Detection of Acute *Corynebacterium bovis* Infection by Environmental Sampling of IVC Rack Exhaust Air Manifolds

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Background and Significance

Corynebacterium bovis is an opportunistic infection of nude (*Foxn1*, nu/nu) mouse populations' worldwide. Identified as the causative agent of nude mouse hyperkeratotic dermatitis or "scaly skin disease", *C. bovis* causes clinical illness of short duration followed by what is believed to be life-long subclinical skin colonization. Despite the limited duration of clinical signs, the impact on xenograft tumor development can be significant leading to delayed, slowed, or failed tumor growth. *C. bovis* plagues many academic and industry research facilities as a bacterial contaminant that is extremely difficult to eliminate.

The rapid spread of *C. bovis* infection among naïve nude mouse colonies requires the use of more rapid methods of detection beyond soiled bedding sentinel programs. The rationale for IVC rack air exhaust system sampling for *C. bovis* detection is based on findings reported by Burr *et al.* 2012 indicating that *C. bovis* is efficiently spread by airborne transmission within air currents of biosafety cabinets. It is believed that *C. bovis* populated skin flakes are distributed by air currents, resulting in airborne transmission. We hypothesized that forced air movement through ventilated cages will effectively carry *C. bovis* contaminated particulate into the IVC exhaust air system for easy diagnostic sampling and rapid detection.

Using swabs of the horizontal exhaust manifold (HEM) of an IVC rack system, we have previously demonstrated detection of mice with established *C. bovis* infections within 24 hrs of cage placement on an IVC rack. Here, we investigated how quickly a new *C. bovis* infections could be detected by HEM sampling following acute *C. bovis* exposure of naïve mice.

IVC Rack Air Supply and Exhaust System

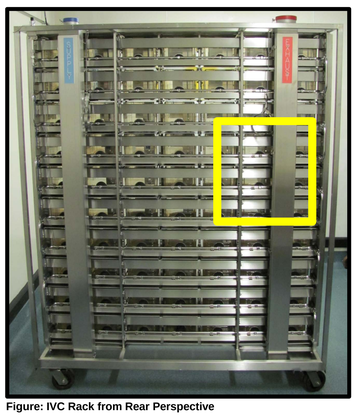


Figure: IVC Rack from Rear Perspective

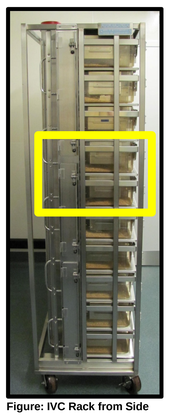


Figure: IVC Rack from Side

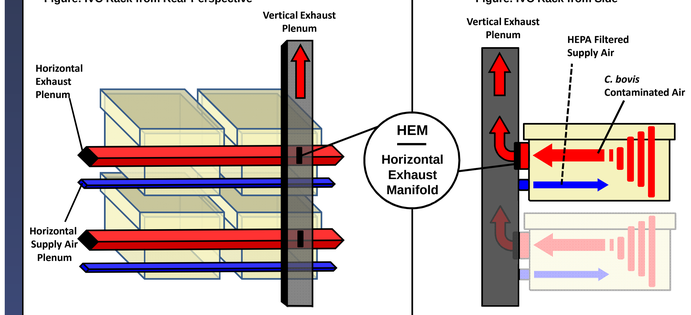


Diagram of the HEM at the junction of the horizontal and vertical exhaust plenums.

Horizontal Exhaust Manifold (HEM) Sampling:
A sterile, dry, non-sticky, non-flocked swab is introduced into the HEM. All 4 sides of the manifold, from front to rear are sampled. To standardize sampling each HEM is sampled for 5 seconds.

Why the HEM? The HEM is a defined location at the union of the vertical and horizontal exhaust plenums. Each row of an IVC rack has a single HEM. Each HEM can be swabbed individually to determine the *C. bovis* status of each row of a rack. Similarly a common swab could be used to sample all HEMs to determine the *C. bovis* status of the entire rack.

Hypotheses

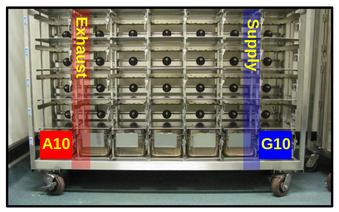
- **Testing of an IVC system's horizontal exhaust manifold (HEM) can be used to rapidly detect nude mice with early *C. bovis* infections.**
- **The number of mice per cage will not affect the speed of detecting *C. bovis* DNA at the HEM.**
- **The cage position of exposed mice in relation to the HEM will not affect the speed of detecting *C. bovis* DNA at the HEM.**

Experimental Goals

1. How quickly can a newly acquired *C. bovis* infection be detected in the horizontal exhaust manifold (HEM)?

2. Will the number of mice in a cage affect how quickly an early infection is detected?

3. Will cage position on the rack (proximity to the HEM) affect how quickly an early infection is detected?



Materials and Methods

Racks, Caging, and Swabs: Allentown Inc., 70 cage, single-sided, individually ventilated MicroVent racks and JAG 75 cages were used. IVC racks were sampled at the HEM using a single, sterile, dry foam swab (Becton Dickinson, BBL™ CultureSwab™ EZ , Ref# 220144).

