Journal of the American Association for Laboratory Animal Science
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Vol 52, No 1
January 2013
Pages 28–33

PCR Testing of a Ventilated Caging System to Detect Murine Fur Mites

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Rodents housed in microisolation caging are commonly monitored for infectious agents by the use of soiled bedding sentinels. This strategy relies on the successful transmission of rodent pathogens from the index rodents via soiled bedding to sentinel cages and the subsequent infection or colonization of sentinel rodents. When the prevalence of a pathogen is low or the target agent is not readily transmitted by soiled bedding, alternative testing methodologies should be used. Given the continued prevalence of institutions self-reporting murine fur mites and with the advent of a new sensitive and specific PCR assay for mites, we sought to determine whether the exhaust system of an individual ventilated caging (IVC) system could be used for monitoring the rack's rodent population for mites rather than relying on the responses of sentinels. We deployed single cages of mice (*Mus musculus*) that were known to be infested with either *Radfordia affinis* or *Myobia musculi* on a 70-cage rack, sampled the horizontal exhaust manifolds weekly, and used the new PCR assay to test these samples for mite DNA. We detected the presence of fur mites at a 94.1% probability of detection within 4 wk of placement. Therefore, we recommend swabbing and testing the shelf exhaust manifolds of IVC racks rather than relying on soiled-bedding sentinels as an indicator of the mite status of the rodents on that rack.

Abbreviations: qPCR, quantitative PCR; IVC, individual ventilated caging.

The use of sentinel animals is typically a key element of the overall health surveillance program for laboratory rodent colonies. For institutions that use microisolation caging systems, whether individually ventilated or static, the transfer of soiled bedding from colony animals is a typical method for exposing sentinel rodents to unwanted infectious agents that may be present in the colony. However, when the prevalence of colony infection is very low or when the agent being monitored is not readily transmitted in the bedding, the likelihood of false-negative results is increased when relying solely on this method of sentinel exposure.[1,15] The use of complementary diagnostic methodologies is warranted in these situations.[15] These techniques must be highly sensitive and specific to reliably detect the agent of interest.

Laboratory rodent health-surveillance programs typically include monitoring for murine fur mites. In a 2006 survey, 30% and 40% of research institutions self-reported the presence of *Myocoptes musculinus* and *Myobia musculi*, respectively, in their mouse colonies.[2] This finding indicates that fur mite infestations remain an important issue for many research facilities. Both the clinical signs of acariasis and its potential effects on research that involves affected mice are well established. The clinical signs include, but are not limited to, pruritis, alopecia, ulcerative dermatitis, and pyoderma.[7] Elevations in IgE levels and inflammatory cytokines as well as alterations in hematologic values are just some of the research-compromising effects that can occur.[6,7,10,14] The use of soiled-bedding sentinels to effectively detect murine fur mites as part of the health-monitoring program has been controvertible. One institution reported the ability to transmit and infest sentinel mice with fur mites exposed to as little as 2.5% known-contaminated soiled bedding administered every week.[13] Another study demonstrated positive transmission (50% of the cages after 4 mo of exposure) when sentinel mice received a cupful of contaminated bedding twice weekly.[16] A third study repudiated those findings, in that the investigators were able to detect mites in only 3% of their animals after they had received 100% contaminated soiled bedding for 12 wk; mites were not detected in 0% of animals that received 11% to 50% contaminated, soiled bedding for 12 wk.[7]

In 2011, 2 commercial rodent diagnostic laboratories (University of Missouri Research Animal Diagnostic Laboratory and Charles River Laboratories International) began to offer a new diagnostic assay for detecting murine fur mites. The laboratories identified the mite species of interest and sequenced their DNA to develop both sensitive and specific PCR assays. These tests provide animal facilities with an alternative method to identify and speciate fur-mite infestations in rodent colonies. Shortly after these assays became available, our animal facility began to use and submit samples for fur-mite PCR analysis. We received positive PCR results for both *Radfordia* spp. and *Myobia* spp. fur mites in some, but not all, of the samples from mouse sentinels that we had submitted. These results were confirmed by direct visualization by using either the fur pluck or tape test methods. Prior to these positive results, our facility had only 2 known outbreaks (several rooms in 2007 in one building and a single room in early 2010 in a new building, which was populated by a relocated room of the 2007 outbreak). In both instances, the rooms were treated and subsequently confirmed as mite-negative by direct visualization of colony and sentinel animals. Our rodent sentinel monitoring program, which relied on testing methods that required direct visualization of the parasites or eggs on sentinel pelts, had not detected any other fur mite infestations in other rooms during the current or since the previous outbreaks.

