AIX-MARSEILLE UNIVERSITE

FACULTE DES SCIENCES MEDICALES ET PARAMEDICALES

ECOLE DOCTORALE DES SCIENCES DE LA VIE ET DE LA SANTE

THÈSE DE DOCTORAT

Présentée publiquement et soutenue devant

LA FACULTÉ DES SCIENCES MEDICALES ET PARAMEDICALES

L'INSTITUT HOSPITALO-UNIVERSITAIRE MEDITERRANEE INFECTION

Le 01 Juillet 2021

Par Madame Basma OUARTI

DEVELOPPEMENT D'OUTILS INNOVANTS POUR L'IDENTIFICATION ET LA CLASSIFICATION DES ARTHROPODES

Pour obtenir le grade de Docteur d'Aix-Marseille Université

Biologie-Santé, Spécialité : Pathologies Humaines - Maladies Infectieuses

Membres du Jury de la Thèse

Mr. Le Docteur Arezki IZRI	Université de Paris	Examinateur
Mme. Le Professeur Christelle POMARES	Université de Nice	Rapporteur
Mr. Le Docteur Pascal DELAUNAY	Université de Nice	Rapporteur
Mr. Le Professeur Philippe PAROLA	Aix- Marseille Université	Directeur de thèse

Laboratoire d'accueil

Aix- Marseille Univ, VITROME, AMU, IRD, SSA IHU-Méditerranée Infection, Marseille, France
19-21 Boulevard Jean-Moulin, IHU Méditerranée Infection

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AVANT-PROPOS

Le format de présentation de cette thèse correspond à une recommandation de la spécialité Maladies Infectieuses et Microbiologie du Master des Sciences de la Vie et de la Santé qui dépend de l'Ecole Doctorale des Sciences de la Vie de Marseille. Le candidat est amené à respecté des règles qui lui sont imposées et qui comportent un format de thèse utilisé dans le Nord de l'Europe et qui permet un meilleur rangement que les thèses traditionnelles.

Par ailleurs, la partie introduction et bibliographie est remplacée par une revue publiée dans un journal scientifique afin de permettre une évaluation extérieure de la qualité de la revue et de permettre à l'étudiant de commencer le plutôt possible une bibliographie exhaustive sur le domaine de cette thèse. Par ailleurs, la thèse est présentée sur article publié, accepté où soumis associée d'un bref commentaire donnant le sens général du travail.

Cette forme de présentation a paru plus en adéquation avec les exigences de la compétition internationale et permet de se concentrer sur des travaux qui bénéficieront d'une diffusion internationale.

Pr. Didier RAOULT, Directeur de l'IHU Méditerranée Infection

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TABLE DES MATIERES

Résumé

L'entomologie médicale et vétérinaire est une discipline scientifique qui s'intéresse à l'étude des insectes et plus largement des arthropodes impliqués en pathologie humaine ou animale. Les arthropodes tels que les poux, les tiques et les puces ont ainsi une grande importance en santé humaine et animale. La surveillance épidémiologique des vecteurs, leur identification et la détection des microorganismes associés, demeurent des éléments clés dans les stratégies de lutte et de contrôle des maladies vectorielles.

Nos travaux de thèse ont porté dans un premier temps sur l'identification des arthropodes en faisant appel à plusieurs méthodes d'identification : Identification morphologique, identification moléculaire et spectrométrie de masse (MALDI-TOF MS pour l'identification des poux). En second lieu, nous nous sommes penchés sur l'étude des microorganismes associés aux arthropodes (poux, tique et puces) et/ou leurs hôtes.

Ainsi, au cours de nos investigations, nous avons pu après une identification morphologique et moléculaire, mettre au point l'identification par MALDI-TOF MS des poux humains d'élevage et de 13 espèces de poux d'animaux collectés en France et dans plusieurs régions d'Algérie par le biais de la spectrométrie de masse MALDI-TOF MS. Nous avons travaillé sur des poux congelés à -20°C ainsi sur des poux conservés en alcool à 70%. La recherche par la suite des microorganismes associés aux poux par les outils de biologie moléculaire (qPCR, PCR standard et la phylogénie) a permis de révéler le portage de plusieurs bactéries comme *Coxiella burnetii*, *Anaplasma ovis* et une potentielle nouvelle Anaplasmataceae sp.

Dans le deuxième volet de cette thèse, nous avons travaillé sur la détection des microorganismes chez des petits mammifères (rongeurs) et leurs ectoparasites associés (Tiques molles) au Sénégal, ainsi que sur des tiques dures collectées sur les végétations en Slovaquie et enfin des puces d'hérisson collectées sur des patients originaires de Strasbourg.

Les résultats des travaux de cette thèse ont confirmé pour la première fois l'efficacité et la précision du MALDI-TOF MS a identifié correctement et rapidement plusieurs espèces de poux quel que soit leurs modes de conservation. Aussi cette étude a contribué à enrichir et compléter le répertoire des microorganismes chez plusieurs arthropodes (poux, tiques et puces) provenant de plusieurs régions Algérie, Sénégal, Slovaquie et Strasbourg.

Abstract

Medical and veterinary entomology is a scientific discipline that is interested in the study of insects and more broadly of arthropods involved in human or animal pathology. Arthropods such as lice, ticks and fleas are thus of great importance to human and animal health. Epidemiological surveillance of vectors, their identification and detection of associated microorganisms, remain key elements in vector-borne disease control strategies.

Our thesis work focused initially on the identification of arthropods using several identification methods: Morphological identification, molecular identification and mass spectrometry (MALDI-TOF MS for the identification of lice). Secondly, we focused on the study of microorganisms associated with arthropods (lice, ticks and fleas) and/or their hosts. Thus, during our investigations, we were able after a morphological and molecular identification, to develop the identification by MALDI-TOF MS of human lice and 13 species of animal lice collected in France and in several regions of Algeria through MALDI-TOF MS. We worked on lice frozen at -20°C and on lice preserved in 70% alcohol. The subsequent search for microorganisms associated with lice using molecular biology tools (qPCR, standard PCR and phylogeny) revealed the carriage of several bacteria such as *Coxiella burnetii*, *Anaplasma ovis* and a potential new Anaplasmataceae sp.

In the second part of this thesis, we worked on the detection of microorganisms in small mammals (rodents) and their associated ectoparasites (soft ticks) in Senegal, as well as on hard ticks collected on vegetations in Slovakia and finally on hedgehog fleas collected on patients from Strasbourg. The results of this thesis confirmed for the first time the efficiency and accuracy of MALDI-TOF MS to correctly and rapidly identify several species of lice regardless of their storage conditions. Also, this study contributed to enrich and complete the repertoire of microorganisms in several arthropods (lice, ticks and fleas) from several regions Algeria, Senegal, Slovakia and Strasbourg.

Chapitre I : Introduction

L'entomologie médicale et vétérinaire est la science qui s'occupe de l'étude de tous les arthropodes parasites qui impacte la santé humaine et animale. Les arthropodes constituent plus de 80% de toutes les espèces animales connue sur terre. Près d'un million ont été décrites et des millions d'autres en attente de découverte [1]. Certaines sont des vecteurs, c'est à dire qu'ils sont hématophages et responsables de la transmission biologique active d'un microorganisme d'un vertébré à un autre [2,3]. Selon les données fournies par l'organisation mondiale de la santé (OMS) plus de 17% des maladies infectieuses sont à transmission vectorielle (World Health Organization 2020). Les arthropodes d'intérêt médical traités dans cette thèse sont les poux, les tiques et les puces qui sont affiliés à 3 sous embranchements distingués : Hexapodes, les chélicérates et les mandibulates respectivement[4,5]. Ces trois groupes d'arthropodes contribuent à la transmission d'une grande variété d'agents pathogènes responsables de maladies infectieuses anciennes ou émergentes et ré-émergentes [6–8]. Citant principalement chez les poux le typhus épidémique, la fièvre des tranchées, la fièvre récurrente et la peste causées par des *Rickettsia prowazekii, Bartonella quintana, Borrelia recurrentis* et *Yersnia pestis* [9,10]. Chez les tiques la maladie de Lyme causée par *Borrelia burgdorferi* et les rickettsioses du groupe boutonneux [11], et chez les puces la peste bubonique, le typhus murin et la maladie des griffes du chat provoquées par *Yersinia pestis*, *Rickettsia typhi* et *Bartonella henselae* [7,12].

Une identification précise des arthropodes est un préalable indispensable pour toute étude épidémiologique des maladies transmises par ces vecteurs « Mieux connaître pour mieux lutter ». L'approche morphologique basée sur des critères d'identification morphologique représente le principal outil d'identification des arthropodes. Cette dernière nécessitant une expertise entomologique et une documentation spécifique, peut être limitée notamment par la qualité des échantillons qui peuvent être endommagés ou immatures, ou en raison de l'absence de critères cruciaux ou de clés d'identification [13].

Depuis plus de 20 ans, des méthodes alternatives telles que les approches moléculaires ont été développées pour identifier les arthropodes [14,15]. En dépit de la qualité des échantillons analysés et peu importe le stade de développement, leurs résultats ne sont pas affectés et restent précis. Nonobstant, la méthode moléculaire reste limitée par la nécessité d'un équipement spécifique et couteux, le temps requis relativement long pour la préparation des échantillons et surtout l'inexistence d'un système unique capable d'identifier tous les arthropodes [16–19], auxquels s'ajoute la base de données de séquences (NCBI) qui peut être non exhaustive à cause des séquences inexistantes, incomplètes ou de mauvaise qualité [3].

Récemment, un nouvel outil d'identification et qui est actuellement en cours de progression, la technique MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) a été exploité en entomologie. C'est une technique d'ionisation qui permet de caractériser un échantillon à partir du spectre de masse moléculaire des différentes protéines présentes. Elle utilise une matrice qui absorbe l'énergie induite par un faisceau laser pour créer avec une fragmentation minimale des ions à partir de grosses molécules.

Cette technique caractérisé par sa rapidité, sa précision et son faible coût, a été appliquée dans un premier temps dans le diagnostic clinique et l'identification des microorganismes à savoir des bactéries, des champignons , des levures, des parasites et helminthes [20]. Plus récemment cette technique a été utilisée dans le domaine de l'entomologie médicale et vétérinaire, notamment dans l'UMR VITROME , où elle a montré toute sa capacité à identifier rapidement et correctement de nombreux arthropodes quel que soit leur mode de conservation (frais, alcool, congélation) ou l'origine (terrain ou laboratoire) et ce à partir d'une partie du corps qui est déterminée au cas par cas [21–26]. Cet outil a aussi été utilisé pour l'identification du repas sanguins des arthropodes [27–29], et aussi leurs statuts infectieux lors des modèles expérimentaux [30,31].

Dans un premier temps de nos travaux originaux de cette thèse nous nous somme intéressés aux données de la littérature sur les poux et leurs maladies transmises en Afrique. Ainsi, dans la première partie du travail nous présentons une revue de la littérature intitulée « Lice and Lice Borne diseases in Humans in Africa » afin de donner un aperçu sur les agents pathogènes circulant chez les poux et d'alarmer les professionnels de leurs impacts sur la santé humaine ainsi qu'estimer la pertinence des poux comme un large éventail de vecteurs potentiels en Afrique. Cette revue représente l'introduction de la thèse (Article1). Nous avons également effectué une enquête sur l'infestation des ruminants par les poux d'animaux dans un but de mettre à jour les informations disponibles sur les espèces de poux rencontrées chez les ruminants dans le nord-est de l'Algérie (Article 2).

Dans la deuxième partie de cette thèse intitulée « Utilisation du MALDI-TOF MS pour l'identification des poux », notre objectif été la mise au point de l'outil MALDI-TOF MS pour identifier des poux collectés sur des animaux en l'Algérie (conservé à -20°C ou dans l'alcool), et aussi des poux humains élevés au laboratoire en France (Article 3 et 4).

La troisième partie de nos travaux de la thèse « Épidemiologie moléculaire des microorganismes associés aux animaux et aux arthropodes » a porté sur la détection des bactéries potentiellement transmissibles chez les arthropodes (poux d'animaux, tiques dure et molles et des puces d'hérisson) provenant de plusieurs régions à savoir (Algérie, Sénégal, Slovaquie et Strasbourg), et aussi de compléter le répertoire des agents pathogènes associés à ces arthropodes on utilisant des outils moléculaires (PCR en temps réel, PCR standards, séquençage et la phylogénie) (Article 5,6,7 et 8).

Aussi, nous avons recherché la présence des agents pathogènes chez les rongeurs du Sénégal en utilisant les mêmes outils moléculaires (Article 7).

Enfin, une étude supplémentaire réalisée en collaboration sera présentée dans le chapitre « Annexe » (Article 8).

Article N°1 : Revue de la littérature

Lice and lice-borne diseases in humans in Africa.

Basma Ouarti, Maxime Descartes Mbogning Fonkou, Linda Houhamdi and Philippe Parola.

(Article à soumettre à Acta Parasitologica)

Préambule

Près de cinq milles espèces de poux sont connues comme des parasites obligatoires, hautement spécifiques et permanents d'oiseaux et de mammifères. Ces insectes sont responsables de l'affection de la santé publique et animale dans le monde. Les poux humains (*Pediculus humanus*) ont la capacité de transmettre beaucoup de pathologies telle que le typhus épidémique, la fièvre des tranchées, la fièvre récurrente et potentiellement la peste. En Afrique les poux et leurs maladies associées restent mal connues, peu négligés et sous-estimés.

L'objectif de notre revue été d'actualiser de façon exhaustive en Afrique les agents bactériens d'importance médicale identifiés chez les poux humains et qui peuvent jouer un potentiel rôle dans leurs transmissions non seulement pour donner un aperçu sur la situation épidémiologique dans cette région mais aussi d'alarmer les professionnels de leurs impacts sur la santé humaine afin d'estimer la pertinence des poux comme un large éventail de vecteurs potentiels en Afrique.

Notre revue se veut pédagogique en décrivant les prévalences des pathogènes détectés dans chaque espèce de poux et chaque région du signalement.

Un total de 28 espèces de bactéries a été signalé chez les poux de corps et/ ou chez des poux de tête dans plusieurs régions en Afrique et notamment sur des zones qui sont normalement indemnes telle que l'Afrique du nord. Par ailleurs, beaucoup d'études expérimentales et épidémiologiques ont suggéré la possibilité d'une compétence vectorielle chez les poux de tête.

En Afrique cette large diversité bactérienne détectée chez les poux et leurs signalements dans des zones vierge devrait attirer l'attention des professionnels sur les risques qui peuvent être engendré à cause de la propagation des poux et leurs maladies associées lors d'une épidémie.

Lice and lice-borne diseases in humans in Africa

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Content

Abstract

Lice are highly host-specific insects. Human lice include: 1) *Pediculus humanus humanus* (body lice) which are known to be vectors of serious human bacterial infectious diseases including epidemic typhus, relapsing fever, trench fever, and plague, 2) *Pediculus humanus capitis* (head lice) that frequently affect children, and 3) *Phtirius pubis*, commonly known as crab lice. In Africa, human infections transmitted by lice remained poorly known and, therefore, underestimated, perhaps due to the lack of diagnostic tools and professional knowledge. In this paper we review current knowledge of the microorganisms identified in human lice in the continent of Africa, in order to alert health professionals to the importance of recognising the risk of lice-related diseases.

Keywords: Lice; Africa; *Rickettsia*; *Bartonella*; *Borrelia*; *Yersinia*.

Introduction

Lice are wingless insects belonging to the Order of Phthiraptera and Sub-order of Anoplura. They are also referred to as sucking lice and include nearly 540 species [1].

Humans are parasitised by two genera of blood-sucking lice; *Pediculus* and *Phthirus* [2]. *Phthirus pubis*, are pubic lice, which are mainly sexually transmitted and not known as vectors of human infectious pathogens [3]. The genus *Pediculus* includes two ecotypes; *Pediculus humanus capitis* (*P. humanus capitis*), head lice, and *Pediculus humanus humanus* (*P. humanus humanus*), body lice [4]. These two ecotypes are morphologically very similar but ecologically and nutritionally different [5,6]. *P. humanus capitis* lay their nits at the base of the hair shafts, and feed on human blood every four to six hours. Head lice infestations often affect school-aged children and are transmitted by direct contact regardless of hygiene conditions [7]. They cause strong pruritus that leads to irritation and sores that can become infected [6]. The role of head lice as a vector of infectious agents remains unclear [8]. In contrast, *P. humanus humanus* lays eggs in clothes, and feeds less frequently on blood than head lice, although they such a greater volume of blood [6,9–11]. They are more resistant to harsh environmental conditions, and can survive up to 72 hours without nutrition [6]. Their infestation is mainly related to poor hygiene and living conditions, and overcrowding, which explains why the population most likely to be infested are refugees, homeless people and prisoners [6].

Using current genomic tools, it has been confirmed that the human body louse genome is the smallest of these insects, with very significant genomic characteristics [12], and the head louse has been suggested to be the ancestor of body lice [6]. The differentiation between them took place only recently, as these species remained genetically and phylogenetically compatible [10]. Moreover, it has been suggested that under adequate conditions, head lice can transform into body lice ecotypes [12,13]. Using mitochondrial DNA analysis, Light *et al.* classified *Pediculus humanus* into several divergent clades with different geographical origins [9]. In addition, head

66 lice have been classified into five mitochondrial clades (A, B, C, D, and E) [4,14,15], while
67 body lice have been classified into only two clades, A and D [4]. Several hypotheses have
68 suggested that these clades of lice have existed since the displacement of hominid ancestors to
69 regions other than Africa [10].
70 The problem posed by body lice is not only related to the parasitism by the louse itself, known
71 as "body pediculosis", which is manifestated by skin lesions and infections due to pruritus and
72 caused by antigens injected during the bite of the lice [16,17], but also to the fact that *P.*
73 *humanus humanus* are known to be vectors of three serious re-emerging human bacterial
74 infections: epidemic typhus, relapsing louse fever and trench fever [16,18–20].Identification of
75 these insects thus remains essential to ensure surveillance and prevention of these diseases.
76 Morphological identification is currently the main tool for identifying arthropods. However,
77 this requires entomological expertise and specific documentation, sometimes limited by the
78 quality of the samples that may be damaged or immature, by the lack of documentation, and/or
79 the absence of criteria for distinction [21]. Currently, alternative methods such as molecular
80 approaches have been developed to identify arthropods. Regardless of the quality of the samples
81 analysed and the stage of development, their results are not affected and remain accurate.
82 However, the use of molecular methods is limited by the need for specific and expensive
83 equipment, the relatively long time required to prepare the samples and, above all, by the lack
84 of a single system capable of identifying all arthropods [22]. Another recent identification tool
85 is Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-
86 TOF MS), which is an ionisation technique that enables a sample from the molecular mass
87 spectrum of the different proteins present to be characterised. This technique uses a matrix that
88 absorbs the energy induced by a laser beam to create ions from large molecules with minimal
89 fragmentation [23]. It was initially used in the clinical diagnosis and identification of a number
90 of microorganisms including bacteria, fungi, yeasts and parasites.[24–26]. Recently, MALDI-

TOF MS has been successfully used in the field of medical entomology [23], and had shown its ability to quickly and correctly identify many arthropods, such as mosquitoes, ticks, fleas and even lice [27–30].

In Africa, disease surveillance and diagnosis programmes on lice are rare, although lice-borne diseases such as louse-borne relapsing fever in East Africa, and epidemic typhus, represent regular threats and remain endemic in some regions, [17,31,32]. In this review, we aim to update knowledge of the multiple bacteria transmitted by human lice in Africa to alert professionals to their potential impact on human health.

Data collection

We conducted a systematic literature search on PubMed and Google scholar to screen studies and reports about lice and their associated microorganisms in Africa, published before March 2021. Our key words included: Louse, Lice, Phthiraptera, Anoplura, Hoplopleuridae, Polyplacidae, Amblycera, Ischnocera, Rhyncophthirina, Pediculidae, microorganisms (Protozoa, parasite, parasites, bacterium, bacteria, fungus, fungi, virus, archaea, microbiome, microbiology) and African countries (Supplementary Data 1). Reviews, meta-analysis and systematic reviews were excluded. Only studies that detected bacteria in human lice in Africa were included. Our initial PubMed and Google scholar search retrieved 507 studies. After removing duplicates, we reviewed the titles and abstracts of 505 studies. Following this analysis, the full text of 74 studies were selected for eligibility. Fifty-four of these studies were then excluded because either the lice sample was not analysed to find the microorganisms, or the lice samples had not been not collected in Africa (Figure1). Ultimately, a total of 20 studies analysed lice samples to identify microorganisms and were included in this systematic review (Supplementary Data 1).

116 ***Rickettsia***

117 *Rickettsia* are obligate Gram negative bacteria, which include 31 recognised species, 17 of
118 which are pathogenic for humans and/or animals [33]. Several haematophagous arthropods can
119 harbour or transmit rickettsiae, including ticks, fleas and lice [34]. Phylogenetically, the genus
120 *Rickettsiae* is divided into four groups: ancestral, transient, typhus group (TG) and spotted fever
121 [33]. *Rickettsia prowazekii* is a bacterium responsible for epidemic typhus and its main vector
122 is the human body louse [6,35–38]. The main reservoir for this bacterium is human beings [36].
123 In humans, the rickettsioses of the TG have spread all over the world [35]. Clinically, epidemic
124 typhus is manifested mainly by fever with severe headaches and a macular, maculopapular or
125 petechial rash, which can be difficult to detect on dark skin. However, there are no pressure
126 ulcers at the sites of the lice bite. In severe cases, the appearance of gangrene in the distal
127 extremities, pulmonary involvement, neurological damage (meningitis, hemiplegia, neuritis,
128 etc.) and cardiac complications can be observed [16].

129 During the First World War, epidemic typhus re-emerged. Russia suffered from a great
130 epidemic during the revolution during which 25 million people were infected and three million
131 died [6]. During the Second World War, epidemic typhus was widespread in North Africa [36],
132 southern Italy [39], and Eastern and Central Europe [40]. Typhus then slowly disappeared,
133 except for African epidemics and a few sporadic cases that were reported in America [41,42]
134 and China [43]. Since 1995, this disease resurfaced in Rwanda and Burundi during the civil
135 wars that caused a proliferation of body lice resulting in 100,000 people being infected [44].
136 Sporadic cases have since occurred in North Africa [36] and North America [39], and Russia
137 experienced a large epidemic [45].

138 Recently, *R. prowazekii* has been detected in *P. humanus humanus* collected from refugee
139 camps and from prisoners in the east of Africa, Burundi and Rwanda, with a minimum
140 prevalence of 7.3% maximum prevalence of 21.2% (Table1), and an overall prevalence of

141 5.57% (Table 3) [46,47]. This may be due to the poverty and civil wars which gave rise to non-
142 compliance with hygiene standards, and an increase in human body lice infestation [46,47]. In
143 these two countries, there was also a concomitant reappearance of the typhus epidemic. No
144 cases of typhus have been indicated in North Africa now for several years [48], which could be
145 explained by the fact that natural immunisation after infection is lasts for life. The resurgence
146 of typhus is known as Brill-Zinsser's disease, and can give rise to a new epidemic [49]. The
147 risk of the disease spreading and leading to an epidemic increases with lice infestations, as was
148 the case in Burundi [44], and the risk of typhus spreading from a single infected patient can
149 trigger an epidemic in a given population [36]. Human head lice are therefore considered as a
150 potential vector [50].
151 It has been suggested that under appropriate conditions, human body lice have the ability to
152 acquire, maintain and transmit other rickettsiae species such as *Rickettsia rickettsii* and *R.*
153 *conorii* to humans [51]. Moreover, *R. aeschlimannii* was detected in *P. humanus capitis* in Mali
154 with a prevalence of 0.67% (Table 2) [50]. Recently, and for the first time in Africa, *R.*
155 *aeschlimannii* was detected in human head lice collected from patients at health centres in two
156 rural villages in Mali [50]. In addition, all these rickettsial species indicate emerging
157 rickettsiosis in humans [33].
158 However, the presence of *Rickettsia* DNA in human head lice did not prove that they were
159 vectors. It may simply be the bacteria that were acquired after a bacteraemic blood meal,
160 although this alone does not rule them out of being potential vectors.
161
162 ***Bartonella***
163 *Bartonella* are Gram-negative bacteria, several species of which are zoonotic and harmful to
164 humans and animals. They are associated with several reservoir hosts (dog, cats, rodents and
165 bats) [52]. Few haematophagous ectoparasites are known to be vectors of *Bartonella*: *B.*

henselae and *B. quintana* are transmitted by *Ctenocephalides felis* [53] and the human body lice *P. humanus humanus* [6], respectively. Body lice transmit the bacteria to humans through their infected excrement [37].

