Comparative Immunology, Microbiology and Infectious Diseases 50 (2017) 29–33

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Transmission of *Coxiella burnetii* to cage mates using murine animal model

Yassina Bechah*, Didier Raoult

Aix Marseille Université, Unité de Recherche sur les Maladies Infectieuses Transmissibles et Emergentes (URMITE), UM63, CNRS 7278, IRD 198, Inserm 1095, WHO Collaborative Center for Rickettsioses and Other Arthropod Borne Bacterial Diseases, Faculté de Médecine, 27 bd Jean Moulin, 13385 Marseille Cedex 5, France

ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form 4 November 2016
Accepted 14 November 2016

Keywords:
Coxiella burnetii
Animal
Aerosol route
Transmission
Cage
Mates
Feces
Urine

ABSTRACT

Aerosols from the products of abortions of infected animals with Coxiella burnetii were known to be the main source of infection in humans with this bacterium. However, little is known about the excretion of C. burnetii in the feces and urine of infected mice, or the dynamic of transmission between infected and healthy mice. To investigate whether C. burnetii can be excreted in the feces and urine of infected mice and whether transmission to uninfected cage mates occurs, male mice were inoculated with C. burnetii using a "whole body aerosol system" and feces and urine were collected different time points post-infection from these mice. One hour post exposure to aerosols, uninfected mice was placed with infected mice and the transmission was monitored using blood, and organs biopsies collected after sacrifice of contact mice different time points post-contact. Bacterial DNA was not detected in the feces and urine of infected mice at 3, 7, 14 and 28 days post-inoculation suggesting that C. burnetii was not excreted in the feces and urine and consequently they cannot be source of contamination. However, based on the positive PCR results for lungs, blood, spleen, tracheal lymph nodes and cervical lymph nodes, some of the contact animals were considered contaminated at 8 days post-contact. These results indicated that transmission of C. burnetii to contact animals occurs, and it is unlikely that feces and urine act as source of this transmission. Further experiments are needed to clarify the exact mode of contamination.

1. Introduction

Coxiella burnetii, an intracellular bacterium, is the causative agent of a zoonosis, Q fever, whose distribution is worldwide [1]. Birth materials of farm infected animals (goats, sheep, and cattle) are the principal source for *C. burnetii* contamination of humans and environment. However, birth products of domestic animals have also been reported as a source. Usually, human contamination with this bacterium occurs via the aerosol route after contact with infected animals or contaminated dust but infection via the digestive route after absorbing inadequately pasteurized dairy products was also reported [2,3]. The excretion of *C. burnetii* in infected ruminants via feces, urine, semen, milk and vaginal mucus was also reported [4–7], and human cases of infection are linked to these shedding paths.

In literature, the role of wild vertebrates in the introduction of *C. burnetii* in the domestic cycle has been reported [8,9], especially from rodents, however an attempt to demonstrate transmission within rats failed [10].

Mice have been used to study *C. burnetii* infection since the discovery of this organism. These models resulted in the systemic distribution of bacterium, including liver, lung, heart and spleen, and mice displayed ruffled fur and lethargy, but no mortality [11–14]. The abortifacient potential of *C. burnetii* and the risk of endocarditis developing in pregnant mice have been also reported [15]. More recently our team reported that live *C. burnetii* persisted in murine adipose tissue for prolonged periods after apparent clinical cure [16]. The presence of *C. burnetii* DNA in the bone marrow of patients with Q fever was also reported without knowing if the bacteria are alive [17]. However little is known about the excretion of *C. burnetii* in the feces and urine of infected mice, or the dynamic of transmission between infected and healthy mice.

The purpose of this study consisted of investigating the presence of *C. burnetii* in the feces and urine of infected mice and then to evaluate whether secondary cases caused by infectious mice occur in uninfected mice.

* Corresponding author.
E-mail address: yassina.bechah@univ-amu.fr (Y. Bechah).

http://dx.doi.org/10.1016/j.cimid.2016.11.009
0147-9571/

2. Materials and methods

2.1. Ethics statement

Adult male BALB/c mice obtained from Charles River laboratories, were handled according to the rules of Decree N° 2013–118, February 7, 2013, France and the experimental protocol (reference APAFIS #725-2015052815417382) was approved by the Ethics Committee "C2EA-14" of Aix-Marseilles University, France and the "French Ministry of National Education, Higher Education and Research".

2.2. Bacterial preparation

C. burnetii organisms (Z3055 isolate, related to the clone of Q fever outbreak in the Netherlands) [18] were cultured in vero cells. Cell monolayers were infected for 7–10 days after which bacteria were harvested, sonicated, purified, and titrated as previously described (16). Bacterial viability was assessed using the LIVE/DEAD BacLight kit (Molecular Probes).

2.3. Study design overview

Mice were exposed to *C. burnetii* aerosols or PBS aerosols as negative controls using Inhalation Exposure System "whole-body" type A4224 (Glas-Col LLC, Terre Haute, USA). 90 BALB/c mice were placed in specific cages and transferred to the device for aerosolization. Then 5 ml of a pure PBS solution or one containing 5×10^7 of *C. burnetii* phase I were introduced into the venturi vial to generate aerosols for the period of one hour. This procedure was repeated twice. One hour after exposure to aerosols, a contact mouse (uninoculated) was randomly placed with five inoculated mice (Fig. 1). A total of 18 contact mice were placed, then the cages were transferred to an isolator and housed in a specific facility. The mice were fed sterile food and water ad libitum. The animals were observed twice a day for any signs of discomfort or distress.

