Molecular detection of microorganisms in lice collected from farm animals in Northeastern Algeria

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Abstract

Lice (Phthiraptera) are highly specific insects organized into four suborders (Anoplura, amblycera, ischnocera and rhynchophthirina). Lice may affect human and animal health. Our objective was to study the bacterial community of lice collected in Algeria. Using molecular tools, we were able to identify by real time PCR the presence of *Coxiella burnetii* DNA in 1% (3/300) *Linognathus africanus* and in 0.3% (1/300) *Linognathus vituli* collected from goats and cattle respectively. We also detected the presence of Anaplasmataceae bacteria in *Bovicola bovis, L. vituli* from cattle and in *L. africanus* from goats. By standard PCR's and sequencing, we were able to identify *Anaplasma ovis* in *L. africanus* as well as a novel Anaplasmataceae sp genotype corresponding probably to a new genus within this family.

Keywords: Anaplasmataceae, *Coxiella burnetii*, Phthiraptera, Cattle, Sheep, Goats.

1. Introduction

Lice are ectoparasites insects known for their high host-specificity [1,2]. There are nearly 4,500 species of lice grouped in four suborders: Anoplura (sucking lice), ischnocera (chewing lice of birds and mammals), amblycera (chewing lice of birds and mammals) and rhynchophthirina (chewing lice of elephants and warthogs) [1,3,4]. Over the course of human history, lice have been recognised as a major public health problem and the body louse *Pediculus humanus humanus* can transmit many diseases to humans including typhus, relapsing fever, trench fever and plague [5]. In veterinary medicine, pediculosis in animals causes severe anaemia, skin damage, and necrosis which have economic and health consequences [6,7]. Few studies have been conducted on animal lice in Algeria. Available studies are limited to inventories of mammal and poultry lice species [8].

Only one molecular study has demonstrated the presence of *Rickettsia slovaca* DNA on wild boar lice *Haematopinus suis* in Algeria [9]. Other studies have shown the presence of *Coxiella burnetii*, the agent of Q fever, in *Pediculus humanus capitis* [10] . DNA of *Acinetobacter baumannii* has also been detected in head lice collected from Nigerien refugees and *Acinetobacter johnsonii*, *Acinetobacter variabilis* and *A. baumannii* collected from head lice in schoolchildren [10]. Epidemiological investigations have often overlooked the possibility that animal lice can be vectors of bacteria [11]. The aim of our study was to broaden our knowledge of animal lice in Algeria and to study their bacterial diversity using molecular tools.

2. Materials and Methods

2.1 Capture of lice in the field and study areas

The study was carried between 2015 and 2017 in three areas of Northeastern Algeria: El Tarf, Souk Ahras and Guelma. Lice were collected on three seasons (autumn, winter, spring) from 11 Cattle, 9 sheep, 5 goats and 6 poultry in five small traditional rural farms: one in El Tarf in the commune of Ain el assel (36 ° 47'11" N, 8 ° 22'57 "E), two in Souk Ahras in the commune of Machrouha (36 ° 21'26 "N, 7 ° 50' 08" E) and two in Guelma in two communes Oued Cheham (36 ° 22'44 "N, 7 ° 45' 52" E) and Bouchegouf (36 ° 28'18 "N, 7 ° 43' 47" E) respectively.

For mammals, animals have been examined carefully by inspecting their wool or hair from different parts of the body. Once the lice were found, a comb brushing was applied to collect them on cattle and goats, concerning sheep, the lice were recovered using tweezers. For poultry, the feathers of the head, neck, legs, wing and body were carefully examined and lice were collected using an entomological clamp. The lice taken from the same animal were recovered and stored in dry tube at -20 ° C.

2.2 Identification of lice

We performed the morphologic identification of lice as previously described in our laboratory [12]. The morphological identification keys, namely Wall [13] and Pajot [14], were used to identify the lice. Mass spectrometry (MALDI-TOF MS) was also used to identify the lice, as described [12].