Currently, *B. quintana* is considered as being an agent responsible for bacillary angiomatosis, trench fever, endocarditis and chronic lymphadenopathy [6]. Trench fever is considered as a re-emerging disease [54] and it is characterised mainly by fever with a relapse five days later and severe and persistent leg pain [16]. It has been clinically recognised since the First and Second World Wars [47,55], with epidemics reported in Russia, Europe, Africa, China and Mexico, 30 years later, new outbreaks of this disease are rare and few cases have been reported [6,55]. Recently, through the use of paleomicrobiology, the DNA of *B. quintana* was detected in the dental pulp of a man who died more than 4,000 years ago [56]. During the First World War, it was suggested that body lice were involved in the outbreak of trench fever, as they had been identified in the soldiers clothing [57]. Later, in 1920, the role of lice in the appearance of the disease was formally demonstrated [58]. However, the relationship between the human host and *B. quintana* remained unclear. In addition, *B. quintana* infection of body lice is irreversible, and it persists until their death even after the disappearance of all symptoms of the disease [6,59], which represented a great danger of spreading of this disease through the presence of vectors such as lice [57,58,60,61].

In recent decades, trench fever has been reported in North Africa [62], Egypt [63] and in the capital of Ethiopia, Addis Ababa [6]. Currently, the infection caused by *B. quintana* has been recognised as a re-emerging disease among poor populations, including homeless people in modern cities [60,64] or in countries that are undergoing profound sociological changes, such as the wars that took place in Rwanda and Burundi [44,65,66]. These circumstances often lead to poor hygiene and living conditions that can encourage the proliferation of lice infestations and consequently the spread of the disease [6]. Recently, 13 studies reported trench fever in

191 Africa between 1999 and 2019 in the following regions: Congo, Ethiopia, Congo Brazzaville,
192 Gabon, Madagascar, Mali and Senegal [46,47,50,67–76]. Between 1999 and 2016, 8 studies
193 reported the presence of *B. quintana* in human body lice collected from homeless people,
194 residents of refugee camps and individuals mono-infested by plague, with a prevalence varying
195 between 2.3% and 93.9% in the following regions: Algeria, Burundi, Congo, Democratic
196 Republic of Congo, Ethiopia, Kenya, Madagascar, Rwanda and Zimbabwe [46,47,67–
197 70,75,76] (Table 1) (Figure 2).
198 Ten studies revealed the presence of *B. quintana* in human head lice collected from healthy
199 individuals, migrants, patients, and people living in poor conditions, with a prevalence varying
200 between 0.5% and 19.4% (Table 2) and with an overall prevalence of 2.6% [50,67–75] (Table
201 3) (Figure 3). It has therefore been suggested that human head lice should be considered as a
202 new potential vector for the transmission of *B. quintana*.
203
204 ***Borrelia***
205 *Borrelia recurrentis* is the causative agent of louse-borne recurrent fever in humans. This
206 spirochete is transmitted to human beings by human body lice [6]. When a louse feeds on a
207 blood meal infected with *B. recurrentis*, these bacteria pass from the louse's digestive tract to
208 its haemolymph, where they proliferate. The body louse retains the *B. recurrentis* infection
209 throughout its life [6]. However, the salivary glands and ovaries are not affected. The infection
210 is transmitted to humans by crushing an infected louse against the skin, and the bacterium has
211 the ability to pass through the skin and healthy or injured mucosa [6]. In addition, lice can
212 excrete live *B. recurrentis* in their faeces, encouraging the very rapid spread of the bacteria
213 during epidemics [77]. Clinically, this disease is manifested mainly as a relapsing fever with
214 states of relapse throughout the course of the disease. During the febrile phase, the spirochete

is easily identified in blood smears. Between these phases, the smears are negative because the bacteria are sequestered inside the organs [6].

During the First and Second World Wars, relapsing fever wreaked havoc, with half a million cases reported in Serbia, and 13 million infections and five million deaths in Russia and Eastern Europe during the civil wars [78]. In Africa, hundreds of thousands of cases have been reported in West Africa, one million cases have been reported in North Africa (Algeria, Tunisia, Morocco and Libya) [16], and just over a million cases have been reported in Egypt [79]. Since then, no major epidemics have been reported, other than in endemic areas such as Ethiopia [80], Somalia, Eritrea [81,82], and Sudan [81,83]. In West Africa, central and eastern Africa, new cases of recurrent fever were identified between 2011 and 2018 in several regions, including Gabon, Congo Brazzaville and Ethiopia [70,73,84,85]. These studies reported the presence of DNA of several species of the genus *Borrelia* in human head lice collected from healthy individuals and from febrile patients. Prevalence rates ranged from 0.2% to 22.9% (Table 2) [70,73,84,85], with an overall prevalence of 0.19%, 2.60% and 0.16% for the species *Borrelia* spp., *B. recurrentis* and *Borrelia theileri*, respectively (Figure 3) (Table 3). Regarding human body lice, a single study conducted in Ethiopia in 2011 detected the presence of *B. recurrentis* with a prevalence of 6.27% in lice collected from a febrile patient [70] (Table 3) (Figure 2). Interestingly, the presence of the genus *Borrelia* in human head lice collected from different regions in Africa was also reported [70,84]. Based on these results, it was suggested that human head lice may be considered as a potential new vector for the *Borrelia* species.

Yersinia pestis

Yersinia pestis is a Gram-negative bacterium of the genus *Yersinia*. It is responsible for plague, one of the most serious zoonotic diseases in human history which caused several major pandemics between the 6th and 8th centuries and between the 14th and 18th centuries [86,87].

This infectious disease can take several forms (sporadic cases, grouped cases, small or large epidemics), which can affected many humans at the same time [87]. In 2018, a recent study confirmed, using genotyping and sequencing of several intergenic "spacers", that the three historic plague pandemics were caused by the same biotype *orientalis* [86].

In recent analyses of ancient genomes, it was revealed that rodent fleas were not responsible for the transmission of plague [20]. However, it has been suggested that human lice have the ability to transmit *Y. pestis* from one human being to another through their infected faeces [20]. The vector role of lice has been proven in an experimental model, and it has been shown that a single bacterial meal is sufficient to infect the lice with this bacterium, ensuring widespread infection [88]. Recently, Barbieri *et al*, suggested that human lice may have played a fundamental role in the transmission of plague between humans during past epidemics and pandemics [20]. In 1998 and 2005, the DNA of *Y. pestis* was detected in the dental pulp of the remains of ancient humans suspected of having been infected with plague [89,90]. This hypothesis is extremely important in the context of the re-emergence of lice in populations at risk, such as homeless populations in developed countries, and poor populations in underdeveloped countries [91].

Currently, Africa, Asia and America are considered to be the natural foci of the re-emergence of plague in the world [92]. In northern Africa, a plague epidemic affected Morocco between 1940 and 1945. During this epidemic, it was mentioned that body lice played a major role in the transmission of plague between humans [93]. In Algeria in 2003, sporadic cases of plague reappeared after six decades of absence. These cases were first reported in humans then in ectoparasites captured from rodents [94,95]. Five years later, another outbreak was reported in Libya and researchers suggested that the bacteria had been reactivated as a result of the outbreak in the neighbouring region [96]. In South Africa, in 2010, two studies conducted in Congo reported the presence of *Y. pestis* DNA in head and body lice collected from patients with

typical bubonic plague symptoms and from people living in six different localities in a highly plague-endemic area near Rethy in the Orientale Province of the Democratic Republic of Congo. These positive cases were reported with an overall prevalence of 0.09% and 0.49% in head and body lice, respectively [68,75] (Table 3).

When all patients were found to have typical symptoms of the plague[68], a field assessment was conducted by collecting head and body lice in highly endemic areas. Other hypothesis suggested that there have been co-infections of *B. quintana* and *Y. pestis* for centuries [31], which was proven in a recent study in which they were able to find DNA of both in the dental pulp of a skeleton dating from between the 11th and 15th centuries, excavated in Bondy, France [31].

In Africa, two studies in Congo reported the presence of *Y. pestis* in head and body lice with an overall prevalence of 0.09% and 0.49%, respectively. In addition, human body lice can be pandemic vectors of *Y. pestis* [18,19]. In endemic areas such as the Congo, it is necessary to control the infestation of lice to limit the spread of plague during an epidemic [68].

Coxiella burnetii

Coxiella burnetii is a Gram-negative zoonotic bacterium that causes Q fever, an infectious disease that is widespread throughout the world except New Zealand. It is a notifiable disease in several countries, including the United States [97]. This bacterium is resistant to harsh environmental conditions and can be harboured by mammals, birds, reptiles and arthropods [98]. Domestic mammals, such as sheep, cattle and goats, are their main reservoir. This bacterium is responsible for a wide range of clinical signs in humans: fever, pneumonia, hepatitis, infective endocarditis, and neurological signs [97].

C. burnetii is transmissible to humans mainly by the inhalation of infected aerosols that circulate in the environment following the parturition or abortion of infected animals, their

infected by-products, or by arthropods [99–101]. The epidemiological situation of this disease differs from one geographical region to another depending on whether or not the disease is notifiable [97].

In Africa, Q fever is underestimated due to a lack of diagnostic techniques. This disease was first reported in 1955 in nine countries across Africa [102]. The highest seroprevalences were reported in regions with high concentrations of livestock, namely Mali, Burkina Faso, Nigeria and the Central African Republic [103]. In humans, the seroprevalence rates of the disease differ from one country to another: rates of 1%, 16%, 15%, 26% and 24.5% have been reported in Chad, Egypt, Algeria, Namibia, Senegal, respectively [104–107]. *C. burnetii* is responsible for 5% of severe pneumonia cases in Tanzania [108], between 1% and 3% of endocarditis cases reported in Algeria and Tunisia, and in 5% of acute fever cases in Burkina Faso [109]. In domestic ruminants, seroprevalence rates have increased in many African regions, including 55% reported in cattle in Niger [110], and 33% in sheep, 23% in goats and 70% in camels in Egypt [111].

It has been suggested that the circulation of *C. burnetii* in animals may be due to the presence of haematophagous arthropods [97]. These arthropods are infected by this bacterium during their bacterial blood meal, at all stages of their biological development [112]. *Coxiella burnetii* has been detected in many arthropods, including lice [113]. Until now, lice have not yet been recognised as vectors of *C. burnetii*, although experimental evidence has shown that lice may play a potential role in the transmission of *C. burnetii.* According to field surveys, it has been reported that, in epidemic areas, lice have transmitted *C. burnetii* to guinea pigs three months after an epidemic [113]. For the first time in Algeria, between 2014 and 2016, the presence of *C. burnetii* was reported in DNA from human lice collected from Nigerian refugees and homeless people, with a prevalence rate of 8.1% and 1.9% in head and body lice, respectively [76,114] (Tables 1 and 2) (Figures 2 and 3). Another study in Mali revealed the presence of *C.*

315 *burnetii* with a prevalence of 1.2% in head lice of patients presenting at health centres [50]
316 (Table 2) (Figure 3). In Africa, an overall prevalence of 0.64% and 1.9% of *C. burnetii* has been
317 reported in *P. humanus capitis* and *P. humanus humanus,* respectively (Table 3) (Figure 4). In
318 contrast, attempts were made to detect *C. burnetii* in the poorest regions of Ethiopia and Gabon,
319 but the results were negative [71,85] (Table 2). Finally, a single study in Algeria used real time
320 PCR to identify the presence of DNA of *C. burnetii,* with rates of 1% (3/300) in *Linognathus*
321 *africanus* lice and 0.3% (1/300) in *Linognathus vituli* lice taken from goats and cattle,
322 respectively [115].
323 *C. burnetii* has been detected in ticks, bedbugs, flies and lice [113], but until now the role of
324 these arthropods in the natural cycle of *C. burnetii* is still unknown. In Africa, the role of lice
325 in the transmission of Q fever should be the subject of future work.
326
327 ***Acinetobacter***
328 *Acinetobacter* spp. are non-motile Gram-negative bacteria, found in the environments, soil,
329 vegetables, urine, faeces, and on the skin of humans and animals [46,116–121]. Several species
330 of the genus *Acinetobacter* are known to be opportunistic bacteria and pathogenic to humans
331 [122]. As molecular techniques progress, the classification of the *Acinetobacter* genus is
332 constantly improving [123,124]. Other species of *Acinetobacter* have now emerged and are
333 considered as pathogenic for humans, such as *Acinetobacter pittii*, *Acinetobacter lwoffii* and
334 *Acinetobacter soli* [122,125,126], although *A. baumannii* remains the most pathogenic species
335 for humans. *A. baumannii* is responsible for epidemics and acquired infections including both
336 nosocomial and severe community infections [127]. In several regions of the world, it has been
337 responsible for various types of manifestations, particularly in immunocompromised patients
338 [122], with skin [127], urinary tract [116], pneumonia and meningitis [119,121] infections.

Moreover, *A. baumannii* has developed resistance to several antimicrobials and can stay alive for long periods of time, even in harsh environmental conditions [116,128,129].

21% of body lice collected worldwide have tested positive for *A. baumannii*, prompting researchers to suggest that the presence of this bacterium in body lice is potentially a natural global phenomenon [130]. This has been shown in an experimental study carried out on infected rabbits [131], where it was demonstrated that when male lice fed on bactaeriemic rabbits, they had the potential to receive the infection caused by *A. baumannii* and *A. lwoffii* and remain infected throughout their lives, following a single infected blood meal, and were also able to spread live bacteria in their faeces [131]. These infected lice can infect humans through contamination of the bite sites or by crushing an infected louse on the human skin.

Several studies have shown the presence of *A. baumannii* in both body and head lice in homeless people, including in Algeria, France, Thailand, Madagascar, Georgia and the United States [132–135]. Recently, the presence of *Acinetobacter* in human lice was considered as an emerging pathogen [122]. In Africa, a total of seven studies detected the presence of 15 species of *Acinetobacter* in *Pediculus humanus* between 2010 and 2019 in North Africa, Central Africa, West Africa [76,84,85,114,136–138] (Table 1-2) (Figure 2-3). A total of 14 species have been detected in *P. humanus capitis* (Figure 3).

Regarding *Acinetobacter* spp., four studies detected its presence with a prevalence ranging from 2.8% to 37.3% (Table 2), and with an overall prevalence of 20.67% (Table 3) [84,85,136,137]. This bacterium has been detected in Algeria, Congo, Gabon and Senegal with prevalence varying between 2.3% and 54% in head lice collected from schoolchildren, Nigerian refugees and from individuals and patients from other geographic communities (Table 2). By pooling the results, the overall prevalence of this bacterium in human head lice was deemed to be 5.3% (Table 3). *A. johnsonii* has been reported in three studies carried out in Algeria and Congo on head lice collected from schoolchildren, patients and healthy individuals, and its prevalence

364 varied between 1.7% and 60% (Table 2), with an overall prevalence of 6.21% (Table 3)
365 [114,136,137]. *A. variabilis* has been reported in two studies carried out in Algeria with a
366 prevalence between 1.6% and 30% (Table 2) [114,136] and an overall prevalence of 12.50% in
367 head lice (Table 3). Concerning *A. guillouiae, A. junii, A. lwoffii, A. nosocomialis, A. pediculi,*
368 *A. pittii, A. schindleri, A. soli, A. towneri* and *A. ursingii*, their prevalence in head and/or body
369 lice was 1.1%, 6.20%, 1.51%, 1.17%, 1.10%, 1.66%, 2.85%, 1.66%, 0.67% and 4.86%,
370 respectively (Table 3) [84,137].
371 In 2014 and 2016, a single study in Algeria detected, for the first time, the presence of six
372 species of *Acinetobacter* (*Acinetobacter* spp, *A. baumannii*, *A. berezeniae*, *A. johnsonii*, *A.*
373 *nosocomialis* and *A. variabilis*) in human body lice collected from homeless people, with an
374 overall prevalence of 46.95%, 17.74%, 5.77%, 9.83%, 3.85% and 3.85%, respectively (Table
375 3) (Figure 2) [76].
376 All these reports and data from Africa suggest that human body lice may be a potential vector
377 of *Acinetobacter* spp. It has also been suggested that lice are a potential preferred host for
378 *Acinetobacter* spp. [84,132,139] and that human head lice are potential vectors of *Acinetobacter*
379 and may be can be associated with lice [136]. To date, however, human head lice are still not
380 considered as a vector of *Acinetobacter,* despite their detection in naturally infected lice [136].
381 In addition, it has been suggested that human lice may be reservoirs of *Acinetobacter*, which
382 could potentially explain their emergence [136]. However, other studies are needed to confirm
383 this hypothesis.
384
385 ***Anaplasmataceae***
386 *Anaplasma* spp. are obligate intracellular bacteria within the family Anaplasmataceae [140].
387 Some *Anaplasma* species, such as *Anaplasma phagocytophilum*, *Anaplasma marginale*.
388 *Anaplasma centrale*, *Anaplasma ovis*, *Anaplasma platys* and *Anaplasma bovis,* are responsible

for “anaplasmosis” which is harmful to humans and/or other animals and has a wide range of clinical signs in humans and animals [140]. Their transmission to different hosts (humans and animals) requires the presence of arthropods, mainly ticks [141,142].

Anaplasma phagocytophilum is an obligate Gram-negative bacterium which is attracted to neutrophils. This bacterium was first identified in 1994 [143,144]. It is widespread in several regions of the world including the United States, Europe, Asia and Africa, and affected humans and many species of animals, including domestic mammals such as sheep, cattle and goats, carnivores, rodents, insectivores, birds, and reptiles [145]. *A. phagocytophilum* causes human granulocytic anaplasmosis (HGA), which clinically varies from an asymptomatic manifestation to very severe febrile states responsible for 30% of hospitalisations [145]. It also causes anaplasmosis in ruminants, sheep, cattle, goats, carnivores and horses [145–150].

A. phagocytophilum is transmitted primarily by several species of hard ticks of the genera *Ixodes* in Europe, United States, Asia and Russia [151]. Other genera of ticks have also been reported to transmit this bacterium species, mainly *Dermacentor*, *Haemaphysalis* and *Amblyomma*, but their role as vector is still not certain [140,152]. In North Africa, many species of *Anaplasma* have been found in animals and ticks using molecular tools [153]. Serological tests have also reported the presence of *Anaplasma* spp., *A. phagocytophilum*, *A. marginale* in carnivorous, equines, dromedaries and humans in Morocco, Algeria, Tunisia, Egypt and Sudan [154–158]. In Tunisia, *A. phagocytophilum* was detected with a rate of 0.6% in cattle, 0.9% in dogs, 13% in horses and 41.7% in cattle in Algeria [159–162]. In North Africa, no molecular reports have indicated the presence of *Anaplasma* spp. in humans [153]. In West Africa, some studies have detected the presence of *A. phagocytophilum* DNA in Niger in carnivorous animals [163], and in febrile sheep in two regions of Senegal [164]. In West Africa, no studies have reported the presence of *A. phagocytophilum* in humans [153].

In North Africa, *A. phagocytophilum* was first detected in human body lice collected from homeless people in three different cities in north Algeria (Tizi Ouzou, Algiers and Boumerdes), with a prevalence of 0.76% (Table 1) (Figure 2) [76]. Interestingly, a potentially new species of *Anaplasma* spp. was also detected in head lice collected from patients in Mali with a prevalence of 0.3% [50]. In terms of similarity, this new *Anaplasma* species was 83% closer to *A. phagocytophilum,* already detected in Senegal [164].

Concerning *A. ovis*, this species is responsible for anaplasmosis ovis in small domestic and wild ruminants [165–168]. It was first identified in sheep in 1912 [169], and later became widespread in the tropics and subtropics. It has been reported in Africa, Europe, Asia, and the United States [170]. *A. ovis* is transmitted primarily by ticks, and potentially by other arthropods such as biting flies, *Melophagus ovinus* [165,171–173]. In Cyprus, one human patient was found to carry *A. ovis* following a tick bite [174]. In North Africa, *A. ovis* has mainly been detected in sheep, with a prevalence of 93.8%, 61.7% and 42.7% in Tunisia, Algeria and Sudan, respectively [142,170,175,176]. This bacterium has also been detected in Algeria in two species of ticks [176]. In addition, other reports in West Africa have noted the presence of this *Anaplasma* species in the blood of sheep and dogs in Senegal [164,177]. Finally, only one study in Algeria detected the presence of *A. ovis* in goat lice, and one new potential case of Anaplsmataceae sp. has been reported in cattle lice [115].

Regarding the other species of *Anaplasma,* including *A. marginale*, *A. centrale, A. platys* and *A. bovis*, no studies have yet reported their occurrence in human lice in Africa.

Ehrlichiae

Ehrlichiae are Gram-negative bacteria attracted mainly to monocytes and granulocytes. They belong to the order Rickettsiales and the family of Anaplasmataceae [169,178]. These microorganisms are widespread in many parts of the world and are responsible for

438 "ehrlichiosis" [141]. The genus *Ehrlichia* includes five species, all of which are pathogenic to
439 humans and/or animals namely: *E. canis*, *E. chaffeensis*, and *E. ewingii* which affects dogs and
440 humans, and *E. muris* and *E. ruminantium* which affect domestic ruminants [141,178]. Each of
441 these bacteria is transmitted by specific species of ticks [178].
442 Human monocytic ehrlichiosis is mainly caused by *E. chaffeensis*, which was first identified in
443 the United States [179]. Other cases were later reported in blood from patients in Venezuela
444 and Cameroon [180,181]. This infectious disease manifests itself mainly as a feverish state,
445 thrombocytopenia, pain in the joints, lymphadenopathy, vomiting, diarrhoea and skin rashes
446 [141,182]. Based on serological surveys, it has been suggested that human ehrlichiosis has
447 always been present in Africa, but as a rare infection [183]. In sub-Saharan Africa, *Ehrlichiae*
448 is currently considered as an emerging disease [184]. For example, in West Africa in 1992,
449 human cases of monocytic ehrlichiosis were diagnosed in 765 blood sera sampled from
450 residents of Côte d'Ivoire, Burkina Faso, Mali, Central African Republic, Angola, Zimbabwe,
451 Mozambique and the Comoros Islands [183]. A few years later, another case was reported of a
452 Malian woman who was diagnosed in North America after returning from Mali [185]. In 2003,
453 on the Cameroonian coast in Central Africa, a detection rate of 10% of *E. chaffeensis* DNA was
454 reported in febrile patients [181]. In South Africa, human cases were infected with *E.*
455 *ruminantium*, which is an endemic organism in sub-Saharan Africa [186]. This has also been
456 detected in sheep in Gambia and Senegal [164,187]. In Africa, several species of *Ehrlichia*
457 (namely *E. ruminantium*, *E. canis*, *E. chaffeensis*, *E. ewingii*) have been detected on several
458 species of ticks in the regions of Niger, Côte d'Ivoire, Burkina Faso and Mali [188–192].
459 According to our literature review, only two studies have reported the presence of *Ehrlichia* in
460 human lice in Africa. The first was in Ethiopia, where *E. muris* was detected for the first time
461 in lice collected from patients with a prevalence of 7.1% and 6.2% in body and head lice,
462 respectively (Tables 1 and 2) (Figures 2 and 3) [73]. The second was in Mali, where a potential

463 new species of *Ehrlichia* was detected in *P. humanus capitis* collected from patients with a
464 prevalence of 2.22% (Table 2) (Figure 3) [50].
465 Phylogenetic research revealed that both head and human body lice have the same symbiont
466 *Candidatus Riesia pediculicola* that co-evolved with them [193]. These endosymbiont bacteria
467 have been a target for new symbiotic therapy techniques [194].
468
469 **Conclusion**
470 A total of 28 species of bacteria have been detected in human lice, of which 25 have been
471 reported in *P. humanus capitis* (Table 3), and 12 have been reported in body lice (Table 3)
472 (Figure 4). Based on our review, it can be assumed that lice can acquire several species of
473 bacteria, some of which are highly pathogenic to humans, and a large number of which were
474 reported in several non-endemic regions. Moreover, many experimental and epidemiological
475 studies suggested the possible vectoring competence of human head lice to transmit human
476 infectious diseases. According to our analysis of data collected in Africa, we noted that there
477 are more species of bacteria identified in human head lice than in body lice (Figure 4). These
478 findings may alert us to the fact that human head lice might be considered as a potential new
479 vector of bacteria. Body lice, in addition to being vectors of three human infectious diseases
480 (epidemic typhus, relapsing fever and trench fever), have been demonstrated to be a potential
481 vector for other human infectious pathogens, such as plague and Q fever. However, it remains
482 important to explore the differences between human body and head lice, in order to keep them
483 under control, which may prevent the spread of their associated diseases.

