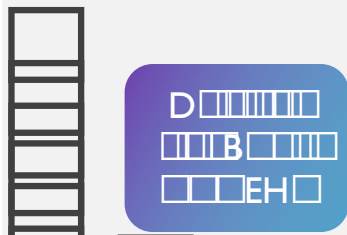
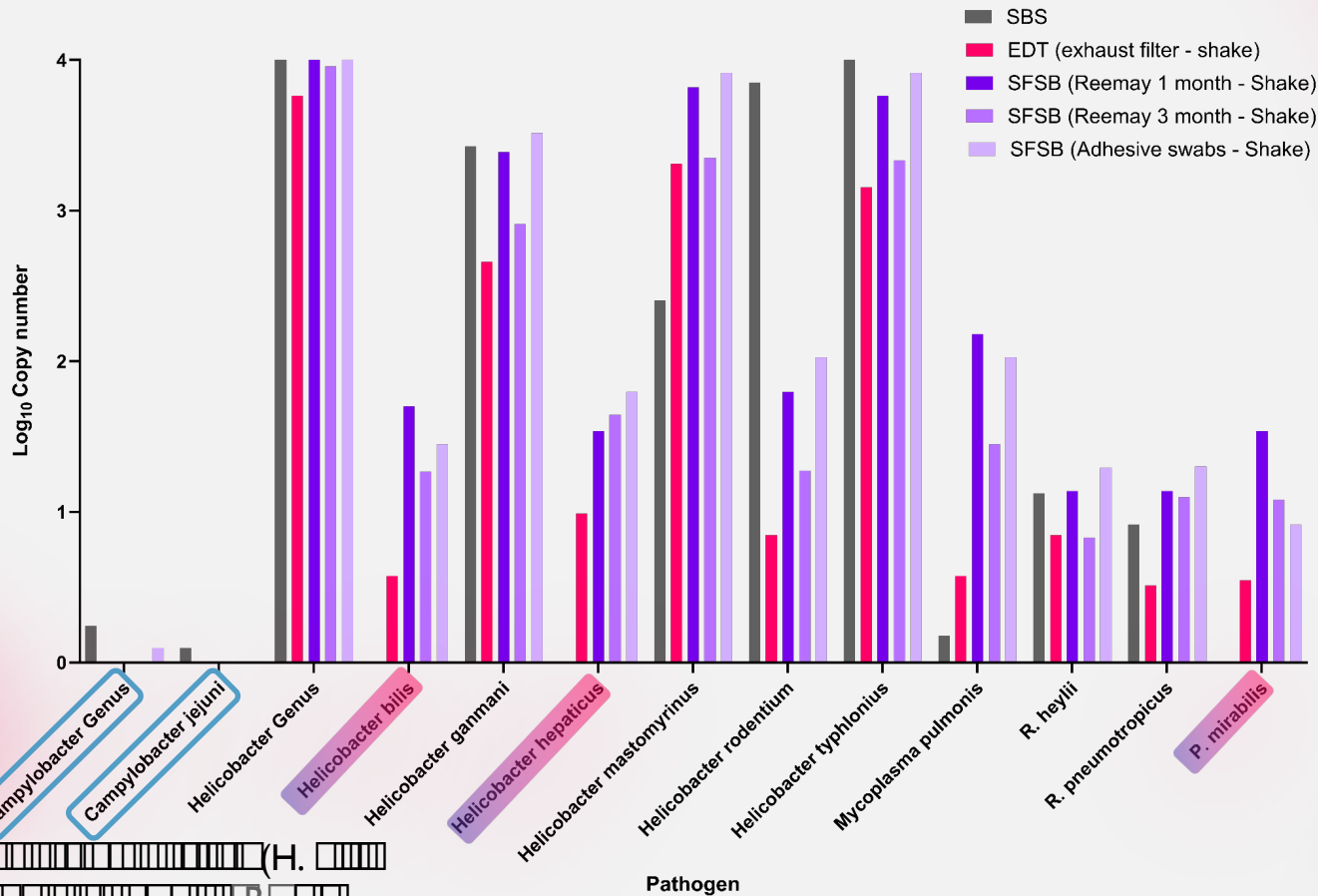
EDT and SFSB detected more viruses than SBS

- 

- SBS
- EDT (exhaust filter - shake)
- SFSB (Reemay 1 month - Shake)
- SFSB (Reemay 3 month - Shake)
- SFSB (Adhesive swabs - Shake)