2.3 DNA extraction

Following morphological identification [13,14], the lice DNA was extracted from the whole abdomens using the EZ1 DNA tissue extraction kit (Qiagen, Hilden, Germany) according to a protocol described previously in our laboratory [12,15]. The DNA of all the samples was eluted at 100 µl.

2.4 Molecular pathogen screening for lice

Each extracted DNA sample was tested in order to detect the presence of bacterial microorganisms (*Anaplasma* spp., *Borrelia* spp., *Bartonella* spp., *C. burnetii* and *Rickettsia* spp.) using the Real-Time PCR CFX96 system (Bio-Rad, Marnes-la-Coquette, France) and the LightCycler^{R} 480 Probes Master mix (Roche Diagnostics, Indianapolis, USA). All samples were screened for specific sequences of bacterial microorganisms with primers/probes listed in (Table 1). The real time PCR reaction mixture is detailed in (Table 1). For each reaction a positive and negative controls accompanied each molecular assay [16].

Two negative controls were used in each real time PCR plate and positive controls corresponded to dilutions of DNA extracts from strains of cultured bacteria (Table 1). The bacterial DNA of *C. burnetii* was initially detected by specific real time PCR with primers and specific probes designed to amplify the spacers *IS1111* and *IS30A* [16,17]. Samples were considered positive when the cycle threshold value was Ct ≤ 35. This value allows us in most cases to have an amplicon by the standard PCR visible. Also, this is the usual value used in several publications[16,18,19]. For Anaplasmataceae all lice that were considered to be positive in real time PCR were subjected to amplification using standard PCR's and sequencing to identify the bacterial species [15,20], with a primer targeting the Anaplasmataceae *23S* gene. To further explore the identity of the Anaplasmataceae species detected in lice, all samples were also tested with an additional PCR *Ehrlichia* genus-specific set of primers targeting part of the gene for heat shock protein (*groEL*) (Table 1).

The amplified products were detected by electrophoresis migration in 1.5% agarose gel stained with SYBR Safe™ and visualised using the ChemiDoc™ MP ultraviolet imager (Bio-Rad, Marnes-la-Coquette, France). The products were then purified using a NucleoFast 96 PCR plate (Macherey-Nagel EURL, Hoerd, France) as recommended by the manufacturer. Sequencing was performed using a Big Dye Terminator kit and an ABI PRISM 3130 Genetic Analyzer (Applied BioSystems, Courtaboeuf, France). All obtained sequences

were analysed and assembled using ChromasPro, version 1.34 (Technelysium Pty, Ltd., Tewantin, Queensland, Australia). All sequences were compared to the GenBank database using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) as previously used [21]. Phylogenetic analyses and tree construction were performed using the maximum likelihood method implemented on MEGA software version 7.0.21 with 1,000 bootstrap replications [22].

3. Results

3.1 Collection, morphological identification and molecular detection of bacteria in lice

The results of the morphological identification of the lice are detailed in (Table 3), these species of lice were confirmed by MALDI-TOF MS [12].

The results of the detection of microorganisms in lice using real-time PCR and standard PCR's are detailed in (Tables 3-2) respectively.

3.2 Phylogenetic analysis

Phylogenetic analysis shows that Anaplasmataceae bacterium from *Bovicola bovis* forms a separate clade located between the *Anaplasma* and *Wolbachia* genera, based on the analysis of the *23S* gene (Fig. 1), and between the *Anaplasma* and the *Ehrlichia* genera, using the *Ehrlichia groEL* gene (Fig. 2). In both cases the bootstrap value are low. All sequences obtained during this study were submitted to GenBank under the following accession numbers: For *23S* rRNA gene, *Anaplasma ovis* (MT408585.1) and three similar sequences Anaplasmataceae sp. (MT408586.1), and for the *Ehrlichia groEL* gene, three similar sequences Anaplasmataceae sp. (MT410711). The *A. ovis* species detected in our study is identical to *A. ovis* (KY498325.1) on the phylogenetic tree (Fig. 1).