484 **Table 1.** Prevalence of bacterial species detected in human body lice in different countries of
485 Africa.
486
487 **Table 2.** Prevalence of bacterial species detected in human head lice in different countries of
488 Africa.
489
490 **Table 3.** Comparison of the prevalence of bacterial species between human head and body lice
491 in Africa.
492
493 **Figure 1.** Flowchart summary of the selection process.
494
495 **Figure 2.** Prevalence of bacterial species detected in human body lice in different countries of
496 Africa.
497
498 **Figure 3.** Prevalence of bacterial species detected in human head lice in different countries of
499 Africa.
500
501 **Figure 4.** Comparison of the prevalence of bacterial species detected in human head and body
502 lice in Africa.
503

Author Contributions

BO wrote the initial draft of the manuscript, MDM analysed the data and wrote the data collection part, PP and LH added their contributions and comments. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Table 1. Prevalence of bacterial species detected in human body lice in different countries of Africa.

The bacterial genera were listed in alphabetical order.

Bacterium genus	*Bacterium species*	Country	Collection period	Population	Total number of tested lice	Number of lice tested positive (%)	Lice Host	Reference	Region
Acinetobacter	*Acinetobacter spp.*	Algeria	2014 - 2016	Homeless people	524	246 (46.9%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Acinetobacter baumannii	Algeria	2014 - 2016	Homeless people	468	83 (17.7%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Acinetobacter berezeniae	Algeria	2014 - 2016	Homeless people	468	27 (5.8%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Acinetobacter johnsonii	Algeria	2014 - 2016	Homeless people	468	46 (9.8%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Acinetobacter nosocomialis	Algeria	2014 - 2016	Homeless people	468	18 (3.8%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Acinetobacter variabilis	Algeria	2014 - 2016	Homeless people	468	18 (3.8%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
Anaplasma	*Anaplasma phagocytophilum*	Algeria	2014 - 2016	Homeless people	524	4 (0,76%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
Bartonella	*Bartonella quintana*	Algeria	2014 - 2016	Homeless people	524	70 (13,4%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
			2001	Homeless people	33	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Batna
			2000	/	21	0 (0%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Burundi	2001	Refugee	33	31 (93.9%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	/
			2000	Refugee	111	100 (90.1%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	/
			1999	1) During typhus outbreak : jail and refugee camp 2) After typhus outbreak : refugee cap	164	19 (11.6%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
			1998	Refugee	38	8 (21.1%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	/
		Congo	2010	/	154	50 (32.5%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
			1999	Refugee	7	0 (0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
		Democratic Republic of Congo	2010	Inhabitants	143	48 (33.6%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.14-0686	Six different localities in a highly plague-endemic area near the Rethy Health District, Orientale Province
				Patients with typical symptoms of bubonic plague	154	50 (32.5%)	Body lice	https://dx.doi.org/10.3201%2Feid1903.121542	Six different localities in a highly plague-endemic area near the Rethy Health District, Orientale Province
		Ethiopia	2011	Inhabitants	62	7 (11.3%)	Body lice	https://doi.org/10.3201/eid1905.121480	Bahir Dar Hospital
				Inhabitants	424	76 (18%)	Body lice	https://dx.doi.org/10.3201%2Feid1712.110453	Different location altitudes in Ethiopia
		Kenya	1999	/	37	27 (73.0%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Madagascar	2009 2011 - 2012	/	22	1 (4.5%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Rwanda	2011	/	166	149 (89.8%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
			2001	Patients	262	6 (2.3%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	~~Jail~~
		Tunisia	2000	Homeless people	3	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Sousse

		Zimbabwe	1999	Homeless people	12	2 (16.7%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	Harare
Borrelia	*Borrelia spp.*	Algeria	2014 - 2016	Homeless people	524	0 (0%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Borrelia recurrentis	Burundi	1999	1) During typhus outbreak | Burundi jail | Burundi refugee camp 2) After typhus outbreak (refugee cap)	164	0 (0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
		Congo	1999	Inhabitants	7	0 (0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
		Democratic Republic of Congo	2010	Patients	154	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid1903.121542	highly plague-endemic area near the Rethy Health District, Province Orientale,
		Ethiopia	2011	Patient with louse-borne relapsing fever	62	25 (40.3%)	Body lice	https://doi.org/10.3201/eid1905.121480	Bahir Dar Hospital, Ethiopia
		Zimbabwe	1999	Homeless people	12	0 (0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
Coxiella	*Coxiella burnetii*	Algeria	2014 - 2016	Homeless people	524	10 (1.9%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
Ehrlichia	*Ehrlichia muris*	Ethiopia	2013	Patients	14	1(7.1%)	Body lice	doi.org/10.1089/vbz.2019.2500	Rural area
Rickettsia	*Rickettsia spp.*	Algeria	2014 - 2016	Homeless people	524	0 (0%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	Three different cities: Algiers, Tizi Ouzou and Boumerdès
	Rickettsia prowazekii	Algeria	2001	Homeless people	33	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Batna
		Burundi	2001	Refugee	33	7 (21.2%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Refugee camp
			2000	Refugee	111	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Refugee camp
			1999	1) During typhus outbreak | Burundi jail | Burundi refugee camp 2) After typhus outbreak (refugee cap)	164	23 (14.0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
			1998	Refugee	38	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Refugee camp
		Congo	1999	Inhabitants of refugee camps	7	0 (0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	/
		Democratic Republic of Congo	2010	Patients had symptoms typical of bubonic plague, and their illnesses were reported as suspected bubonic plague.	154	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid1903.121542	highly plague-endemic area near the Rethy Health District, Province Orientale,
		Ethiopia	2011	Patient with louse-borne relapsing fever	62	0 (0%)	Body lice	https://doi.org/10.3201/eid1905.121480	Bahir Dar Hospital, Ethiopia
		Rwanda	2001	Jail	262	19 (7.3%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Jail
		Tunisia	2000	Homeless people	3	0 (0%)	Body lice	https://dx.doi.org/10.3201%2Feid0812.020111	Sousse
		Zimbabwe	1999	Homeless people	12	0 (0%)	Body lice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC84482/	Harare
Yersinia	*Yersinia pestis*	Algeria	2014 - 2016	Homeless people	524	0 (0%)	Body lice	https://doi.org/10.1371/journal.pntd.0006397	03 localities: Algiers, Tizi Ouzou and Boumerdès located
		Democratic Republic of Congo	2010	Patients had symptoms typical of bubonic plague, and their illnesses were reported as suspected bubonic plague.	154	2 (1.3%)	Body lice	https://dx.doi.org/10.3201%2Feid1903.121542	highly plague-endemic area near the Rethy Health District, Province Orientale,
				Infected individuals	143	2 (1.4%)	Body lice	https://dx.doi.org/10.4269%2Fajtmh.14-0686	living in six different localities in a highly plague-endemic area near the Rethy Health District, Orientale Province, Democratic Republic of the Congo

Table 2. Prevalence of bacterial species detected in human head lice in different countries of Africa.

The bacterial genera were listed in alphabetical order.

Bacterium genus	*Bacterium Species*	Country	Collection period	Population	Lice tested	Lice tested Positive (%)	Lice Host	Species of lice	Références	Region
Acinetobacter	*Acinetobacter spp.*	Algeria	2013 - 2014	schoolchildren's scalps and hair.	64	17 (26.6%)	Head lice	Pediculus humanus capitis	https://doi.org/10.1016/j.cimid.2017.06.003	4 different locations inAlgiers, the regions of El Madania, ElAchour, Chéraga and Tassala ElMerdja
		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	235 (37.3%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	03 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	5 (2.8%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
		Gabon	2018	Patients	557	39 (7.0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
	Acinetobacter baumannii	Algeria	2015 - 2016	Schoolchildren	40	4 (10.0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2013 - 2014	Schoolchildren's scalps and hair.	64	9 (14.1%)	Head lice	Pediculus humanus capitis	https://doi.org/10.1016/j.cimid.2017.06.003	4 different locations inAlgiers, the regions of El Madania, ElAchour, Chéraga and Tassala ElMerdja
			2016	Nigerien refugees	37	20 (54.0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria
		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	22 (3.7%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	6 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	15 (8.3%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
		Gabon	2018	Patients	557	13 (2.3%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Senegal	2010 - 2011	Healthy conditions	354	14 (4.0%)	Head lice	/	https://doi.org/10.1371/journal.pone.0039495	5 were collected in Keur Massar (12.5% of the lice collected in Keur Massar), 4 in Rufisque (7.5%), 3 in Malika (5.3%), and 2 in Dielmo (10.0%).
	Acinetobacter guillouiae	Democratic Republic of Congo	2019	Patients	181	2 (1.1%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
	Acinetobacter johnsonii	Algeria	2015 - 2016	Schoolchildren	40	24 (60.0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	school children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2013 - 2014	Schoolchildren's scalps and hair.	64	7 (10.9%)	Head lice	Pediculus humanus capitis	https://doi.org/10.1016/j.cimid.2017.06.003	4 different locations inAlgiers, the regions of El Madania, ElAchour, Chéraga and Tassala ElMerdja
			2013 - 2014	Schoolchildren's scalps and hair.	4	2 (50.0%)	Head lice	Phthirus pubis	https://doi.org/10.1016/j.cimid.2017.06.003	4 different locations inAlgiers, the regions of El Madania, ElAchour, Chéraga and Tassala ElMerdja
		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	19 (3.2%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	7 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	3 (1.7%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
	Acinetobacter junii	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	37 (6.2%)	head lice	/	https://doi.org/10.1371/journal.pntd.0005142	4 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Acinetobacter lwoffii	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	9 (1.5%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	9 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Acinetobacter nosocomialis	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	7 (1.2%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	10 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Acinetobacter pediculi	Democratic Republic of Congo	2019	Patients	181	2 (1.1%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
	Acinetobacter pittii	Democratic Republic of Congo	2019	Patients	181	3 (1.7%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa

	Acinetobacter schindleri	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	17 (2.8%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	8 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Acinetobacter soli	Democratic Republic of Congo	2019	Patients	181	3 (1.7%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
	Acinetobacter towneri	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	4 (0.7%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	11 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Acinetobacter ursingii	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	597	29 (4.9%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	5 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Acinetobacter variabilis	Algeria	2015 - 2016	Schoolchildren	40	12 (30.0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2013 - 2014	Schoolchildren's scalps and hair.	64	1 (1.6%)	Head lice	Pediculus humanus capitis	https://doi.org/10.1016/j.cimid.2017.06.003	4 different locations inAlgiers, the regions of El Madania, ElAchour, Chéraga and Tassala ElMerdja
Anaplasma	*Anaplasma spp.*	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	0 (0%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	16 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
		Ethiopia	2013		30	0 (0%)		14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
		Algeria	2015 - 2016	Schoolchildren	40	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2016	Nigerien refugees	37	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria
		Gabon	2018	Patients	691	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Mali	2013	Patients	600	2 (0.3%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
Bartonella	*Bartonella spp.*	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	0 (0%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	19 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
	Bartonella quintana	Algeria	2015 - 2016	Schoolchildren	40	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2016	Nigerien refugees	37	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria
			2000	/	9	0 (0%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
			2002	Schoolchildren	18	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid0812.020111	Schoolchildren
			2002	Schoolchildren	20	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid0812.020111	Schoolchildren
		Congo	2010	/	35	6 (17.1%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	0 (0%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	12 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
			2010	Patients	35	6 (17.1%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1903.121542	Highly plague-endemic area near the Rethy Health District, Province Orientale,
			2010	Patients	31	6 (19.4%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.14-0686	Living in six different localities in a highly plague-endemic area near the Rethy Health District, Orientale Province, Democratic Republic of the Congo
		Ethiopia	2011	Patients	271	19 (7%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1712.110453	/
			2011	Patients	35	1 (2.9%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1905.121480	Bahir Dar Hospital, Ethiopia

			2010	Street beggars	65	6 (9.2%)	Head lice	/	https://doi.org/10.1016/j.cimid.2011.09.007	Poorer regions of Jimma
		Gabon	2018	Patients	691	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Madagascar	2009, 2011 - 2012	/	75	2 (2.7%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Mali	2013	Patients	600	3 (0.5%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
			2010	/	92	0 (0%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Senegal	2010 - 2011	Persons living in poor conditions	274	19 (6.9%)	Head lice	/	https://doi.org/10.1089/vbz.2011.0845	Different locations : KaolackCity, Dakar City and itssuburbs, and two villages in the Fatick region, Dielmo and Ndiop
			2011	/	381	2 (0.5%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.13-0707	/
		Ethiopia	2013	/	30	4 (13.3%)		14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
		Senegal	2011	Patients	148	2 (1.4%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.13-0685	Dielmo (13°45′ N/16°25′ W), and Ndiop (13°41′ N/16°23′ W; 400 inhabitants)
Borrelia	*Borrelia spp.*	Algeria	2015 - 2016	Schoolchildren	40	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2016	Nigerien refugees	37	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria
		Ethiopia	2010	Street beggars of poorer regions	65	0 (0%)	Head lice	/	https://doi.org/10.1016/j.cimid.2011.09.007	Poorer regions of Jimma
		Gabon	2018	Patients	691	3 (0.4%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Mali	2013	Patients	600	0 (0%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
	Borrelia recurrentis	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	10 (1.6%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	15 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2010	Patients	35	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1903.121542	Highly plague-endemic area near the Rethy Health District, Province Orientale,
		Ethiopia	2013	/	30	1 (3.3%)		14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
			2011	Patient	35	8 (22.9%)	Head lice	/	https://doi.org/10.3201/eid1905.121480	Bahir Dar Hospital, Ethiopia
	Borrelia theileri	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	1 (0.2%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	14 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
Coxiella	*Coxiella spp.*	Ethiopia	2013	/	30	0 (0%)		14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
	Coxiella burnetii	Algeria	2016	Nigerien refugees	37	3 (8.1%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
		Ethiopia	2010	Street beggars of poorer regions	65	0 (0%)	Head lice	/	https://doi.org/10.1016/j.cimid.2011.09.007	Poorer regions of Jimma
		Gabon	2018	Patients	691	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville

		Mali	2013	Patients	600	7 (1.2%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
Ehrlichia	*Ehrlichia spp.*	Mali	2013	Patients	600	14 (2.3%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
		Ethiopia	2013	/	30	0 (0%)	/	14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
	Ehrlichia muris	Ethiopia	2013	/	16	1 (6.2%)	/	14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
Francisella	*Francisella spp.*	Ethiopia	2013	/	30	0 (0%)	/	14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
Moraxellaceae bacterium	*Moraxella new species*	Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	202	6 (2.97%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	/
Rickettsia	*Rickettsia spp.*	Algeria	2015 - 2016	Schoolchildren	40	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2016	Nigerien refugees	37	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria
		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	0 (0%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	17 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
		Ethiopia	2010	Street beggars of poorer regions	65	0 (0%)	Head lice	/	https://doi.org/10.1016/j.cimid.2011.09.007	Poorer regions of Jimma
		Gabon	2018	Patients	691	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Ethiopia	2013	/	30	0 (0%)	/	14 p. humanus humanus / 16 p. h capitis	https://doi.org/10.1089/vbz.2019.2500	Rural area
	Rickettsia aeschlimannii	Mali	2013	Patients	600	4 (0.6%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
	Rickettsia prowazekii	Algeria	2002	Schoolchildren	18	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid0812.020111	Schoolchildren
			2002	Schoolchildren	20	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid0812.020111	Schoolchildren
		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	0 (0%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	18 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
			2010	Patients	35	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1903.121542	Highly plague-endemic area near the Rethy Health District, Province Orientale,
		Ethiopia	2011	Patient	35	0 (0%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1905.121480	Bahir Dar Hospital, Ethiopia
		Gabon	2018	Patients	691	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Mali	2013	Patients	600	0 (0%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.
Yersinia	*Yersinia pestis*	Algeria	2015 - 2016	Schoolchildren	40	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	School children in 5 elementary schools at 3 different location in eastern Algiers, north Algeria, including the regions of Bab Ezzouar, El Mohammadia and Bordj el kiffan
			2016	Nigerien refugees	37	0 (0%)	Head lice	/	https://dx.doi.org/10.1186%2Fs13071-018-2930-5	Bab Ezzouar Nigerien refugees camp, East Algiers, Algeria

		Congo-Brazzaville	2015	Apparently healthy authochthonal individuals (pygmies)	630	0 (0%)	Head lice	/	https://doi.org/10.1371/journal.pntd.0005142	13 different villages: i) Thanry-Ipendja, ii) Pokola, iii) Béné-Gamboma
		Democratic Republic of Congo	2019	Patients	181	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-019-3540-6	Monkole Hospital Center located in Kinshasa
			2010	Patients	35	1 (2.9%)	Head lice	/	https://dx.doi.org/10.3201%2Feid1903.121542	Highly plague-endemic area near the Rethy Health District, Province Orientale,
			2010	Patients	31	1 (3.2%)	Head lice	/	https://dx.doi.org/10.4269%2Fajtmh.14-0686	Living in six different localities in a highly plague-endemic area near the Rethy Health District, Orientale Province, Democratic Republic of the Congo
		Ethiopia	2010	Street beggars of poorer regions	65	0 (0%)	Head lice	/	https://doi.org/10.1016/j.cimid.2011.09.007	Poorer regions of Jimma
		Gabon	2018	Patients	691	0 (0%)	Head lice	/	https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04293-x	Franceville
		Mali	2013	Patients	600	0 (0%)	Head lice	/	https://dx.doi.org/10.1371%2Fjournal.pone.0184621	Two rural Malian villages, Donéguébougou (12°48'85"N 7°58'22"W) and Zorocoro (12°44'75"N 80°04'50"W), situated in close proximity in the Koulikoro region in a savanna zone.

Table 3. Comparative of bacterial species prevalence between human head and body lice in Africa.

The bacterial genera were listed in alphabetical order.

Bacterium genus	*Bacterium Species*	Head Lice			Body Lice		
		Number of lice tested	Number of lice tested Positive	% of positive	Number of lice tested	Number of lice tested Positive	% of positive
Acinetobacter	*Acinetobacter* spp.	1432	296	20,67	524	246	46,95
	Acinetobacter baumannii	1830	97	5,30	468	83	17,74
	Acinetobacter berezeniae	/	/	/	468	27	5,77
	Acinetobacter guillouiae	181	2	1,10	/	/	/
	Acinetobacter johnsonii	886	55	6,21	468	46	9,83
	Acinetobacter junii	597	37	6,20	/	/	/
	Acinetobacter lwoffii	597	9	1,51	/	/	/
	Acinetobacter nosocomialis	597	7	1,17	468	18	3,85
	Acinetobacter pediculi	181	2	1,10	/	/	/
	Acinetobacter pittii	181	3	1,66	/	/	/
	Acinetobacter schindleri	597	17	2,85	/	/	/
	Acinetobacter soli	181	3	1,66	/	/	/
	Acinetobacter towneri	597	4	0,67	/	/	/
	Acinetobacter ursingii	597	29	4,86	/	/	/
	Acinetobacter variabilis	104	13	12,50	468	18	3,85
Anaplasma	*Anaplasma* spp.	2209	2	0,09	/	/	/
	Anaplasma phagocytophilum	/	/	/	524	4	0,76
Bartonella	*Bartonella quintana*	3698	76	2,06	2370	644	27,17
Borrelia	*Borrelia* spp.	1614	3	0,19	524	0	0,00
	Borrelia recurrentis	730	19	2,60	399	25	6,27
	Borrelia theileri	630	1	0,16	/	/	/
Coxiella	*Coxiella* spp.	30	0	0,00	/	/	/
	Coxiella burnetii	1574	10	0,64	524	10	1,91
Ehrlichia	*Ehrlichia* spp.	630	14	2,22	/	/	/
	Ehrlichia muris	16	1	6,2	14	1	7.1
Francisella	*Francisella* spp.	30	0	0,00	/	/	/
Moraxellaceae	*Moraxella* spp.	202	6	2,97	/	/	/
Rickettsia	*Rickettsia* spp.	1674	0	0,00	524	0	0,00
	Rickettsia aeschlimannii	600	4	0,67	/	/	/
	Rickettsia prowazekii	2210	0	0,00	879	49	5,57
Yersinia	*Yersinia pestis*	2310	2	0,09	821	4	0,49