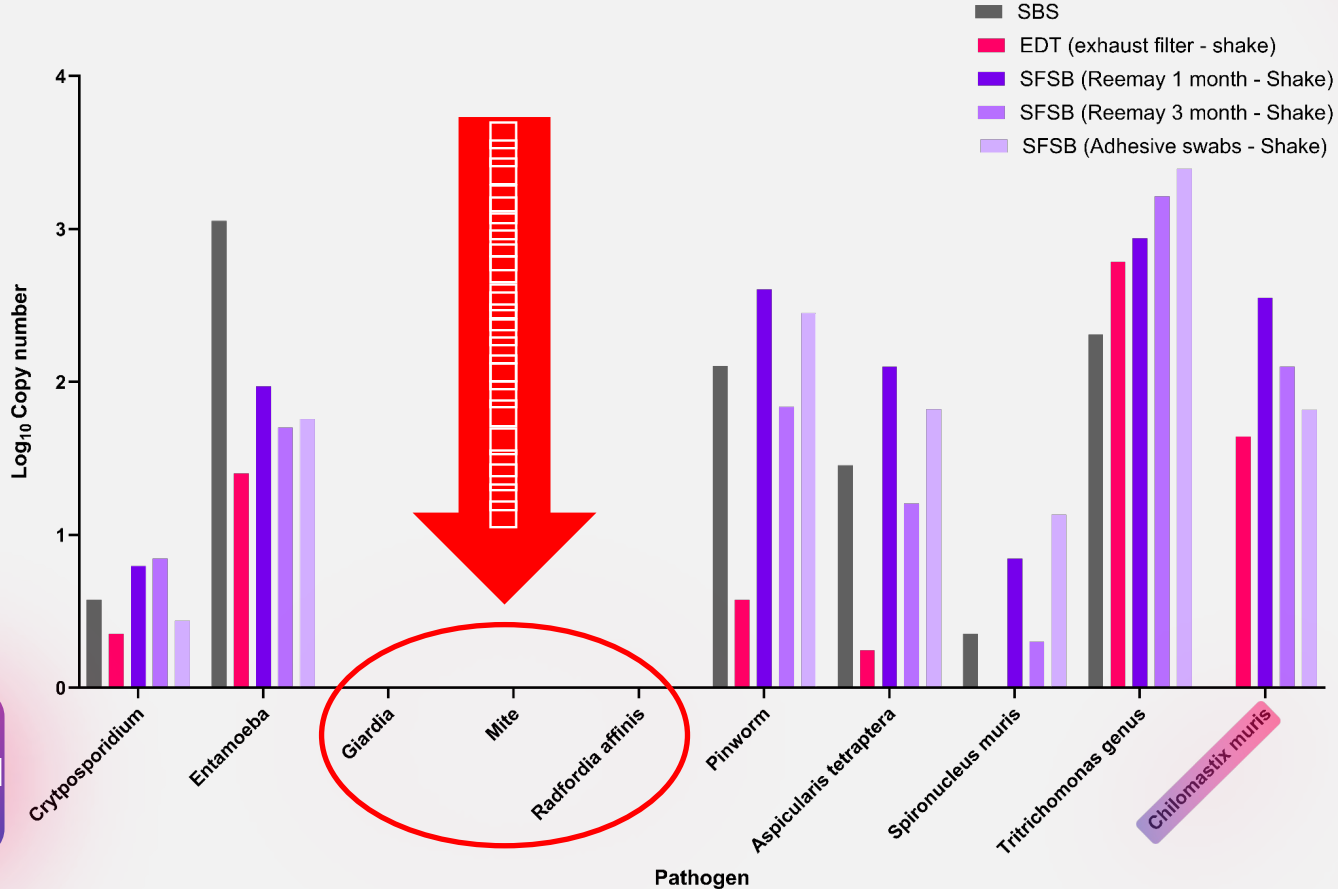


EDT and SFSB detected 3 more bacteria than SBS; SBS detected Campylobacter where EDT and SFSB did not



• **AB** [grid of 15 cells] (H. [grid of 15 cells])
 [grid of 15 cells] H. [grid of 15 cells] [grid of 15 cells] B [grid of 15 cells]