4. Discussion

Anoplura lice frequently move between hosts and puncture the skin in several places during each blood meal [23,24], transmitting pathogens to susceptible hosts [11]. For the first time in Algeria, we detected the presence of *C. burnetii*, *A. ovis* and a novel Anaplasmataceae sp. bacterium in animal lice. Q fever is a zoonosis reported worldwide with the exception of New Zealand [25]. It is caused by *C. burnetii*, which is an obligate

intracellular bacterium [26]. The clinical manifestations of Q fever in humans depends on both the virulence of the infecting strain and specific risks factors in the infected patient. Two form of infection are known (Acute and Persistent chronic infection) [27]. *C. burnetii* can be hosted by several vertebrate or invertebrate hosts [27].

In humans and animals, the main route of transmission of this disease is through the respiratory tract [26,27]. The animal reservoirs of *C. burnetii* favoring human epidemics are domestic ruminants (cattle, sheep and goats). These reservoirs can eliminate the bacteria without having symptoms [27]. Arthropods such as ticks have been shown to play a role in the transmission of *C. burnetii* in animals [26,28]. In north Africa such as Tunisia and Algeria, 1 to 3% of infectious endocarditis is caused by *C. burnetii* [29]. In Algeria cases of human and veterinary infection caused by this bacterium have been reported [30]. For example, in the northeast and south-eastern region of Algeria DNA of *C. burnetii* has been detected in several species of ticks, on the blood of small ruminants [16,28,31], in dogs and cats spleens [32] and at one human case signaled in the northwest of Algeria[33].

Here, for the first time in Algeria we detected the presence of *C. burnetii* in lice collected from cattle and goats. These lice may have acquired the bacteria during their feeding on bacteraemic host or during a mixed infestation where they co-fed with other infected arthropods. This phenomenon was already described in other hematophagous arthropods such as ticks and fleas [34,35]. However, so far these results cannot be considered proof of vector competence of lice for the transmission of *C. burnetii*. Greater attention should be paid to lice because they may play a part in the epidemiology of *C. burnetii* infection.

Anaplasma spp. are intracellular bacteria belonging to the order Rickettsiales and the Anaplasmataceae family [36]. In recent years, many new species that affect human and animal health have been recognised [21]. *Anaplasma* spp. have been detected in many species of ticks of various genera (*Ixodes*, *Dermacentor*, *Rhipicephalus* and *Amblyomma*), and some of them are recognized vectors [37]. In tropical and subtropical regions of the world *A. ovis* is the main cause of anaplasmosis widely transmitted by ticks and it is much more likely to be found in small ruminants [36,38]. A study was carried out in Algeria in an area where bovine anaplasmosis has never been reported. The authors were able to identify three genetic variants of *Anaplasma phagocytophilum*, *Anaplasma platys* and *Anaplasma* sp."variant 4" in bovine blood [39]. Also, other studies have reported the presence of *A. ovis* in ticks taken from sheep and goats [16,40]. In Hungary, the presence of *Anaplasma* spp., *Rickettsia* and Haemotropic mycoplasma was detected for the first time in lice of ruminants and pigs [11].

Our study revealed the presence of *A. ovis* in 1/4 *L. africanus* which is a hematophagous louse of goats. As discussed above this does not mean that these lice act as vectors but confirms the presence of the bacteria in Algeria. Also, 3/4 of *B. bovis* which is mallophagous louse revealed the presence of a probable new genotype of a yet undescribed bacterium within Anaplasmataceae.

Occasionally mallophaga lice feed on blood and as lice move from one host to another during mating and feeding activities [41], they can ingest blood during feeding due to pre-existing lesions or desquamation lesions or injuries induced by the louse himself [42]. It can explain the presence of blood borne pathogens as the genera of *Ehrlichia* and *Anaplasma* bacteria in these lice.

