Indhu *et al. Zoonoses* (2024) 4:3
DOI 10.15212/ZOONOSES-2023-0042

SHORT COMMUNICATION

Rodents/Shrews and their Ectoparasites are not Associated with the Enzootic Maintenance and Transmission of *Coxiella burnetii* to Livestock and Humans in Puducherry, India

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ABSTRACT

Objectives: "Q fever," which is caused by *Coxiella burnetii*, is endemic in India. In addition to livestock, rodents have also been reported to be associated with enzootic maintenance, favoring pathogen transmission. Currently, however, no data are available on the role of rodents in "Q fever" transmission in India.

Methods: A cross-sectional study was undertaken in 39 Puducherry villages to screen *Coxiella burnetii* in synanthropic rodents (rats and shrews) and their ectoparasites (ticks, mites, and fleas) by real-time and conventional PCR protocols targeting the pathogen specific *IS 1111* and *com 1* genes.

Results: One hundred forty animals were trapped (107 shrews and 33 rats). The ticks, mites, and fleas infesting the rodents were identified as *Rhipicephalus sanguineus*, *Leptotrombidium deliense* and *Schoengastiella* spp., and *Xenopsylla cheopis*, respectively. PCR screening of the DNA extracted from the rodent/shrew blood samples and their ectoparasites tested negative for *C. burnetii*.

Conclusions: Synanthropic rodents, such as rodents/shrews and their ectoparasites do not have a pivotal part in the enzootic maintenance and spread of Q fever to humans and livestock in Puducherry.

Keywords: Rodents, ectoparasites, *C. burnetii*, PCR, Q fever, *Suncus murinus*, *IS 1111* gene

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Received: October 17 2023
Revised: November 9 2023
Accepted: November 27 2023
Published Online: January 4 2024

INTRODUCTION

Coxiella burnetii infection causes "Query (Q) fever" in humans and animals. Q fever is ranked as one of the top 13 priority zoonoses globally and has been designated as one of the most contagious diseases [1]. *C. burnetii* infects a wide variety of mammals (particularly rodents), reptiles, and birds [2]. Cattle, sheep, and goats are the primary reservoir hosts and are responsible for the majority of human infections. The primary mode of transmission in humans involves inhalation of aerosolized bacteria spread from infected reservoirs, while the primary mode of transmission in animals involves inhalation of infectious organisms

and ingestion of contaminated feed and bedding [3]. In addition to aerosol transmission, ticks have been shown to exhibit vector competence in transferring pathogen to its hosts. Additionally, transstadial and transovarian transmission of *C. burnetii* in ticks has been reported. Moreover, ticks aid in the transmission of the pathogen to wild and domestic animals [4]. Natural infections have been reported in > 40 species of Ixodidae and Argasidae ticks [5]. The brown dog tick, *Rhipicephalus sanguineus*, has tested positive for *C. burnetii* [6]. Transmission of the Q fever agent by tick bites in humans has not been established; however, there are reports of humans acquiring Q fever infection by crushing the tick between the fingers [7] and *via* dried faeces containing spore-like forms of *C. burnetii* [3].

Rodents have been reported as reservoirs for Q fever; however, rodent contribution to pathogen maintenance, transmission, and geographic spread remains to be elucidated. Small rodents serve as an important intermediate linking the sylvatic and domestic cycles, thereby contributing to *C. burnetii* transmission from rodents-to-livestock and incidentally to humans [8]. In this study we have clarified the role of synanthropic rodents/shrews and their ectoparasites in the epidemiology of Q fever under natural settings in Puducherry, India. Such data are essential to assess the mode of maintenance and spread of *C. burnetii* and risk of human infection in the future.

MATERIALS AND METHODS

This study was conducted in 39 villages within the Union Territory of Puducherry (Fig 1). The Institutional Animal Ethics Committee (IAEC-2018/ ICMR-VCRC/P-2) approved the study. Rodents and shrews were trapped in randomly selected villages using Sherman traps.

