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Research Article

Examination of vertical transmission of *Bartonella quintana* in body lice following multiple infectious blood meals

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One sentence summary: Vertical transmission of *Bartonella quintana* from infected adult lice to their eggs is not transovarial, but rather involves nontransovarial contamination with feces.
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Abstract

Bartonella quintana is a re-emerging louse-borne pathogen. Horizontal transmission from the body louse vector (*Pediculus humanus humanus)* to a human host occurs through contact with infectious louse feces containing a high concentration of the bacteria. However, questions have remained about whether vertical transmission from infected vectors to their progeny, which could significantly influence the dynamics of transmission to humans, occurs in body lice. To address this subject, we performed a series of controlled laboratory experiments that examined the presence of *B. quintana* on the surface of and within eggs produced by female body lice that were provisioned multiple infectious blood meals to recapitulate the natural pathogen acquisition process. Our results demonstrate that *B. quintana* DNA can be detected from the surface of eggs by qPCR due to vertical transfer of infectious feces to the egg sheath during or after oviposition. However, viable *B. quintana* could not be cultured from the hemolymph of adult female lice or from within eggs that were surface sterilized, indicating a lack of true transovarial transmission. Based on this evidence, vertical transfer of *B. quintana* from infected adult lice to their eggs probably has a limited impact on the dynamics of transmission to humans.

Keywords: lice, louse, vector, *Bartonella quintana*, vertical, transmission

Introduction

The bacterium *Bartonella quintana* is a neglected, re-emerging louse-borne pathogen that can cause trench fever, endocarditis, and bacillary angiomatosis (Anderson and Neuman 1997, Cheslock and Embers 2019). Infections primarily occur among socioeconomically vulnerable and medically underserved populations such as the homeless. *Bartonella quintana* is acquired by the body louse vector (*Pediculus humanus humanus*) during blood feeding on a bacteremic human host and subsequently replicates in the lumen of the louse gut (Ito and Vinson 1965, Seki et al. 2007, Kim et al. 2017). Louse feces expelled after subsequent blood meals contain a high concentration of viable *B. quintana,* and inoculation of infectious feces into abraded skin or mucus membranes is the primary route of transmission to a human host (Byam and Lloyd 1920, Bruce 1921).

Many vector-borne viral and bacterial pathogens (e.g. *Rickettsia* spp.) can be vertically maintained from one generation of the vector to the next after horizontal acquisition in a blood meal. This typically occurs via transovarial passage from an infected female to its offspring but can also take place through nontransovarial contamination of eggs and/or larvae by the excreta of infected adults (Burdorfer and Varma 1967). The vertical transmission process can amplify the infectious vector population without the need for additional pathogen acquisition events from an infected host, thus playing an important role in the dynamics of horizontal transmission to humans.

There is evidence of vertical transmission of *Bartonella spp.* in diverse arthropods. A *Bartonella washoensis*-like bacterium has been detected in ovary tissue from multiple rodent flea species, supporting likely transovarial transmission (Brinkerhoff et al. 2010.) However, in fleas, vertical transmission of *Bartonella henselae* is not transovarial (Chomel 2011, Morick et al. 2011, Bouhsira et al. 2013). Rather, studies of *Xenopsylla ramesis* have shown that nontransovarial vertical transmission involves infection of larvae by gut voids and feces from adults (Morick et al. 2013). In the tick *Rhipicephalus sanguineus*, detection of *B. henselae* DNA in an artificial blood meal fed upon by larvae derived from infected adults suggested vertical transmission (Wechtaisong et al. 2021). Similarly, both eggs and larvae produced by *Ixodes ricinus* infected with *B. henselae* were found to contain DNA from the pathogen (Król et al. 2021). Moreover, in the deer ked *Lipoptena cervi*, *Bartonella schoenbuchensis* is passed from females to developing larvae at a high rate (De Bruin et al. 2015). In contrast, no form of vertical transmission of *Bartonella bacilliformis* appears to take place in sand flies (*Lutzomyia* spp.; Battisti et al. 2015).