Phylogenetic analysis shows that this amplicon forms a distinct line on the phylogenetic tree (Fig. 1.2). As for the moment this is the only representative of this group and bootstrap value are low in the both genes trees, we do not have enough data to classify this genotype in a specific genus. We don't know also the microbiological characteristics of this bacterium nor their isolate. Hence, it is difficult to attribute it to a well-defined genus. Phylogenetic proximity to *Wolbachia* makes suggest possible endosymbiotic role of this microorganisms.

Further research and investigation should therefore be conducted in order to be able to isolate other genes.

Conclusions

Pediculosis in animals deserves more attention and lice should be evaluated as potential vectors for arthropod-borne pathogen. Further research will be necessary to fully understand the ability of lice to harbour pathogens.

Author Contributions

P.P. designed the experiments; B.O. collected the samples and performed the experiments; B.O. and O.M. analysed the data; B.O. wrote the manuscript. All authors approved the final version of the manuscript.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Table 1.

Representation of primers and probes used for real-time PCR and standard PCR's in this study and the protocol of real-time PCR reaction mixture, the positive and negative control.

Table 2.

BLAST analysis of *Anaplasma* spp. *23S* rRNA and *Ehrlichia* (*groEL*) sequences obtained from tested lice.

Table 3.

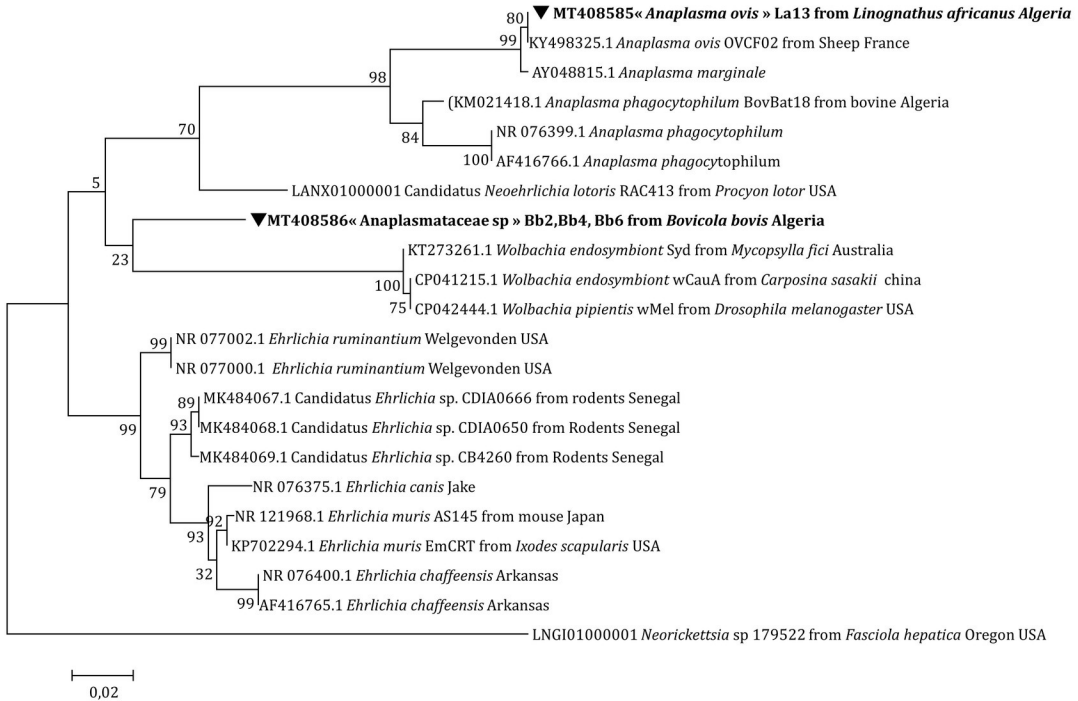
Collection, morphological identification of mammalian and poultry lice and molecular detection of bacteria in lice using real-time PCR.

