

# First report of *Coxiella burnetii* and *Borrelia burgdorferi* sensu lato in poultry red mites, *Dermanyssus gallinae* (Mesostigmata, Acari), related to urban outbreaks of dermatitis in Italy

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## Abstract

The poultry red mite (PRM), *Dermanyssus gallinae*, is a nonburrowing haematophagous nest-dwelling ectoparasite of birds; occasionally it bites humans, inducing dermatitis. The possibility that this parasite may also be involved in transmission of pathogens is an additional concern. We investigated the presence of zoonotic agents in PRMs from bird nests and pets, and related them to urban outbreaks of dermatitis. A total of 98 PRMs from 12 outbreaks of PRM dermatitis that occurred in Italian cities from 2001 to 2017 were molecularly investigated for detection of *Coxiella* spp. (16S rRNA), *Chlamydomphila* spp. (16S rRNA), *Rickettsia* spp. (17 kDa protein-encoding gene), *Borrelia burgdorferi* sensu lato (*groEL* gene) and *Bartonella* spp. (16S–23S rRNA intergenic spacer). Of the 12 tested mite pools, one was positive for *Coxiella burnetii* (100% identity) and two for *B. burgdorferi* sensu lato (99% with *Borrelia afzelii*). For the first time, the presence of *B. burgdorferi* sensu lato and *C. burnetii* is reported in PRMs from urban areas. Birds, mainly pigeons, can harbour both pathogens. Therefore, birds and their nest-dwelling PRMs may play a role in the epidemiology of these infections.

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## Introduction

Zoonotic haematophagous mites belonging to the *Dermanyssidae* and *Macronyssidae* families (Mesostigmata, Acari) are well known for their capacity to attack and bite humans when their natural primary hosts (birds or rodents) are not available [1,2]. In particular, the cosmopolitan poultry red mite (PRM) *Dermanyssus gallinae* (De Geer, 1778) represents relevant veterinary and medical issues [3]. In Europe, most human cases of dermanyssosis are due to exposure to infested poultry in industrial or rural farms. Thus, dermanyssosis is considered an occupational hazard for poultry industry operators [4].

However, an increasing number of PRM dermatitides is being reported from urban dwellers. Urban outbreaks are usually linked to infested bird nests, mainly those of pigeons, located on window ledges or in air conditioners located in close proximity to windows [3,5–7]. More occasionally, humans may come in contact with PRM through infested pets such as canaries and gerbils [7,8].

After the mite bite and the inoculation of salivary fluids, subjects develop erythematous, papular eruptions usually associated with itching. Apart from its potential role in eliciting allergic reactions, the possibility that *D. gallinae* may act as a vector of infectious diseases should not be overlooked. However, although this vector role remains to be definitively confirmed, a large number of microorganisms have been found associated to PRMs [3,9], including pathogens such as *Salmonella enterica* [9], *Chlamydia psittaci* [10] and avian influenza A virus [11]. However, the available information is scarce, as it is mainly focused on poultry and animal pathogens, and such analyses have not been performed on PRMs causing human urban outbreaks, with the exception of a study that revealed the



**FIG. 1.** Skin eruptions caused by *Dermanyssus gallinae* (poultry red mite) bites in 54-year-old woman.

presence of *Bartonella quintana* in *Dermanyssus* sp. mites during an outbreak of trench fever in the Czech Republic [12].

We investigated the presence of zoonotic agents in PRMs collected from urban patients with pruritus and dermatitis.

## Materials and methods

### Red mite collection

During 2016 and 2017, a total of 98 PRM samples related to 12 outbreaks of itching dermatitis were sent to the Medical Entomology Laboratory of the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata. Parasites were manually collected in 12 dermanysiosis foci from a hospital, a public office and ten private residences, for a total of 31 subjects. The possible origin of mites were pigeon nests (10/12), sparrow nests (1/12) and pet canaries (1/12). All patients had pruritic, erythematous papules that in some cases were relieved by systemic and/or local antihistaminic and corticosteroid treatment (Fig. 1). Collected mites were identified according to morphologic keys [1,2,13] and stored in plastic vials in 70% ethanol.