Questions have remained about the occurrence of vertical transmission of *B. quintana* in lice due to some seemingly conflicting reports (Amanzougaghene et al. 2020). Initial investigations into the transmission mechanisms of trench fever prior to the isolation of *B. quintana* indicated that feces from offspring of female lice fed on infected humans were not infectious, arguing against stable vertical transmission of the causative agent (Bruce

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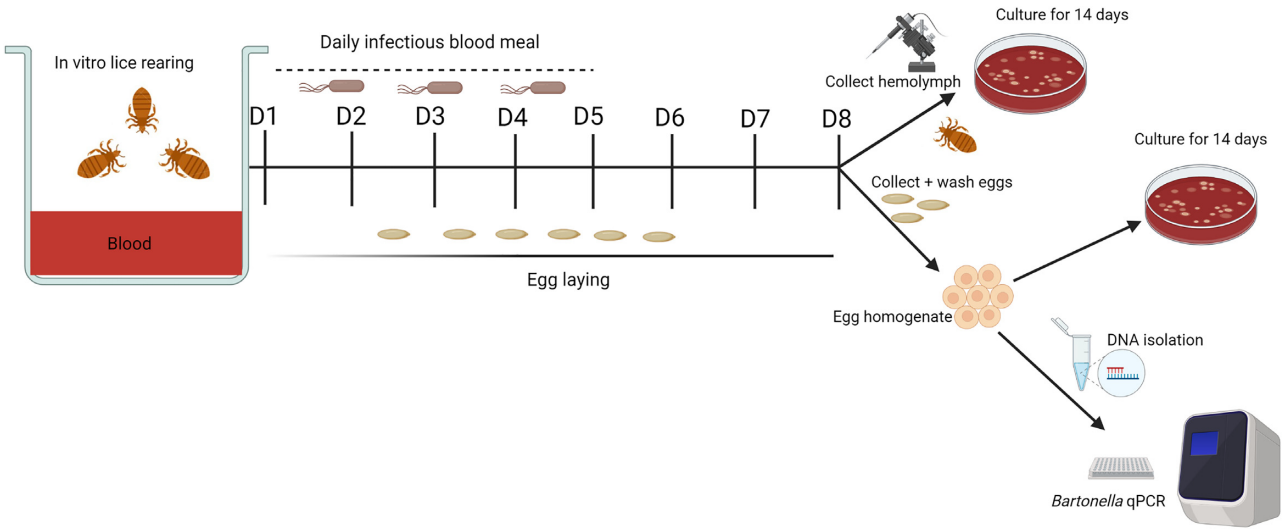

Figure 1. Experimental schematic. Created with BioRender (publication license CH23TPXR29).

1921). A more recent laboratory study that employed both qPCR and culture-based detection did not find *B. quintana* in eggs produced by adult female lice that were fed a single infectious blood meal (Fournier et al. 2001). Nonetheless, the experimental design did not mimic the natural feeding behavior of lice, which take multiple daily blood meals from their host, and the lice in the aforementioned study harbored low internal bacterial loads (7–20 CFU/louse). On the other hand, a later field study intriguingly detected *B. quintana* DNA in louse eggs collected from an infested human subject, indicating the possibility of some form of vertical transmission (Angelakis et al. 2011). However, it was unclear whether the eggs in this study were washed to remove surface contaminants and whether the detected *B. quintana* was viable, as culture was unsuccessful. A follow-up report extended the findings of Angelakis et al. (2011) by demonstrating qPCR detection of *B. quintana* in first instar nymphs hatched in the laboratory from eggs collected from homeless persons but again did not definitively exclude surface contaminants nor determine bacterial viability (Drali et al. 2014).

Our present work aimed to specifically address the unresolved question of whether any form of vertical transmission of *B. quintana* takes place in the body louse vector. Critically, we employed a controlled experimental design capable of discerning between nontransovarial vertical transmission via fecal contamination of eggs and true transovarial passage following administration of multiple infectious blood meals to recapitulate the natural pathogen acquisition process (Fig. 1).