Animals: Female, 6-7 weeks old, Hsd:Athymic Nude mice (*Foxn1*™) were naturally infected by exposure to a soiled cage containing a *C. bovis* infected mouse for 1 min. Only one mouse per cage was exposed. For experimental cages containing 5 mice, intra-cage transmission was relied upon to spread the infection. On days 1,3 and 5, post exposure a sterile, dry swabs were used to serially sample the skin and oral cavity of the exposed mouse within each cage to confirm infection. Mice were considered infected once *C. bovis* was detected by qPCR.

Early *C. bovis* Infection Detection at the HEM: A single cage containing either 1 *C. bovis* exposed mouse or 1 *C. bovis* exposed mouse housed with 4 naïve mice, was placed on row 10 of the rack at either the bottom left cage position (A10; closest to the HEM) or the bottom right cage position (G10: furthest from the HEM). Prior to *C. bovis* exposed cage placement, the HEMs were sampled using a single foam swab and confirmed *C. bovis* negative. Following cage placement the HEM of row 10 was swabbed daily for 11 days. As an environmental control, the HEM of row 9 of the experimental rack and row 10 of a control rack was swabbed at the end of the sampling period. Swabs were submitted for qPCR detection of *C. bovis.*

Air Supply and Exhaust: Rack ventilation remained consistent for the study with supply air delivered at 12.3 ± 1.0 ft³/min, exhaust air of 27.9 ± 2.4 ft³/min, and 41.1 ± 1.8 air changes/hr at the cage level. Air flow did not differ for position A10 and G10.

Quantitative PCR (qPCR): *C. bovis* primers and probe sequences:
C. bovis F 5'-AACGCGAAGAACCTTACCTGG-3'
C. bovis R 5'-ACCACCTGTGAACAAGCCCA-3'
and the probe 6FAM-GGCAGGACCGGCGTGGAGA-TAMRA.

Experimental Results

Detection of Early *C. bovis* Infection

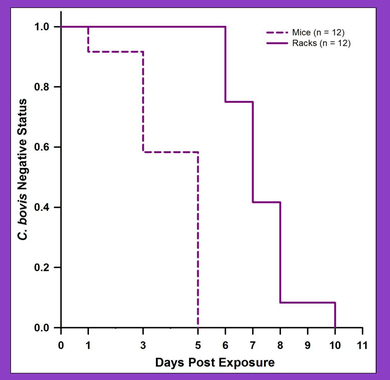


Figure 1: Detection of Early C. *bovis* Infection. Post *C. bovis* exposure, all nude mice were *C. bovis* positive by PCR on day 5 with a mean of 4.0 ± 1.3 days. Immediately after mouse exposure, cages containing exposed mice were placed on racks for *C. bovis* detection by HEM sampling. *C. bovis* DNA was detected at the HEM of all racks by day 10 with a mean of 7.3 ± 1.1 days.

Cage Density on *C. bovis* Detection

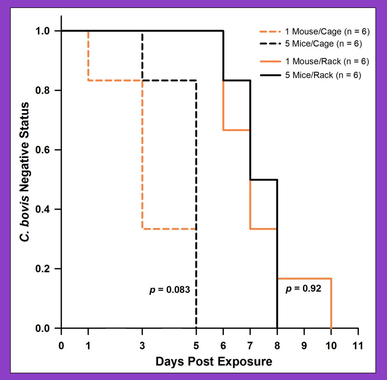


Figure 2: The Impact of Cage Density on Early *C. bovis* Detection. The number of infected mice/cage did not demonstrate a significant effect on the time to *C. bovis* detection at the HEM. The mean day of *C. bovis* detection at the HEM for individually housed and group housed mice was 7.3 ± 1.5 and 7.3 ± 0.8 days, respectively.

Rack Position on *C. bovis* Detection

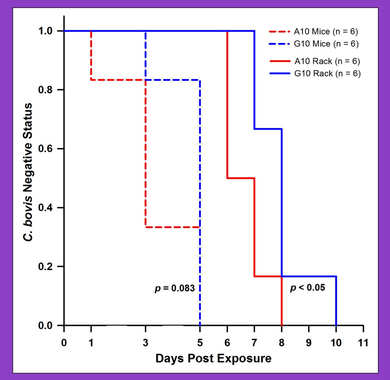


Figure 3: The Impact of Rack Position on Early *C. bovis* Detection. For cage position analysis, data sets between 1 and 5 mice/cage were combined for both cage locations due to the lack of significance of cage density of the time to detect *C. bovis* at the HEM. The number of infected mice per cage had a significant effect on the time required to detect C. bovis at the HEM ($p < 0.05$). The mean day of *C. bovis* detection at the HEM for cages of exposed mice placed at position A10 and G10 was 6.7 ± 0.8 and 8.0 ± 1.1 days, respectively.