### DNA extraction and molecular analyses

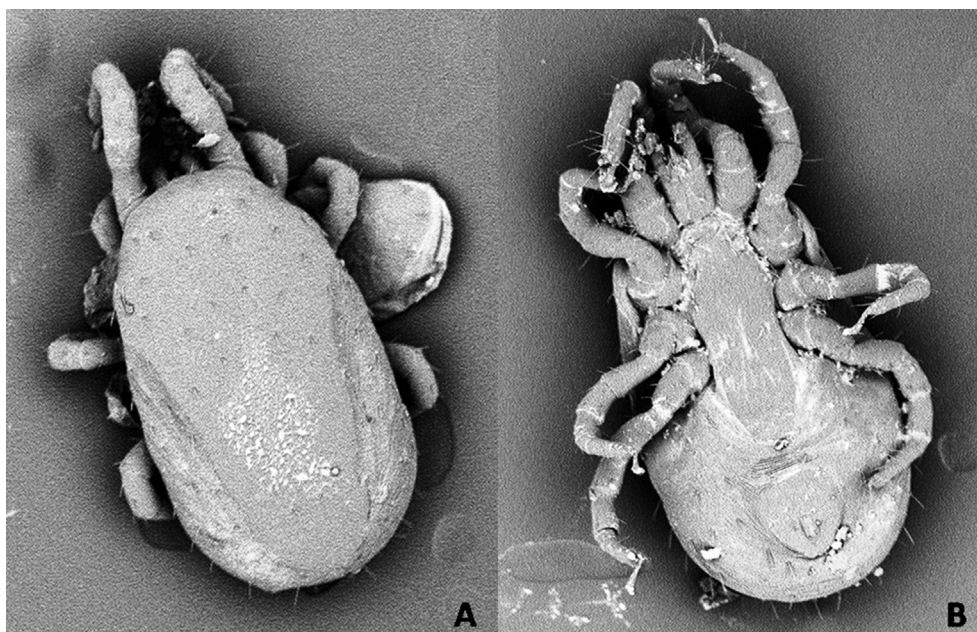
Mites from each focus were pooled, for a total of 12 studied pools. The origin and size of each pool are listed in Table 1. Total DNA was extracted from each pool by using the DNeasy Blood and Tissue Kit (Qiagen, Milan, Italy) following the manufacturer's instruction. Before DNA extraction, arthropods were ground in lysis buffer with sterile mortars and pestles. Five distinct PCR assays were performed, using specific primers for *Coxiella* spp. (16S rRNA) [14], *Chlamydia* spp. (16S rRNA) [15], *Rickettsia* spp. (17 kDa protein-coding gene) [16], *Borrelia burgdorferi* sensu lato (*groEL* gene) [17] and *Bartonella* spp. (16S–23S rRNA intergenic spacer) [18]. Amplicons were sequenced, and nucleotide sequences were compared to GenBank using BLAST ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)).

Samples previously tested positive for *Bartonella henselae*, *Coxiella endosymbiont of Rhipicephalus bursa*, *Rickettsia raouli*, *Chlamydia abortus* and *Borrelia garinii* were used as positive