Materials and methods

Louse infection

Groups of adult female body lice from a colony maintained on aseptic, mechanically defibrinated blood (Hemostat, Dixon, CA) via an artificial membrane feeding system as previously described (Pietri and Ray 2020) were fed blood inoculated with a physiologically relevant dose of *B. quintana*. To realistically recapitulate the natural pathogen acquisition process, the lice were provided an infectious blood meal daily for 5 days. In brief, the JK-7 strain of *B. quintana* (BEI Resources, Manassas, VA) was grown on blood agar plates in a candle extinction jar at 37°C until growth of a confluent layer (i.e. a lawn) was observed. *Bartonella quintana* was harvested using a cell scraper, and the resulting pellet was resuspended in heart infusion broth (HIB). The bacterial suspension was standardized to an OD_{600} of 1.0 in HIB (~5.2×10^7 CFU/ml as determined by plating of serial dilutions on blood agar) and further diluted 1:100 (v/v) in blood to reach a final concentration of ~5.2×10^5 CFU/ml in the experimental blood meal that was immediately administered to lice via the membrane feeding system. Between the daily blood feeds, the lice were maintained in a dedicated incubator under nonsterile conditions. A total of three independent infection replicates were conducted.

Egg collection

Females used in experiments mated with male lice in the colony prior to infectious blood feeds as well as throughout the duration of the experimental period (Fig. 1). Three days after the fifth infectious blood meal was administered, eggs produced by infected females during the experimental period were gently collected with forceps and pooled for analysis. The eggs were washed sequentially with 10% bleach, 70% ethanol, and sterile PBS by vigorous mixing to kill any viable bacteria contaminating their surface. Then, the eggs were homogenized in sterile HIB using a pestle.

Detection of *B. quintana* by qPCR

For detection of *B. quintana* DNA by qPCR, DNA was isolated from a portion of the washed egg homogenate using the DNeasy blood and tissue kit (Qiagen, Germantown, MD) according to the manufacturer's protocol. qPCR was performed on a QuantStudio 3 instrument (Applied Biosystems, Waltham, MA) using the PowerUp SYBR Green Master Mix (Applied Biosystems) with previously published primers targeting the *16S rRNA* gene of *B. quintana* (Minnick et al. 2003). Amplification conditions were set to the instrument default for a fast run: 95°C for 20 seconds, followed by 40 cycles of 1 second at 95°C, and 20 seconds at 60°C. Reactions with no DNA template were run as negative controls. To confirm specific amplification, the PCR product was cloned into a pCR 4-TOPO plasmid in *Escherichia coli* using the TOPO TA Cloning Kit according to the manufacturer's protocol (Invitrogen, Carlsbad, CA), and Sanger sequencing was performed.

Table 1. Summary of results. "+" indicates detection of *B. quintana*. "Negative" indicates *B. quintana* was not detected. "CT" = cycle threshold. "NP" indicates the experiment was not performed.

	Replicate 1	Replicate 2	Replicate 3
# eggs tested	12	20	41
qPCR of washed egg homogenate	+ (CT = 33.98)	+ (CT = 28.66)	+ (CT = 34.25)
Culture of washed egg homogenate	Negative	Negative	Negative
Culture of hemolymph	NP	Negative ($N = 4$)	Negative ($N = 5$)