**EDT and SFSB detected 1 more parasite than SBS;
Moxidectin treatment prevented detection of 3 parasites**



D 
 A B 
 B 



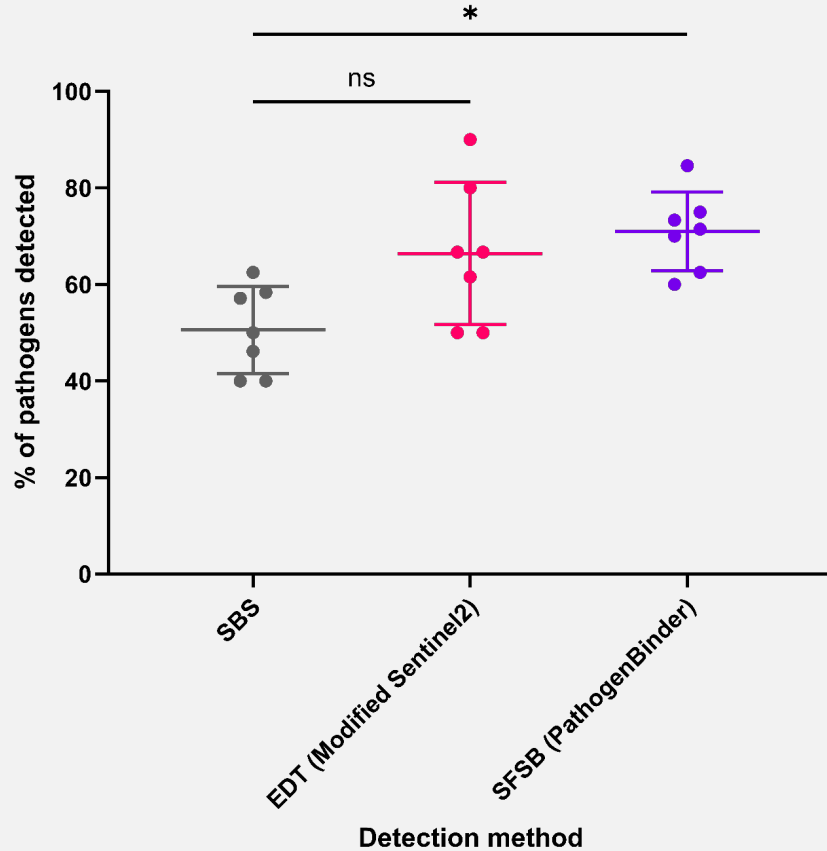
B **ED** **B**



SFSB detected more pathogens than SBS

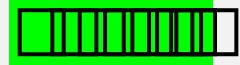
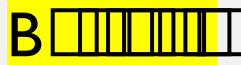
EDT detected pathogens as efficiently as SBS

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







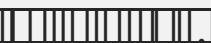
Good, but not perfect:



- B B (1/5)
- C (4/4)
- C (2/6)
- E (6/6)
- H (7/7)
 - H. (5/5)
 - H. (5/5)
- H (2/3)
- (3/6)
- (5/7)
- (4/5)
- 2 (2) (5/5)
- (1/3)

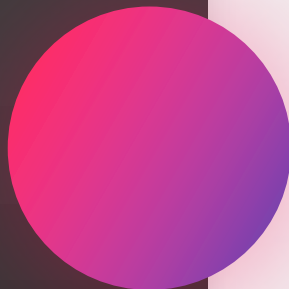
- B B (2/5)
- C (3/4)
- C (6/6)
- E (6/6)
- H (7/7)
 - H. (3/5)
 - H. (5/5)
- (1/3)
- (3/6)
- (6/7)
- (5/5)
- 2 (2) (5/5)
- (3/3)



- C   
- 
 - 100% 
 - A  ED 
 -  C  BB
- B  ED  BB  B   
- D  



- D [diagram], DAC [diagram]; [diagram]
- [diagram], D [diagram]; B [diagram] C [diagram] H [diagram]
- [diagram] A [diagram], D [diagram]; [diagram]
- [diagram] A A; [diagram]
- [diagram] F [diagram] [diagram] A G; [diagram]
- [diagram] H [diagram]; C [diagram]
- C [diagram] [diagram]; C [diagram]
- 3 [diagram]



B [redacted] H [redacted] D [redacted], DAC [redacted] A [redacted]

[redacted]@ [redacted]

[redacted] (901) 448-2762

C [redacted] (901) 295-9118

Environmental Health Monitoring: Mixed Methods & Mixed Results

Wendy R. Williams, DVM, MS, DAACLAM
Director and Attending Veterinarian
Division of Comparative Medicine
Associate Professor of Research
Department of Pathology





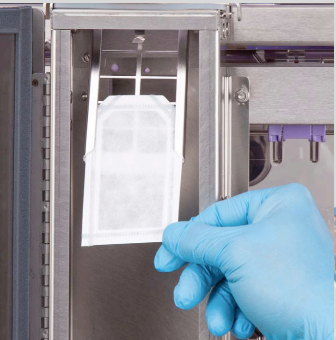
Data supporting
environmental health
monitoring encouraged us
to *slowly* transition from
soiled bedding sentinels.

Our Process

- **Tandem SFSB + EDT + SBS** for 2 years
 - Disposable and reusable cages of several types
 - Shake & Bake and Sentinel™ EAD® and Sentinel2™
- Study comparing **SFSB vs. EDT vs. SBS** in **disposable** cages
- Study evaluating **prevalence and timing** of positive bedding on positive results with **SFSB** and **Helicobacter** in disposable cages

SFSB vs. EDT vs. SBS

- Disposable caging on IVC racks
 - Sentinel2™
 - Shake & Bake
 - SBS
- Samples collected after **3 months**
 - **EDT and SFSB:** PCR (n = 25)
 - **SBS:** Serology + PCR (n = 42)



Results: Both methods of environmental health monitoring detect more agents more frequently than soiled bedding sentinels in disposable caging.