The traps were set by 5:00 pm and retrieved by 6:30 am the next morning. As the trapped rodents and shrews were potential sources of other zoonotic infections, the trapped animals were immobilized by exposure to chloroform to avoid accidental handling injuries. The anesthetized animals were euthanized by injecting an overdose of pentobarbital sodium (250 mg/kg) via the intraperitoneal route. The euthanized rodents were identified after recording their morphologic features [9]. Blood samples (0.5-1.0 ml) were collected from rodents and shrews via direct cardiac puncture using sterile syringes. The ears, snout, axillary regions, and limbs of individual rodents/shrews were examined under a stereo microscope. Ectoparasites were retrieved using thin tweezers and preserved in labelled vials containing 70% ethanol. The ectoparasites were identified based on the morphologic characteristics using standardard taxonomic keys [10-15]. The following formula was used to calculate the ectoparasite index: ectoparasite index = total number of ectoparasites collected/total number of animals examined.

A commercially available DNA extraction kit (GenElute Blood Genomic DNA kit; Sigma-Aldrich, St. Louis, MO, USA) was used to extract DNA from the ectoparasites and rodent blood samples following the manufacturer's protocols. Preliminary screening for *C. burnetii* in rodent/shrew and ectoparasite DNA was carried out using real-time PCR by targeting the 70-bp fragment of the *IS 1111* gene [16]. The samples that tested negative by real-time PCR were re-screened for *C. burnetii* according to the published protocol of Dhaka et al. [17] and De Bruin et al. [18] and Zhang et al. [19] to amplify the *IS1111* and *com 1* genes, respectively. A *C. burnetii*-positive DNA sample (kindly provided by Dr. Stephen Selvaraj, Professor of Microbiology at MGAMRI, Puducherry) was used as a PCR-positive control and standardization of all PCR assays.

RESULTS

Details of the trapped rodents/shrews and ectoparasites

In this study a total of 724 traps were placed and 140 animals were trapped (a trap-positive rate of 19.34%). Of the 140 animals trapped, 33 were identified as *Rattus rattus* and 107 were identified as *Suncus murinus*. Tick infestation was noticed in 15 animals, of which 11 were *S. murinus* and 4 were *R. rattus*. Mite infestation was detected in 89 trapped animals, of which 79 were *S. murinus* and 10 were *R. rattus*. Flea infestation was detected in one *R. rattus*. In total, 57 ticks, 3290 mites, and 6 fleas were collected. The tick-, mite-, and flea-positive rates are shown in Table 1. The retrieved ticks were identified as *Rhipicephalus sanguineus*, the mites were identified as *Leptotrombidium deliense* and *Schoengastiella* spp., and the fleas were identified as *Xenopsylla cheopis*.

Molecular detection of *C. burnetii* in rodent/shrew and ectoparasite DNA samples

The DNA samples from 140 rodents and shrews, 45 ticks (9 pools), 1375 mites (55 pools), and 6 fleas (1 pool) tested *C. burnetii* negative using real-time and conventional PCR methods (Fig 2A-D).

DISCUSSION

The lack of *C. burnetii* in the present study is in agreement with the results obtained by Sahu et al. [20], who reported that of 38 rodents collected from paddy fields adjoining the goat farms in Chattishgarh and Odisha, none were positive for *C. burnetii* [20]. Pluta et al. [21] did not detect *C. burnetii* among 119 rodents trapped from 3 Q fever endemic areas in southern Germany. Similarly, Minichova et al. [22] reported zero prevalence of *C. burnetii* in rodents in Slovakia. However, Reusken et al. [23] reported the presence of *C. burnetii* in black and brown rats trapped from animal farms located close to bulk milk-positive goat farms associated with a Q fever outbreak in The Netherlands. Gonzalez et al. [24] reported the presence of *C. burnetii* in micromammals, such as *Apodemus* spp., *Crocidura* spp. and *Rattus rattus* in Spain. Alotaibi et al. [25] demonstrated *C. burnetii* DNA in 17.5% of rodents trapped in Saudi Arabia. The absence of *C. burnetii* in rodents and