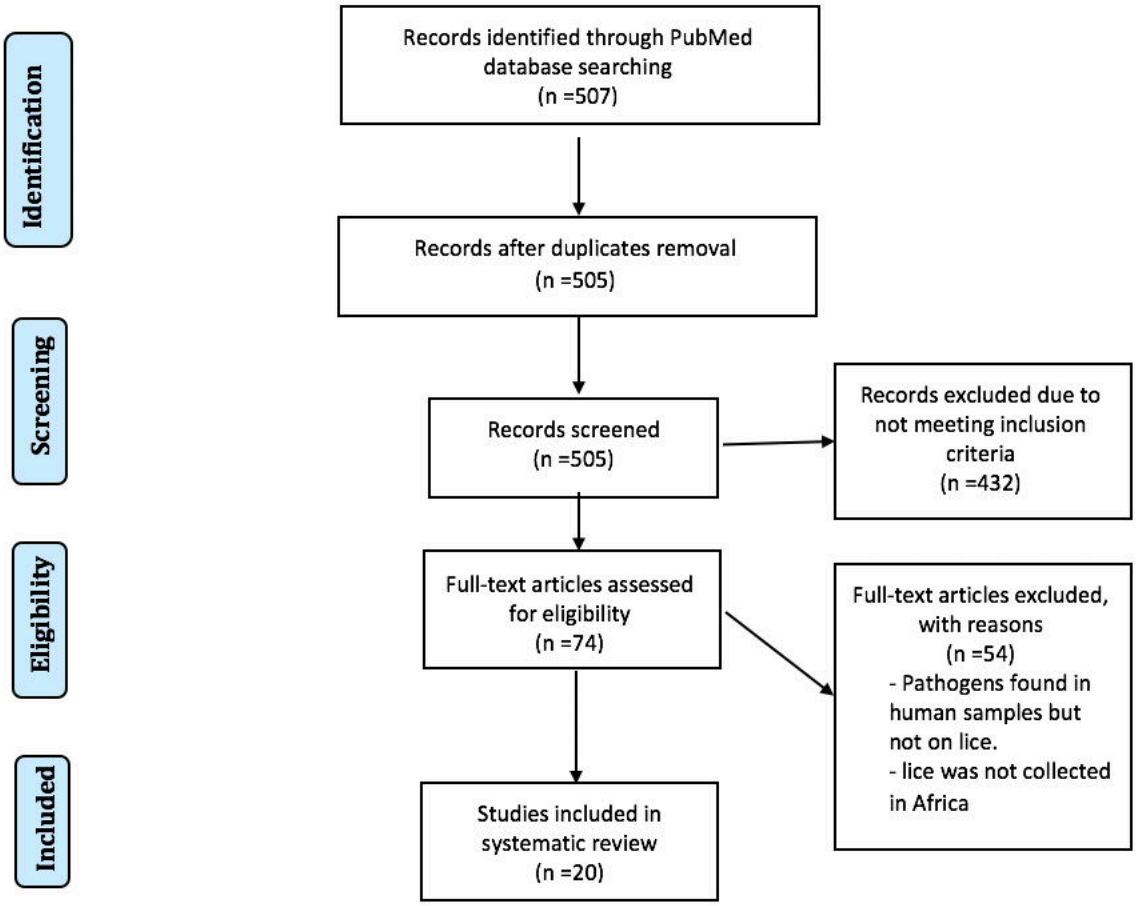


Figure 1. Flowchart summary of the selection process

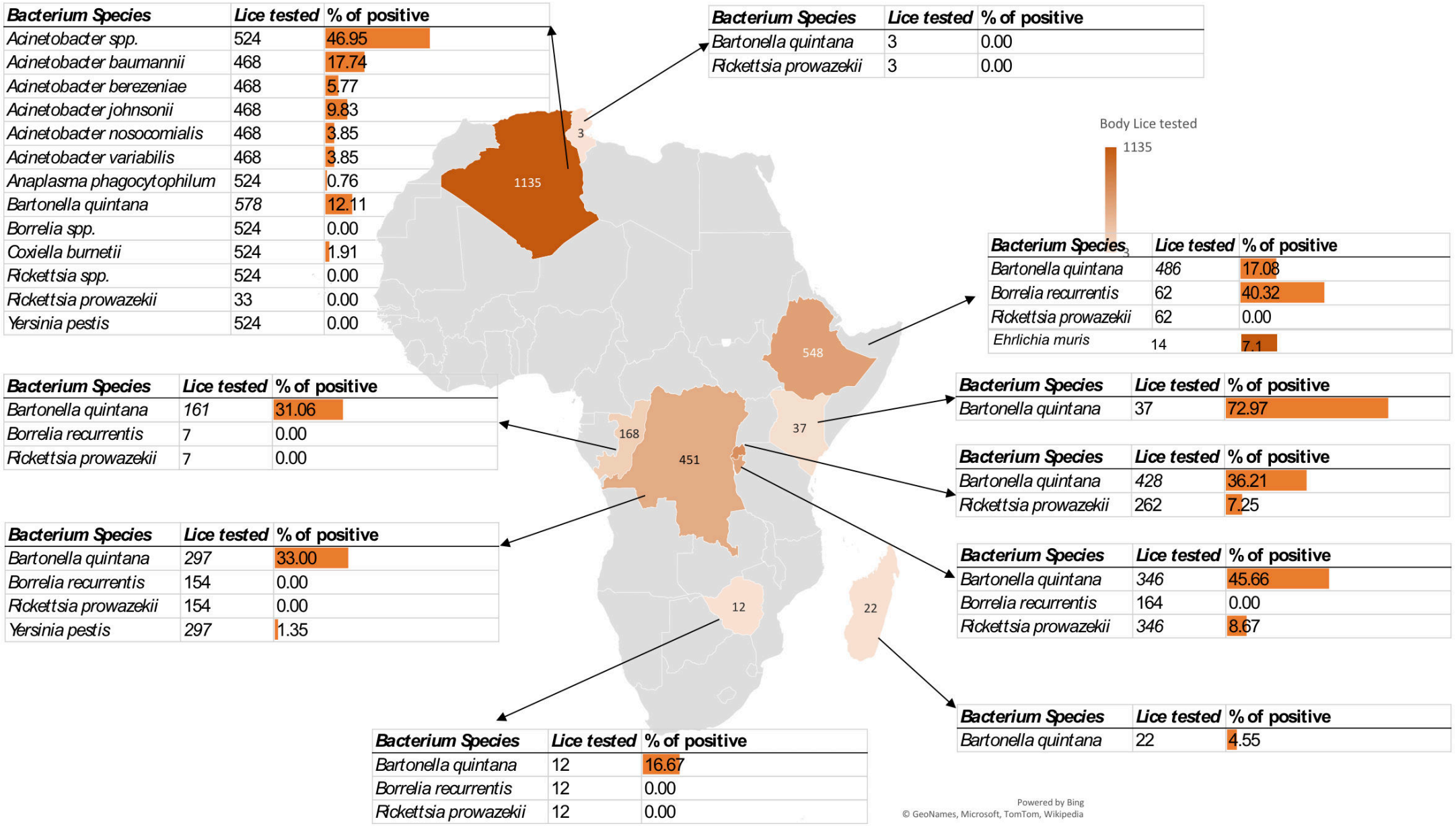


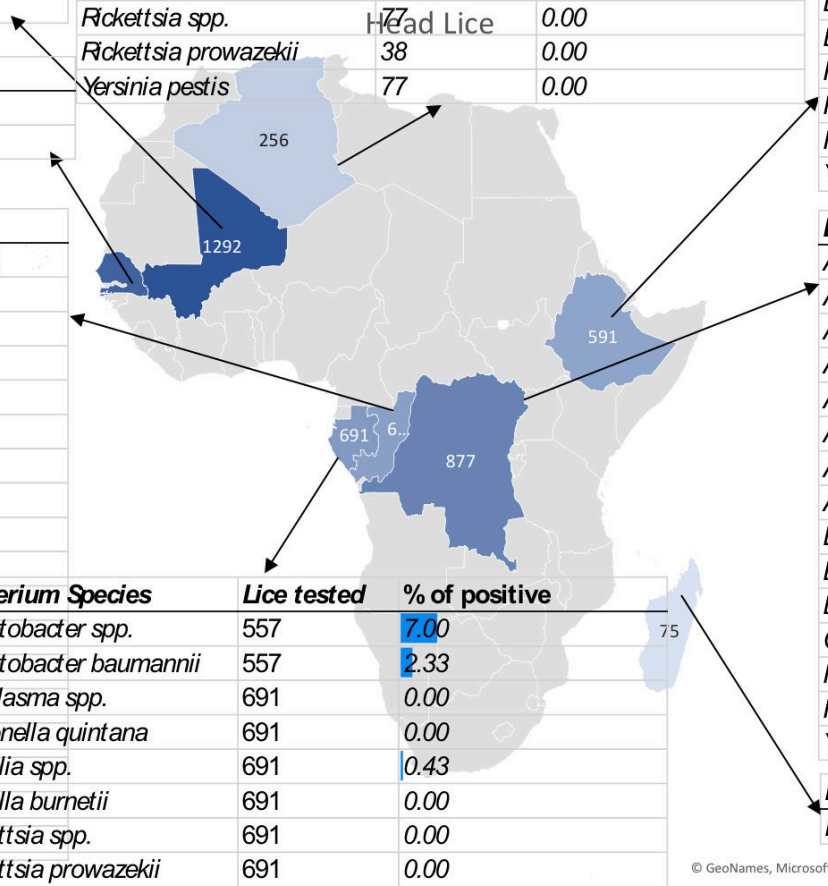
Figure 2. Prevalence of bacterial species detected in human body lice in different countries of Africa

Bacterium Species	Lice tested	% of positive
Anaplasma spp.	600	0.33
Bartonella quintana	692	0.43
Borrelia spp.	600	0.00
Coxiella burnetii	600	1.17
Ehrlichia spp.	600	2.33
Rickettsia aeschlimannii	600	0.67
Rickettsia prowazekii	600	0.00
Yersinia pestis	600	0.00

Bacterium Species	Lice tested	% of positive
Acinetobacter baumannii	354	3.95
Bartonella quintana	803	2.86

Bacterium Species	Lice tested	% of positive
Acinetobacter spp.	630	37.30
Acinetobacter baumannii	597	3.69
Acinetobacter johnsonii	597	3.18
Acinetobacter junii	597	6.20
Acinetobacter lwoffii	597	1.51
Acinetobacter nosocomialis	597	1.17
Acinetobacter schindleri	597	2.85
Acinetobacter towneri	597	0.67
Acinetobacter ursingii	597	4.86
Anaplasma spp.	630	0.00
Bartonella spp.	630	0.00
Bartonella quintana	630	0.00
Borrelia recurrentis	630	1.59
Borrelia theileri	630	0.16
Moraxella new species	202	2.97
Rickettsia spp.	630	0.00
Rickettsia prowazekii	630	0.00
Yersinia pestis	630	0.00

Bacterium Species	Lice tested	% of positive
Acinetobacter spp.	64	26.56
Acinetobacter baumannii	141	23.40
Acinetobacter johnsonii	108	30.56
Acinetobacter variabilis	104	12.50
Anaplasma spp.	77	0.00
Bartonella quintana	124	0.00
Borrelia spp.	77	0.00
Coxiella burnetii	37	8.11
Rickettsia spp.	77	0.00
Rickettsia prowazekii	38	0.00
Yersinia pestis	77	0.00



Bacterium Species	Lice tested	% of positive
Acinetobacter spp.	557	7.00
Acinetobacter baumannii	557	2.33
Anaplasma spp.	691	0.00
Bartonella quintana	691	0.00
Borrelia spp.	691	0.43
Coxiella burnetii	691	0.00
Rickettsia spp.	691	0.00
Rickettsia prowazekii	691	0.00
Yersinia pestis	691	0.00

Bacterium Species	Lice tested	% of positive
Anaplasma spp.	30	0.00
Bartonella quintana	401	7.48
Borrelia spp.	65	0.00
Borrelia recurrentis	65	13.85
Coxiella spp.	30	0.00
Coxiella burnetii	65	0.00
Ehrlichia spp.	30	0.00
Ehrlichia muris	16	6.62
Francisella spp.	30	0.00
Rickettsia spp.	95	0.00
Rickettsia prowazekii	35	0.00
Yersinia pestis	65	0.00

Bacterium Species	Lice tested	% of positive
Acinetobacter spp.	181	2.76
Acinetobacter baumannii	181	8.29
Acinetobacter guillouiae	181	1.10
Acinetobacter johnsonii	181	1.66
Acinetobacter pediculi	181	1.10
Acinetobacter pittii	181	1.66
Acinetobacter soli	181	1.66
Anaplasma spp.	181	0.00
Bartonella quintana	247	4.86
Borrelia spp.	181	0.00
Borrelia recurrentis	35	0.00
Coxiella burnetii	181	0.00
Rickettsia spp.	181	0.00
Rickettsia prowazekii	216	0.00
Yersinia pestis	247	0.81

Bacterium Species	Lice tested	% of positive
Bartonella quintana	75	2.67

Figure 3. Prevalence of bacterial species detected in human head lice in different countries of Africa

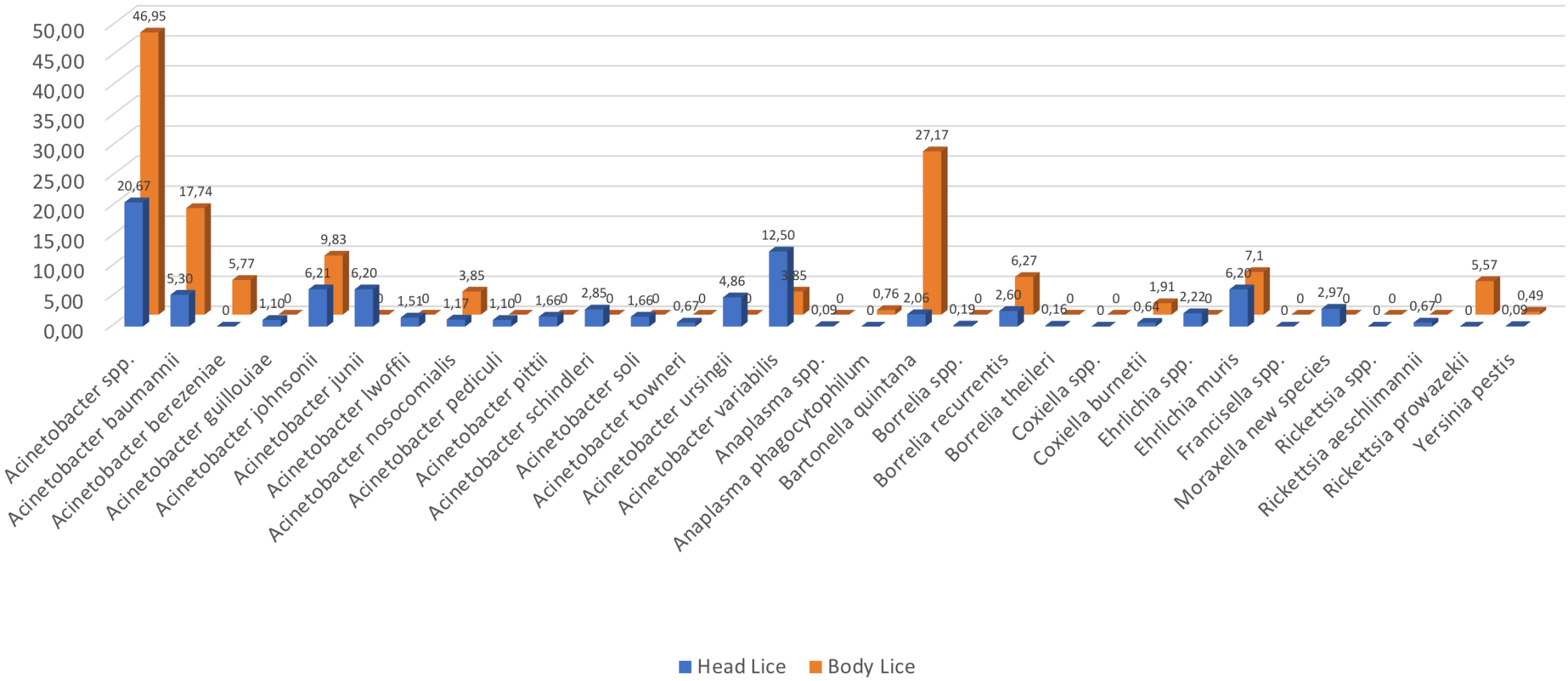


Figure 4. Comparison of the prevalence of bacterial species detected in human head and body lice in Africa

Article N°2:

Survey of ruminant infestation by lice in north-east Algeria.

Basma Ouarti[a,b], Souad Righi[d], Mamadou Lamine Tall[b,c], Mohamed Nadir Meguini[d,e], Khaoula Ouarti[e·], Philippe Parola[a,b] , Ahmed Benakhla[d].*

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Résumé

Les poux sont des insectes très spécifiques à l'hôte appartenant à l'ordre des phthiraptera. L'importance médicale des poux n'est pas seulement liée aux dommages causés par leurs piqûres ou morsures, mais aussi à leur capacité potentielle à transmettre certains agents pathogènes. Le but de cette étude était de mettre à jour les informations disponibles sur les espèces de poux rencontrées chez les ruminants dans le nord-est de l'Algérie. Un total de 16 moutons, 13 bovins et 12 chèvres ont été examinés pour la présence de poux.

L'étude a été menée de 2014 à 2015 dans quatre fermes d'élevage.

Dans la région de Guelma, les résultats ont révélé la prévalence suivante de l'infestation: 100%, 60% et 66,66% chez les bovins, les ovins et les caprins, respectivement, et à Souk-Ahras, la prévalence était de 71,41%, 63,63% et 66,66% chez les bovins, les ovins et les caprins, respectivement. La présence d'une seule espèce chez les moutons *Bovicola ovis*, de quatre espèces chez les bovins *Bovicola bovis*, *Linognathus vituli*, *Haematopinus eurysternus*, *Solenopotes capillatus* et de deux espèces chez les chèvres *Bovicola caprae*, *Linognathus africanus* a été observée.

Les infestations les plus importantes ont été enregistrées pendant la saison hivernale. Les infestations étaient également plus prononcées chez les femelles que chez les mâles. Les résultats de cette étude contribueront à la lutte contre les poux et les maladies qu'ils transmettent.

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Survey of ruminant infestation by lice in north-east Algeria

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Informations

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Ruminants
Souk-Ahras
Guelma

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Abstract

Lice are highly host specific insects belonging to the order of phthiraptera. The medical importance of lice is not only related to the damage caused by their stings or bites but also to their potential ability to transmit some pathogens. The aim of this study was to update the available information on species of lice encountered in ruminants in the northeast of Algeria. A total of 16 sheep, 13 cattle and 12 goats were examined for the presence of lice. The study was conducted from 2014 to 2015 in four livestock farms. In the region of Guelma, the results revealed the following prevalence of infection: 100%, 60% and 66.66% in cattle, sheep and goats, respectively, and at Souk-Ahras the prevalence was 71.41%, 63.63%, and 66.66% in cattle, sheep and goats respectively. The presence of a single species in sheep *Bovicola ovis*, four species in cattle *B. bovis*, *Linognathus vituli*, *Haematopinus eurysternus, Solenopotes capillatus* and two species in goats *B. caprae*, *Linognathus africanus* was observed. The largest infestations were recorded during the winter season. Infestations were also more pronounced in females than males. The results of this study will contribute towards the control of lice and the diseases transmitted by them.

1. Introduction

Ectoparasites, represent undoubtedly a non-negligible economic scourge for ruminant livestock farming in Africa. They include a wide variety of parasitic arthropods belonging to the class of Arachnida (order of Acari (Ticks and mites) or to the class of Insecta (orders of Siphonaptera (Fleas), Phtiraptera (sucking and chewing lice) and Diptera (suborders of nematocera and brachycera)[1]. Lice are among the ectoparasites that are frequent in livestock farming in Algeria. They are obligate wingless insects that parasitize mammals and birds [1]. Nearly five thousand lice species are known as obligate, highly specific and permanent parasites of birds and mammals [2, 3].

Lice fall within the order of Phthiraptera, which is divided into four suborders, namely anoplura, amblycera, ischnocera, and rhynchophthirina [4].

Anoplura lice (Sucking lice) feed exclusively on mammalian blood (requiring several daily meals) [5] and they can lead to economic losses for livestock, skin damage and anaemia[6].

Mallophaga lice infests mostly birds and incidentally mammals and feed on feathers, skin, blood or secretions from their hosts. They compose the remaining three sub-orders, namely amblycera, ischnocera, and rhynchophthirina [7].

Currently, lice identification is primarily morphological and based on dichotomous keys, which consider the host animal from which the louse was collected [9]. In the world the main species of lice that infest mammals are (*L. vituli* (*Linognathus vituli*), *S. capillatus* (*Solenopotes capillatus*), *H. eurysternus* (*Haematopinus eurysternus*), *H. quadripertusus* (*Haematopinus quadripertusus*) and *B. bovis (Bovicola bovis*)) hosted in cattle, (*B. ovis* (*Bovicola ovis*), *L. ovillus* and *L. stenopsis* (*Linognathus stenopsis*)) found in the sheep and (*B. caprae* (*Bovicola caprae*) *and L. africanus* (*Linognathus africanus*)) in goats.

Lice infestations are more marked in winter, and there is intense multiplication of parasites in this season. This is due to several reasons: More dense coat in winter, Promiscuity of animals under the effect of cold undernourishment, and various

stresses. Contamination is essentially direct, but it can also be indirect through the environment or for example by the tufts of wool transported by the birds [10]. Lice are potential biological or mechanical vectors for many infectious agents, these insects can play a role in the transmission of pathogens from one host to another [11]. We notice that DNA of some bacterial have been detected in animal lice from susceptible hosts citing examples of *Rickettsia slovaca*, *Acinetobacter* species and *Anaplasma platys* detected in wild boar, domestic animals and dogs respectively [12–14]. The detection of these DNAs is not sufficient to say that lice are vectors, but they may play a role in the transmission of pathogens. Considering the importance of the subject, it is more interesting to conduct a study on these Phthiraptera through surveys carried out in the North East of Algeria in order to update information on the species of lice found in this region and to study the influence of receptivity and sensitivity, namely sex and season.

2. Material and methods

2.1. Study area

This study was carried out on four private farms. Farm 1 and 2 are located in the region of Guelma (36 ° 27'0 "N, 7 ° 25'0" E) while Farm 3 and 4 are located in Souk-Ahras region (36 ° 17'11 "N, 7 ° 57'4" E) **(Fig. 1)**. The region of Souk-Ahras is characterised by a sub-humid climate in the north and semi-arid in the south. There is up to 700 mm of rainfall in the north and 250 mm in the south, with an average of 550 mm/year and snowfall at altitude. The Guelma region is characterised by a sub-humid climate in the centre and the north, and semi-arid in the south. Rainfall ranges from 400 to 500 mm/year in the south to nearly 1,000 mm/year in the north **(Fig. 1)**.

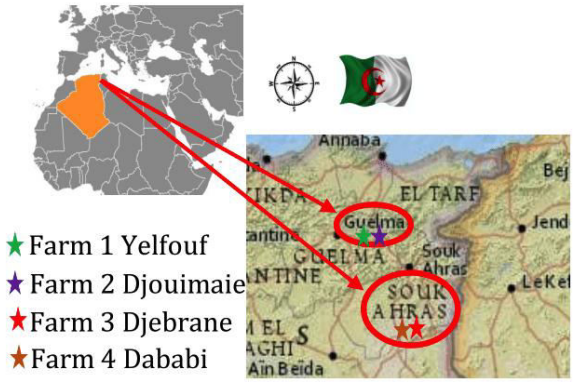


Fig.1: Location of the study area (Souk-Ahras and Guelma regions) and selected farms on the map of Algeria.

2.2. Sample collection

Lice were predominantly collected during the winter period, when they are most active. The survey was performed between 10 October 2014 and 29 April 2015, covering three seasons (winter, autumn, spring). We collected lice from sheep, goats and cattle from four farms in the Souk-Ahras and Guelma regions in Algeria, namely the Yelfouf farm and the Djouimaie farm in the commune of Oued Cham in the region of Guelma and the Djebrane farm and Dababi farm that are located in the municipality of Machrouha in the Souk-Ahras region **(Fig. 1)**.

The animals were carefully inspected by examining the different parts of the body surface to detect the presence of lice. After detecting lice in cattle and goats, they were brushed with a comb for between 10 and 30 minutes depending on the infestation's intensity. Sheep were brushed in the opposite direction to that of the animal's wool, and the skin was visually inspected for lice. Lice collected from the same animal were recovered and stored in identified tubes -20°C to perform additional analyses.

2.3. Identification of lice

The identification of lice took place in the Parasitology Laboratory of the Department of Veterinary Sciences, University of El Tarf in Algeria, using a binocular loupe and confirmed in a laboratory in Marseille, France.
Each louse was rinsed with ethanol (70%) for 15 minutes and then with distilled water for one minute. All body parts of the collected lice were examined under a microscope at x56 magnification (Zeiss Axio Zoom. V16, Zeiss, Marly-le-Roi, France) based on the morphological identification keys established by Wall and Shearer [15] and Pajot [9]. In generally blood sucking lice are morphologically recognisable by the head being generally narrower than the prothorax , as well as by their bite-sucking type mouth parts [15]. They cannot spend more than four days without eating [10]. They move little and very slowly on the skin to find the ideal temperature (29-30°C) [16]. And mallophagous lice are discernible by their head which is wider than their prothorax and have crusher-type mouthpieces located on the head's ventral surface [15]. They move more quickly on the skin surface than anoplura [10].
A part of these lice was identified by molecular biology and by mass spectrometry (MALDI-TOFMS) the details of the protocol were mentioned in a previous study [4].

2.4. Statistical analysis

The rstudio software was used to perform statistical analyses. On the other hand the statistical tests and the graphics [17, 18] were made using the package

ggplot2 and ggpubr respectively (https://cran.rproject.org/web/packages/ggplot2/index.html) and (https://cran.r-project.org/web/packages/ggpubr/index.html). Whatever test is used a difference is declared significant if $p \leq 0.05$.

3. Results and discussion

The results of the survey conducted in the region of Guelma revealed 3 out of 5 sheep (60%), 6 out of 6 cattle (100%) and 4 out of 6 goats (66.66%) were infested with lice **(Table 2)**. In the Souk-Ahras region the infestation rates noted were as follows 7/11 (63.63%) in sheep, 5/7 (71.42%) in cattle, and 4/6 (66.66%) in caprae **(Table 3)**. The results of the morphological identification revealed the presence of seven species of mammalian lice including one species in sheep (*B. ovis*), four in cattle (*B. bovis*, *L. vituli*, *S. capillatus* and *H. eurysternus*) and two species in goats (*B. caprae*, *L. africanus*) **(Fig. 2)**.

In total we examined 16 sheep, 13 cattle and 12 goats located at the four farms. In the region of Guelma our results revealed a prevalence of infestation for farms 1 and 2 respectively: 50%, 50% in cattle, 33.33%, 66.66% in sheep, and 75%, 25% in goats. For the Souk-Ahras region, we have marked the following prevalence for farms 3 and 4 respectively: 40%, 60% in cattle, 57.14%, 42.85% in sheep and 50%, 50% in goats **(Table 1)**.

Table. 1: Lice infestation in sheep, cattle and goats.