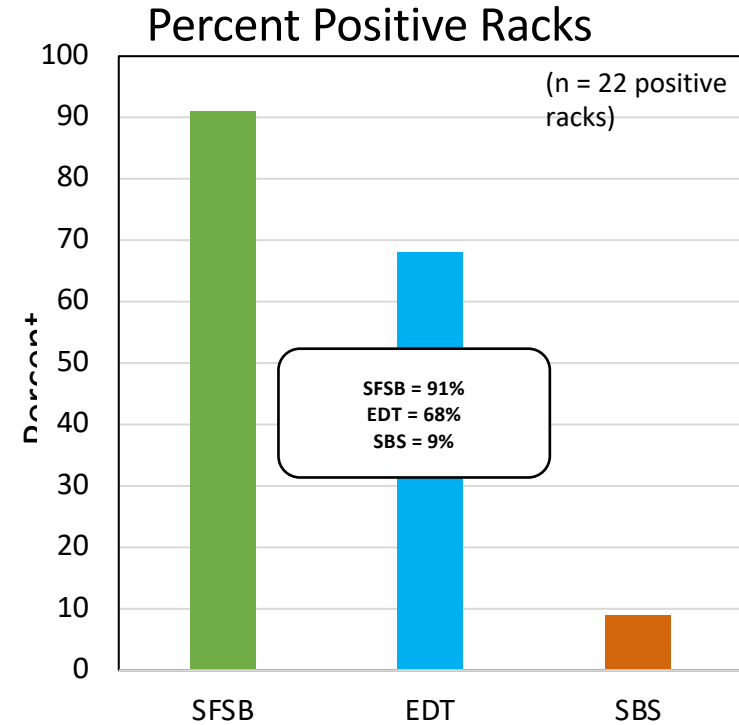
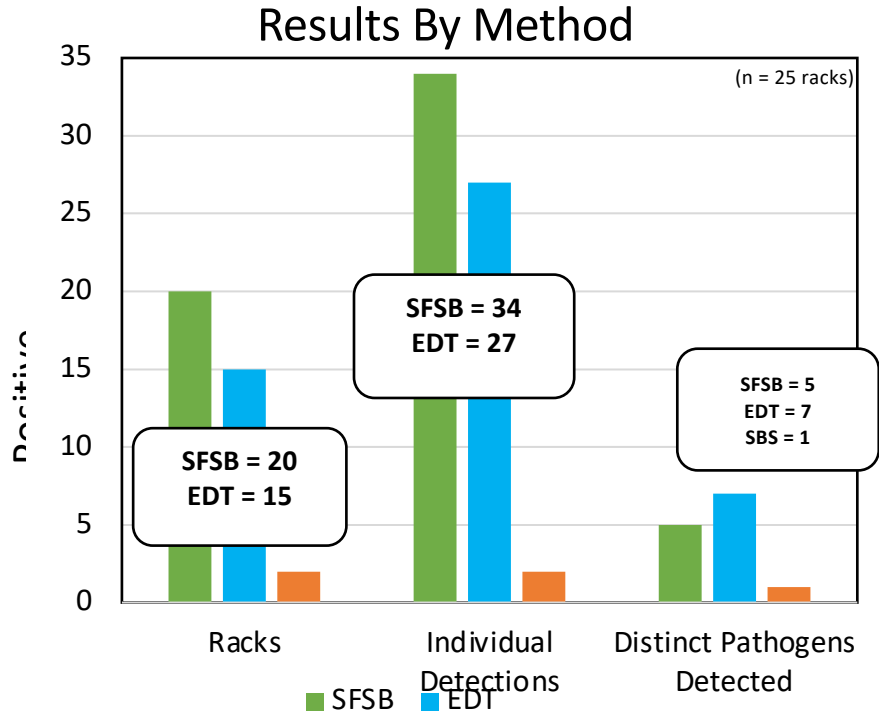
Racks positive for at least one agent by agent and method, and percent positive by agent and method (SFSB, EDT, SBS1 or SBS2).

	Positive Racks By Method			# Positive Detections by Agent			# of Positive Racks	% Positive Racks by Agent†		
	SFSB	EDT	SBS	SFSB	EDT	SBS		SFSB	EDT	SBS
Proteus spp.	+	+	-	9	4	0	16	56	25	0
Rodentibacter spp.	+	+	-	10	12	0	13	77	92	0
Astrovirus	+	+	+	7	1	2	8	88	13	25
Tritrichomonas	-	+	-	0	5	0	5	0	100	0
Entameoba	+	+	-	7	2	0	8	88	25	0
Klebsiella spp.	+	-	-	1	0	0	1	100	0	0
Pseudomonas spp.	-	+	-	0	1	0	1	0	100	0
Staphylococcus spp.	-	+	-	0	1	0	1	0	100	0
MNV	-	+	-	0	1	0	1	0	100	0
TOTAL	20	15	2	34	27	2	22	91	68	9

* + = detected, - = not detected; SBS1 or SBS2: double-sided racks required one sentinel cage per side, if one sentinel was positive, the SBS rack score was +.

† Number of detections divided by positive racks with at least one detection.

Results: Both methods of environmental health monitoring detect more agents more frequently than soiled bedding sentinels in disposable caging.