Received: 16 May 2012. Revision requested: 12 Jun 2012. Accepted: 31 Jul 2012.
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To evaluate the prevalence of mites in our facility, we initiated a survey of all inhouse mouse colonies. Given previous reports questioning the efficacy of soiled-bedding transfer for detecting fur mite infestations and because our sentinels had not all been deployed for the same amount of time, we did not have confidence that only testing sentinel animals (by either PCR or traditional direct visualization methods) would yield accurate results—that is, we felt that this strategy would be prone to false-negative results. Conversely, a decision to sample individual colony animals directly would have been both time- and labor-intensive as well as costly due to the number of tests required.

Given that the vast majority of mice in our facilities are housed in an individually ventilated caging (IVC) system having robust exhaust of unfiltered air from the cages, we speculated that the same mite DNA that was on the skin and fur of the mice would be aerosolized and subsequently accumulate on the inner surfaces of the rack exhaust systems. If so, sampling the rack exhaust system might facilitate the screening of relatively large numbers of cages with relatively few samples. To accomplish this goal, we initially selected the terminal vertical exhaust plenum—the final part of the rack itself—as the sampling site (Figure 1). Using this method, we obtained a single sample from each rack and pooled as many as 10 samples for a single PCR test. In this way, only one PCR test was done for each mouse room. Although we initially obtained several PCR-positive results with this sampling methodology, we subsequently discovered that some racks known to have mite-infested cages returned PCR-negative results. Therefore, we decided that the terminal vertical exhaust plenum was an unreliable sampling site. We speculated that this site might be too far from the cages to detect mite DNA reliably. To determine a more suitable site for testing, we considered sampling a part of the rack that was closer to the cages themselves, with the goal of a locating an enriched source of mite DNA. One possible location is the shelf horizontal air-exhaust manifold located at the terminal section of a row's exhaust system before it enters the vertical exhaust plenum (Figure 2).

The purpose of the current study was to determine whether and at what frequency the presence of murine fur mite DNA could be detected in the shelf horizontal air-exhaust manifold of a mouse IVC rack that contains a known fur-mite–positive cage.

Materials and Methods

Animal housing and care. The vivarium at the Medical College of Wisconsin is part of an AAALAC-accredited animal care and use program. Mice were housed in ventilated microisolation caging on commercial racks (model no. MS75JU70MVPSHR-R, Allentown, Allentown, NJ) and maintained under constant environmental conditions (14:10-h light:dark cycle, an average daily relative humidity of 35%, and a temperature range of 69 to 71 °F [20.6 to 21.7 °C]). The mice were fed a commercial diet (5LOD, PMI Nutrition International, Brentwood, MO) ad libitum and given reverse-osmosis–filtered, hyperchlorinated water via an automatic watering system (Edstrom Industries, Waterford, WI). All cages, wire-bar lids, and filter tops were sanitized and autoclaved prior to use. Bedding material consisted of an autoclaved hardwood bedding (SaniChip, PJ Murphy Forest Products, Montville, NJ) and was enriched with autoclaved shredded paper (Enviro-Dri, Shepard Specialty Papers, Watertown, TN). Our rodent sentinel monitoring program includes a quarterly serologic panel for detecting excluded pathogens as well as endo- and ectoparasitic screenings of our sentinel cages that are receiving soiled bedding from the colony cages on their respective racks. Excluded murine pathogens included cilia-associated respiratory bacillus, ectromelia virus, *Encephalitozoon caniculi*, Hantaan virus, lymphocytic choriomeningitis virus, minute virus of mice, mouse adenovirus, mouse cytomegalovirus, mouse hepatitis virus, mouse parvovirus, mouse pneumonitis virus, mouse rotavirus, mouse thymic virus, *Mycoplasma pulmonis*, pneumonia virus of mice, polyoma virus, Prospect Hill virus, reovirus, Sendai virus, Theiler murine encephalomyelitis virus, and pinworms and fur mites. All cage manipulations and changes were performed in a HEPA-filtered, small-animal cage-changing station (model NU612, NuAire, Plymouth, MN). The hood was disinfected between cages by using a chlorine dioxide solution (Labsan C-Dox, Sanitation Strategies, Holt, MI). Nitrile gloves (High Five Products, Chicago, IL) were worn and changed between cage-handling procedures (including cage changes and qPCR sampling). All activities in the current study were approved by the IACUC.