**TABLE 1.** Cases of dermanysiosis and detected pathogens

Case (date/region)	Collection site	PRM source	No. and sex of affected people	No. of pools (size)	Pool positive	Detected pathogen species	GenBank accession no.
2017/09 Puglia	Bedroom	Pigeon nest	1F	1 (5)	–	–	–
2017/06 Puglia	Bedroom	Pigeon nest	1F, 1M	1 (10)	–	–	–
2015/09 Puglia	Bedroom	Pet canary	1F	1 (10)	–	–	–
2011/10 Puglia	Hospital	Pigeon nest	4 (2M, 2F)	1 (10)	–	–	–
2011/10 Puglia	Bedroom	Pigeon nest	3 (2M, 1F)	1 (8)	<i>Borrelia burgdorferi</i> sensu lato	<i>Borrelia afzelii</i>	KY828976
2009/06 Puglia	Bedroom	Sparrow nest	1F	1 (5)	<i>B. burgdorferi</i> sensu lato	<i>B. afzelii</i>	KY828977
2008/05 Basilicata	Bedroom	Pigeon nest	3 (1M, 2F)	1 (10)	<i>Coxiella</i> sp.	<i>Coxiella burnetii</i>	KU215908.1
2007/05 Puglia	Bedroom	Pigeon nest	4 (1M, 3)	1 (10)	–	–	–
2007/03 Campania	Bedroom	Pigeon nest	1M	1 (5)	–	–	–
2005/10 Puglia	Bedroom	Pigeon nest	2 (1M, 1F)	1 (10)	–	–	–
2005/06 Puglia	Public office	Pigeon nest	7 (3M, 4F)	1 (10)	–	–	–
2001/05 Basilicata	Bedroom	Pigeon nest	2 (1M, 1F)	1 (5)	–	–	–
Total			31	98	3 pools		

PRM, poultry red mite.



**FIG. 2.** Scanning electron microscopy photographs of *Dermanyssus gallinae* female collected from outbreak. (A) Dorsal view. (B) Ventral view.

controls. For each PCR reaction, two negative control samples were prepared by adding water and DNA from human specific pathogen-free (SPF) blood instead of DNA from mites.

### Phylogenetic analysis

Nucleotide sequences from the 16S rRNA and *groEL* amplicons of *Coxiella* spp. and *B. burgdorferi* sensu lato, respectively, were separately aligned with the matching sequences from GenBank (Supplementary Tables S1 and S2, respectively) using ClustalW in MEGA7 [19]. Sequences from *Legionella* sp. (RefSeq accession no. NR\_116014) and *Borrelia anserina* (CP013704) were used as outgroups, respectively.

For both organisms, phylogenetic inferences were obtained by maximum-likelihood estimation using PhyML software [19], with 1000 nonparametric bootstrap replicates. The best-fitting evolutionary models were determined by the Model test script [20] implemented for the web at Find Model (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>).

Phylogenetic analysis was carried out using the TN+G model ( $\alpha = 0.24$ ) for the 16S rRNA gene of *Coxiella* spp., and the HKY+G model ( $\alpha = 0.16$ ) for the *groEL* gene of *B. burgdorferi* sensu lato.

## Results

### Morphologic identification of mites

All collected mite specimens were identified as *Dermanyssus gallinae* (De Geer, 1778) (Fig. 2).

