Comparison of Individual Ventilated Cage Rack Cleaning Methods: Tools for Exhaust Air Dust Testing

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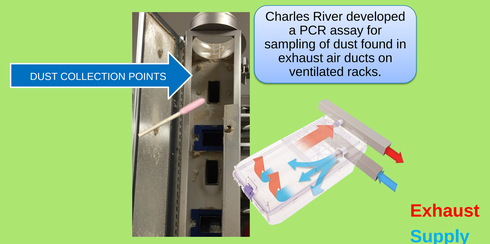
ABSTRACT

At McGill, rodents housed in individually ventilated cages (IVC) have been routinely monitored for pathogenic agents by the use of soiled bedding sentinels. Wanting to take advantage of the recent advances in exhaust air dust testing as an alternative to sentinels unfortunately left us discovering historical fur mite results that did not reflect the current state of our animal facility. In this study we tested 4 different cleaning methods (rack wash only, mechanical wash only, mechanical and rack wash, and mechanical with a bleach solution and rack wash) to best determine the most effective way to remove residue fur mite DNA, bacterial DNA (*Pasteurella pneumotropica*) and rodent non-specific DNA (APOR). We tested, by PCR assay, before cleaning and after cleaning, and determined that all 4 ways were effective in removing fur mite DNA, a mechanical wash with a bleach solution alongside rack wash was most effective to eliminate the dust containing *Pasteurella pneumotropica* and rodent DNA. With this knowledge, we were able to fully implement an effective environmental health monitoring program and eliminating the need of sentinels.

INTRODUCTION

Henderson *et al.* (JAALAS 2013) previously described the efficacy of direct PCR testing of index animals versus soiled bedding sentinels. Some pathogens transfer well to soiled bedding sentinels such as MPV, *Pseudomonas aeruginosa* and Pinworms. However, some pathogens, such as *Pasteurella pneumotropica*, Helicobacter spp. and fur mites, were more effectively found by index animal testing. Our sentinel program previously included sentinel serology and in house bacterial and parasitology screenings.



In December 2014, hoping to embrace the exhaust air dust collection method, we pooled samples of swabbed racks and feces collected and submitted for a complete PCR Panel. The result came out positive for fur mites (*Radfordia affinis*). However, we had pooled both rack swabs with index feces in the same sample. In January of 2015, we reswabbed the racks and submitted index animal feces seperately and we found that only the racks were positive for fur mites, while the index animals were found to be negative.

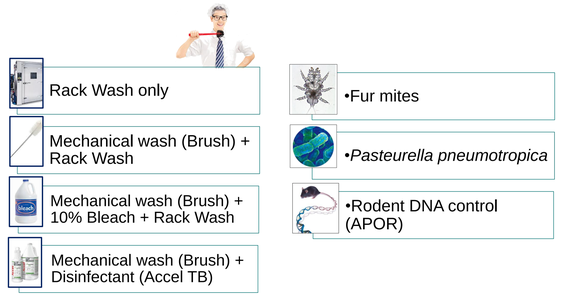
GCC Animal Facility History

- 2011/2013: fur mite outbreak at GCC
- selamectin treatment: 5 cycles
- Animals negative after treatment (PCR)
- All sentinels negative
- No clinical signs of mite infestation

How do we get rid of the DNA in the IVC racks?

MATERIALS

Nineteen Allentown IVC racks were chosen for the project, all of which were positive for *Pasteurella pneumotropica* and 11 of which were positive for *Radfordia affinis*. The racks were divided randomly into 4 different cleaning methods representing both mechanical (scrubbing off dust inside plenums with a bristle brush) and chemical (using 0.5% hydrogen peroxide (Accel TB or Prevail) or chlorine (10% bleach) based solutions) sanitation processes. The exhaust plenums of the racks were swabbed, using sticky swabs, before and after being washed. One swab was used per exhaust plenum, with 10 swabs included per sample. Samples were sent for PCR analysis at Charles River Laboratories.



RESULTS

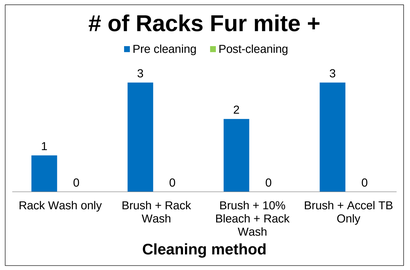


Figure 1. Total number of *Radfordia affinis* positive racks before and after cleaning using 4 different cleaning methods. All 4 cleaning methods were effective in removing all fur mite, *Radfordia affinis,* DNA.