During the course of the study, the airflow to each rack in the room was measured by using a Rack Flow Detector (model RFD, Allentown). The racks had a supply airflow range of 28.9 to 31.8 ft^3/min and an exhaust airflow range of 44.7 to 47.9 ft^3/min. The rack ventilation system supplies uninterrupted positive-pressure air flow to each rodent cage. The blower system is factory-preset to 60 air changes hourly. The rack supply air is equipped with a HEPA-filtered air delivery module. Ambient room air is drawn through a prefilter into the HEPA via a single inlet blower. The HEPA-filtered air supply is directed into a vertical plenum, pressurized, and delivered to a series of horizontal air supply manifolds located on each rack shelf. HEPA-filtered air enters the individual cage through a cage-mounted air supply diffuser. Air is circulated throughout the cage microenvironment, purging each cage with filtered air. The rack also provides for the capture of exhausted cage air that escapes from the cage–lid perimeter. Air is drawn into an exhaust collar surrounding each cage, by using the room's exhaust system. The exhausted air is drawn into each shelf horizontal air exhaust manifold, travels up the vertical exhaust plenum, and then is released into the room exhaust system (Figure 1).

In our facility, racks are washed and autoclaved before they are returned to service. The plenum and manifold doors are opened and manually sprayed to remove gross debris prior to being placed in the rack washer (model MTP 2130, Getinge, Rochester, NY), where they are washed for 5 min (8 min total cycle time) with 180 °F water containing detergent (Labsan 120, Sanitation Strategies). Racks then are autoclaved (model GE 182222 AR2 Steam Sterilizer, Getinge) at 250 °F and 15 psi for 16 min.

Rack exposure. Five cages housing at least 2 mite-infested mice each (Table 1) were selected for the study. Mite infestation was identified initially by PCR and subsequently confirmed as positive by direct observation of live fur mites prior to initiation of the study. A live mite was defined as any mite with moving legs or any other body part during direct examination. Both CD1 (Charles River Laboratories, Wilmington, MA) and an immunocompetent transgenic strain of mice were used. The single cage of CD1 mice was one of our sentinel cages that was positive for *Myobia musculi*; the other 4 cages were donated research mice, which had the HD5Cre strain on a C57BL/6J background, that were positive for *Radfordia affinis*. Each cage was returned to its own 70-cage, single-sided, ventilated rack in the same location (Figure 1). The known-positive cage was placed on the fourth row, closest to the supply side and at the farthest point on the rack from the sampling site (the terminal horizontal exhaust