Farms	Species	Guelma	Souk-Ahras
Farm 1	Cattle	50%	/
	Sheep	33.33%	/
	Goat	75%	/
Farm 2	Catlle	50%	/
	Sheep	66.66%	/
	Goat	25%	/
Farm 3	Cattle	/	40%
	Sheep	/	57.12%
	Goat	/	50%
Farm 4	Catlle	/	60%
	Sheep	/	42.85%
	Goat	/	50%

In Guelma, the infestation rate is higher in winter 66.66% in cattle and sheep, 33.33% in spring and in autumn in cattle and sheep respectively. Concerning goats, we have recorded an infestation rate of 50% in spring, 25% in autumn and winter.

Concerning the region of Souk Ahras, the cattle infestation rates of 40% in winter and spring and 20% in autumn were observed. For sheep, we noted 14.28%, 57.14% and 28.57% in autumn, winter and spring respectively and for goats 50% in spring and 25% in autumn and winter (Fig.3).

In figure 3, as can be observed from, the number of infestation is important during the winter but there is no effect of the season on the infestation at the 5% threshold.

We noticed much higher rates of infestation in females than in males in both regions. On Guelma the infestation rates were as follows (Female versus male): 83.33% vs. 16.66%, 66.66% vs. 33.33%, and 50% vs. 50% in cattle, sheep and goats respectively. On Souk- Ahras the infestation rates were as follows (Female versus male): 80% vs. 20%, 85.71% vs. 14.28% and 75% vs. 25% in cattle, sheep and goats respectively (Fig. 4).

Table 2: Lice infestation in ruminants in northeastern Algeria (Guelma).

Host	Species	Number of infested cattle and rate infestation (%)	Percentage of average infestation rate (%)	Lice Number	Sex	season
cattle	B. bovis	1(16.66)	100	201	Female	Winter
	B. bovis	1(16.66)		100	Male	Winter
	L. vituli	1(16.66)		48	Female	Winter
	H. eurysternus	1(16.66)		4	Female	Winter
	H. eurysternus	1(16.66)		15	Female	Spring
	S. capillatus *	1(16.66)		60	Female	Spring
Total	/	6	6	428	/	/
Sheep	B. ovis	1(20)	60	48	Female	Autumn
	B. ovis	1(20)		59	Male	Winter
	B. ovis *	1(20)		10	Female	Winter
Total	/	3	5	117	/	/
Goat	B. caprae	1(16.66)	66.66	20	Male	Autumn
	B. caprae	1(16.66)		35	Female	Winter
	B. caprae *	1(16.66)		8	Female	Spring
	B. caprae	1(16.66)		4	Male	Spring
Total	/	4	6	67	/	/

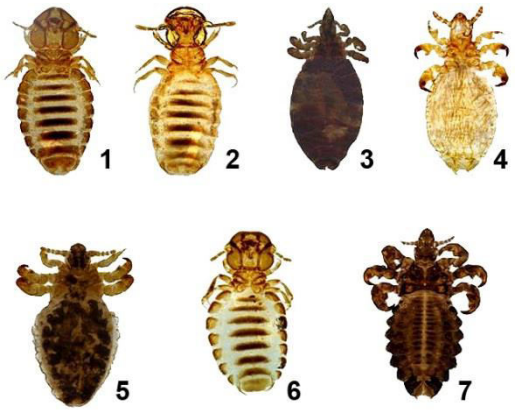


Fig.2: Pictures of lice species with their sex included in this study 1. *Bovicola bovis* ♂, 2. *Bovicola ovis* ♂, 3. *Linognathus vituli* ♀, 4. *Linognathus africanus* ♀, 5. *Solenopotes capillatus* ♂, 6. *Bovicola caprae* ♀, 7. *Haematopinus eurysternus* ♀.

Host	Species	Number of infested cattle and rate infestation (%)	Percentage of average infestation rate (%)	Lice Number	Sex	Seasons
cattle	*B. bovis* *	1(14.28)	71.42	15	Female	Autumn
	B. bovis	1(14.28)		12	Female	Winter
	B. bovis	1(14.28)		10	Male	Winter
	B. bovis	1(14.28)		8	Female	Spring
	B. bovis	1(14.28)		5	Female	Winter
Total	/	5	7	50	/	/
Sheep	*B. ovis*	1(9.09)	63.63	100	Female	Autumn
	B. ovis	1(9.09)		150	Male	Winter
	B. ovis	1(9.09)		100	Female	Winter
	B. ovis	1(9.09)		80	Female	Winter
	B. ovis	1(9.09)		50	Female	Winter
	B. ovis	1(9.09)		48	Female	Spring
	B. ovis	1(9.09)		42	Female	Spring
Total	/	7	11	570	/	/
Goat	*B. caprae*	1 (16.66)	66.66	15	Male	Autumn
	B. caprae	1 (16.66)		20	Female	Winter
	L. africanus	1 (16.66)		5	Female	Spring
	L. africanus *	1 (16.66)		2	Female	Spring
Total	/	4	6	42	/	/

*****Lice identified by molecular biology and mass spectrometry.

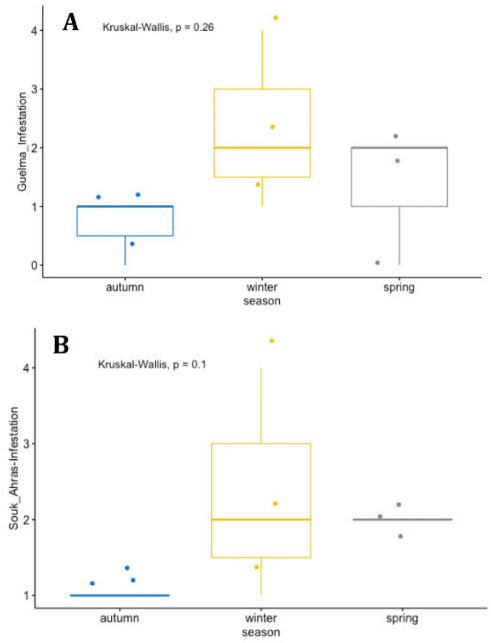
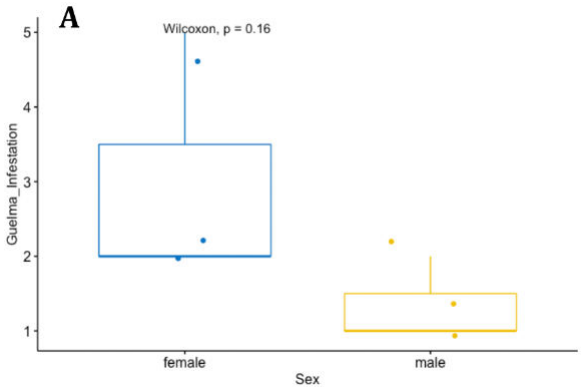


Fig.3: Distribution of the number of lice infestations in the two regions Guelma (A) and Souk Ahras (B) according to the season.



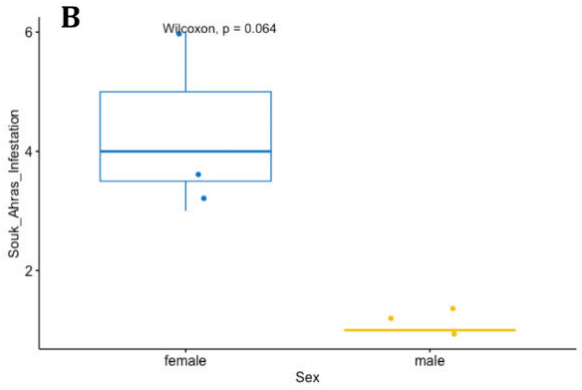


Fig.4: Distribution of the number of lice infestations in the two regions Guelma (A) and Souk Ahras (B) according to sex.

Figure 4 shows that females are more affected in the two regions, but there is not at the 5% threshold the effect of sex on the number of infestations for the two regions.

The prevalence of the infestations recorded in our study is higher compared to the work carried out by Meguini *et al*., in the regions of Souk-Ahras and Guelma [19]. These authors reported rates of 63%, 39% and 53% respectively in cattle, sheep and goats in the Souk-Ahras region and 27%, 24% and 30% in cattle, sheep and goats in the Guelma region [19].

The differences in prevalence found in the studies carried out in the Souk-Ahras and Guelma regions can be attributed to various factors including the breeding conditions, the state of health of the animals examined, and their diet quality. In Tunisia, Gharbi *et al*., reported a prevalence of 4.7% in cattle [20].

Identification by mass spectrometry (MALDI-TOF MS) has become a new tool for identifying arthropods [21]. MALDI-TOF MS is an efficient method for the identification of lice [4].

Only one species of mallophagan lice has been identified in sheep, one species of anoplura and one of mallophagan in goats and three species anoploura and one mallophagan in cattle. Our results are partly in line with those reported by Meguini *et al*, 2018 who, in addition to these species, reported the presence of *L. ovillus* in sheep [19]. *B. ovis* was the most frequently encountered louse in our study (Fig.5). We take note that several species of lice can coexist on the same animal. For example, in small ruminants *B. ovis, L. ovillus* and *L. stenopsis* can coexist [22].

Molecular and mass spectrometric identification carried out on three species of lice has been successfully carried out (Table 2-3).

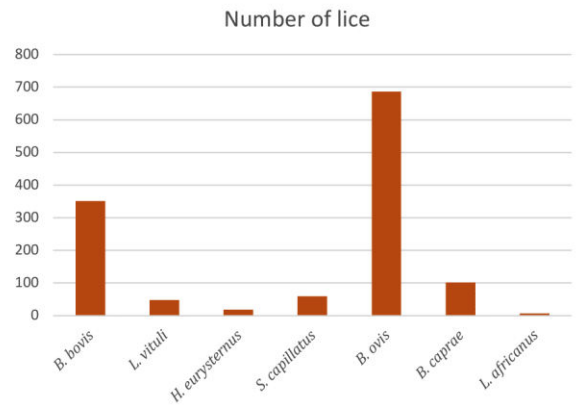


Fig.5: A diagram represents the distribution of species in the two regions Guelma and Souk-Ahras.

In Tunisia, Gharbi et al., 2013 and in Algeria, Meguini et al., 2018, reported also the predominance of *B. bovis*. The predominance of this species can be explained by the fact that this chewing louse is considered to be more adapted to the climate with dry winters and a tropical climate is unfavourable for its development [23].

Three species of anoplura lice (*L. vituli*, *H. eurysternus* and *S. capillatus*) were identified in our cattle survey, and one species (*L. africanus*) identified in Goat. These results are consistent with the findings reported by Meguini *et al.*, 2018, where the presence of these species was also reported. *L. vituli, H. eurysternus, S. capillatus* and *L. africanus* species are cosmopolitan but are more abundant in temperate and cold climates. In general, their distribution is the same as that of their main host (domestic livestock) and they can be found wherever livestock are raised [23]. It should be noted that even in small numbers, lice from sheep and goats can cause damage to the skin and devalue wool and hair. As a result, significant wool losses have been incurred by major wool-producing countries such as Australia, New Zealand and South Africa [24].

The study of the influence of sex on animals' infestation showed a much more marked infestation in females compared to males. This difference can be attributed to the fact that females remain more prone to infestation for physiological reasons. (gestation, parturition and lactation).

We noticed greater infestation rates during the winter. These results corroborate those noted by Gharbi *et al*. [20]. These results are explained by the fact that the winter temperature is much more favorable for the survival of the lice, the animal's hair is longer during this period, favoring infestation by lice, and the fact that the animals huddle together, which facilitates the transmission of the lice from one animal to another [8].

In our study, the p value was greater than the threshold of 5% but this can be explained by the fact that the sample size is small due to the constraints during collection.

Pediculosis in ruminants often reflects a more serious underlying disease or problem because the pelts of sick or undernourished animals are damaged[15].

Small infestations are generally well tolerated and pass unnoticed, but losses can be considerable in significant infestations that can affect the entire body surface and cause considerable stress and itching [10]. In addition, an animal's infestation by a larger number of biting lice can cause significant blood loss and cause anaemia, weakness, a decrease in milk production and weight gain. Hence the need to pay more attention to controlling these insects [24–26].

4. Conclusion

Very little number of works have been carried out in Algeria on lice which continue to affect the country's flocks. This work is therefore a very important step towards understanding the distribution of lice in the north-east region of Algeria.

Considering the economic and medical consequences of mammalian lice, it is imperative to identify these arthropod species in Algeria through other surveys across the country to offer new opportunities for vector surveillance.

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Chapitre II: Utilisation du MALDI-TOF MS pour l'identification des poux

Préambule

L'identification des arthropodes est une étape indispensable dans le domaine de l'entomologie. Les arthropodes sont le plus souvent liés à la santé humaine et animale. Donc leur identification donne une idée sur la répartition épidémiologique des pathogènes vectorisé et entre autres, participe aux mesures de lutte anti-vectorielle.

Les techniques d'identification des arthropodes les plus fréquemment utilisés est l'identification morphologique et moléculaire. Cependant, en plus de prendre du temps, l'identification morphologique nécessite une compétence entomologiste et documentation spécifique. L'identification moléculaire depuis quelques années a connu un grand sucée dans l'identification des arthropodes. Cependant, cette technique présente plusieurs limites à savoir : Ces dosages extrêmement cher, l'absence de gène universel pour identifier tous les arthropodes et enfin la base de données NCBI est non exhaustive.

Récemment une méthode alternative nommé la spectrométrie de masse MALDI-TOF a été émergée dans le domaine de l'entomologie. Cet outil en plus d'être rapide, nécessitant pas de compétences entomologiques, peu coûteux et précis, il l'a montré toutes ça capacité à identifier avec sucée beaucoup d'arthropodes, leur repas sanguins mais aussi la distinction entre des arthropodes infecté ou non par des microorganismes.

Dans notre deuxième chapitres intitulé « Utilisation du MALDI-TOF MS pour l'identification des poux » deux projets ont été réalisés.

L'objectif de notre premier projet était de tester la capacité du MALDI-TOF MS à identifier les spécimens de poux conservés à -20°C et prélevés sur le bétail et la volaille en Algérie.

Le choix de l'ensemble de l'échantillon ou des parties du corps de l'arthropodes est un paramètre crucial pour les analyses MALDI-TOF MS. Dans notre étude nous avons testé deux protocoles de dissection une coupe longitudinale (la moitié du corps du pou) et une coupe transversale (le céphalothorax du pou) pour pouvoir choisir la partie de corps idéale à utiliser pour établir une

bonne identification des poux après avoir créé une base de données avec des spécimens conservé à -20°C. C'est l'utilisation du céphalothorax qui a été la plus efficace pour identifier les poux stockés à -20 C° (Article 3).

Sur le deuxième projet notre but était d'évaluer l'influence de la conservation de l'alcool sur la précision de l'identification par MALDI-TOF MS des poux d'animaux et des poux d'élevage en laboratoire provenant de l'Algérie et de la France respectivement. Ces poux ont été soumis à l'analyses SEP après avoir optimisé les protocoles de préparation des échantillons et créée une base de données avec des spécimens conservés en alcool. Les résultats ont montré que le MALDI-TOF MS est efficace pour identifier des poux conservés dans l'alcool pendant différentes périodes (Article 4).

Article N°3:

Development of MALDI-TOF Mass Spectrometry for the identification of lice isolated from farm animals.

Basma Ouarti[1,2], Maureen Laroche[1,2], Souad Righi[3], Mohamed Nadir Meguini[3,4], Ahmed Benakhla[3], Didier Raoult[2,5], and Philippe Parola[1,2,]*

Parasite 27, 28 (2020), https://doi.org/10.1051/parasite/2020026

Résumé

La Spectrométrie de Masse à Temps de Vol par Désorption/Ionisation Laser Assistée par Matrice est maintenant utilisée pour l'identification rapide des microorganismes isolés à partir d'échantillons cliniques et a récemment été appliquée avec succès pour l'identification des arthropodes. Dans cette étude, cet outil protéomique a été utilisé pour identifier les poux prélevés sur le bétail et la volaille en Algérie. Les spectres MALDI-TOF MS de 408 spécimens adultes ont été mesurés pour 14 espèces, dont *Bovicola bovis*, *B. ovis*, *B. caprae, Haematopinus eurysternus, Linognathus africanus, L. vituli, Solenopotes capillatus, Menacanthus stramineus, Menopon gallinae, Chelopistes meleagridis, Goniocotes gallinae, Goniodes gigas, Lipeurus caponis* et *Pediculus humanus corporis* élevé en laboratoire. Des spectres de bonne qualité ont été obtenus pour 305 échantillons.

L'analyse spectrale a révélé une reproductibilité intra-espèce et une spécificité inter-espèces qui concordaient avec la classification morphologique. Un test à l'aveugle de 248 échantillons a été effectué par rapport à la base de données de notre laboratoire mise à niveau avec de nouveaux spectres et validée à l'aide d'outils moléculaires. Avec des pourcentages d'identification allant de 76 à 100 % et des scores d'identification élevés (moyenne : 2,115), cette étude propose MALDI-TOF MS comme un outil efficace pour distinguer les espèces de poux.

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RESEARCH ARTICLE OPEN ACCESS

Development of MALDI-TOF mass spectrometry for the identification of lice isolated from farm animals

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Abstract – Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is now routinely used for the rapid identification of microorganisms isolated from clinical samples and has been recently successfully applied to the identification of arthropods. In the present study, this proteomics tool was used to identify lice collected from livestock and poultry in Algeria. The MALDI-TOF MS spectra of 408 adult specimens were measured for 14 species, including *Bovicola bovis, B. ovis, B. caprae, Haematopinus eurysternus, Linognathus africanus, L. vituli, Solenopotes capillatus*, *Menacanthus stramineus*, *Menopon gallinae, Chelopistes meleagridis*, *Goniocotes gallinae*, *Goniodes gigas, Lipeurus caponis* and laboratory reared *Pediculus humanus corporis*. Good quality spectra were obtained for 305 samples. Spectral analysis revealed intra-species reproducibility and inter-species specificity that were consistent with the morphological classification. A blind test of 248 specimens was performed against the in-lab database upgraded with new spectra and validated using molecular tools. With identification percentages ranging from 76% to 100% alongside high identification scores (mean = 2.115), this study proposes MALDI-TOF MS as an effective tool for discriminating lice species.

Key words: MALDI-TOF MS, Lice, Phthiraptera, Anoplura, Mallophaga.

Résumé – Développement de la spectrométrie de masse MALDI-TOF MS pour l'identification de poux isolés d'animaux de ferme. La Spectrométrie de Masse à Temps de Vol par Désorption/Ionisation Laser Assistée après Matrice est maintenant utilisée pour l'identification rapide des microorganismes isolés à partir d'échantillons cliniques et a récemment été appliquée avec succès pour l'identification des arthropodes. Dans cette étude, cet outil protéomique a été utilisé pour identifier les poux prélevés sur le bétail et la volaille en Algérie. Les spectres MALDI-TOF MS de 408 spécimens adultes ont été mesurés pour 14 espèces, dont *Bovicola bovis*, *B. ovis*, *B. caprae*, *Haematopinus eurysternus, Linognathus africanus*, *L. vituli*, *Solenopotes capillatus*, *Menacanthus stramineus*, *Menopon gallinae*, *Chelopistes meleagridis*, *Goniocotes gallinae*, *Goniodes gigas*, *Lipeurus caponis* et *Pediculus humanus corporis* élevé en laboratoire. Des spectres de bonne qualité ont été obtenus pour 305 échantillons. L'analyse spectrale a révélé une reproductibilité intra-espèce et une spécificité inter-espèces qui concordaient avec la classification morphologique. Un test à l'aveugle de 248 échantillons a été effectué par rapport à la base de données de notre laboratoire mise à niveau avec de nouveaux spectres et validée à l'aide d'outils moléculaires. Avec des pourcentages d'identification allant de 76 à 100 % et des scores d'identification élevés (moyenne : 2,115), cette étude propose MALDI-TOF MS comme un outil efficace pour distinguer les espèces de poux.

Introduction

Lice are highly host-specific insects [20], belonging to the order Phthiraptera. They are obligate parasites of birds and many species of mammals, including humans [40, 41]. Nearly 5000 species of parasitic lice have been described and classified under four sub-orders: Anoplura, Amblycera, Ischnocera, and Rhynchophthirina. Anoplura (sucking lice) are hematophagous and feed exclusively on mammals [34]. The lice of the other suborders are Mallophaga (chewing lice). These lice mostly infest birds, and secondarily mammals, and feed on feathers, dead skin, blood or secretions from their hosts [18].

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Lice parasitism may be responsible for pediculosis causing mild to severe anemia, and many types of skin damage such as focal necrosis and scars on the skin of heavily infested animals [8, 12]. These have economic consequences especially for livestock farmers [8, 45]. Some sucking lice such as *P. humanus corporis* (*Pediculus humanus corporis*) have the ability to transmit pathogens to humans [17].

The identification of arthropods including lice is an important step for surveillance and control of parasitism as well as transmitted diseases [25]. Currently, lice are mainly identified morphologically based on dichotomous keys that take high consideration of the host animal from which the louse has been collected [32, 47].

Morphological identification requires entomological expertise and specific documentation [50]. For lice and other arthropods, it may be limited by the integrity of the specimen which can be damaged during collection or transport by its fragility or by the absence of distinctive morphological criteria at an immature stage of the life cycle such as ticks [33].

Alternative methods such as molecular approaches have been developed to identify arthropods including lice [19, 27]. These are based on comparative analyses of gene sequences such as the *18S* rRNA or the cytochrome c oxidase subunit I (*COI*) genes widely used for the identification of lice [19, 27]. However, the NCBI GenBank database is still far from comprehensive regarding animal lice gene sequences [24].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an ionization technique that generates specific spectra from protein extracts from organisms [48]. The acquisition of the spectra allows the creation of a database based on reference spectra of the formally identified organism [25]. In recent years, this proteomic approach has revolutionized clinical microbiology for the identification of bacteria and fungi [36, 39].

Recently, MALDI-TOF MS has been evaluated as an efficient tool for the identification of arthropods including ticks [2, 5], mosquitoes [26, 28, 46], culicoides [6, 35], fleas [30, 48], triatomines [25], tsetse flies [15], and phlebotomines [9] in laboratory and field conditions.

The objective of the present study was to test the ability of MALDI-TOF MS to identify lice specimens collected from livestock and poultry in Algeria.

Materials and methods

Ethical considerations

Informal verbal consent was obtained from the owners of the mammals and poultry that were selected for sampling lice directly. Lice were not sampled from protected animals nor from animals in private residences or national parks.

Human lice were reared at IHU Méditerranée Infection on adult female New Zealand white rabbits obtained from Charles River Laboratories. They were handled according to Decree No. 2013–118, 7 February 2013 and as described in the approved experimental protocols (references APAFIS #01077.02 & 2015050417122619). Protocols were approved by the Ethics Committee "C2EA-14" of Aix-Marseille University, France and the French Ministry of National Education, Higher Education and Research.

Field capture and morphological identification of lice

The collection was carried out on mammals and poultry between 2015 and 2017 in three regions of northeastern Algeria: El Tarf (36°46′1.2″ N, 8°19′1.2″ E), Souk Ahras (36°17′11″ N, 7°57′4″ E); and Guelma (36°27′0″ N, 7°25′0″ E) during three seasons (autumn, winter, spring).

For mammals, the animals were examined by parting their wool from sheep and goats and hair from cattle, visually inspecting the skin for lice. In poultry, the head and feathers on the neck, feet, skin, wing feathers, feathers of the belly, and feathers of the croup and of the tail were meticulously examined. In some cases, chickens were sprayed with insecticide and placed on a small spot on a sampling surface for 20 min [4]. Lice collected from the same animal were recovered and stored in the same tube either dry at −20 °C or in 70% ethanol to be transported to Marseille, France for further analyses. For the present study, we used frozen lice only and kept the other lice for future studies. Each louse was rinsed with ethanol (70%) for 15 min, and later in distilled water for one minute. All body parts of the collected body lice were examined using a Zeiss Axio Zoom V16 (Zeiss, Marly-le-Roi, France) microscope. The morphological keys provided by Wall [47] and Pajot [32] were used for morphological identification (Fig. 1). The names of the species of lice and their abbreviations used in this study were chosen according to previously published identification keys [1, 7, 14, 34, 37].