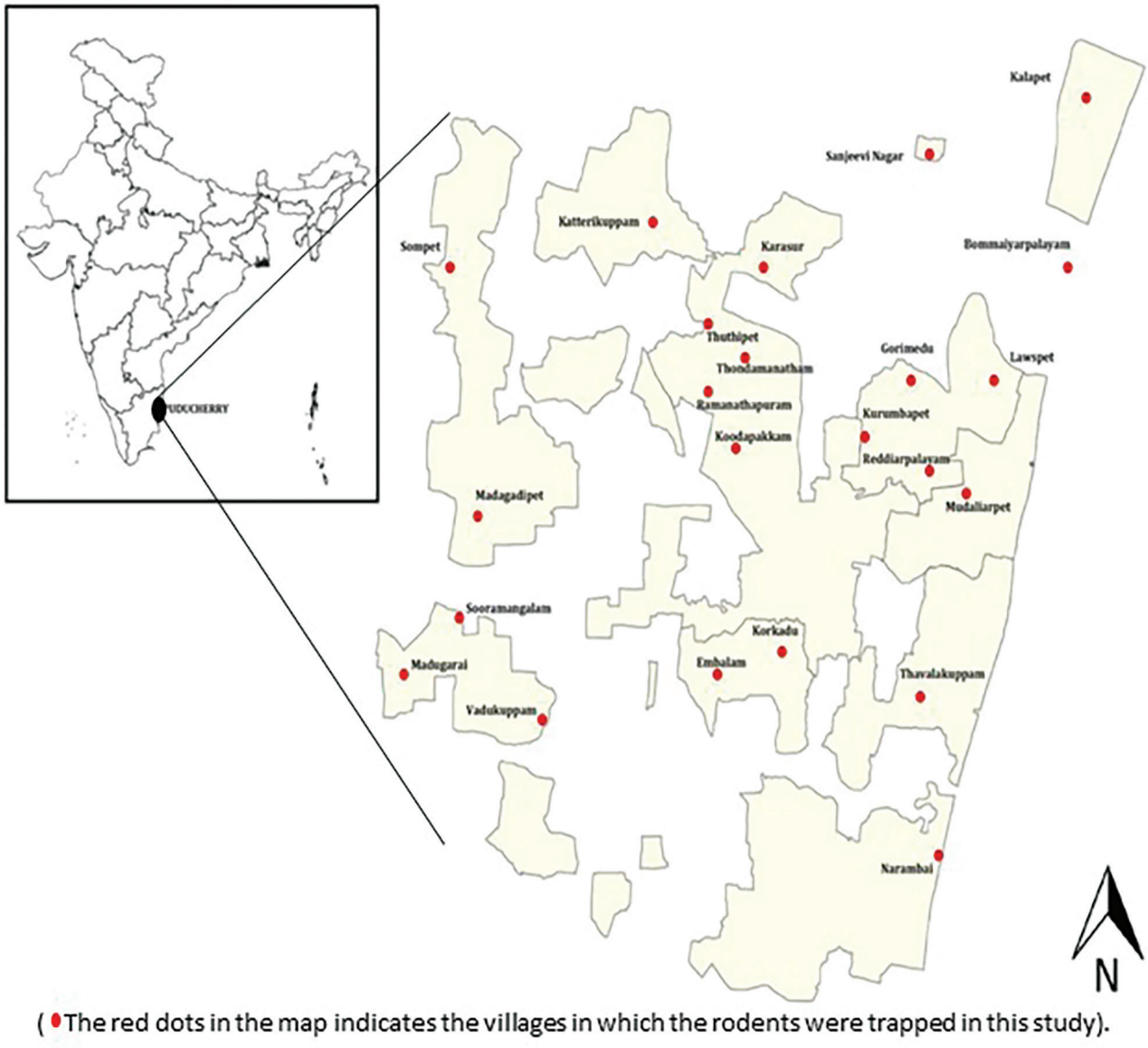

FIGURE 1 | Map representing the villages in which the rodents were trapped in Puducherry.

shrews in the current study could be due to a lack of pathogen exposure from infected livestock or neutralization and clearance of the pathogen by the antibodies developed in exposed rodents/shrews.