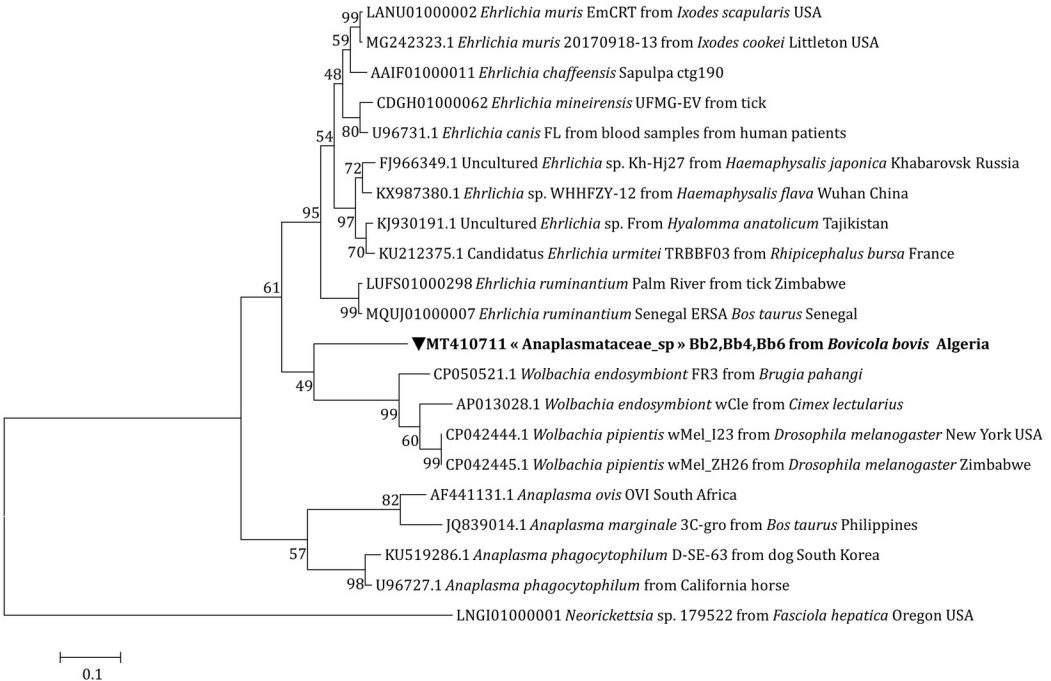
Figure1.

Maximum-likelihood phylogenetic tree of Anaplasmataceae, based on the partial 513-bp *23S* gene.

Figure 2.

Maximum-likelihood phylogenetic tree of *Ehrlichia* spp, based on the partial 633-bp *groEL* gene**.**