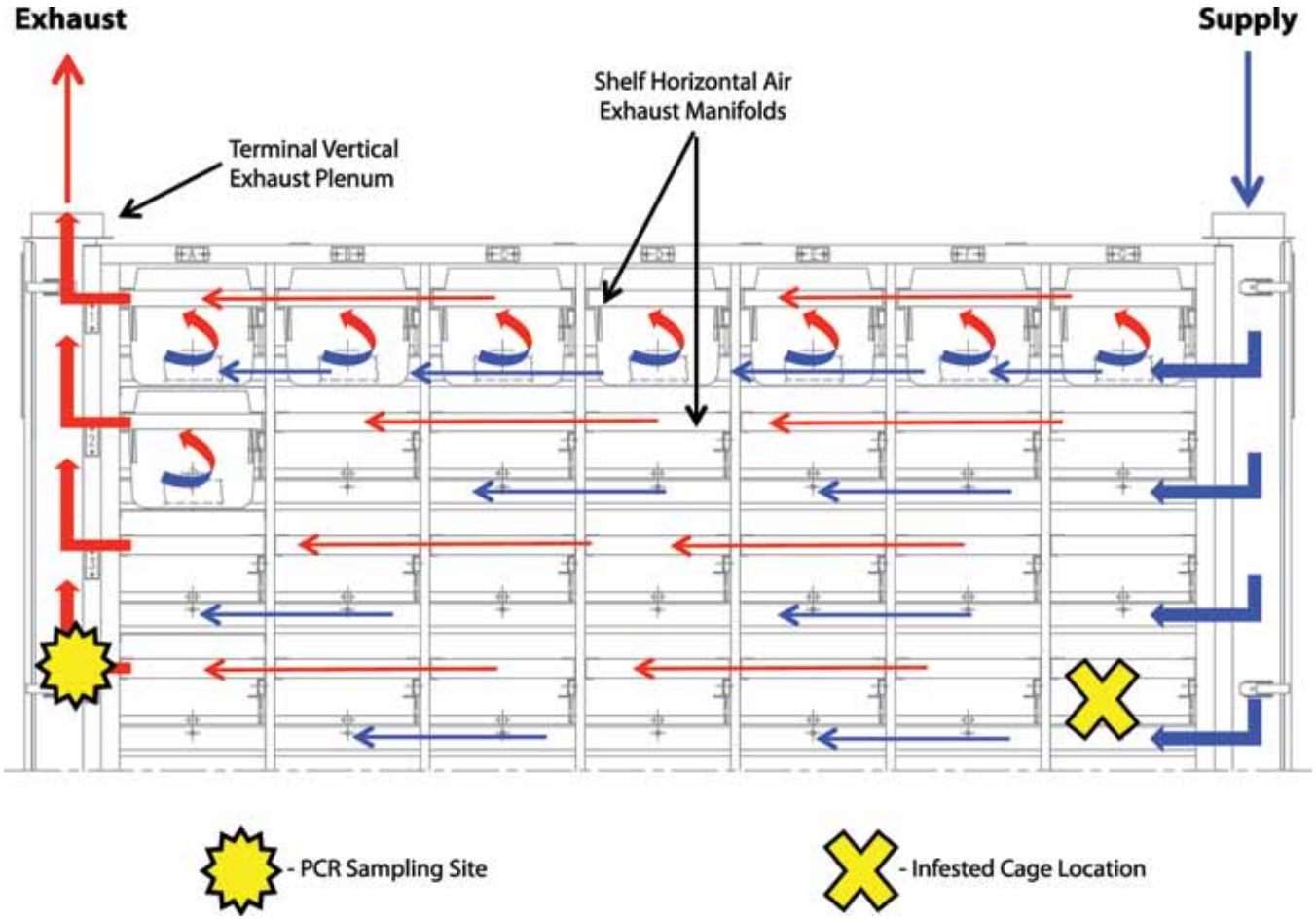

Figure 1. IVC rack schematics, indicating airflow direction, sampling site, and cage location. Blue arrows indicate supply airflow. Red arrows indicate exhaust airflow. Image courtesy of Allentown

Figure 2. View of the backside of the IVC rack, indicating the location of the supply and exhaust vertical plenum covers. The blue-labeled supply cover (left) is closed; the exhaust cover (right) is open, revealing the location of the shelf horizontal exhaust manifolds.

manifold). The other 69 cage slots were filled with complete cage set-ups (including bedding, wire-bar lid, and filter top), but no other mice were housed in these cages during the testing period. All mice were transferred to clean cages at least every 2 wk (or sooner if the cages were excessively soiled). In addition, a single cage containing a trio of CD1 mice that tested both PCR-negative and negative on direct visualization was placed at the same location on a separate IVC rack in the same room and was ventilated by the same interstitial blower unit as were all the other racks in the room. The horizontal exhaust manifold of this negative-control rack was swabbed on the same schedule as were all other racks.

Mite testing. Mice were tested for fur mites by using both direct visualization methods and PCR analysis. The racks were tested for mites by using only PCR analysis.

Direct visualization. Samples for identification of adult mites were collected first by fur plucks from live mice. If the results from fur-pluck samples were inconclusive, then the tape-test method was used for detection. Regardless of the method, samples were obtained from the dorsal rump, dorsal neck and head, and ventral abdominal (including the inguinal) regions. Fur-pluck samples were adhered to a piece of tape and then placed on a glass microscope slide for direct examination.[1] For the tape method, sample-containing tapes were placed directly onto glass slides.[1] All samples were scanned at 40× under a light microscope. At the end of the study, each mouse was examined to ensure that it still harbored live mites. After mice were euthanized by using CO_2, a pelt exam was performed as described previously,[17] except that rather than using transparent shipping tape, we placed each mouse in a culture dish for at least 6 h, with 3 strips of transparent tape covering the dorsal and lateral body areas and a single strip on the ventral abdomen. The strips of tape then were removed, placed on glass slides, and examined for adult mites at 40× power under a light microscope. During both the pre- and postenrollment direct visualization tests, we found at least 1 but not more than 3 adult mites among all the samples from each cage's entire mouse population.