In addition to the molecular evidence, exposure to *C. burnetii* in rodents was also confirmed based on serologic results. Meredith et al. [26] reported an overall seroprevalence of 17.3% among rodents in the UK (range, 15.6%–19.1%) based on species. Hence, a sero-surveillance in rodents/shrews in the current study would have helped confirm exposure to *C. burnetii;* however, this remains a major limitation.

TABLE 1 | Ectoparasite positivity rate and index in the trapped animals.

Species of rodents/ shrews trapped (No. of animals trapped)	Number of animals positive for ticks	Tick positivity rate	Number of ticks collected	Tick index	Number of animals positive for mites	Mite positivity rate	Number of mites collected	Mite index	Number of animals positive for fleas	Flea positivity rate	Number of fleas collected	Flea index
Rattus rattus (n=33)	4	12.12%	7	0.21	10	30.3%	7	0.21	1	3.0%	6	0.18
Suncus murinus (n=107)	11	10.28%	50	0.47	79	73.83%	3283	30.68	0	0.0%	0	0.0

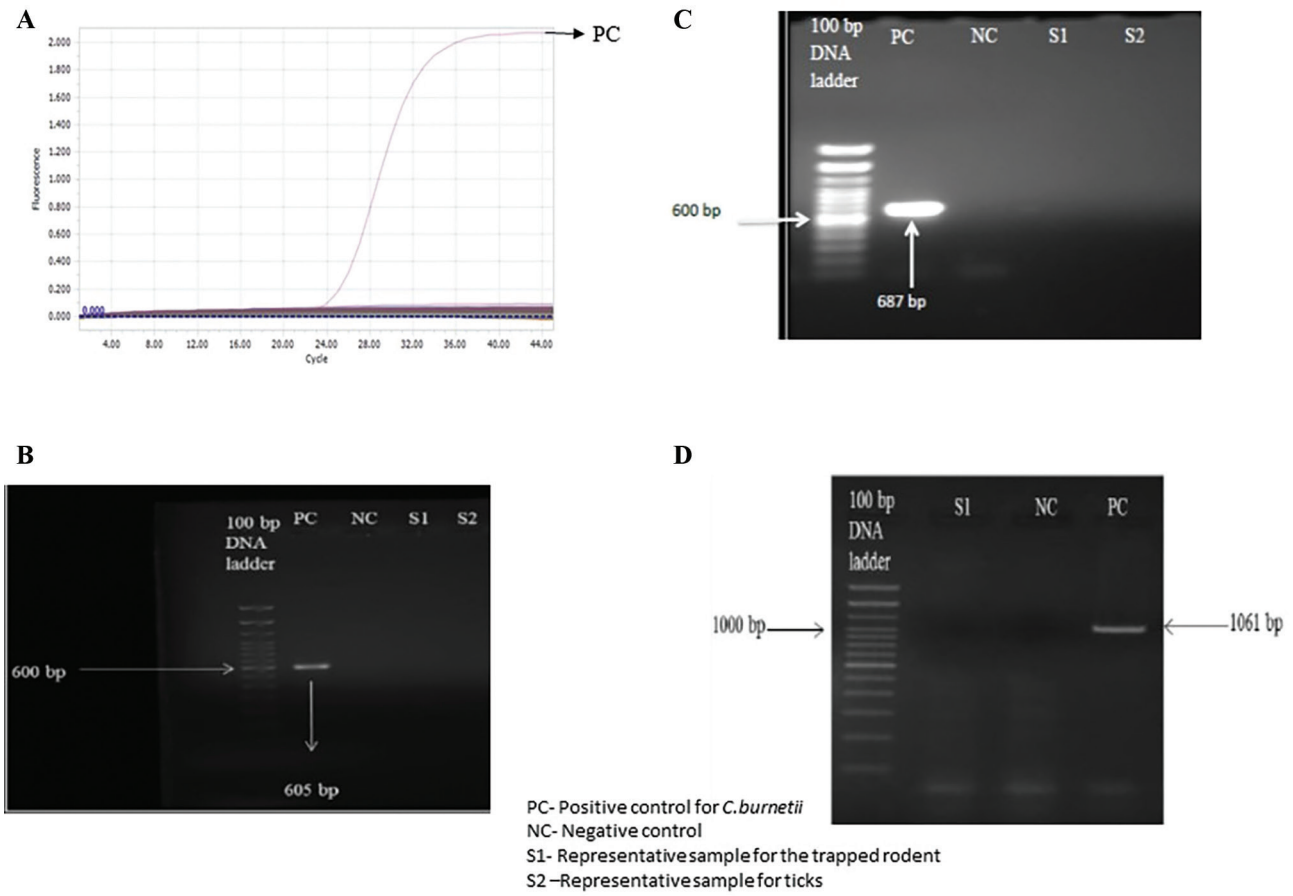

FIGURE 2 | (A) Real-time PCR screening of *C. burnetii* in rodents/shrews and their ectoparasites targeting the *IS 1111* gene. (B) Results of screening *C. burnetii in* rodents and their ectoparasites by PCR targeting the *IS1111* gene. (C) Results of screening *C. burnetii in* rodents and their ectoparasites by trans- PCR targeting the *IS1111* gene. (D) Results of screening *C. burnetii in* a rodent by PCR targeting the *com1* gene.

None of the ticks (n=45), mites (n=1375), and fleas (n=5) infesting rodents and shrews tested positive for *C. burnetii*. Our findings are consistent with earlier reports of *C. burnetii* absence among 8593 tick samples in Slovakia [22]. Similarly, Kamani et al. [27] did not detect *C. burnetii* in rodent ticks (*Rhipicephalus sanguineus*), mites (*Haemolaelaps* spp. and *Hemimerus talpoides*), and fleas (*Xenopsylla cheopis* and *Ctenophthalmus* spp.) in Nigeria. Our findings suggest that the natural foci of *C. burnetii* are limited, which accounted for our negative results in the ectoparasites.