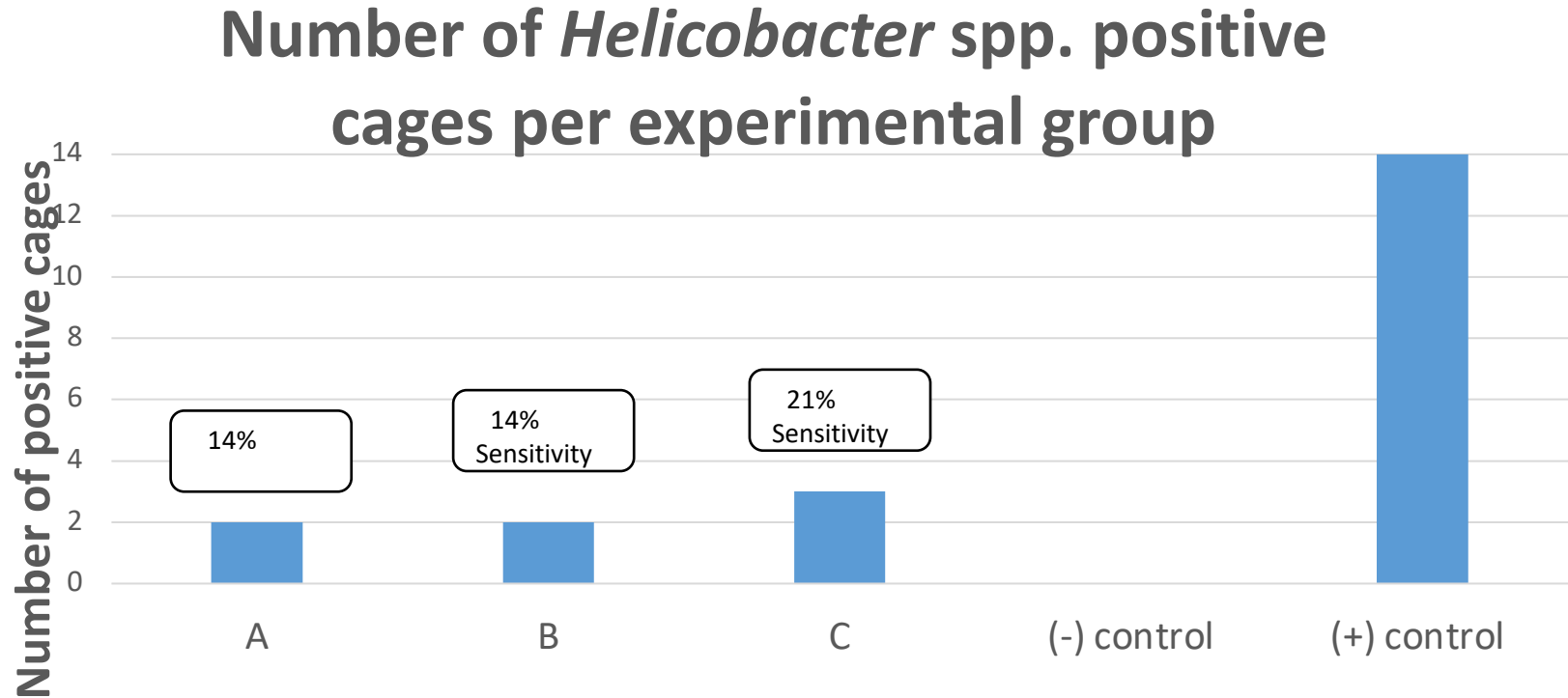


Sensitivity of PCR on **SFSB** for detection of *Helicobacter* species

- Shake & Bake
- Assumption: **10% prevalence** on a known positive rack
- 5 experimental groups, n = 14 cages per group
 - A: positive bedding added at wk 3
 - B: positive bedding added at wk 6
 - C: positive bedding added at wk 9
 - Negative control
 - Positive control
- Filter media removed at 12 wk, submitted for PCR to *Helicobacter* spp.



Results: **SFSB** has **low sensitivity** when identifying *Helicobacter* spp. in the face of **low prevalence**.

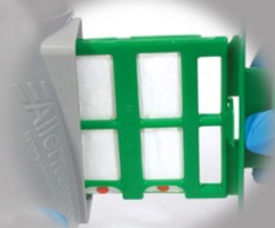


What It All Means

- Both **SFSB and EDT** sampling are **superior to SBS** evaluated by PCR and serology.
- **SFSB** may be preferable to EDT for **viral agents**.
- Bacteria are detected well by both SFSB and EDT.
- Either **SFSB or EDT** may be acceptable for **routine colony surveillance** in disposable cages.

Making the Leap

Stakeholder Interest and Support
Cost Analysis
Know Your Pathogens and Prevalence
Be Flexible
Be Patient



New pathogens and positive results... What to do with them?

It Depends!

Vendors

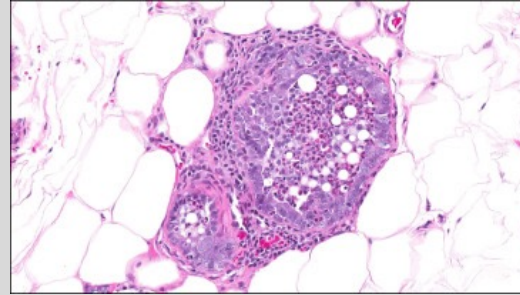
Support

Resources

Choose Your
Battles Wisely

Lessons Learned

- **SFSB and EDT > SBS**
- Be careful what you look for
- Dilution effect and **low prevalence** may hide things
- There's a place for tried and true
 - **Direct testing** imported animals
 - Direct testing suspected outbreaks
 - **Necropsy and histopathology** on weird stuff



Acknowledgments

- Jessica Morrison, U. of Oklahoma HSC
- Treva Lawson, U. of Oklahoma HSC
- Abby Bernardini, U. of Oklahoma HSC
- Shawn Lane, U. of Oklahoma HSC
- Mitch Moore, Allentown
- Brian Bilecki, Allentown
- Ken Henderson, Charles River Laboratories
- Pat Sikes, Charles River Laboratories
- Cheryl Perkins, Charles River Laboratories
- 3RsC

Switching to Sentinel Free Soiled Bedding Testing – Sweat, Blood, Tears, Feces, and Sarcasm

Marnie Silverstein Metzler, DVM, PhD, DACLAM

mgsilver@ncsu.edu

Always start with a good history...