Blood samples from contact mice were collected by orbital puncture for DNA extraction and serology one day before contact, then at 3, 8, 14 and 28 days post-contact with the inoculated animals. At 3, 8, 14 and 28 days post-contact, contact mice were sacrificed and lungs, spleen, tracheal lymph nodes, cervical lymph nodes were collected and conserved at −80 °C until DNA extraction and quantitative real-time PCR (qPCR) assays were performed.

Samples of feces and urine from inoculated mice were also collected at 0, 3, 8, 14, and 28 days post-infection.

2.4. Molecular detection of C. burnetii

DNA was extracted from blood, organ samples, feces and urine specimens using the QIAamp Tissue Kit (Qiagen) in accordance with the manufacturer's recommendations and protocols. qPCR was performed using the CFX96® qPCR Detection System (Bio-Rad, France), and carried out with 5 μl DNA extract with specific primers and probe targeting a fragment of the *C. burnetii* 16 s DNA gene, as previously described [16]. Additionally, an in-laboratory control was included, to check for PCR inhibitors in the DNA extracts from feces targeting a conserved fragment of the 16s rRNA gene, for detecting all bacteria [19], or targeting the synthetic sequence "Tiss" added to urine samples before DNA extraction as previously reported [20].

2.5. Serology

Blood samples were left to clot at room temperature and centrifuged. The serum was collected and titrated for the presence of *C. burnetii* specific antibodies as previously described [15]. In brief, slides with smears of formaldehyde-inactivated *C. burnetii* phase I or phase II organisms were incubated with serial dilutions of serum for 30 mins. Antigens were labeled with FITC-conjugated goat Abs directed against mouse IgG and IgM (Beckman Coulter) at a 1:100 dilution for 30 mins. After washing, the slides were examined by fluorescence microscopy. The starting dilution for the serum sample was 1:25, and samples were tittered to end point.

3. Results

3.1. Mice infection and C. burnetii presence in feces and urine

C. burnetii was inoculated to BALB/c male mice using a "whole-body aerosol system".

Exposed mice demonstrated signs of illness from day 3 post-inoculation (PI) (ruffled fur), persisting for 2–3 days after the mice recovered. Just after exposition to aerosols (day 0), lungs were sampled and analyzed for the presence of *C. burnetii* DNA using qPCR. Bacterial DNA was found in all tested lungs, with about 10^4 DNA copies per lung confirming the ability of the "whole-body aerosol system" to infect mice. DNA copies increased at day 9 PI by about three logs (1.54E + 07 ± 3.38 DNA copies/per/lung), demonstrating significant replication of bacteria compared to day 0 ($p < 0.05$).

The presence of *C. burnetii* DNA in the feces and urine of inoculated mice was also evaluated in 10 mice at days 3, 14 and 28 PI and in 60 mice at day 8 PI. Both the qPCRs for 16 s rRNA of all bacteria in feces and the Tiss sequence in urine, showed cycle threshold values of (14.2 ± 0.5, 261 ± 2, respectively) for samples collected at day 3 PI, of (15.5 ± 1.4, 25.4 ± 3) for those sampled at day 8, of (16.4 ± 6, 23.4 ± 3,3) for the samples collected at day 14 and of (14.7 ± 1.1, 27.5 ± 0.2) for those sampled at day 28. Occasionally, traces of *C. burnetii* DNA were detected in some tested samples of feces and urine; the highest values of these positive samples were < 100 DNA copies: the highest value for feces samples was 82 DNA copies per 5 μl DNA extract, and for urine was 39 DNA copies per 5 μl DNA extract (see detailed results in Table 1).

Our results showed the absence of excretion of *C. burnetii* in the feces and urine of infected mice.

3.2. C. burnetii transmission to cage mates

The transmission of *C. burnetii* to cage mates placed with inoculated mice was monitored by checking the presence of *C. burnetii* DNA in blood and several tissue samples collected from contact mice after sacrifice at 3, 8, 14 and 28 days post-contact.

For all tested kinetics, we did not see any external signs of illness in the control mice placed with the infected mice.

No bacterial DNA was detected in the blood and tissue biopsies collected from contact mice sacrificed at 3, 14 or 28 days post-contact with inoculated mice. However, at day 8 post-contact, 4 of the 12 contact mice tested positive for *C. burnetii* DNA (Table 2). Indeed, the qPCRs results showed that one contact mouse tested positive in blood, lungs, tracheal lymph nodes and cervical lymph nodes with DNA copy values of 7.63E + 02/5 μl DNA, 1.69E +06/5 μl DNA, 2.07E + 04/5 μl DNA and 3.16E + 02/5 μl DNA, respectively.

The other three contact mice tested positive in only one tissue sample each, with DNA copy values of 5.58E + 02/5 μl DNA in spleen for the first, 1.58E + 04/5 μl DNA in blood for the

second, and 5.1E + 03/5 μl DNA in tracheal lymph nodes for the third.

4. Discussion

C. burnetii infects a wide large of domestic and wild mammals, birds, reptiles, arthropods and fish [21–24]. However, livestock (sheep, goats and cattle) and pets are considered the main sources