Table 1. Description of study cages

Cage ID	Mite infestation	Mouse strain	Cage population	Sex of mice	Age of mice at study start
C1	*Myobia musculi*	CD1	2	Female	8 mo
C2	*Radfordia affinis*	HD5Cre	4	Female	1 mo
C3	*Radfordia affinis*	HD5Cre	2	1 Female	8 mo
				1 Male	10 mo
C4	*Radfordia affinis*	HD5Cre	2	Female	7 mo
C5	*Radfordia affinis*	HD5Cre	3	Male	1 mo
C6	Negative control	CD1	3	Female	1 mo

Detection of fur-mite DNA. Samples for quantitative PCR (qPCR) analysis were obtained by direct swabbing of the mice and their cage environments by using adhesive swabs obtained from Research Animal Diagnostic Services at Charles River Laboratories. For the mice, the swabs were rubbed against the lay of the hair, concentrating on the rump, dorsal neck, and ventral abdominal–inguinal regions. For the cage environment, the swabs were rubbed through the bedding around the perimeter of the cage as well as the nesting area. The swabs then were submitted to Charles River for qPCR analysis. The swab samples were coded so the diagnostic lab was blinded to the samples submitted. At the testing laboratory, DNA was isolated from sample swabs by automated magnetic isolation and screened for fur-mite DNA by using 2 qPCR assays specific for the 18S ribosomal RNA genes of the *Myobia* and *Radfordia* genera and the *Myocoptes* genus, respectively. Initial positive results were confirmed by repeat testing with qPCR screening assays and species-specific 18S qPCR assays for *M. musculi*, *R. affinis*, *R. ensifera*, and *Myocoptes musculinus*. A PCR inhibition control was used to monitor samples for PCR inhibition.[11] PCR inhibition was not detected in any samples.

Reporting of PCR results. In the current study, we scored equivocal results as positive because the testing laboratory records 'equivocal' in cases when the screening assay yields approximately 10 or fewer target copies per reaction, providing that these results are confirmed by repeat PCR testing with either the screening or species-specific assays. As such, an equivocal result still indicates that mite DNA was present in the sample.

Rack sampling. Prior to the placement of a mite-positive cage on a new rack, a baseline swab sample was obtained from the rack's horizontal air-exhaust manifold on the row where the cage was to be housed. Samples were collected by using the same adhesive swabs as those provided to us by the diagnostic laboratory. Before opening the vertical exhaust plenum cover on the IVC rack, we disconnected the supply and exhaust hoses from the rack (Figure 2). The cage's row manifold was identified, and all 4 sides of the rectangular manifold were swabbed by using a back-and-forth streaking motion (Figure 3). At each testing, approximately 60 cm^2 of the manifold was swabbed to collect accumulated particles originating from the rack cages. Subsequently, once every 7 d, the racks were screened for the presence of the mite DNA at that row's horizontal exhaust manifold. Once a rack tested mite-positive, it was replaced with a new rack, and the entire process began again. In addition, before its replacement on a new rack, a mite-positive cage was swabbed and the sample submitted for fur-mite qPCR analysis to confirm that the cage remained qPCR-positive.

Statistical analysis. All analyses were performed by using R 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria).[12] The cumulative incidence of fur-mite detection was estimated by using the complement of the Kaplan–Meier estimate.

Figure 3. Close up of shelf horizontal exhaust manifold during sampling.

Results

Of the 17 different racks exposed to cages containing mite-positive mice, 14 tested PCR-positive for the known mite species in the test cage. The 3 remaining racks yielded equivocal results (as defined by the testing laboratory) for both the presence and species of mites in their respective cages. As mentioned previously, we consider that these equivocal results indicate mite infestation. By looking at the combined overall detection rate of rack infestation, we reached a 94.1% detection rate by week 4 (Table 2). During the first week of testing, 10 (8 positive, 2 equivocal) of the 17 racks tested PCR-positive. By week 2, 15 (13 positive, 2 equivocal) of the 17 racks tested PCR-positive, and by week 4, 16 (13 positive, 3 equivocal) of the 17 tested mite PCR-positive. The remaining rack tested PCR-positive by week 9. The negative control rack did not yield PCR-positive results during the entire 22 wk of sampling (Figure 4).