The *IS1111* gene is a transposase-like insertion sequence with a wide range of copy numbers (7–100 copies per genome), offering higher sensitivity of *C. burnetii* detection by PCR. It has been reported that ticks also harbor *Coxiella*-like endosymbionts (CLEs), which may also test positive for the *IS1111* gene by PCR, leading to false-positive reports of *C. burnetii* [28]. Therefore, we also performed screening with PCR targeting the *com1* gene, which encodes outer membrane protein 1 with a single copy in the *C. burnetii* genome [4].To rule out non-specific amplifications, a standard positive control was also used. We believe that the absence of *C. burnetii* in rodents and their ectorparasites might be due to the absence of the pathogen or very low-copy numbers of pathogen DNA.

Given that serologic and molecular evidence in Puducherry indicated exposure to *C. burnetii* in sheep, goats [29], and a buffalo [30], future longitudinal studies with serologic and molecular markers are warranted in rodents. Such investigations will help to delineate the factors facilitating the sustenance and spread of *C. burnetii* in Puducherry.

Overall, our findings indicate that the enzootic maintenance of *C. burnetii* and its transmission *via* ticks in rodents/shrews has a minor role in the tansmission of Q fever to animals and humans in Puducherry compared to the major route of transmission by aerosol from the infected livestock. Further longitudinal studies are warranted to delineate the influence of seasonal variations and the role of rodents and their ectoparasites in Q fever disease dynamics.

ACKNOWLEDGEMENTS

We sincerely acknowledge the extramural grant received from the Indian Council of Medical Research (ICMR), New Delhi to carry out this study. We thank the Dean of Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry for supporting this study by providing all the facilities. The technical assistance provided by Mr. S. Rajkumar and Mrs. S. Pushpa is also acknowledged. This study was funded by the Indian Council of Medical Reseaerch (ICMR), New Delhi (F. No: 81/14/2019-Nano/BMS).

CONFLICTS OF INTEREST

All the authors declare that there are no conflicts of interests.

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