Molecular identification of lice

Following morphological identification, between 8 and 10 specimens of each louse species were selected from at least two animal hosts at each study site. The abdomen of each louse was used for the extraction of DNA using an EZ1 DNA tissue extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Lice DNA was then eluted in 100 µL of Tris EDTA buffer using a DNA extracting EZ1 Advanced XL Robot (Qiagen), as previously described [5]. The DNA was either immediately used or stored at −20 °C until molecular analysis. The DNA extracting EZI (Qiagen) was disinfected after each batch of extraction as per the manufacturer's recommendations in order to avoid cross-contamination.

SAIDG (5′ – TCTGGTTGATCCTGCCAGTA – 3′) and SBIDG (5′ – ATTCCGATTGCAGAGCCTCG – 3′) primers were used to amplify partial 539 base pair *18S* rRNA gene sequences for species-level molecular identification of the lice, as previously described [21]. The DNA samples tested were successfully amplified using an automated DNA thermal cycler (Applied Biosystems, Foster City, CA, USA). The cycling program consisted of 15 min at 95 °C followed by 39 cycles of denaturing at 95 °C for 30 s, annealing at 58 °C for 30 s, extension of 1 min at 72 °C, followed by a final cycle of 5 min at 72 °C and sampling while held at 4 °C. A mix without

Figure 1. Photographs of habitus of 13 species of lice collected from three regions of northeastern Algeria: *Solenopotes capillatus* (1); *Menopon gallinae* (3); *Menacanthus stramineus* (4); *Lipeurus caponis* (5); *Haematopinus eurysternus* (6); *Bovicola caprae* (7); *Goniocotes gallinae* (8); *Goniodes gigas* (9); *Linognathus vituli* (10); *Chelopistes meleagridis* (11); *Linognathus africanus* (12); *Bovicola bovis* (13); *Bovicola ovis* (14). Laboratory specimens of *Pediculus humanus corporis* (2) were also used in this study.

DNA was used as a negative control. The amplification products were then subjected to electrophoresis through a 1.5% agarose gel stained with SYBR Safe™ and visualized with the ChemiDoc™ MP ultraviolet imager (Bio-Rad, Marnes-la-Coquette, France).

The positive samples were purified, sequenced using a Big Dye Terminator kit and an ABI PRISM 3130 Genetic Analyzer (Applied BioSystems, Courtaboeuf, France). The obtained sequences were analyzed and assembled using ChromasPro, version 1.34 (Technelysium Pty, Ltd., Tewantin, QLD, Australia).

BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was used for sequence alignment.

All sequences were compared to the GenBank database using BLAST analysis and new sequences were deposited in GenBank (Table 3).

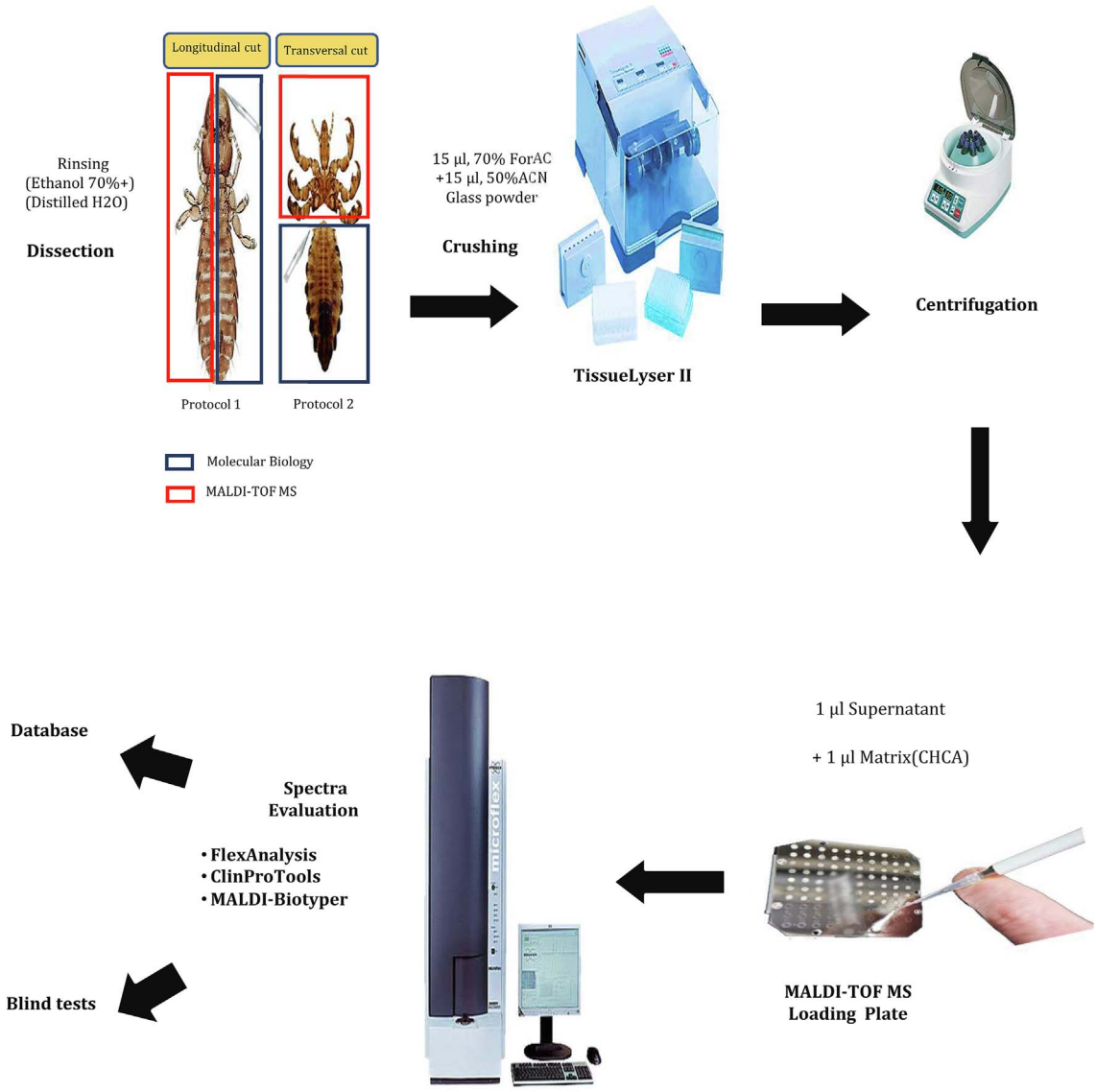

Figure 2. An explanatory flowchart of the MALDI-TOF MS protocol. ACN: Acetonitrile, ForAC: Formic acid, CHCA: α-cyano-4-hydroxycinnamic acid.

Sample preparation for MALDI-TOF MS analysis

Two protocols were tested to assess which body part was relevant for MALDI-TOF MS analyses. In protocol 1, the louse was longitudinally cut into two equal parts, one used for MALDI-TOF MS analysis and the other for molecular biology. In protocol 2, a transverse section was performed to separate the cephalothorax and the legs for the MALDI-TOF MS and spectra obtained were tested. We used the abdomen for molecular biology.

Body halves (protocol 1) or cephalothorax-legs (protocol 2) of the selected specimens were individually homogenized using a TissueLyser II device (Qiagen, Hilden, Germany) with three 60-second cycles at a frequency of 30 Hz, in 15 µL of 50% (v/v) acetonitrile (Fluka, Buchs, Switzerland) and 15 µL of 70% (v/v) formic acid (Sigma, Lyon, France) with glass powder (Glass beads, acid-washed G4649, ≤106 µm, Sigma, Lyon. France) in 1.5 mL micro-tubes.

In both protocols, after homogenization of the sample, a quick spin centrifugation at 10,000 rpm for 1 min was performed to pellet debris and 1 µL of supernatant from each sample was deposited on the MALDI-TOF MS target plate in quadruplicate (Bruker Daltonics, Wissembourg, France) and covered with 1 µL of CHCA matrix solution composed of saturated α-cyano-4-hydroxycinnamic acid (Sigma), 50% acetonitrile (v/v), 2.5% trifluoroacetic acid (v/v) (Aldrich, Dorset, UK) and high-performance liquid chromatography (HPLC)-grade water. After drying for several minutes at room temperature, the target was placed in the MALDI-TOF MS [30] (Fig. 2).

Following comparison of the spectra quality obtained when using protocol 1 and protocol 2, protocol 2 (using cephalothorax-legs) was chosen for further analyses. The validity of

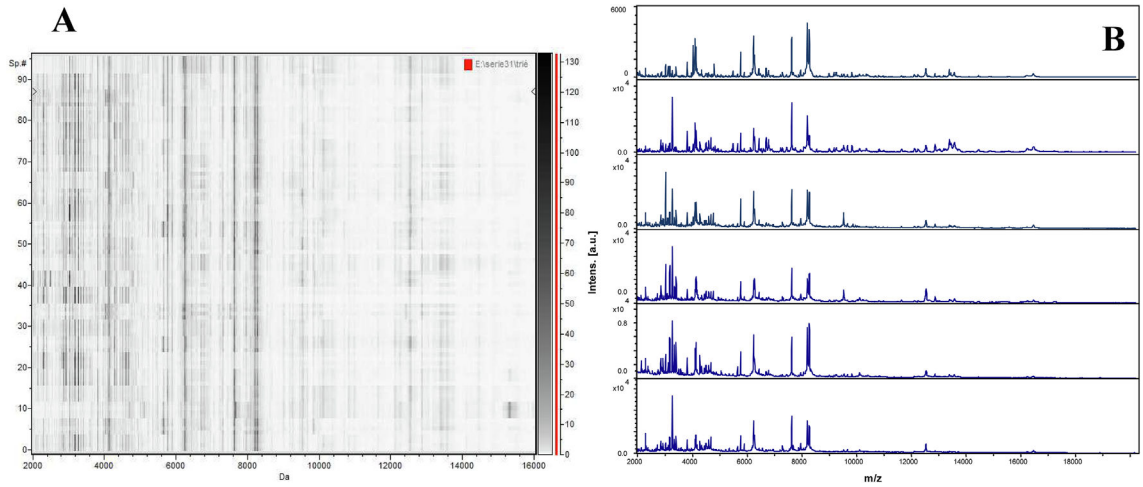

Figure 3. MALDI-TOF analysis of *Pediculus humanus corporis*. (A) MS profiles of 24 specimens using the gel view tool of ClinProTools software, (B) MS profiles of different specimens of *Pediculus humanus* corporis using FlexAnalysis software.

Table 1. Sampling regions, number of parasitized animals, and specimens of lice collected from sheep, cattle, goats, and poultry in Algeria.

Host	Souk-Ahras	Guelma	El Tarf	Total number of specimens collected
Cattle (n = 20)	1066	748	/	1814
Sheep (n = 23)	256	300	96	747
Goats (n = 14)	288	289	229	806
Poultry (n = 13)	615	225		840
Total	2225	1562	325	4112

Table 2. Morphological identification of lice collected from Algeria and stored at −20 °C before being tested by MALDI-TOF MS.

Hosts	Morphological ID	Souk-Ahras	Guelma	El Tarf	Total
Cattle (n = 20)	*Bovicola bovis*[b]	49	56	/	105
	Haematopinus eurysternus[a]	401	278	/	679
	Linognathus vituli[a]	26	19	/	45
	Solenopotes capillatus[a]	646	339	/	985
Sheep (n = 25)	*Bovicola ovis*[b]	289	111	95	495
Goats (n = 18)	*Bovicola caprae*[b]	258	105	85	448
	Linognathus africanus[a]	145	111	106	362
Poultry (n = 17)	*Menacanthus stramineus*[b]	46	162	3	211
	Menopon gallinae[b]	199	83	132	414
	Chelopistes meleagridis[b]	20	/	/	20
	Goniocotes gallinae[b]	101	92	82	275
	Goniodes gigas[b]	2	/	10	12
	Lipeurus caponis[b]	30	17	14	61
Total	13 species	2212	1373	527	4112

[a] Anoplura.
[b] Mallophaga.

the spectra obtained with protocol 2 was confirmed by testing 24 fresh lice *P. humanus corporis* from laboratory rearing by MALDI-TOF MS. Reproducibility and spectra quality was confirmed using FlexAnalysis v.3.3 software and the gel view tool of ClinProTools 2.2 software (Bruker Daltonics, Leipzig, Germany) (Fig. 3). Non-engorged fresh *P. humanus corporis* lice were later used as controls for each MALDI-TOF MS assay.

MALDI-TOF MS parameters

Protein mass profiles were obtained using a Microflex LT MALDI-TOF Mass Spectrometer (Bruker Daltonics), using Flex Control software (Bruker Daltonics), with the parameters described previously [49]. The profiles of the spectra obtained were viewed using FlexAnalysis v.3.3 software and exported to ClinProTools v.2.2 and MALDI-Biotyper v.3.0 software

Table 3. Results of the molecular identification based on the partial *18S* rRNA of lice: BLAST analysis and sequences deposited on the NCBI GenBank database.

Morphological identification	N	Molecular identification by BLAST (accession number)	Identity level with GenBank	Sequences deposited in GenBank *18S* with accession numbers
Bovicola bovis[b]	17	*Bovicola bovis* (JX184911.1)	100%	MH377332.1
Haematopinus eurysternus[c]	11	*Haematopinus tuberculatus* (GU569180.1)	99.21%	MH377326.1
Linognathus vituli[b]	13	*Linognathus vituli* (JX401573.1)	99%	MH377328.1
Solenopotes capillatus[b]	17	*Solenopotes capillatus* (JX184910.1)	100%	MH377327.1
Bovicola ovis[b]	20	*Bovicola ovis* (GU569184.1)	100%	MH377330.1
Bovicola caprae[c]	20	*Bovicola bovis* (JX184911.1)	99.79%	MH377331.1
Linognathus africanus[c]	15	*Linognathus vituli* (JX401573.1)	95.86%	MH377329.1
Menacanthus stramineus[a]	19	*Menacanthus* sp (AF385066.1)	99.79%	MH377333.1
Menopon gallinae[c]	5	Menoponidae sp (JQ309930.1)	100%	MH377334.1
Chelopistes meleagridis[a]	9	*Rhynonirmus* sp. (AF385048.1)	98.98%	MH377335.1
Goniocotes gallinae[a]	5	*Goniodes aff. dissimilis/gigas* (AY077767.1)	97.69%	MH469486.1
Goniodes gigas[a]	2	*Goniodes aff. dissimilis/gigas*(AY077767.1)	97.69%	MH469487.1
Lipeurus caponis[a]	6	*Rhynonirmus* sp. (AF385048.1)	99.79%	MH469485.1
13 species	159	/	/	/

N: Number of specimens used for molecular biology.
[a] Species with sequences not available in GenBank.
[b] Species with correct identification using the *18S* rRNA gene.
[c] Species with incorrect molecular identification.

(Bruker Daltonics) for data processing (smoothing, basic subtraction and peak selection) and cluster analysis.

Creation of a reference spectra database

In order to obtain reference spectra and upgrade our arthropod database, a subgroup of lice specimens identified both morphologically and using molecular tools were subjected to MALDI-TOF MS (Tables 2 and 3). Lice species from the same genus were run on the same MALDI-TOF MS target plate to rule out any plate bias. Intra-species reproducibility and inter-species specificity of MALDI-TOF MS spectra were visually evaluated using the gel view, dendrogram and principal component analysis tools of ClinProTools 2.2 and MALDI-Biotyper v3.0. (Bruker Daltonics). Dendrograms are based on the results of Composite Correlation Index (CCI) matrices. CCIs are calculated by dividing spectra into intervals and comparing these intervals across a dataset. The composition of correlations of all intervals provides the CCI which is used as a parameter that defines the distance between spectra. A CCI match value of 1 represents complete correlation, whereas a CCI match value of 0 represents an absence of correlation [25]. Spectral dendrograms were created to assess the profile diversity within each species and high-quality spectra from separate clusters were selected using FlexAnalysis software v.3.3. (Bruker Daltonics) to update the reference spectra database.

Reference spectra were selected based on intensity, overall spectrum quality and intra-species reproducibility. For each reference sample, a main spectrum profile (MSP) was created using the automated function of MALDI-Biotyper software v.3.3. (Bruker Daltonics). Spectra from a spot of lower quality were sometimes removed to obtain a high-quality MSP. MSPs were created on the basis of an algorithm using peak position, intensity and frequency data. Between two and nine new reference spectra per species were added to the lice database in our laboratory [26].

Blind tests and cluster analysis

New specimens of lice collected at different study sites were tested. Each spectrum obtained by MALDI-TOF MS analysis as described above was subjected to a blind test analysis against the upgraded database. The significance of the identification was determined using the log score values (LSV) given by MALDI-Biotyper software v.3.3. corresponding to a signal intensity level of the mass spectra of the query and reference spectra. The LSV range was from 0 to 3. LSVs allow for good evaluation of reproducibility between a queried spectrum and a reference spectrum, as they result from a thorough comparison of the position of peaks and the intensity between those two spectra (MALDI BioTyper Help, Bruker). In order to visualize MALDI-TOF MS profile similarities and distances, hierarchical clustering of the mass spectra of all tested species was performed using the dendrogram function of MALDI-Biotyper software v.3.3. Although no threshold has been definitively validated for arthropod identification using MALDI-TOF MS, LSVs $\geq$ 1.8 were considered adequate for relevant identification, as reported in pioneer papers [30, 44]. Percentages of included spectra are reported in Table 4.

Results

Lice collection and morphologic identification

A total of 4112 lice were collected from several livestock farm animals and stored at −20 °C: a total of 23 sheep, 20 cattle, 14 goats, and 13 poultry (Table 1).

Table 4. MALDI-TOF MS identification of included louse species. Specimens were included based on the quality of their spectra (intensity, overall spectrum quality, and intra-species reproducibility).

Host	Species	Percentage of included specimens	Number of spectra added as reference	Number of specimens used for the blind test	LSVs obtained from blind tests against database & mean	Percentage of correct identification
Mammal lice	*Haematopinus eurysternus*	18/20 (90%)	4	14	[1.91–2.9] – 2.213	14/14 (100%)
	Solenopotes capillatus	59/68 (86.76%)	6	53	[1.719–2.707] – 2.023	52/53 (98.11%)
	Linognathus vituli	25/35 (71.42%)	5	20	[1.632–2.511] – 2.075	20/20 (100%)
	Linognathus africanus	17/21 (80.95%)	4	13	[1.704–2.644] – 2.046	13/13 (100%)
	Bovicola caprae	33/45 (73.33%)	8	25	[1.546–2.857] – 1.852	19/25 (76%)
	Bovicola bovis	29/40 (72.5%)	5	24	[1.728–2.873] – 2.11	23/24 (95.83%)
	Bovicola ovis	57/96 (59.37%)	9	48	[1.813–2.837] – 2.198	48/48 (100%)
Poultry lice	*Goniocotes gallinae*	5/5 (100%)	3	2	[2.34–2.393] – 2.366	2/2 (100%)
	Goniodes gigas	2/2 (100%))	1	1	1.932	1/1 (100%)
	Menopon gallinae	13/16 (81.25%)	4	9	[1.809–2.613] – 2.166	9/9 (100%)
	Menacanthus stramineus	6/10 (60%)	3	3	[2.306–2.608] – 2.456	3/3 (100%)
	Chelopistes meleagridis	15/18 (83.33%)	4	11	[1.703–2.046] – 1.824	11/11 (100%)
	Lipeurus caponis	2/8 (25%)	1	1	2.107	1/1 (100%)
Human lice	*Pediculus humanus corporis*	24/24 (100%)	0	24	[1.979–2.328] – 2.251	24/24 (100%)
	14	305/408 (74.75%)	57	248	2.115	240/248 (96.77%)

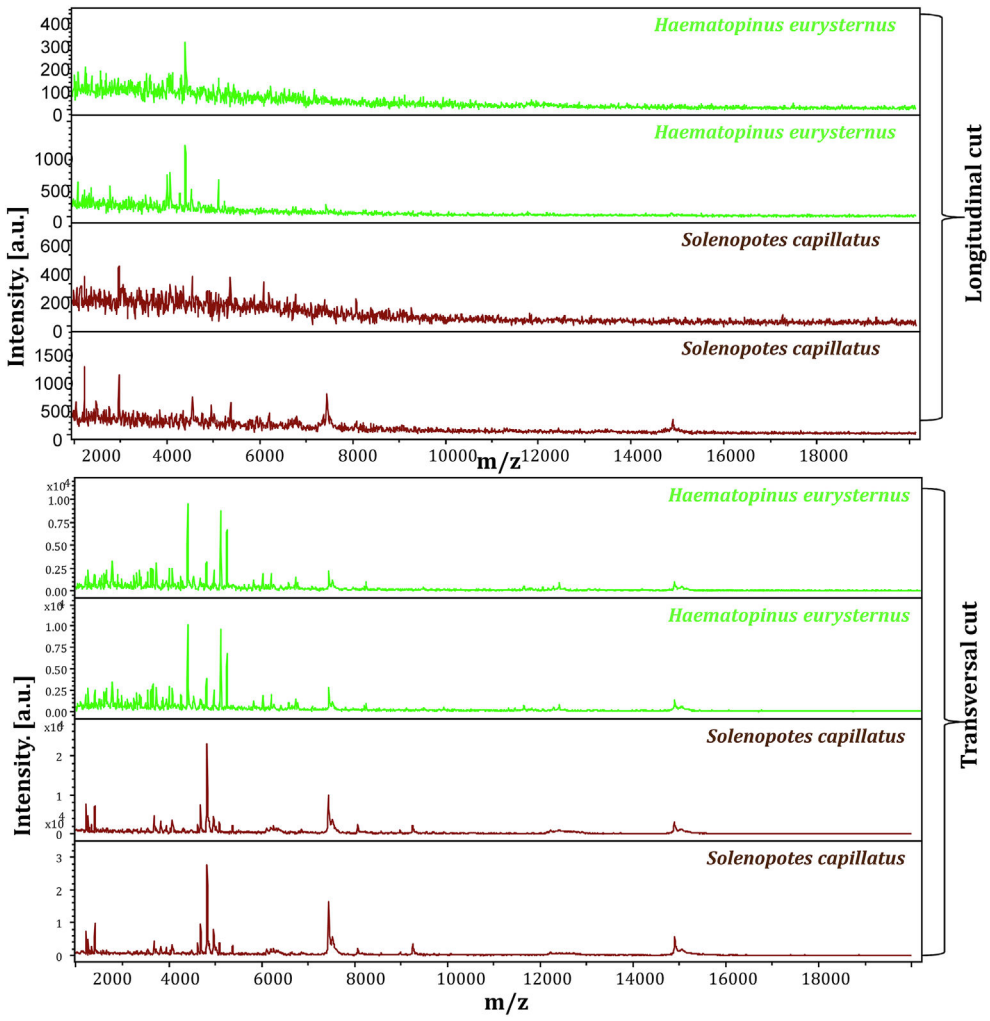

Figure 4. Comparison between the spectra obtained using protein extracts from (A) the cephalothorax and legs; (B) a longitudinal cut of the louse. Spectra were visualized using FlexAnalysis software.