Six different racks with a cage of mice infested with *M. musculi* were tested; 5 of the 6 racks were PCR-positive within 1 wk. The remaining rack was mite-positive by the second week (6 positive, 0 equivocal). For the mice infested with *R. affinis*, we tested 11 different racks by using 4 different cages of mice. All but one rack (10 of 11; 7 positive, 3 equivocal) were PCR-positive for mites within 4 wk of placement, with 9 (7 positive, 2 equivocal) of those 10 racks becoming PCR-positive within either 1 or 2 wk of being placed on the rack. One *R. affinis* rack did not yield a mite-positive result until week 9.

At the end of the study, all 6 cages of mice (the 5 mite-containing cages and the negative control cage) were swabbed and assayed for fur-mite DNA. All 5 mite-containing cages tested qPCR-positive for their respective mite species, whereas the negative control remained qPCR-negative. Subsequently, all mice were euthanized by CO_2 asphyxiation and their pelts ex-

Table 2. Cumulative probability of detection of fur mites from infested racks by week

Week	Percentage	95% Confidence interval
1	58.8%	33% to 82%
2	88.2%	64% to 99%
3	88.2%	64% to 99%
4	94.1%	71% to 100%
5	94.1%	71% to 100%
6	94.1%	71% to 100%
7	94.1%	71% to 100%
8	94.1%	71% to 100%
9	100.0%	80% to 100%
10	100.0%	80% to 100%

The cumulative probability of detection is the probability of detection at or before any given time. For all weeks, control racks yielded a percentage of 0.0% with a 95% confidence interval of 0% to 98%.

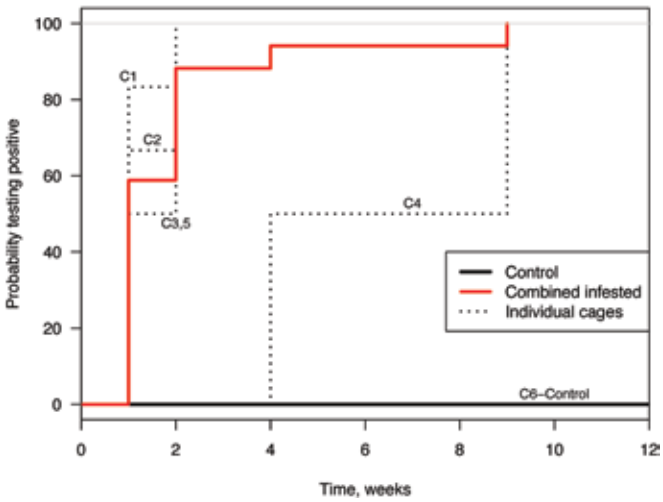

Figure 4. The cumulative probability of fur-mite detection by using the complement of the Kaplan–Meier estimate. C1 through C6 represent the individual test cages. The control cage (C6) was monitored for an additional 10 wk and remained negative.

amined for the presence of adult mites as previously described. Among the 5 mite-containing cages, 4 remained positive for live mites; we found 1 to 3 live adult mites among all of the mice in each of these 4 cages. We were unable to find live mites in one cage (C5; Table 1), in which the mice were infested with *Radfordia affinis*.

Because the censoring time exceeded the longest time to detection, the estimated cumulative incidence of fur-mite detection was equal to the observed proportion of experimental runs with a positive result at or before a given week. Figure 4 shows this cumulative incidence visually, whereas Table 2 gives the estimates with exact binomial-distribution–based 95% confidence intervals.

Discussion

The results of the current study demonstrate that the shelf exhaust manifold of IVC racks is a reliable sampling site for the detection of fur-mite infestations by qPCR methodology. This collection and assay process is sensitive enough to detect (with 94.1% probability) a single infested cage on a rack's row within a 4-wk time period (Table 2). Previous research using the various conventional diagnostic methods, including fur pluck, skin scrape, tape test, and pelt exam, for detecting fur mites have demonstrated variable success in their detection rates.[1,9,13,17] In addition, it is important to recognize that these same direct examination methods can result in false-negative results if the infestation is very light.[1,8,15] The use of the rack's exhaust system for detecting murine pathogens is not a novel concept and has proven to be effective for detecting both mouse hepatitis virus and Sendai virus via PCR analysis of gauze filters placed in the exhaust system.[3] But to our knowledge, our method of using the mite PCR assay in this manner to detect fur mites has not been published previously.