- Small rodent census: 2000 mice, 100 rats
- Trimester program consisting of soiled bedding sentinels
- Outbred mice (NOT excluded - *Strep* sp, *K. oxytoca*, *K. pneumoniae*, *P. pneumotropica*, *P. aeruginosa*, *P. mirabilis*, and *S. aureus*)
- Samples from sentinel cage
 - Serology
 - Fecal PCR – pinworms and *Helicobacter*
 - Environmental swabs



Road map to...

9/21 Start at
NCSU as
Clinical
Veterinarian

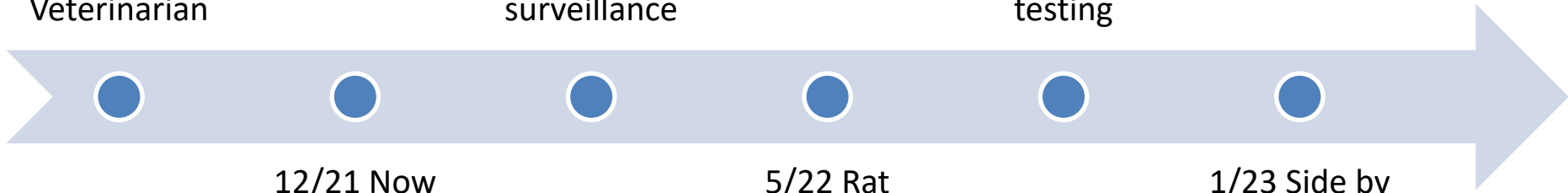
1/22 Mouse
SBS
trimester
surveillance

9/22 Start
SFSB
comparison
testing

12/21 Now
the only
Veterinarian
at NCSU

5/22 Rat
DCS added
to trimester
surveillance

1/23 Side by
side by side
comparison



Recipe for...

EAD and SFSB media set up to run side-by-side with SBS over a 4 months sampling period. Three racks were chosen for testing in a “dirty” room recently cleared of MHV



Exhaust Air Duct
Testing¹



Soiled Bedding
Sentinels²



Sentinel Free Soiled
Bedding³



1. Allentown Sentinel™ 2. IDEXX BioResearch's Opti-Spot® 3. Charles River, PathogenBinder™

Infectious disease PCR testing showed differences in agents detected

	SBS	EAD	SFSB
Norovirus	+	+	+
<i>Campylobacter</i>	NT	+	+
<i>K. pneumoniae</i>	-	+	+
<i>Helicobacter</i> genus	+	+	+
<i>H. bilis</i>	-	+	+
<i>H. ganmani</i>	+	+	+
<i>H. hepaticus</i>	-	+	+
<i>H. mastromyrinus</i>	-	+	+
<i>H. typhlonius</i>	+	+	+