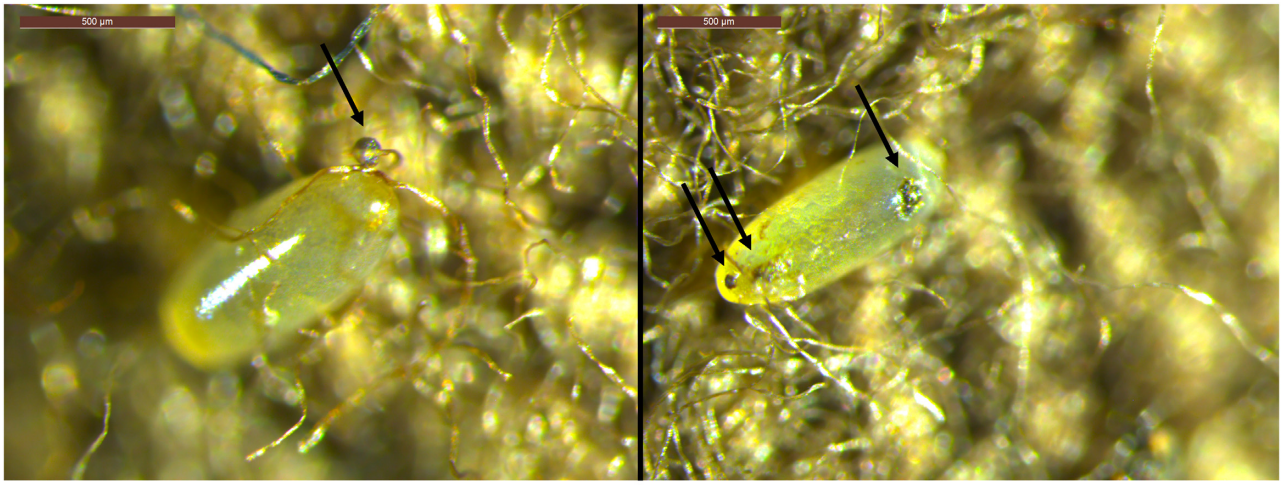

Figure 2. Fecal contamination of the louse egg sheath. After oviposition, fecal contamination was frequently visualized on the egg sheath by stereomicroscopy (black arrows). Scale bars = 500 µM.

Detection of B. quintana by culture

For detection of viable *B. quintana* via culture, the remaining washed egg homogenate not used for qPCR was plated directly on blood agar and incubated in a candle extinction jar at 37°C for 14 days before checking for growth. A homogenate of washed eggs produced by lice fed sterile blood served as a negative control, and swabs of the surface of unwashed eggs were also cultured. In two of the infection replicates, hemolymph from the adult female lice was analyzed. The hemolymph was collected from the lice into sterile PBS at end of the experimental period using a pulled glass capillary needle as previously described (Potts et al. 2022) and examined for the presence of *B. quintana* by direct culture as above.

Stereomicroscopy of eggs

To examine whether *B. quintana* may contaminate the egg surface (sheath) by passive transfer in the feces, separate intact eggs were visualized by stereomicroscopy using a Leica M165FC instrument (Leica, Wetzlar, Germany) and photographed with a DFC 310FX camera.

Results

The results of all *B. quintana* detection experiments are summarized in (Table 1). In replicate 1, *B. quintana* DNA was detected in the washed egg homogenate by qPCR. The mean cycle threshold (CT) value of duplicate reactions was 33.98, and Sanger sequencing revealed an amplicon of the expected length that was a 100% match (65/65 nucleotides, 0 gaps, and e-value = $4e^{-26}$) with the *16S rRNA* gene of numerous strains of *B. quintana* present in GenBank (e.g. strain MF1-1, sequence ID AP019773.1). However, culture of the washed egg homogenate did not yield growth of *B. quintana* or any other organisms after 14 days, consistent with the negative control of washed eggs derived from lice fed sterile blood. On the other hand, cultures of swabs from the surface of unwashed eggs were replete with fast growing commensal bacteria. These results importantly confirmed that louse embryos are free of culturable bacteria and that our washing protocol was effective at killing contaminating bacteria commonly associated with lice (e.g. *Staphylococcus* spp.) (Agany et al. 2020) that was present on the surface of the eggs.

In replicate 2, qPCR detection of *B. quintana* was again achieved from the washed egg homogenate (mean CT value of triplicate reactions = 28.66), but culture resulted in no growth. In replicate 3, the results of the previous replicates held consistent. Further, in replicates 2 and 3, no *B. quintana*-like colonies were apparent after culture of hemolymph from adult females. However, it should be noted that several colonies of a fast-growing, hemolytic commensal were apparent in all hemolymph cultures, which could have affected the growth of any *B. quintana* present in the samples. We did not identify these bacteria in the present study, but we have previously isolated multiple species of *Staphylococcus* from the guts of body lice and suspect these same bacteria may sporadically contaminate the hemolymph.