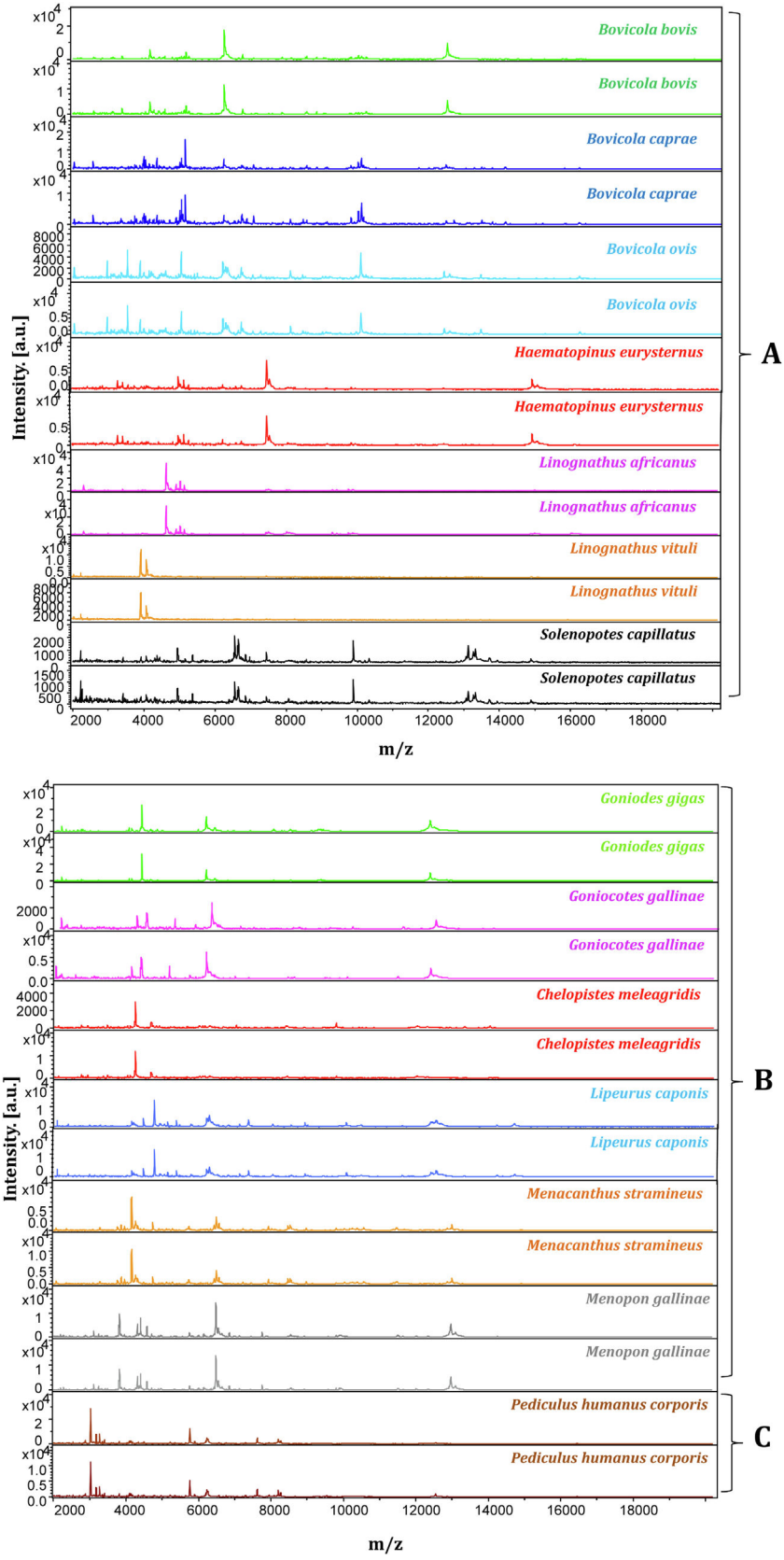

Figure 5. Good quality spectra of different species of mammalian and poultry lice visualized using FlexAnalysis software. (A: Mammal lice / B: Poultry lice / C: Human lice).

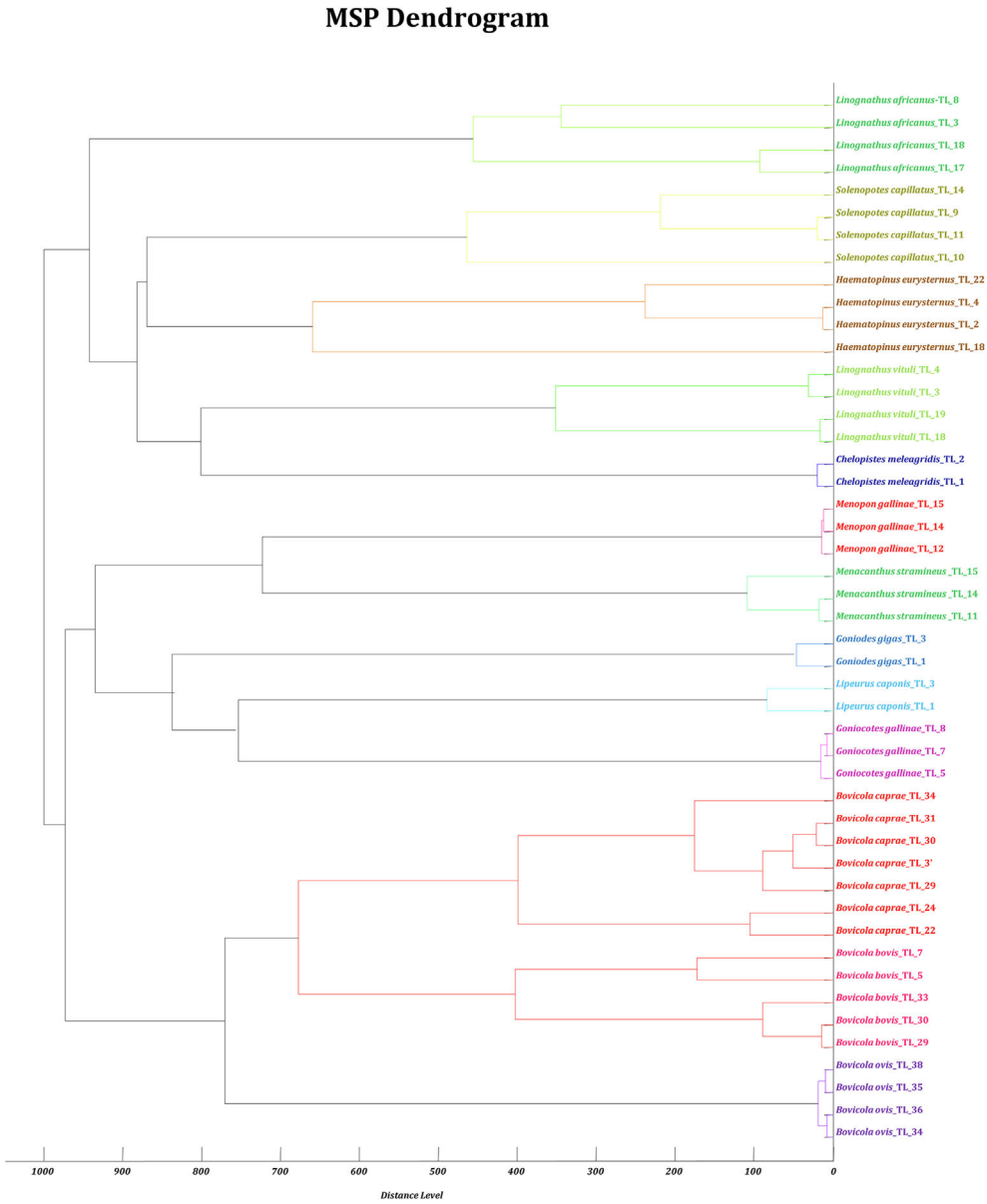

Figure 6. Dendrogram constructed using MALDI-Biotyper software v.3.3 including 2–7 random MS spectra representative of the 13 distinct species of lice.

On the basis of morphological criteria, 13 species of lice were morphologically identified including four species of sucking lice and 9 chewing lice (Table 2). Seven species were collected on mammals including four from cattle with *B. bovis* (*Bovicola bovis*) (n = 105), *H. eurysternus* (*Haematopinus eurysternus*) (n = 679), *L. vituli* (*Linognathus vituli*) (n = 45) and *S. capillatus* (*Solenopotes capillatus*) (n = 985), two from goats with *B. caprae* (*Bovicola caprae*) (n = 448) and *L. africanus* (*Linognathus africanus*) (n = 362), and one species from sheep namely *B. ovis* (*Bovicola ovis*) (n = 495). Six other lice species were collected from poultry including *G. gallinae* (*Goniocotes gallinae*) (n = 275), *Li. caponis* (*Lipeurus caponis*) (n = 61), *M. gallinae* (*Menopon gallinae*) (n = 414), *Me. stramineus* (*Menacanthus stramineus*) (n = 211), *C. meleagridis* (*Chelopistes meleagridis*) (n = 20) and *Go. gigas (Goniodes gigas)* (n = 12) (Table 2). Genera abbreviations were modified in this study to properly differentiate genera with the same initials. A list of abbreviations is provided.

Molecular identification of lice

Of the 4112 lice morphologically identified, 159 lice specimens preserved at −20 °C and belonging to 13 species were randomly selected to be included in the study.

Randomly selected specimens of each species were subjected to molecular identification targeting the *18S* rRNA gene. A GenBank request revealed that *18S* rRNA gene reference sequences were available for 8 of the 13 lice species. Amongst

Table 5. Hosts and distribution of lice in the orders Mallophaga and Anoplura.

Order/suborder/family	Valid name	References	Host
Mallophaga, Ischnocera, Philopteridae	*Goniodes gigas* (Taschenberg, 1879) (=*Goniocotes gigas* Taschenberg, 1879)	[34]	Poultry
Mallophaga, Ischnocera, Trichodectidae	*Bovicola bovis* (Linnaeus, 1758) (= *Damalinia bovis* Linnaeus, 1758)	[14]	Cattle
Mallophaga, Ischnocera, Trichodectidae	*Bovicola ovis* (Schrank, 1781) (=*Damalinia ovis* (Schrank, 1781)	[14]	Sheep
Mallophaga, Ischnocera, Trichodectidae	*Bovicola caprae* (Gurlt, 1843) (=*Damalinia caprae* (Gurlt, 1843)	[14]	Goat
Mallophaga, Ischnocera, Philopteridae	*Chelopistes meleagridis* (Linnaeus, 1758) (= *Goniodes meleagridis* (Linnaeus, 1758)	[14, 34]	Poultry
Mallophaga, Amblycera, Menoponidae	*Menacanthus stramineus* Nitzsch, 1818	[42]	Poultry
Mallophaga, Ischnocera, Philopteridae	*Lipeurus caponis* Linnaeus, 1758	[42]	Poultry
Mallophaga, Amblycera, Menoponidae	*Menopon gallinae* Linnaeus, 1758	[42]	Poultry
Mallophaga, Ischnocera, Philopteridae	*Goniocotes gallinae* de Geer, 1778	[38]	Poultry
Anoplura, Haematopinidae	*Haematopinus eurysternus* Nitzsch, 1818	[42, 47]	Cattle
Anoplura, Linognathidae	*Solenopotes capillatus* Enderlein, 1904	[42]	Cattle
Anoplura, Linognathidae	*Linognathus vituli* Linnaeus, 1758	[42]	Cattle
Anoplura, Linognathidae	*Linognathus africanus* Kellogg & Paine, 1911	[47]	Goat

these eight available sequences on GenBank, four had an average quality and the remaining four were of very poor quality. No sequences were available for five species including *Li. caponis, C. meleagridis, G. gallinae, Go. gigas*, and *Me. stramineus* (Table 3).

For 13 species of lice, the BLAST analysis of *18S* rRNA reference sequences of the lice specimens of the same species demonstrated high identity ranging from 99% to 100%, supporting correct morphological identification (Table 3). The sequences obtained for each species of lice were corrected and blasted to reveal the intra-species similarity of the sequence of the *18S* RNA gene. Sequence alignment using BioEdit software revealed that all sequences from the same species were identical and thirteen *18S* rRNA gene good quality consensus sequences were deposited in the NCBI GenBank database (Table 3).

MALDI-TOF MS analyses

A total of 427 lice preserved at −20 °C were tested by MALDI-TOF MS using two protocols.

An analysis of the spectral profiles using FlexAnalysis software showed that the spectra obtained using the second protocol provided MALDI-TOF MS profiles of higher intensity and superior quality to those obtained with the first protocol (Fig. 4). Based on spectra quality MALDI-TOF MS, the second protocol provided good intra-species reproducibility and inter-species specificity between specimens of the same species and variability between different species. This protocol was therefore selected for further MALDI-TOF MS analyses (Fig. 5) to create a reference spectra database.

Therefore, protocol 2 was used for 408 specimens concerning the following species: *B. ovis*, *B. bovis*, *B. caprae*, *L. vituli*, *L. africanus*, *H. eurysternus*, *S. capillatus, C. meleagridis, Go. gigas, Me. stramineus, M. gallinae, G. gallinae, Li. caponis*, and *P. humanus corporis*. Samples were subjected to blind test analysis against the upgraded database (Table 4).

MALDI-TOF MS identification was considered correct when there was concordance between the morphological identification and molecular identification, when the latter was possible, that is when sequences were available in GenBank and were considered reliable.

In this study, we obtained 305 specimens with good quality spectra, of which 57 spectra were added as reference spectra and 248 specimens used for the blind test with an average LSV of 2.115 and correct identification percentages between 76% and 100% (Table 4). In all, 103 of the 408 samples (25.25%) tested had poor quality spectra and these were removed for this proof-of-concept (Supplementary Data 1). Nevertheless, specimens with low quality spectra were correctly identified with an average percentage of 61.11% and with low LSVs, highlighting the quality of the database created (Supplementary Data 1).

The controls of fresh and non-engorged fresh *P. humanus corporis* lice were well identified at each test. The intra-species reproducibility and inter-species specificity of the MALDI-TOF MS profiles were further objectified using MALDI-Biotyper software cluster analysis. Dendrogram analysis revealed specific clustering on distinct branches of lice according to species. Lice belonging to the same genus were grouped in the same part of the MSP dendrogram (Fig. 6).

Discussion and conclusion

The morphological identification of lice is very complex because the species are morphologically close to one another. For the first time, MALDI-TOF MS was used as an additional tool for lice identification.

In this study, we successfully identified 14 species of lice using MALDI-TOF MS. Morphological identification was molecularly confirmed by targeting a fragment of louse *18S* rRNA gene sequences. The choice of the *18S* rRNA gene is based on previous results that proved the relevance of this gene for louse identification and the presence of reference sequences in GenBank [21]. However, 5/13 of the lice species studied in this work had no sequence available in GenBank, highlighting the drawbacks of using molecular biology alone for louse identification. Only 4/13 species of lice presented correct

identification using the *18S* rRNA gene. The remaining 4/13 species of lice resulted in incorrect identification despite the fact that their reference sequences were present in GenBank. Further analysis of the GenBank reference sequences of each of these species revealed that they were all of poor quality.

This study allowed us to add five new sequences that did not exist on GenBank, and eight additional complementary sequences for which a reference was already available (Table 3). Five of the 13 sequences of lice namely *Go. gigas, B. bovis, B. ovis, B. caprae,* and *Chelopistes meleagridis* were already published under new genera (Table 5) [14, 34, 38, 42, 47].

A preliminary MALDI-TOF MS database containing the spectra of 14 species was hereby created and the database will be regularly updated with the spectra of new specimens. The spectra files are available on request and transferable to any Bruker MALDI-TOF MS device. The MALDI-TOF MS arthropod database can be shared through scientific collaboration projects; it will be possible to freely query this database online in the future.

For use in entomology, the choice of arthropod body parts to be used for the MALDI-TOF MS test is a very important criterion. For ticks and mosquitoes, MALDI-TOF MS identification of the arthropod species is based on leg spectra. Other body parts had to be carefully selected for other arthropods when the legs did not provide satisfactory spectra [10, 13, 22]. Here, the spectral profiles generated from the cephalothorax-legs of the lice subjected to MALDI-TOF MS were reproducible. Spectral analysis highlighted intra-species reproducibility and inter-species specificity, which was consistent with the morphological classification. In addition, hierarchical clustering based on the MALDI-TOF MS spectra revealed that all of the specimens from the same species were grouped in the same branch. Our results demonstrated that the use of the body of a louse without the abdomen was the best sample for distinguishing lice species using the MALDI-TOF MS approach. There are many advantages to selecting this part of the body, for example avoiding the influence of the intestinal contents on the MALDI-TOF MS spectra [50]. Moreover, using a small body part for MALDI-TOF MS allows further analyses of the remaining parts of the arthropod, such as the detection of microorganisms [10] or the identification of blood meals of the arthropods [31]. Higher quality spectra resulted from the cephalothorax-legs part of the louse compared to when it was dissected longitudinally (Fig. 4). This can be explained by the fact that some parts of arthropods yield better spectral qualities than others, as has been demonstrated by several studies [10, 13, 22, 43].

In this study, we included only good quality spectra. Indeed, at this stage, only high-quality spectra can be included to validate the results and create a reliable database.

The number of specimens with low quality spectra can be explained by the fact that all these samples had to be frozen and thawed several times for various analyses including long morphological identification, molecular biology, and MALDI-TOF MS assays. These repeated thawing steps could have caused protein alterations responsible for the poor quality of the spectra. This hypothesis is supported by the fact that the groups of samples that were manipulated first have a greater number of high-quality spectra. This should not be an issue when applied to entomological studies since molecular biology is not always required and when a comprehensive MALDI-TOF MS database is available, the quality of the spectra will be improved. Nevertheless, many specimens with low quality spectra were correctly identified, reaching 100% correct identification for some species such as *Menopon gallinae* (average: 61.11%) (Supplementary Data 1). The performance of the identification despite the many thawing steps is a validation of the quality of the database created, which will be continuously strengthened with new field specimens.

MALDI-TOF MS enables the identification of lice without any entomological knowledge [25], as long as the database is comprehensive. Furthermore, the MALDI-TOF MS sample preparation method is simple and the speed of data analysis makes it possible to obtain quick and reliable identification results [50].

This study points to new possibilities for improving the knowledge of animal lice in Algeria by using several identification tools. We have also illustrated the limitations of molecular biology with the lack of comprehensiveness of the NCBI GenBank database, which is a major setback to using this method. To circumvent these limitations, we have deposited our new sequences in the NCBI GenBank database (Table 3).

This fast and accurate low-cost tool identifies not only the different immature stages of the arthropod's life cycle [6, 13], but also the origin of blood meal sources from arthropods [31].

Recently, preliminary studies have examined the ability of MALDI-TOF MS to detect *Plasmodium* parasites in *Anopheles* mosquitoes [23], differentiate ticks infected or not infected with *Borrelia* spp. or spotted fever group *Rickettsia* spp. [3, 10, 11, 43, 49], and fleas infected or not with *Bartonella* spp. [10].

The MALDI-TOF MS detection of louse-borne bacteria could provide new opportunities for vector surveillance, particularly in Algeria where all these louse species are present [16].

Previous studies reported the detection of *Rickettsia slovaca* in *Haematopinus suis* from Algeria [51]. It was later demonstrated that lice could acquire the bacterium *R. slovaca* after feeding on a bacteremic boar which does not yet prove that they are vectors, but would require epidemiological studies to be carried out [51]. It would be interesting to attempt to detect louse-associated bacteria such as *Bartonella quintana* or *Borrelia recurrentis*. Using the proposed protocol, the abdomen of the lice can be used for molecular screening of microorganisms.

This study confirmed that MALDI-TOF MS is a faster and cheaper method for identifying lice stored at −20 °C. In the field, alcohol is a more widely-used method of conserving the samples, especially in countries with limited resources [5]. It has been shown that MALDI-TOF MS is reliable for identifying arthropods preserved in alcohol, such as ticks[5], mosquitoes [29], and fleas [10]. Therefore, it would be interesting in the future to set up a MALDI-TOF MS protocol for identifying lice kept in alcohol [52]. It would also be interesting to assess whether MALDI-TOF MS can be used to differentiate lice which are infected or not infected by louse-borne microorganisms.

Conflict of interest

Each individual author has no conflict of interest to disclose.

Authors' contribution

PP and ML designed the experiments. BO collected the samples and performed the experiments. BO and ML analyzed the data. BO wrote the manuscript. All authors approved the final version of the manuscript.

Abbreviations

MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
CHCA matrix	a-Cyano-4-Hydroxycinnamic Acid
MSP	Main Spectrum Profile
LSVs	Log Score Values
H	Haematopinus
L	Linognathus
S	Solenopotes
B	Bovicola
G	Goniocotes
Li	Lipeurus
M	Menopon
Me	Menacanthus
C	Chelopistes
Go	Goniodes

Supplementary materials

Supplementary material is available at https://www.parasite-journal.org/10.1051/parasite/2020026/olm

Supplementary Data 1. MALDI-TOF MS identification of louse species excluded from the final analyses.

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Article N°4:

Identification of Lice stored in alcool using MALDI-TOF MS.

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Résumé

Les poux posent des problèmes majeurs de santé publique et vétérinaire, avec des conséquences économiques. Leur identification est essentielle et nécessite le développement d'une stratégie innovante. Le MALDI-TOF MS a récemment été proposé comme un outil rapide, peu coûteux et précis pour l'identification des arthropodes. L'alcool est l'une des méthodes de stockage les plus fréquemment utilisées et permet de conserver les échantillons pendant de longues périodes à température ambiante. Plusieurs études récentes ont rapporté que l'alcool modifie les profils protéiques résultant de l'analyse de la SEP.

Après des études préliminaires sur les poux congelés, le but de cette recherche était d'évaluer l'influence de la conservation de l'alcool sur la précision de l'identification des poux par la SEP MALDI-TOF. À cette fin, des poux conservés dans l'alcool pendant des périodes variables ont été soumis à l'analyse de la SEP et les protocoles de préparation des échantillons ont été optimisés. La reproductibilité et la spécificité des spectres de MS obtenus sur ces arthropodes nous ont permis de mettre en œuvre la base de données des spectres de MS de référence (DB) avec les profils protéiques de sept espèces de poux stockés dans l'alcool.

Des tests en aveugle ont révélé une identification correcte de 93,9% de *Pediculus humanus corporis* (Linnaeus, 1758) et de 98,4% des autres espèces de poux collectées sur le terrain. Cette étude a démontré que le MALDI-TOF MS pouvait être utilisé avec succès pour l'identification des poux stockés dans l'alcool pendant différentes durées.

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Research

Identification of Lice Stored in Alcohol Using MALDI-TOF MS

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Abstract

Lice pose major public and veterinary health problems with economic consequences. Their identification is essential and requires the development of an innovative strategy. MALDI-TOF MS has recently been proposed as a quick, inexpensive, and accurate tool for the identification of arthropods. Alcohol is one of the most frequently used storage methods and makes it possible to store samples for long periods at room temperature. Several recent studies have reported that alcohol alters protein profiles resulting from MS analysis. After preliminary studies on frozen lice, the purpose of this research was to evaluate the influence of alcohol preservation on the accuracy of lice identification by MALDI-TOF MS. To this end, lice stored in alcohol for variable periods were submitted for MS analysis and sample preparation protocols were optimized. The reproducibility and specificity of the MS spectra obtained on both these arthropod families allowed us to implement the reference MS spectra database (DB) with protein profiles of seven lice species stored in alcohol. Blind tests revealed a correct identification of 93.9% of *Pediculus humanus corporis* (Linnaeus, 1758) and 98.4% of the other lice species collected in the field. This study demonstrated that MALDI-TOF MS could be successfully used for the identification of lice stored in alcohol for different lengths of time.

Key words: lice, MALDI-TOF MS, storage method, identification

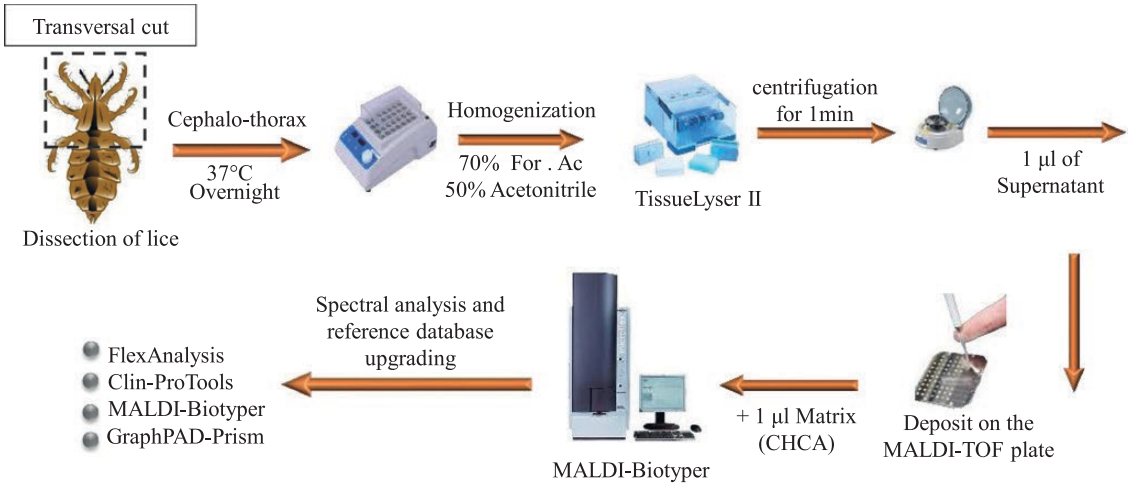
Résumé

Les poux posent des problèmes majeurs de santé publique et vétérinaire, avec des conséquences économiques. Leur identification est essentielle et nécessite le développement d'une stratégie innovante. Le MALDI-TOF MS a récemment été proposé comme un outil rapide, peu coûteux et précis pour l'identification des arthropodes. L'alcool est l'une des méthodes de stockage les plus fréquemment utilisées et permet de conserver les échantillons pendant de longues périodes à température ambiante. Plusieurs études récentes ont rapporté que l'alcool modifie les profils protéiques résultant de l'analyse de la SEP. Après des études préliminaires sur les poux congelés, le but de cette recherche était d'évaluer l'influence de la conservation de l'alcool sur la précision de l'identification des poux par la SEP MALDI-TOF. À cette fin, des poux conservés dans l'alcool pendant des périodes variables ont été soumis à l'analyse de la SEP et les protocoles de préparation des échantillons ont été optimisés. La reproductibilité et la spécificité des spectres de MS obtenus sur ces arthropodes nous ont permis de mettre en œuvre la base de données des spectres de MS de référence (DB) avec les profils protéiques de sept espèces de poux stockés dans l'alcool. Des tests en aveugle ont révélé une identification correcte de 93,9% de *Pediculus humanus corporis* (Linnaeus, 1758) et de 98,4% des autres espèces de poux collectées sur le terrain. Cette étude a démontré que le MALDI-TOF MS pouvait être utilisé avec succès pour l'identification des poux stockés dans l'alcool pendant différentes durées.