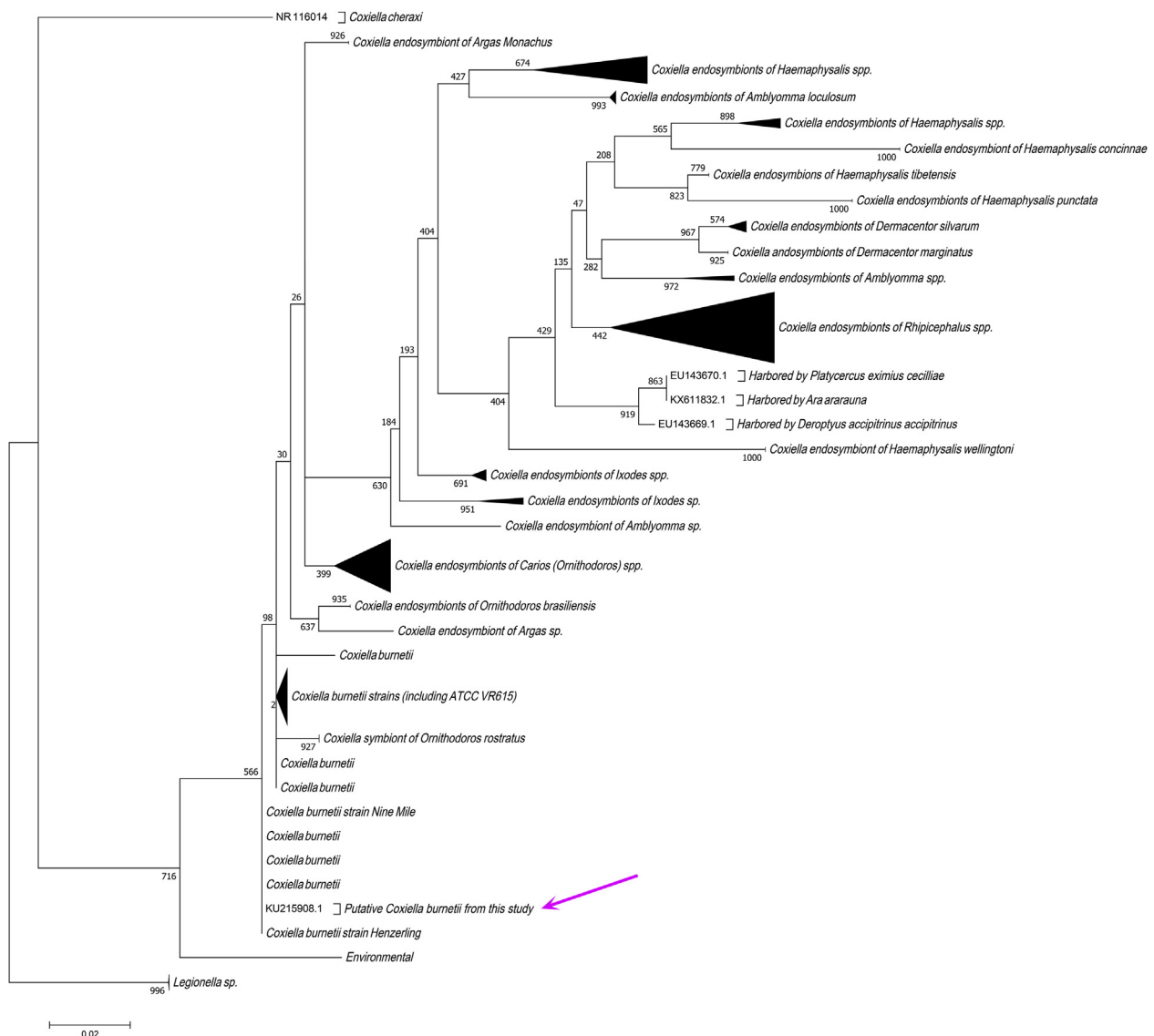
### Mite-borne pathogens detected in examined red mite pools

Pathogen detection revealed that one pool out of 12 was positive for *Coxiella* sp., as were two pools for *B. burgdorferi* sensu lato (Table 1). The *Coxiella* sp. amplicon exhibited 100% identity with *Coxiella burnetii*. The sequence was submitted to GenBank under accession number KU215908. The phylogenetic analysis confirmed the identification of *C. burnetii*, as shown in Fig. 3.

The two positive pools were made of mites collected in a pigeon nest and a sparrow nest, respectively. The nucleotide sequences from the two *B. burgdorferi* sensu lato amplicons submitted to GenBank under accession numbers KY828976 and KY828977, respectively, were identical but exhibited a 99% identity with *Borrelia afzelii*. The phylogenetic analysis also confirmed the identification, as demonstrated in Fig. 4.