▼ MT408585« Anaplasma ovis » La13 from Linognathus africanus Algeria
KY498325.1 Anaplasma ovis OVCF02 from Sheep France
AY048815.1 Anaplasma marginale
(KM021418.1 Anaplasma phagocytophilum BovBat18 from bovine Algeria
NR 076399.1 Anaplasma phagocytophilum
AF416766.1 Anaplasma phagocytophilum
LANX01000001 Candidatus Neoehrlichia lotoris RAC413 from Procyon lotor USA
▼MT408586« Anaplasmataceae sp » Bb2,Bb4, Bb6 from Bovicola bovis Algeria
KT273261.1 Wolbachia endosymbiont Syd from Mycopsylla fici Australia
CP041215.1 Wolbachia endosymbiont wCauA from Carposina sasakii china
CP042444.1 Wolbachia pipientis wMel from Drosophila melanogaster USA
NR 077002.1 Ehrlichia ruminantium Welgevonden USA
NR 077000.1 Ehrlichia ruminantium Welgevonden USA
MK484067.1 Candidatus Ehrlichia sp. CDIA0666 from rodents Senegal
MK484068.1 Candidatus Ehrlichia sp. CDIA0650 from Rodents Senegal
MK484069.1 Candidatus Ehrlichia sp. CB4260 from Rodents Senegal
NR 076375.1 Ehrlichia canis Jake
NR 121968.1 Ehrlichia muris AS145 from mouse Japan
KP702294.1 Ehrlichia muris EmCRT from Ixodes scapularis USA
NR 076400.1 Ehrlichia chaffeensis Arkansas
AF416765.1 Ehrlichia chaffeensis Arkansas
LNGI01000001 Neorickettsia sp 179522 from Fasciola hepatica Oregon USA
80
99
98
70
84
100
5
23
100
75
99
99
89
93
99
79
92
93
32
99
0,02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LANU01000002 Ehrlichia muris EmCRT from Ixodes scapularis USA
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MG242323.1 Ehrlichia muris 20170918-13 from Ixodes cookei Littleton USA
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AAIF01000011 Ehrlichia chaffeensis Sapulpa ctg190
CDGH01000062 Ehrlichia mineirensis UFMG-EV from tick
80
U96731.1 Ehrlichia canis FL from blood samples from human patients
54
72
FJ966349.1 Uncultured Ehrlichia sp. Kh-Hj27 from Haemaphysalis japonica Khabarovsk Russia
KX987380.1 Ehrlichia sp. WHHFZY-12 from Haemaphysalis flava Wuhan China
95
97
KJ930191.1 Uncultured Ehrlichia sp. From Hyalomma anatolicum Tajikistan
70
KU212375.1 Candidatus Ehrlichia urmitei TRBBF03 from Rhipicephalus bursa France
61
LUFS01000298 Ehrlichia ruminantium Palm River from tick Zimbabwe
99
MQUJ01000007 Ehrlichia ruminantium Senegal ERSA Bos taurus Senegal
▼MT410711 « Anaplasmataceae_sp » Bb2,Bb4,Bb6 from Bovicola bovis Algeria
49
CP050521.1 Wolbachia endosymbiont FR3 from Brugia pahangi
99
AP013028.1 Wolbachia endosymbiont wCle from Cimex lectularius
60
CP042444.1 Wolbachia pipientis wMel_I23 from Drosophila melanogaster New York USA
99
CP042445.1 Wolbachia pipientis wMel_ZH26 from Drosophila melanogaster Zimbabwe
82
AF441131.1 Anaplasma ovis OVI South Africa
JQ839014.1 Anaplasma marginale 3C-gro from Bos taurus Philippines
57
KU519286.1 Anaplasma phagocytophilum D-SE-63 from dog South Korea
98
U96727.1 Anaplasma phagocytophilum from California horse
LNGI01000001 Neorickettsia sp. 179522 from Fasciola hepatica Oregon USA
0.1