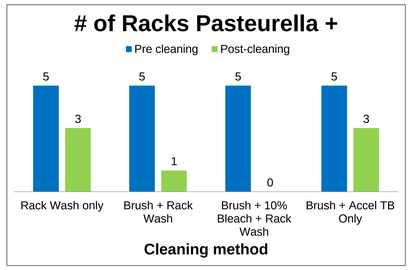


Figure 2. Total number of *Pasteurella pneumotropica* positive racks before and after cleaning using 4 different cleaning methods. Using the rack wash only (no mechanical/chemical cleaning) or the brushing with Accel TB only (no final rack washing) were the least effective in removing the bacterial DNA. Combining brushing with bleach solution and rack wash was the most effective in removing *Pasteurella pneumotropica* DNA.

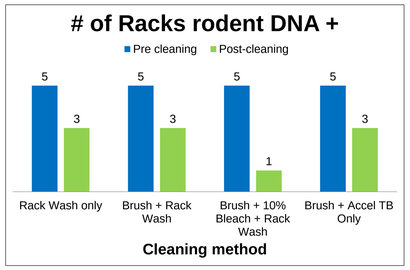


Figure 3. Total number of racks positive for rodent DNA housekeeping gene *(APOR)* before and after cleaning using 4 different cleaning methods. Combining brushing with bleach solution and rack wash was the most effective in removing non-specific rodent DNA.

Rack #	Fur mites	*P. pneumotropica* – Heyl	*P. pneumotropica* – *Jawetz*	APOR	Fur mites	*P. pneumotropica* – Heyl	*P. pneumotropica* – *Jawetz*	APOR
	PRE-CLEANING				**POST-CLEANING**			
Rack Wash					**Rack Wash**			
1	0	811	6579	1630	0	2	25	0
2	3275	811	26561	1630	0	1	0	50
3	0	1630	1630	1630	0	0	0	3275
4	0	811	285	811	0	3	0	3
Average	818.75	1015.75	8763.75	1425.25	0	1.5	6.25	832
Mechanical Wash (brush) + Rack Wash					**Mechanical Wash (brush) + Rack Wash**			
5	0	811	6579	811	0	0	0	0
6	6579	3275	13219	811	0	0	0	3
7	0	811	3275	811	0	0	0	0
8	6	404	26561	811	0	0	17	3
9	50	3275	1630	811	0	0	0	3275
Average	1327	1715.2	10252.8	811	0	0	3.4	656.2
Mechanical Wash (brush) + Bleach 10% + Rack Wash					**Mechanical Wash (brush) + Bleach 10% + Rack Wash**			
10	0	3275	6579	1630	0	0	0	12
11	25	1150	811	811	0	0	0	0
12	0	4642	404	811	0	0	0	0
13	404	6579	26561	3275	0	0	0	0
14	0	4642	3275	1630	0	0	0	0
Average	85.8	4057.6	7526	1631.4	0	0	0	2.4
Mechanical Wash (brush) + Accel TB					**Mechanical Wash (brush) + Accel TB**			
15	0	3275	404	404	0	0	0	0
16	25	4642	201	811	0	0	0	2
17	12	1150	285	404	0	2	0	0
18	404	9326	26561	3275	0	2	25	3275
19	0	9326	37649	3275	0	3	17	50
Average	88.2	5543.8	13020	1633.8	0	1.4	8.4	665.4

Table 1. DNA copy numbers before and after cleaning for each sanitation method.. Parasite, bacterial and non-specific rodent DNA (APOR) copy numbers were all reduced after cleaning. *Pasteurella pneumotropica* - Heyl and Jawetz represent the different biotypes.

5 IVC racks	*P. pneumotropica* – Heyl	*P. pneumotropica* – *Jawetz*	APOR
	Post cleaning using Brush + Prevail + Rack Wash		
Average copy numbers	0	0	0

Table 2. DNA copy numbers post-cleaning for cleaning method consisting of brushing, Prevail disinfection (0.5% hydrogen peroxide) and rack wash. 5 dirty IVC racks were processed and *Pasteurella pneumotropica* and non-specific rodent DNA (APOR) copy numbers were all found to be negative after cleaning.

DISCUSSION

With these results, we have concluded that the most effective sanitation method to remove parasite (*Radfordia affinis*), bacterial (*Pasteurella pneumotropica*), and non-specific rodent DNA (APOR) was to use a three-step process. The first step consists of mechanically removing the attached dust particles using a bristle brush, the second step consisted of spraying the plenums with a disinfectant spray, and the third step consisted of washing the IVC rack in the rack washer using a standard rack wash cycle. This method was proved to remove the pathogenic DNA residues.

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CLEANING IVC RACKS

CONCLUSION

Following the validation of the Allentown IVC rack cleaning method, we were successful in eliminating residual environmental DNA thus reducing probabilities of false positives. As a result, we are now able to use environmental sampling as a replacement for sentinels and converted the GCC animal facility into a sentinel-free zone! Now relying on environmental and index mice PCR monitoring, we have developed a rack cleaning SOP and schedule (110 racks currently at the GCC). Not only does this reduce the number of mice used (360 mice/6months), but economical savings were made as well (16,000$ in sentinel per diem alone).

ACKNOWLEDGEMENTS

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