## Discussion

*Dermanyssus gallinae* is well known for being associated with a number of microorganisms, but to date little attention has been paid to its potential role as a vector or reservoir of human pathogens. Our findings demonstrate that zoonotic pathogens might be associated to *D. gallinae*. In particular, the detection of *C. burnetii* in mites causing human dermatitis may represent a major concern, as the pathogen is the agent of the Q fever, a worldwide zoonosis [20]. Previous studies noted that birds living in urban environments, mostly pigeons,



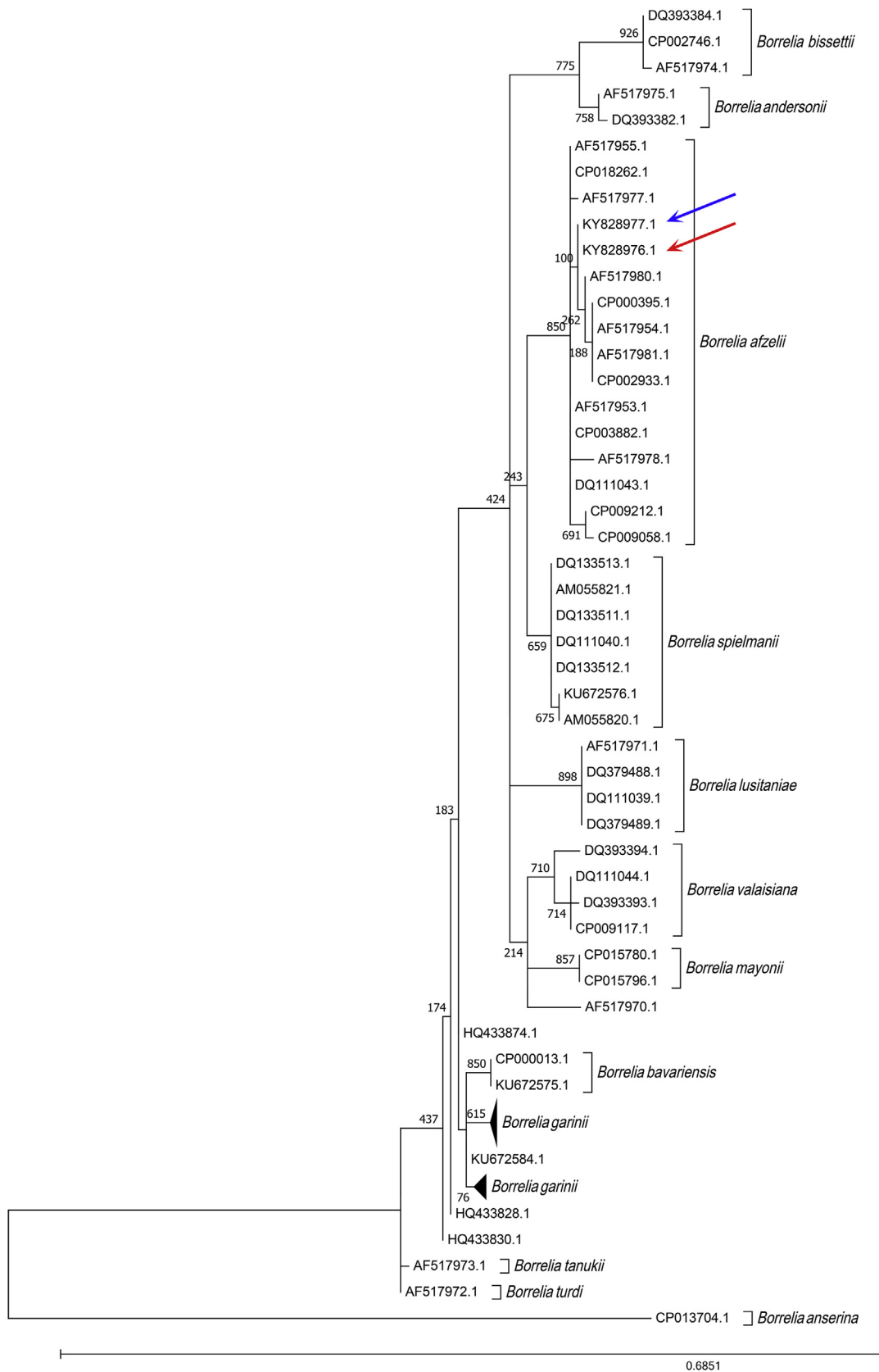
**FIG. 3.** Maximum-likelihood phylogenetic analysis of 16S rRNA sequence from *Coxiella* sp. detected by PCR and sequencing. Bootstrap values are shown at nodes. Details about sequences included in analysis are provided in [Supplementary Table S2](#). Arrow indicates position of *Dermanyssus gallinae*. *Coxiella burnetii* strain was detected in this study.

