G-500900-001

Journal of Veterinary
Science & Technology

Miller et al., J Vet Sci Technol 2016, 7:4
http://dx.doi.org/10.4172/2157-7579.1000343

Short Communication Open Access

Exhaust Air Particle PCR Detects *Helicobacter hepaticus* Infections at Low Prevalence

Manuel Miller*, **Bärbel Ritter**, **Julia Zorn** and **Markus Brielmeier**

Research Unit Comparative Medicine, German Research Center for Environmental Health GmbH, Neuherberg, Germany

Abstract

Reliable detection of unwanted organisms in experimental animal colonies is an essential requirement for biomedical research. Since most rodents are housed in Individually Ventilated Cage (IVC) systems which prevent aerogenic transmission of pathogens, soiled-bedding sentinels are commonly used for routine health monitoring. In this study, we investigated whether testing of Exhaust Air Particle (EAP) samples by real-time PCR (EAP PCR) might be a valid alternative to SBS for the detection of *H. hepaticus* infections. EAP PCR detected *H. hepaticus* infections at a minimal prevalence of 1/63 cages within one week. EAP PCR provides a sensitive, simple, and reliable alternative to SBS for *H. hepaticus* identification in laboratory mouse colonies.

The 3R (Replacement, Reduction, Refinement) are the principle framework for the ethical use of laboratory animals in biomedical research [1]. "Replacement" refers to the preferred use of alternatives over the use of laboratory animals whenever possible to achieve the same scientific purpose. Health monitoring programs for laboratory mouse colonies housed in Individually Ventilated Cage (IVC) systems most commonly use Soiled-Bedding Sentinels (SBS) as representatives if housing conditions, immunodeficiency or insufficient numbers of animals do not allow the direct sampling of resident animals [2]. This method results in high numbers of used animals, since SBS are usually euthanized for testing. In addition, since transmission of many pathogens is insufficient to SBS and infections are often overseen [3-5], alternative monitoring approaches should be investigated. Testing of particles that accumulate on exhaust air prefilters in IVC racks via PCR has been shown to have the potential to reduce or even replace SBS. Several studies tested the feasibility of that method for mouse hepatitis virus, Sendai virus, and mouse parvovirus [3] as well as *Radfordia affinis* and *Myobia musculi* [6]. Recently, we have demonstrated that the method is superior to SBS monitoring for detection of *P. pneumotropica* [7]. *Helicobacter hepaticus* is one of the most prevalent bacterial pathogens in experimental animal facilities [8]. Compton et al. failed to detect *H. hepaticus* on gauze pieces placed in the exhaust prefilter of IVC systems [3]. Accordingly, the recommendations for health monitoring of rodent colonies by the Federation of European Laboratory Animal Science Associations [2] mention that *H. hepaticus* cannot be easily transferred via exhaust air dust. Here, we re-investigated whether exhaust air particle (EAP) PCR is suitable for detection of *H. hepaticus* infections at low prevalence. To this end, we identified 15 *H. hepaticus*-positive, immunocompetent female mice by testing single fecal samples with a sensitive and specific real-time PCR and housed them in groups of five animals per cage. Mice came from colonies found positive during standard microbiological monitoring. A *H. hepaticus*-negative colony consisted of immunocompetent male AVM: ICR mice housed in groups of two to five animals per cage. All procedures were in accordance with German Animal Welfare Legislation (TierSchG, TierSchVersV, VersTierMeldV). All animals were housed in the 63 cage experimental IVC rack (Seal Safe Plus, Tecniplast, Buggugiate, Italy) in IVC cages (GM 500, Tecniplast, Buggugiate, Italy), with a cage density and environmental conditions according to directive 2010/63/EU. Air-handling units were run with 60 air changes per hour in positive pressure mode (15-22 Pa). Autoclaved wood fiber (Lignocel ¾ S, J. Rettenmaier & Söhne GmbH, Rosenberg, Germany) and nesting material (Crinkle Nature, J. Rettenmaier & Söhne GmbH) were used for bedding and nesting. Sterile-filtered tap water and an irradiated standard diet for rodents (Altromin 1314, Altromin Spezialfutter GmbH, Lage, Germany) were available ad libitum. Using a decreasing number of *H. hepaticus*-positive mice within the experimental IVC rack, we investigated whether exhaust air dust analysis is suitable for the reliable detection of *H. hepaticus*. Prior to the start of the experiment the microbiological state of the negative colony was confirmed by testing 10 fecal samples per cage. The cleaned and autoclaved experimental IVC rack connected to a cleaned air-handling unit was then populated with the *H. hepaticus*-negative colony. One gauze piece (2 × 2 cm) was pinned directly above the opening of the exhaust air hose on a new exhaust air prefilter within the air handling unit. This gauze piece and the prefilter were removed and analyzed after a baseline testing week, to exclude any remaining DNA contamination within the IVC rack or air-handling unit, and replaced with a new prefilter containing two new gauze pieces before adding the *H. hepaticus*-positive mice to the experimental rack. Three rounds of EAP-PCR each with a baseline test in-between were performed with 3, 2, and 1 cage(s) with each 5 *H. hepaticus*-positive mice. One gauze piece was collected one week after the positive mice entered the experimental rack, the second gauze piece and a sample (2 × 2 cm) cut out of the prefilter material with disinfected forceps and scissors were collected after two weeks. After three weeks in total, the rack was washed and autoclaved and a new experimental round was launched. At the end of the three experimental rounds, the negative colony was re-tested to exclude *H. hepaticus* transmission within the rack. DNA isolation from gauze pieces and prefilter material was performed using a modified phenol/chloroform extraction method. DNA isolation of fecal samples was performed using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) with minor amendments [7]. A *H. hepaticus* specific real-time PCR [9] was performed using the Rotor Gene Q instrument (Qiagen, Hilden, Germany). DNA template (2 µl) was added to a reaction