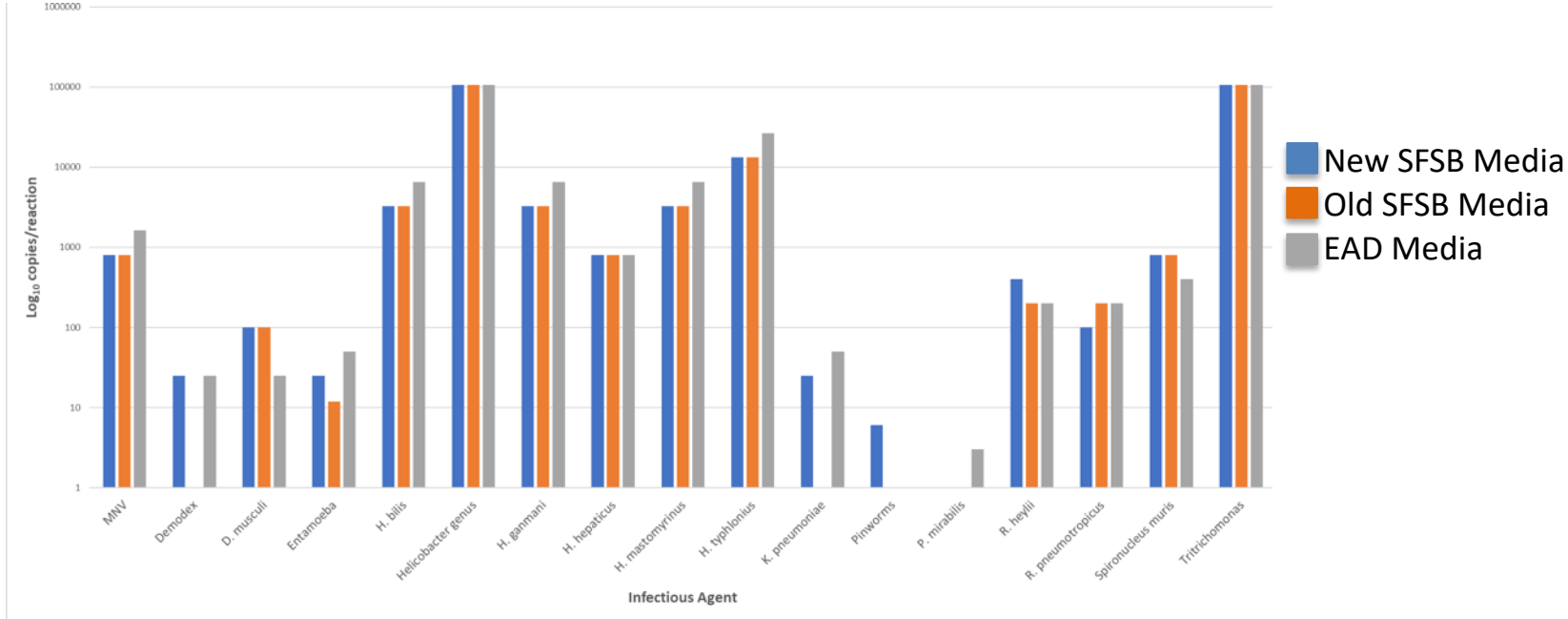
	SBS	EAD	SFSB
<i>P. mirabilis</i>	+**	+**	-
<i>R. heylii</i>	NT	+	+
<i>R. pneumotropicus</i>	NT	+	+
<i>Spiroucleus muris</i>	+	+	+
<i>Tritrichomonas</i>	+	+	+
<i>Demodex</i>	NT	+	+
<i>Entamoeba</i>	+	+	+
Pinworms	-	-	+*

NT: Not Tested

*: Confirmatory testing was negative

** : not excluded for in sentinel mice

Copy numbers and direct contact transmission explain most of the variation



Upcoming Changes to Rodent Health Surveillance > Inbox x



Marnie Metzler <mgsilver@ncsu.edu>
to group-rodent, Kathryn

Wed, Feb 15, 3:42 PM ☆ ↶ ⋮

Dear Rodent Users,

Historically, infectious agent screening of rodent colonies here and at other institutions was performed by the transfer of soiled-bedding from research rodents to SPF soiled-bedding sentinels.

However, the transition of most vivaria for these cage types. ... rly via soiled-bedding to sentinel rodents

Established environmental sampling reduce the use of sentinel rodents. N

FANTASTIC, Marnie!

There is a very well detailed white paper health surveillance we compared this

Thanks for taking this initiative!

types. During the last round of rodent live than our current practice.

As a result, I would like to improve our PCR testing for non-traditional vendors



replace direct serology and fecal/fur swab

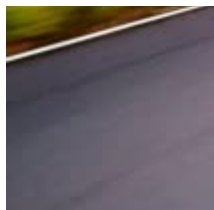
Below are the results of our comparison

Please let me know if you have any questions

Ph.D. (she / her / hers)

Professor of Virology and Alumni Assn Distinguished Graduate Professor
Department Head

Department of Molecular Biomedical Sciences





RESET

August 2023
Other rooms test negative for *Syphacia* ...

Sept 2023
“Rodent supervisor” resigns, auditing reveals ACTs not trained on SFSB

May 2023
•Room 142 tests positive for *Syphacia*
•No rat samples

May 2023
Room 142 confirmatory testing positive for *Syphacia*

Where do we go from here?

- Global staff retraining occurred end of Sept
- SFSB boxes reset for November sampling
- Room 142 started on Fenbendazole diet
- Decontamination and additional room treatment pending November sampling



Lessons Learned, Questions to Ask

- Timing is everything
 - Is the time right for your institution?
 - Can you leverage new leadership?
 - Are your investigators ready for change?
- Make sure your team is ready
 - Do you have a trusted staff?
 - Are you be prepared to be very hands on?
- Be prepared to find all the potholes and all the pathogens
 - Don't look if you don't want to find – but, really it's there anyways...
 - Do you have the resources to test/treat?



Use Your Resources



- Talk to colleagues
- Talk to the labs/vendors
- AALAS presentations/posters
- The 3Rs Collaboration

Final Thoughts

- Communicate well, communicate often
- Be present
- Be patient
- Be flexible
- Don't be afraid to ask for help

mgsilver@ncsu.edu



Acknowledgements

- NCSU Laboratory
Animal Resources
- Sarah Kinlaw
- Jenny Estes
- Jessie LeGrand
- Ken Henderson
- Cheryl Woods
- Laurie Comfort

3RsC Resources



Caroline Winn

AALAS 2023-10-25
12:30-2pm

The 3RsC has extensive resources to help make replacing sentinels easier

Rodent Health Monitoring

[Overview](#)

[Presentations](#)

[Publications](#)

[Editable Slide Deck](#)

[SOPs](#)

[Cost Analysis](#)

[Sanitation](#)

[How to Switch](#)

[Call for Research Projects](#)

[FAQs](#)

Our SOPs can help you start

STANDARD OPERATING PROCEDURE Environmental Health Monitoring Exhaust Dust Testing (EDT) in IVC Allentown Rack System

For Allentown IVC ventilated racks without cage level filtration, either Sentinel™ media or sticky swabs can be used to sample debris from the exhaust plenum of the rack over 3 month periods.