Real-time PCR and standard PCR's specificity	Targeted sequences	Primers f, r (5'-3') and probes p (FAM-TAMRA)	Amplicon size for standard PCR's	The real-time PCR reaction mixture	Negative control mixture	Positive control mixture	Annealing temperature	References
Anaplasmataceae	*23S* rRNA (TtAna)	f_TGACAGCGTACCTTTTGCAT r_TGGAGGACCGAACCTGTTAC p_GGATTAGACCCGAAACCAAG	/	. 10 µl of Master mix (Roche Diagnostics, Indianapolis, USA). . 3 µl of distilled water. . 0.5 µl of each reverse, forward primers (The final concentration of the primers used is 0.5 mM). . 0.5 µl of the probe. . 0.5 µl Uracil-DNA Glycosylase (UDG). . 5 µl of DNA extract for each qPCR plate. The final reaction volume is a 20 µl.	. 5 µl of DNA extracted from uninfected lice from our laboratory colony. . 15 µL of the qPCR reaction mix.	*Anaplasma phagocytophylum* (for the detection of *Anaplasma* spp.)	60°C	[43]
Coxiella burnetii	(*IS1111*) Intergenic spacer	f_CAAGAAACGTAACGCTGTGGC r_CACAGAGCCACCGTATGAATC p_CCGAGTTCGAAACAATGAGGGCTG	/			*C. burnetii* (for the detection *C. burnetii)*	60°C	[44]
	(*IS30A*)	f_ CGCTGACCTACAGAAATATGTCC r_ GGGGTAAGTAAATAATACCTTCTGG p_CATGAAGCGATTTATCAATACGTGTATG	/					[17]
Borrelia spp.	(*ITS4*)	f_GGCTTCGGGTCTACCACATCTA r_CCGGGAGGGGAGTGAAATAG p_TGCAAAAGGCACGCCATCACC	/			*Borrelia crocidurae* (for the detection of *Borrelia* spp.	60°C	[45]
Bartonella spp.	(*ITS2*)	f_GATGCCGGGGAAGGTTTTC r_GCCTGGGAGGACTTGAACCT p_GCGCGCGCTTGATAAGCGTG	/			*B. elizabethae* (for the detection of *Bartonella* spp.)	60°C	[46]
Rickettsia spp.	*gltA*(RKND03)	f_GTGAATGAAAGATTACACTATTTAT r_GTATCTTAGCAATCATTCTAATAGC p_CTATTATGCTTGCGGCTGTCGGTTC	/			*R. montanensis* (for the detection of *Rickettsia* spp.)	60°C	[47]
Standard PCR's								
Anaplasmatacae	*23S* rRNA gene	f_ATAAGCTGCGGGGAATTGT r_TGCAAAAGGTACGCTGTCAC	513	/	/	/	55°C	[39]
Ehrlichia spp.	*groEL* gene	f-GTTGAAAARACTGATGGTATGCA r-ACACGRTCTTTACGYTCYTTAAC	633	/	/	/	50°C	[48]

Host	Primers	Species lice	Molecular identification by BLAST	Percent Identity	Query Cover	Accession Number
Goat	Anaplasmatacae *23S* rRNA gene	*Linognathus africanus*	*Anaplasma ovis*	100%	100%	CP015994.2
Cattle	Anaplasmatacae *23S* rRNA gene	*Bovicola bovis* 2/4/6	*Ehrlichia ruminantium*	91.72%	100%	NR_077002.1
Cattle	*Ehrlichia groEL*	*Bovicola bovis 2/4/6*	*Ehrlichia canis*	77.12%	100%	MN216188.1

Host (mammal and poultry lice)	**morphological identification**	**Number of specimens of lice**	**Real-time PCR Primers**	**Results of bacteria detected in lice using real-time PCR**	**Percentage of positive bacteria detected in lice using real-time PCR**
Cattle	*Bovicola bovis*[b]	27 (9%)	Anaplasmataceae *23S* rRNA	Anaplasmataceae spp.	5/300 (1.6%)
	Haematopinus eurysternus[a]	36 (12%)	/	/	/
	Linognathus vituli[a]	43 (14.3%)	. IS1111/ IS30A .Anaplasmataceae *23S* rRNA	*Coxiella burnetti* Anaplasmataceae spp.	1/300 (0.3%) 1/300 (0.3%)
	Solenopotes capillatus[a]	34 (11.3%)	/	/	/
Goats	*Linognathus africanus*[a]	35 (11.7%)	. IS1111/ IS30A .Anaplasmataceae *23S* rRNA	*Coxiella burnetti* Anaplasmataceae spp.	3/300 (1%) 4/300 (1.3%)
	Bovicola caprae[b]	26 (8.7%)	/	/	/
Sheep	*Bovicola ovis*[b]	46 (15.3%)	/	/	/
Poultry	*Goniocotes gallinae*[b]	3 (1%)	/	/	/
	Goniodes gigas[b]	3 (1%)	/	/	/
	Menopon gallinae[b]	12 (4%)	/	/	/
	Menacanthus stramineus[b]	23 (23%)	/	/	/
	Lipeurus caponis[b]	6 (2%)	/	/	/
	Chelopistes meleagridis[b]	6 (2%)	/	/	/
Total	13 species	300	/	/	/

[a] Anoplura.

[b] Mallophaga.

Note: All real time PCR tests were negative for the detection of *Borrelia* spp., *Rickettsia* spp., and *Bartonella* spp.