Rack Position and Cage Density on *C. bovis* Copy Number

Figure 4

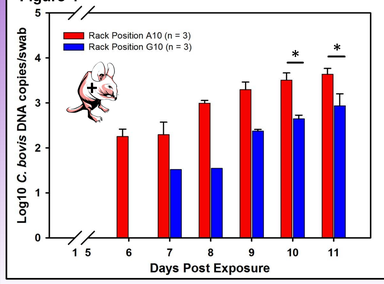


Figure 5

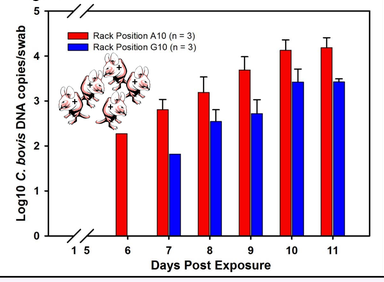


Figure 4: Copy number detected at the HEM with 1 mouse/cage at rack position A10 vs. G10. Consistently more *C. bovis* DNA was detected at the HEM when the cage was placed in position A10. However, this was only statistically significant on days 10 and 11 post exposure (* $p < 0.05$) with 1 mouse/cage.

Figure 5: Copy number detected at the HEM with 5 mice/cage at rack position A10 vs. G10. As seen with 1 mouse/cage, more *C. bovis* DNA was consistently detected at the HEM with 5 mice/cage when the cage was placed in position A10. However, this was not statistically significant at any time point.

Experimental Infection: For cages with 5 mice/cage, only 1 mouse in the cage was exposed to *C. bovis*. Intra-cage transmission was used to spread the infection. This may explain why significantly more *C. bovis* DNA is not detected at the HEM with 5 mice/cage as compared 1 mouse/cage until day 11 (data not shown).

Conclusions

- **HEM sampling reliably detected *C. bovis* from all racks housing experimentally infected mice 6 - 10 days (7.3 ± 1.1 days) post exposure (Figure 1).**
- **Cage density did not significantly effect the amount of time required to detect *C. bovis* at the HEM (Figure 2, $p = 0.92$).**
- **Cage position on the rack significantly effect the amount of time required to detect *C. bovis* at the HEM (Figure 3, $p < 0.05$).**
- **Mice directly exposed to a soiled cage containing an infected mouse required 4.0 ± 1.3 days after exposure to test positive by PCR for *C. bovis* with combined oral and dermal swabs.**
- **The cage position closest to the HEM consistently resulted in a higher copy number of *C. bovis* DNA, but only significant at 2 time points during the 11 days tested (Figure 4, $p < 0.05$).**
- **6-10 days post *C. bovis* exposure may provide a working approximation of the time prior to airborne bacterial shedding of *C. bovis* from infected mice.**

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Acknowledgements

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nude mouse artwork by Jane Wang, 2006

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Abstract – P9

Detection of Acute *Corynebacterium bovis* Infection by Environmental Sampling of IVC Rack Exhaust Air Manifolds

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Environmental sampling for rodent pathogens is gaining momentum for the enhancement and potential replacement of traditional sentinel monitoring programs. *Corynebacterium bovis* is an opportunistic infection of nude (*Foxn1*, nu/nu) mice that spreads rapidly, and is not detected by traditional sentinel programs. We investigated how quickly post-exposure *C. bovis* could be detected in nude mice by quantitative PCR swabs collected from the horizontal exhaust manifold (HEM) of an individually ventilated caging (IVC) system. We also determined if cage row position or animal housing density would have an effect on time to detection. Female nude mice were naturally infected by exposure to a soiled cage of a *C. bovis* infected mouse. Exposed mice were then either housed singly or with 4 naïve nude mice in sterile caging. Cages of 1 or 5 nude mice were placed in the first or last cage position on the bottom row of 70 cage IVC racks. Daily sterile, dry swabs were used to serially sample the skin and oral cavity of exposed mice and the corresponding HEM for *C. bovis* detection. Rack ventilation remained consistent for the study with supply airflow delivered at 12.3 ± 1.0 ft^3/min, exhaust airflow of 27.9 ± 2.4 ft^3/min, and 41.1 ± 1.8 air changes/hr at the cage level. Cage position on the row had a significant effect on the time required for *C. bovis* detection. The first cage position closest to the HEM required 6.7 ± 0.8 d (n = 6) as compared to 8.0 ± 1.1 d (n = 6) for the last position on the row ($p < 0.05$). The time required for mice to test positive for *C. bovis* post-exposure was 4.0 ± 1.3 d (n = 12). The time to mouse infection post-exposure and housing density did not have a significant effect on the time to *C. bovis* detection at the HEM. These findings suggest that HEM sampling can be utilized for routine surveillance of acute *C. bovis* infections in nude mouse colonies, irrespective of cage row position and housing density.

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