1. Frequency of Diagnostic Sample Collection

- Media are collected once every three months (i.e. quarterly)
- Multiple media or swabs may be pooled together for submission, and media or swabs may also be pooled with other samples such as swabs from resident animals. Note that pooling may not be ideal due to possible dilution of any positive sample(s). Please contact your testing laboratory for details on how pooling can work in your situation.

2. Materials Needed

- Gloves
- 50ml Sterile conical tubes
- The Sentinel™ media & The Sentinel™ media holder OR Sticky Swabs
- Step stool or mobile ladder (to access the exhaust hose when placing media)

3. Procedure for using Sentinel™ media

- Label the conical tube with:
 - Date in
 - Date out (3 months later)
 - Room number
 - Racks number(s)
- Place media for the first time
 - Turn the blower off and open the vertical exhaust plenum door
 - Put new gloves on and insert media, beveled side first and the red dot facing away from you into the metal holder at the top of the open exhaust plenum. Close the vertical exhaust plenum door.
 - Turn on the blower.



- Collect media every 3 months
 - Ensure the media has been in place for approximately 3 months prior to collection by checking the dates

- Wearing clean gloves, place soiled media in the conical tube by rolling/folding media paper to easily insert into the tube with the red dot facing inward.
- After handling media, remove and change gloves immediately to prevent accidental cross-contamination of other items in the room.
- Close conical tube lid. Close plenum door, replace rack to starting position, re-attach hoses.
- Once the samples are collected, a new media is placed in the holder. At the discretion of the end user, such as if the holder will be moved between racks, the media holder may be washed prior to placing a new media to remove any old dust that may remain.
- Multiple media may be pooled together for submission, and media may also be pooled with other samples such as swabs from resident animals. Please contact your testing laboratory for details on how pooling can work in your situation.

4. Procedure for manual plenum swabbing

- Label conical tubes with
 - Room number
 - Rack number
 - Date of collection
- Collect plenum swabs every 3 months
 - Ensure that the rack has not been cleaned in the past 3 months to ensure there is enough dust.
 - Open the side panel of the Allentown rack to expose the horizontal exhaust plenums.
 - Using a sticky swab, rotate the swab inside the plenums. The same swab can be reused for several plenums.
 - Insert the tip of the swab inside a clean tube. A maximum of 10 swabs are permitted per tube. Up to 3 racks be pooled per sample.

5. Changing or moving racks within a collection cycle

- It is up to user discretion to transfer media to the new rack, dispose, or submit for testing. If choosing to transfer media, it is critical to ensure that the collection media stays with the cages. Contact your caging manufacturer or diagnostic lab for additional guidance.

6. Decommissioned racks

- Label conical tube with
 - Room number
 - Rack number
 - Date of collection
- Follow steps for either media testing or manual plenum swabbing as listed above. Samples may be sent out to be tested or pooled with other filters to be collected at another time.

7. Minimum exposure time

- Sentinel™ media** that has been in a rack that houses animals for at least 30 days is eligible for submission. This includes new racks put into service at least 30 days before collection and racks that were decommissioned after the last quarterly media collection.

Advice on How to Switch

- 1. Reference the 3RsC Website & determine which method(s) are right for your Institution**
- 2. Reach out to your diagnostic vendors to determine their EHM preferences**
- 3. Gather an internal advocacy group & create a plan to switch**
 - 1. Hybrid? Gradual? Parallel? Cold Turkey?**
 - 2. Order any supplies**
- 4. Communicate to everyone & train!**
- 5. See the PDF from AALAS 2022 & CALAS 2023 for case studies**

Institutions have made the change



A cautionary note on the recent article, “Assessing Methods for Replacement of Soiled Bedding Sentinels in Cage-Level Exhaust IVC Racks”

You may have seen this recent article that concludes that SBS is superior to EHM and are now concerned about switching. Unfortunately, this article suffers from severe flaws. The 3RsC EHM Initiative will be writing a letter to the editor describing them and asking for retraction or extensive editing. What is wrong exactly?

Mainly, SBS & SFSB are not compared on equal grounds and therefore the conclusions made are inherently faulty and therefore should not be construed to contradict a literature base of over 40 studies supporting EHM.

- SFSB samples were pooled at the room level while SBS taken at the rack level
- SBS samples were supplemented with DCS (therefore bolstering the sensitivity of SBS), but described as “SBS” > no true conclusions can be drawn about SBS alone
- Substandard, outdated media was used for SFSB
- Large amount on incomplete data sets
- Inappropriate use of statistics for the conclusions drawn