Racks were exposed by placing a single cage on a row of an IVC rack to determine whether the assay would detect the lowest possible infestation on a row (that is, 1 of 7 cages). Furthermore, the cage was placed in the position on the shelf farthest from the sampling site to test the method's sensitivity. This strategy may explain why 3 of the 17 positive assays were reported as equivocal. However, as previously indicated, we scored the equivocal results as positive for the current study. Equivocal results still indicate the presence, albeit in low copy numbers, of mite DNA in the collected sample. Therefore, from a practical perspective, an equivocal result may warrant the same action on the part of the animal facility as would a positive result.

Because we enrolled mite-positive cages as they became available to us, we did not attempt to standardize each cage (for example, strain, age, sex, mite species, cage population, age at the time of mite infestation). Therefore, 3 cages had 2 mice per cage, and the remaining cages had 3 to 4 mice per cage (Table 1). We did not attempt to quantify the mite burden in each cage. Our criteria for enrollment were both a positive qPCR assay and visualization of at least one adult mite from that cage's mice. We had 2 strains of mice that were presented to us for study and 2 different species of mites. The CD1 mice were infested with *M. musculi*, and the other 4 mite-positive cages contained HD5Cre mice that were infested with *R. affinis*.

The 2 racks exposed to an adult pair of HD5Cre mice infested with *R. affinis* (cage C4) did not return qPCR positive results until weeks 4 and 9 of the study. Although the mice in this cage remained qPCR-positive throughout the study and yielded at least one adult mite on direct examination prior to and at the conclusion of the study, these colony-raised mice were 7 mo old at the time of study enrollment and likely had been infested for most of their life. Because mite populations are known to increase during new infestations and then decrease to equilibrium during weeks 8 to 10 of infestation, we suspect this cage likely had a low-level infestation, leading to less mite DNA present and thus requiring more time until detection.[4,5] Cage C4 was not the same cage in which no adult mites were found at the terminal pelt examination.

The testing methodology we describe here not only takes advantage of the sensitivity and specificity of the mite PCR assay but also uses the ventilation system of the IVC rack itself for monitoring and identification of the mite status of the entire rack's mouse population. Each shelf (or row) can be sampled by using an individual swab; the swab samples can then be pooled and submitted as a single sample for each rack (for example, as many as 10 swabs for a 10-row rack). This sampling method likely would save considerable time and labor and therefore would be a cost-saving measure for an institution using this assay. In addition, this strategy allows the user to sample the entire rack (a maximal 70-cage sample size for this rack model) as compared with either only sampling a single sentinel cage or taking random samples (that is, a 10% population sample size) from that rack's cages.

We presume that similar sampling methods would also be effective for other IVC rack makes or models as long as the air exhausted from the cages is not filtered before reaching the sampling site. One should also consider the distance of infested cages from the sample site (in other rack styles), in light of our prestudy sampling experience in which sampling at the terminal rack exhaust plenum did not reliably detect mite infestations. Obviously, our method would not be applicable to screening animals housed in static microisolation caging. However, in such caging configurations, one could consider obtaining samples from the underside of the rim of the microisolation lid or swabbing both the soiled bedding sentinels and random colony animals on the rack. Regardless, we believe that sampling only soiled bedding sentinels for PCR and traditional direct visualization are not the most reliable ways to detect fur-mite infestations in mouse colonies. Future aspects to consider include evaluation of the sensitivity of each shelf (or row) on the IVC rack as its own individual zone. The results of this assessment might be useful if different mite species were on different rows or for screening or narrowing down an infestation to a specific row of cages, rather than having to sample all of the individual cages on a rack.

In summary, our studies using the murine fur mite PCR assay indicate that the horizontal shelf manifolds of IVC racks can reliably be used as the primary sampling site for monitoring the rodent population on the rack. This method does not rely on the infestation of a sentinel cage, and it accommodates surveying of the entire rack population by using a very sensitive and specific PCR assay.

Acknowledgments

The statistical analysis was supported by grant 1UL1RR031973 from the CTSA program of NIH. We would like to thank Kami Young, Lisa King, Tracy Smelter, and Frank Bartell for their technical assistance. We would also like to thank Dipica Haribhai and Jennifer Ziegelbauer for their help in providing the HD5Cre mice.

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