***Corresponding author:** Manuel Miller, Research Unit Comparative Medicine, German Research Center for Environmental Health GmbH, Neuherberg, Germany, Tel: 498931874838; E-mail: manuel.miller@helmholtz-muenchen.de

Received May 20, 2016; **Accepted** May 27, 2016; **Published** June 01, 2016

Citation: 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: 343. doi:10.4172/2157-7579.1000343

Copyright: © 2016 Miller M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

mixture consisting of 4 µl of 5× HOT FIREPol® Probe qPCR Mix Plus (Solis BioDyne, Tartu, Estonia), 300 nM of each primer, 250 nM probe, and ultrapure water in a 20 µl total reaction volume. The thermocycling parameters were: initial denaturation at 95°C for 15 min, and 40 cycles with denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s. Prior to the beginning of the experiment as well as after three rounds, each cage containing *H. hepaticus* negative mice was tested via feces real-time PCR with all samples tested negative. No cross-contamination occurred between the infected and non-infected colonies. In all three experimental rounds the absence of residual *H. helicobacter* DNA in IVC rack was confirmed by baseline testing with all samples tested negative. In all instances, with prevalences of 3, 2 or 1 cage(s) per rack, the presence of the *H. hepaticus*-positive mice was detected by EAP- PCR already after one week of gauze exposure to exhaust air and confirmed after two weeks by analyzing the second gauze piece and a prefilter sample. We demonstrated that EAP-PCR is a reliable strategy to identify *H. hepaticus* infections in mouse colonies housed in IVC systems even at low prevalence. The alternative testing method of EAP-PCR has the potential to constitute a major contribution to the 3R in two ways. First, the number of sentinels needed for microbiological monitoring might be reduced; second better microbiological information helps to improve experimental conditions and thereby contributes to refinement. The number of agents with proven detectability by EAP-PCR increases rapidly. We are therefore convinced that this simple and reliable alterative monitoring method has the potential to become a broadly used tool for health monitoring surveillance of rodent populations.

References

1. Russell WMS, Burch RL (1959) The Principles of Humane Experimental Technique. Johns Hopkins Bloomberg School of Public Health.
2. Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, et al. (2014) FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim 48: 178-192.
3. Compton SR, Homberger FR, Paturzo FX, Clark JM (2004) Efficacy of three microbiological monitoring methods in a ventilated cage rack. Comp Med 54: 382-392.
4. Artwohl JE, Cera LM, Wright MF, Medina LV, Kim LJ (1994) The efficacy of a dirty bedding sentinel system for detecting Sendai virus infection in mice: a comparison of clinical signs and seroconversion. Lab Anim Sci 44: 73-75.
5. Scharmann W, Heller A (2001) Survival and transmissibility of *Pasteurella pneumotropica*. Lab Anim 35: 163-166.
6. 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.
7. Miller M, Ritter B, Zorn J, Brielmeier M (2016) Exhaust Air Dust Monitoring is Superior to Soiled Bedding Sentinels for Detection of *Pasteurella pneumotropica* in Individually Ventilated Cage Systems. J Am Assoc Lab Anim Sci.
8. Pritchett-Corning KR, Cosentino J, Clifford CB (2009) Contemporary prevalence of infectious agents in laboratory mice and rats. Lab Anim 43: 165-173.
9. Fischer G (2004) Helicobacter infections in rodents: establishment of new real-time PCR assays, cultivation and infection experiments with selected Helicobacter species. Thesis. Vetsuisse-Fakultät Universität Zürich, Institut für Labortierkunde.

OMICS International: Publication Benefits & Features

Unique features:

- Increased global visibility of articles through worldwide distribution and indexing
- Showcasing recent research output in a timely and updated manner
- Special issues on the current trends of scientific research

Special features:

- 700+ Open Access Journals
- 50,000+ editorial team
- Rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at major indexing services
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: www.omicsonline.org/submission/

Citation: 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: 343. doi:10.4172/2157-7579.1000343