Interestingly, visualization of eggs by stereomicroscopy revealed frequent contamination of the egg sheath with small amounts of louse feces (Fig. 2). In some instances, the fecal contamination appeared to be on the external surface of the egg sheath, indicating it was deposited after the sheath hardened, whereas in other instances it appeared to be partially integrated within the sheath, suggesting it was deposited on the egg during or rapidly after oviposition before the sheath hardened (Kim et al. 2021).

Discussion

Our work comprehensively re-examined the possibility of vertical transmission of *B. quintana* in the body louse vector, ultimately reconciling conflicting results from previous laboratory and field studies and uncovering strong evidence against the occurrence of true transovarial transmission.

Our results are consistent with and build upon the observations of Angelakis et al. (2011), who detected *B. quintana* DNA from field-collected louse egg samples. That is, our ability to detect *B. quintana* DNA by qPCR from washed eggs laid by infected females, along with the lack of detection of viable bacteria in their hemolymph or within eggs by culture, together indicate that *B. quintana* can be present as a surface contaminant of the egg sheath rather than incorporated within the developing embryo. Indeed, stereomicroscopy revealed frequent fecal spotting on the sheath of eggs laid by female body lice. It is not surprising that these fecal spots can result in positive qPCR findings even after washing the eggs, given the high concentration of *B. quintana* known to be excreted in the feces (Seki et al. 2007, Kim et al. 2017). Based on our culture results, our washing protocol appears to be effective at killing viable bacteria present on the egg sheath, but it is unlikely that washing could remove all contaminating traces of genomic DNA. Nonetheless, if transovarial transmission were taking place, one would expect viable *B. quintana* to be present within the egg even after surface washing as well as within the hemolymph of infected females, as the hemolymph serves as a route of dissemination from the gut to the ovaries for vertically transmitted pathogens.

Fecal contamination of the egg sheath may further explain how *B. quintana* DNA was previously detected from first instar louse nymphs hatched in the laboratory from eggs collected from homeless persons (Drali et al. 2014). It is plausible that contamination could transfer from the egg sheath to nymphs as they eclose. A similar phenomenon appears to occur in bed bugs experimentally infected with *B. quintana* (Leulmi et al. 2015). In bed bugs, immunohistochemistry found that *B. quintana* was present only in the gut of adults and not in the ovaries. Yet, viable *B. quintana* could be cultured from a suspension of eggs laid by infected insects and its DNA could be detected from eclosed nymphs. The authors attributed these observations to contact with infectious feces. Given that Fournier et al. (2001) failed to detect even DNA from the pathogen on eggs produced by female body lice after a single infectious blood meal, we speculate that nontransovarial vertical transfer only happens when sufficiently high doses of *B. quintana* are consumed. The phenomenon may, therefore, be of natural relevance as the load of *B. quintana* in human subjects varies dramatically (Rolain et al. 2002) and lice typically consume daily infectious blood meals.

While vertical nontransovarial transfer by fecal contamination may occur in nature, it probably has limited implications for transmission to humans. Unfed nymphs eclosing from contaminated eggs are unlikely to become contaminated with sufficient bacteria to be infectious by the established vector-borne mechanism. Unlike flea larvae, louse nymphs do not consume conspecific feces and, therefore, there is no efficient route for *B. quintana* that contaminates the egg sheath or cuticle to enter the gut of nymphs to replicate. Thus, adult lice probably do not amplify the infectious vector population as would occur with transovarial transmission. No less, a small possibility that nontransovarial vertical transfer of *B. quintana* could contribute to stercorarian transmission in some rare occasions cannot be completely excluded. For example, instances of body louse eggs discovered on clothes in secondhand markets have been reported (De Liberato et al. 2019). Since *B. quintana* can remain viable in louse feces for months (Bruce 1921), clothes harboring eggs contaminated with *B. quintana* could potentially facilitate inoculation of the pathogen into a new host in the absence of live lice.

While the findings we report here provide strong evidence against transovarial transmission, further high-resolution imaging studies (e.g. FISH) to rule out the presence of *B. quintana* in the ovaries and embryos of infected lice could provide additional support for our conclusion (Perotti et al. 2007).

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Conflict of interest statement. None declared.

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