can harbour *C. burnetii* [21,22] and may be the source of Q fever outbreaks that typically occurred in people not in contact with ruminants, the classical source of infection [20]. In 1955, Zemskaia and Pchelkina [23] showed that *D. gallinae* could acquire infection while feeding on infected animals and that *C. burnetii* survived about 6 months in live PRMs and about 1 year in dead mites. As a nidicolous mite, *D. gallinae* may also come in contact with *C. burnetii* through contaminated nesting materials such as bird faeces. Observations in poultry farms showed that chickens usually remove mites by picking, and other avian species, including pigeons, also adopt the same strategy to remove parasites. Therefore, ingestion

of infected *D. gallinae* may represent a further route for the transmission of *C. burnetii* in avian species, as well as in some mammals [24].

By considering those factors, one may speculate that the almost ubiquitous distribution of *D. gallinae* might contribute to the almost global diffusion of *C. burnetii*. Confirming such a hypothesis would require accurate and comprehensive analysis of urban mites to promptly detect the possible diffusion of this pathogen.

Similar conclusions may be drawn after the detection of *B. afzelii* in naturally infected PRMs infesting humans. *Borrelia afzelii* is a genospecies of *B. burgdorferi* sensu lato, and it is one



**FIG. 4.** Maximum-likelihood phylogenetic analysis of *groEL* gene from *Borrelia burgdorferi* sensu lato detected by PCR and sequencing. Bootstrap values are shown at nodes. Details about sequences included in analysis are provided in [Supplementary Table S1](#). Blue and red arrows show position of *Borrelia afzelii* strains detected in this study.

of the agents of Lyme borreliosis in humans [25]. This disease is a multisystemic infection caused by spirochetes belonging to the *B. burgdorferi* sensu lato complex. In Europe, most genospecies of that group are specifically host associated [26]. For example, rodents and insectivores are usually infected by *B. afzelii* or *B. garinii* [27], while birds are frequently associated with *B. garinii* or *B. valasiana* [28]. However, some authors suspect that birds may also play a role in the transmission of *B. afzelii*.

Although Lyme disease is considered essentially to be a tick-borne disease [29], other species of blood-sucking parasites are able to spread the spirochetes [30–32]. This study extends the group of potential Lyme borreliosis vectors by also including *D. gallinae*. Overall, our study demonstrates the association between *D. gallinae* and pathogens such as *C. burnetii* and *B. afzelii* that are potentially dangerous for animals and humans. These findings should focus attention on the frequent infestation of synanthropic birds, mainly pigeons, by *D. gallinae*, including in urban areas [22].

To our knowledge, no study has yet been carried out to ascertain the ability of *D. gallinae* to transmit disease fever related to *C. burnetii* or Lyme borreliosis to humans, but this possibility should be no longer be neglected. Unfortunately, no data were available about the serologic response of patients bitten by infected *C. burnetii* and *B. afzelii*, respectively, and no blood or biosamples were collected from these patients, which prevented further analysis from being performed. Therefore, we suggest that physicians, dermatologists and clinicians in general consider carrying out more in-depth investigations in cases of human dermanysiosis.

## Conflict of interest

None declared.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.nmni.2018.01.004>.

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