Mots-clés: Poux, MALDI-TOF MS, méthode de stockage, identification.

Graphical abstract

Protocol established for MALDI-TOF MS analysis of lice in alcohol 70%.



Lice are apterous insects that belong to theorder of Phtiraptera. They are mandatory ectoparasites of mammals and birds. More than 4,900 species of lice have been described in the literature (Johnson et al. 2004). Lice are separated into two suborders: Anoplura (sucking lice) and Mallophaga (chewing lice). Two genera are recognized within the human sucking lice order (Phthiraptera: Anoplura), *Pthirus* and *Pediculus*. Each genus is presented by one species, *Phtirius pubis* (Linnaeus, 1758) and *Pediculus humanus* (Linnaeus, 1758) (Louni et al. 2018, Amanzougaghene et al. 2020). Both louse species are obligate blood-feeding parasites that have gorged on human blood for thousands of years (Amanzougaghene et al. 2020). The sucking lice feed exclusively on the blood of mammals (Boyd and Reed 2012). Chewing lice infest birds, mammals and feed mainly on epidermal debris from their hosts (Shao et al. 2015).

Lice such as the body louse (*Pediculus humanus humanus*) and head louse (*Pediculus humanus capitis*) (Linnaeus, 1758), which live in clothing and on the human scalp, respectively, are ectoparasites of great concern to public health (Louni et al. 2018). Lice have also been reported to be a veterinary problem, inducing economic loss, notably for livestock (Titchener 1985). These consequences can be summarized in some countries around the world by blood spoliation, anemia, stress, hair loss, severe pruritus, decreased appetite and milk production, focal necrosis, and scarring on the skin of heavily infested animals (Domínguez-Peñafiel et al. 2011, Álvarez-Ortega and Peña-Santiago 2013). Body lice are very common in people living in unstable and impoverished conditions (Brouqui 2011), unlike head lice, which infest school children, regardless of the level of hygiene (Izri et al. 2010). Outside of their biotopes, the two species of lice are morphologically indistinguishable (Drali et al. 2013). Body lice transmitted infections form are part of the epidemics that have been described during wars throughout history (Raoult and Roux 1999). They are known to be vectors of human disease including *Bartonella quintana*, the agent of trench fever, *Borrelia recurrentis*, the agent of louse-borne relapsing fever, *Rickettsia prowazekii*, the agent of epidemic typhus (Raoult et al. 1998, Amanzougaghene et al. 2020), *Yersinia pestis*, the causal agent of plague and are also responsible for phtiriosis, otherwise known as pediculosis (McNair 2015). The potential of head lice as a disease vector has not yet been clearly established, despite the detection of the DNA of several pathogens in head lice (Mana et al. 2017, Amanzougaghene et al. 2020).

Morphological identification requires entomological expertise and accurate identification is sometimes not possible, notably for damaged or immature specimens, due to the lack of crucial criteria or identification keys (Dupuis et al. 2018). To overcome these drawbacks, molecular approaches, such as sequencing the *18S*rRNA gene, have been developed for lice identification (Whiting 2002). The sequencing of cytochrome c oxidase subunit I (*COI*) gene of lice has also been used for their identification (Johnson et al. 2003). Recently, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS) has been evaluated as a rapid tool for the identification of arthropods, including ticks (Boyer et al. 2017), mosquitoes (Vega-Rúa et al. 2018), culicoides (Sambou et al. 2015), and phlebotomine sand flies (Lafri et al. 2016). MALDI-TOF MS has also been successfully applied for the identification of frozen lice (Ouarti et al. 2020). The large majority of the arthropod families submitted to MALDI-TOF MS for identification have been performed on fresh or frozen specimens (Yssouf et al. 2016). However, arthropod collection sites are often far from laboratories, which require the use of storage methods other than freezing, methods which are less restrictive for sample preservation. Alcohol at 70% or 90% (v/v) is one of the storage methods frequently used, preserving samples for long periods at room temperature (Song et al. 2016). Recent studies have reported a modification of MS profiles between fresh or frozen specimens compared to counter specimens preserved in alcohol buffer (Nebbak et al. 2016, 2017). However, the stability over time of MS profiles from some tick and flea species stored in alcohol underline that MALDI-TOF MS could also be used for the identification of arthropods stored in this buffer (Diarra et al. 2017, Zurita et al. 2019).

The purpose of this study was to assess the influence of alcohol preservation on the accuracy of identification of lice by MALDI-TOF MS. For this purpose, one laboratory-reared louse species, *P. humanus corporis*, was preserved in alcohol for periods ranging from 2 mo to 1 yr to assess the efficacy of MALDI-TOF MS for the correct identification of these arthropods. In addition, seven lice species from field, stored in alcohol from between a few months to

several years, and which had been morphologically identified, were submitted to MALDI-TOF MS to assess the intraspecies reproducibility and interspecies specificity of resulting MS spectra.

Materials and Methods

Arthropod Rearing and Storing

Adult *P. humanus corporis* lice were reared in a climatic chamber (25°C, relative humidity 80–90%) and successive generations were obtained by allowing the lice to feed on rabbits, as previously described (Mana et al. 2017). Two hundred adult specimens were sedated at –20°C prior to being stored in 70% (v/v) alcohol at room temperature. Fifteen specimens stored in alcohol were collected every month for MS analysis between months 2 and 12.

Field Collection of Arthropods

Seven lice species (*Bovicola caprae* (Gurlt, 1843), *Goniocotes gallinae* (Geer, 1778), *Goniodes gigas* (Taschenberg, 1879), *Goniodes dissimilis* (Denny, 1842), *Haematopinus eurysternus* (Nitzsch, 1818), *Menacanthus stramineus* (Nitzsch, 1818), and *Menopon gallinae* (Linnaeus, 1758), collected from animals from the 'El-Taref' area (Algeria) were identified using morphological keys (Meguini et al. 2018). Between 9 and 15 specimens per species were included, representing a total of 75 specimens. All lice collected were immediately stored in 70% (v/v) alcohol prior to morphological identification and being sorting by species. Details about number of specimens per species, storage duration, and mammal host origin are available in Table 1.

Sample Preparation for MALDI-TOF Analysis

The cephalo-thorax for some specimens was retained for MALDI-TOF MS tests (Yssouf et al. 2013). The abdomens of each specimen were frozen at –20°C for later molecular biology analysis. The cephalo-thorax was placed in an oven at 37°C overnight, to evaporate organic solvents (Diarra et al. 2017). The cephalo-thorax of each louse specimen were then homogenized using TissueLyser (Qiagen) with a pinch of glass powder (Sigma, Lyon, France) as disruptor. The parameters selected for sample homogenization were six cycles of 60 s at a frequency of 30 Hertz. Then microliters of homogenization buffer were used for the lice conserved in alcohol (70%v/v). The homogenization buffer was composed of a 50/50 (v/v) mix of formic acid (70%v/v) (Sigma) plus acetonitrile (50% v/v) (Fluka, Buchs, Switzerland). Two fresh *P. humanus corporis* specimens were homogenized using the same automated conditions, and used as positive controls.

Molecular Identification of Lice

The two specimens per species definitely identified by morphological criteria and selected for MS DB creation, were selected (Table 1). The abdomen was used for DNA extraction using a Qiagen kit (Qiamp DNA mini kit, Hildesheim, Germany) according to the manufacturer's instructions, after mechanical homogenization. Species-level molecular identification attempts of the lice were performed by sequencing a fragment of the 18SrRNA gene (Ouarti et al. 2020). Primers SAIDG: (5′-TCTGGTTGATCCTGCCAGTA-3′) and SBIDG: (5′-ATTCCGATTGCAGAGCCTCG-3′) were used to amplify the 18SrRNA gene and LCO1490: 5′-GGTCAACAAATCAT AAAGATATTGG-3′; HC02198: 5′-TAAACTTCAGGGTGACCAA AAAATCA-3′) (Folmer et al. 1994) for molecular identification of lice in this study. Abdomens of two *P. humanus corporis* specimens freshly collected or stored in alcohol during 6–12 mo were used as extraction and PCR controls.

Sample Loading on MALDI-TOF Target Plates

After sample homogenization, centrifugation at 200 g was performed for 1 min to pellet debris, and 1 µl of supernatant from each sample was loaded on the MALDI-TOF MS target plate in quadruplicate (Bruker Daltonics, Wissembourg, France) and covered with 1 µl of CHCA matrix solution, composed of saturated alpha-cyano-4-hydroxycynnamic acid (Sigma, Lyon, France), 50% acetonitrile (v/v), 2.5% trifluoroacetic acid (v/v) (Aldrich, Dorset, UK), and HPLC-grade water. After drying for several minutes at room temperature, the target was placed in the Microflex LT MALDI-TOF Mass Spectrometer (Bruker Daltonics) for analysis. To control the matrix quality, sample loading, and MALDI-TOF apparatus performance, the matrix solution was loaded in duplicate on to each MALDI-TOF plate, with and without two fresh lice prepared under the same conditions, which served as grinding controls.

MALDI-TOF MS Parameters

Protein mass profiles were generated using a Microflex LT MALDI-TOF Mass Spectrometer (Bruker Daltonics, Germany), with detection in the linear positive-ion mode at a laser frequency of 50 Hz within a mass range of 2–20 kDa. The acceleration voltage was 20 kV, and the extraction delay time was 200 ns. Each spectrum corresponded to ions obtained from 240 laser shots performed in six regions of the same spot, and automatically acquired using the Auto Xecute method of the flex Control v2.4 software (Bruker Daltonics). The spectrum profiles were visualized with flex Analysis v3.3 software and were exported to ClinPro Tools v2.2 and MALDI-Biotyper v3.0 (Bruker Daltonics, Germany) for data processing (smoothing,

Table 1. Overview of lice origins, subgroup selected for MALDI-TOF MS reference database creation a priori blind tests for MS identification and Log-score values for each species

Species	Host	Storage time (years)[a]	Total number of specimens	Number of specimens included in the database and sequenced	Species identified by MALDI-TOF MS	LSVs
Bovicola caprae	Goats	(2014) 5	10	2	*B. caprae*	[2.18–2.43]
Goniocotes gallinae	Poultry	(2014) 5	9	2	*G. gallinae*	[2.01–2.25]
Goniodes gigas	Poultry	(2015) 4	9	2	*G. gigas*	[2.03–2.35]
Goniodes dissimilis	Poultry	(2015) 4	15	2	*G. dissimilis*	[1.77–2.30]
Haematopinus eurysternus	Cattle	(2014) 5	11	2	*H. eurysternus*	[2.00–2.24]
Menacanthus stramineus	Mammals	(2015) 4	10	2	*M. stramineus*	[1.82–2.13]
Menopon gallinae	Poultry	(2016) 3	11	2	*M. gallinae*	[1.91–2.39]
Total			75	14		

[a]All specimens were stored in 70% (v/v) alcohol.

baseline subtraction, and peak picking) and evaluation with cluster analysis.

Spectral Analysis and Reference Database Upgrading

Intraspecies reproducibility and interspecies specificity of MS spectra were visually compared using the average spectra (main spectrum profile [MSP]) obtained from the four spectra of each sample tested using the flex Analysis v3.3 and ClinPro Tools v2.2 software (Bruker Daltonics). To create a database specific to lice samples preserved in alcohol, reference spectra (MSP) were created by combining the spectra replicate results from each selected specimen using the automated function of the MALDI-Biotyper software v3.0. (Bruker Daltonics). At least two specimens per species were used. MSPs were created on the basis of an unbiased algorithm using information on the peak position, intensity and frequency data. For *P. humanus corporis*, MS spectra from two specimens that had been freshly collected or stored in alcohol for 2, 4, and 6 mo were selected to create reference MS spectra. A total of eight *P. humanus corporis* specimens were then included in the database. Concerning the seven lice species collected in the field and stored in alcohol, MS spectra from two specimens per species were included in the reference MS spectra database, representing a total of 14 specimens. These 14 specimens were subjected to standard PCR using 18SrRNA and mCOI genes as previously described (Folmer et al. 1994, Ouarti et al. 2020). The MS spectra from the specimens included in the database were used for clustering analysis and to increment our homemade database using MALDI-Biotyper v.3.0. Software (Bruker Daltonics, Germany). Cluster analyses (MSP dendrogram) were performed to determine how organisms are related to one another. The setting parameters were as follows: distance measure by correlation, linkage by average, the score threshold value for a single organism was 300 (arbitrary unit), and for related organisms was 0 (arbitrary unit).

Blind Test for Study Validation

Blind tests were performed with new louse specimens which were either laboratory reared or collected in the field. Total spectra of 226 specimens encompassing eight lice species were tested against the upgraded homemade MS reference spectra database. The level of identification significance was determined using the log score values (LSVs) given by the MALDI-Biotyper software v.3.3, corresponding to a matched degree of signal intensities of mass spectra of the query and the reference spectra. LSVs ranging from 0 to 3 were obtained for each spectrum of the samples tested. Based on previous studies (Yssouf et al. 2013, Dieme et al. 2014), an LSV greater than 1.8 was considered as the cut-off for species identification. Data were analyzed using GraphPad Prism software version 5.01 (GraphPad, San Diego, CA).

Results

Assessment of Lice Preservation Modes and Duration Compatibilities With MALDI-TOF MS Analyses

Fifteen lice specimens from *P. humanus corporis* species, stored in alcohol from between 2 and 12 mo were submitted for MS analysis. MS spectra from fresh *P. humanus corporis* specimens were particularly different from those of counter species stored in alcohol (Fig. 1(i)). MS spectra from lice stored for the same length of time in alcohol and between different time points presented visually similar MS spectra. To verify the reproducibility of MS spectra whatever the length of time they spent in alcohol, an MSP dendrogram was performed using two specimens per storage condition and duration (Fig. 1(ii)). Specimens stored in alcohol were grouped in a distinct branch from those freshly submitted to MS from the same species (i.e., *P. humanus corporis*). Moreover, no ordination of the MS spectra was noted according to the length of time the sample was stored in alcohol. The mixing of the samples revealed that these MS profiles appeared to be stable, independently of the length of time they were stored in the alcohol buffer.

To confirm these results, MS spectra from 15 specimens per time point were queried against the homemade MS spectra reference database upgraded with MS profiles from six *P. humanus corporis* samples stored in alcohol for 2, 4, and 6 mo (two specimens per time point). Of the 165 MS spectra queried against the database, 97.6% (n = 161/165) of them were correctly identified at species level, matching with MS spectra from *P. humanus corporis* stored in alcohol (Fig. 1(iii)). The four MS spectra which were not identified came from specimens stored for three and 10 mo, and were of very low quality (low MS peak intensity and diversity), explaining why they were not correctly identified (LSVs < 1.2). The LSVs of the remaining samples which were correctly identified (n = 161) ranged from 1.34 to 2.65. To consider the identification as reliable, LSVs should reach the threshold of 1.8. Here, 93.9% (n = 155/165) of MS spectra succeeded in reaching this threshold (LSVs > 1.8). Although the LSV threshold of 1.8 is considered for identification relevant of arthropods, for microorganism identification LSVs upper or equal to 1.7 and 2.0 were established for genus and species identification, respectively. The application of these thresholds revealed that 96.4% (159/165) of *P. humanus corporis* were identified at least at the genus level and 63.0% (104/165) at the species level. It is interesting to note that high LSVs were also obtained for MS spectra corresponding to specimens stored in alcohol for which respective time points were not included in the database. These results underlined the reproducibility of the MS spectra between these specimens, independently of the length of time the specimens were preserved in ethanol.

MS Database Creation and Blind Tests for Lice Identification by MALDI-TOF MS

To assess whether species-specific MS profiles could be obtained for lice preserved in alcohol for long periods, 75 specimens from seven lice species stored in alcohol for between 3 and 5 yr, were included in this study (Table 1). A subgroup of 14 lice which had been morphologically identified, including two specimens per species, were selected for evaluating intraspecies reproducibility and interspecies specificity of MS spectra (Table 1). To confirm their morphological identification, molecular assays were done. Unfortunately, neither 18SrRNA nor mCOI target gene sequences were successfully amplified using DNA extract from 14 lice stored long time in alcohol. Conversely, PCR products were obtained from DNA extracts of *P. humanus corporis* specimens freshly collected or stored in alcohol during 6–12 mo, for both gene targets. The sequencing and BLAST of these gene products matched at 99% of identity and 100% coverage with *P. humanus corporis* (18S RNA: FJ267398.1 and COI: KP143370). A visual analysis of the MS profiles from the cephalothorax of lice using the Flex Analysis software indicated that they seem to be reproducible between specimens from the same species (Fig. 2(i)). Moreover, the MS profiles appeared to be distinct between lice species. The intraspecies reproducibility and interspecies specificity of MS profiles was objectified by cluster analysis. Cluster

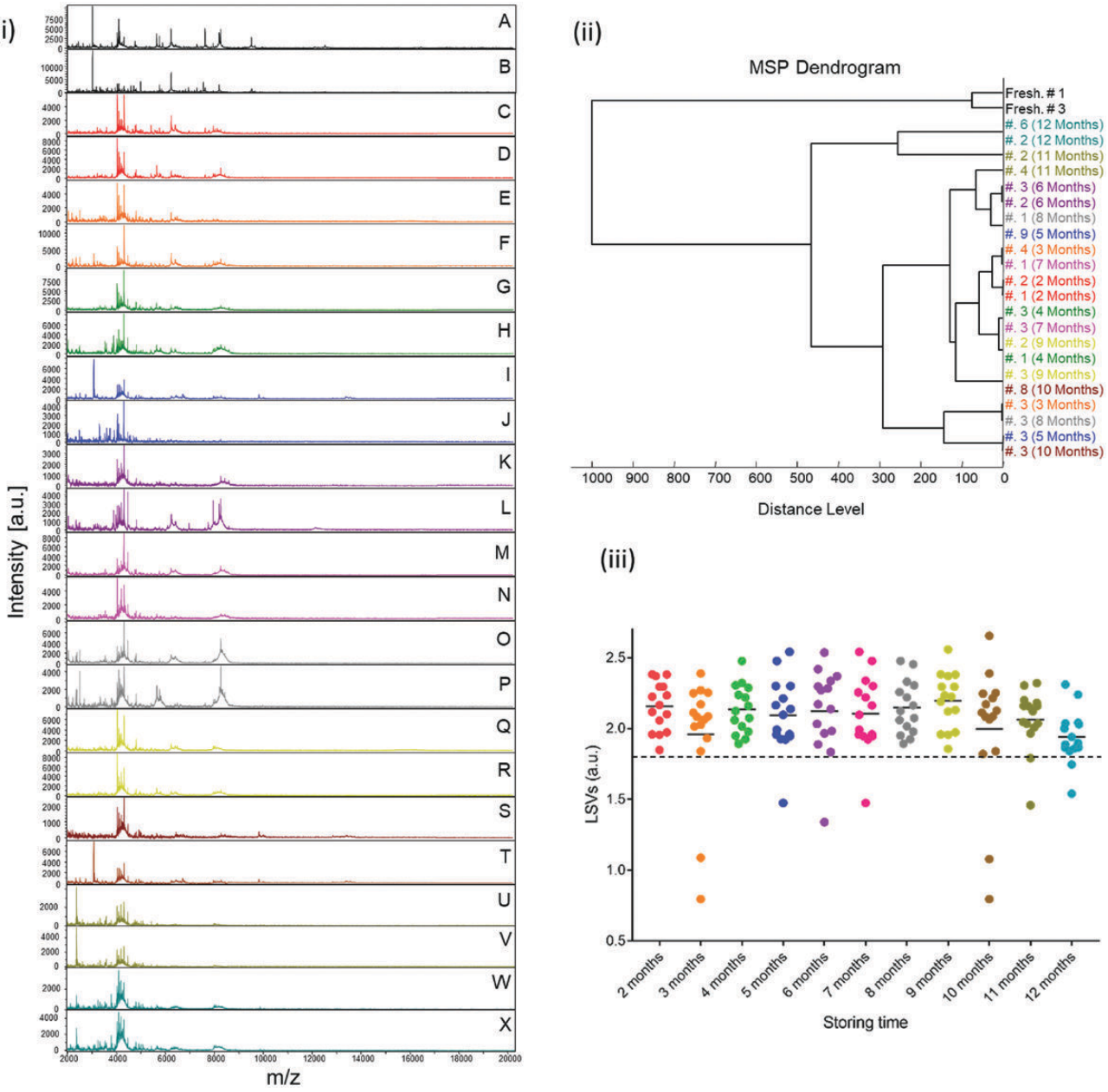


Fig. 1. Consequences of storage mode and duration on mass spectrometry (MS) spectra of lice cephalo-thorax and their reliable identification. (i) Representative MS spectra of cephalo-thorax of adult *Pediculus humanus corporis* fresh (A, B) or stored for 2 (C, D), 3 (E, F), 4 (G, H), 5 (I, J), 6 (K, L), 7 (M, N), 8 (O, P), 9 (Q, R), 10 (S,T), 11 (U,V), or 12 (W, X) months in alcohol 70% v/v. (ii) Reproducibility and specificity of MALDI-TOF MS spectra from *P. humanus corporis* lice. Two specimens per storing mode (fresh vs alcohol) and time of storing (from 2 to 12 mo in alcohol) were used to construct the MSP dendrogram. #: specimens number. The dendrogram was created using Biotyper v3.0 software and distance units correspond to the relative similarity of MS spectra. (iii) Comparison of LSVs obtained for 15 *P. humanus corporis* specimens stored in alcohol tested monthly against the upgraded homemade MS reference database. Dashed line represents the threshold value for reliable identification (LSV > 1.8). a.u., arbitrary units; m/z, mass-to-charge ratio.

analysis revealed clustering around distinct branches of lice according to species (Fig. 2(ii)). However, lice from the same genus (e.g., *Goniodes*) were not all clustered on the same branch of the MSP dendrogram. Interestingly, MS spectra from *P. humanus corporis*, stored in alcohol for 6 mo, were separated on the MSP dendrogram from the other lice species, underlining the specificity of their MS spectra. Based on these results, the spectra of these 14 specimens from these seven new species were used to increment the homemade MS reference spectra database. The 61 remaining lice were submitted for MS analysis. Resulting MS profiles were then used to query the homemade MS reference spectra database (Fig. 2(iii)). Overall, the query against the upgraded MS reference spectra database revealed LSVs ranging from 1.77 to 2.43 with 100% classification, corroborating the morphological classification. An LSV threshold for considering an identification as relevant was established at 1.8 (Yssouf et al. 2013), and only one *G. dissimilis* specimen did not reach this threshold and could not be validated. The rate of relevant identification was ultimately established at 98.4% (n = 60/61). If the thresholds established for micro-organisms identification were taken into account for the lice, 100% (61/61) and 78.69% (48/61) reached the 1.7 and 2.0 cut-off LSVs corresponding to genus and species classification, respectively.

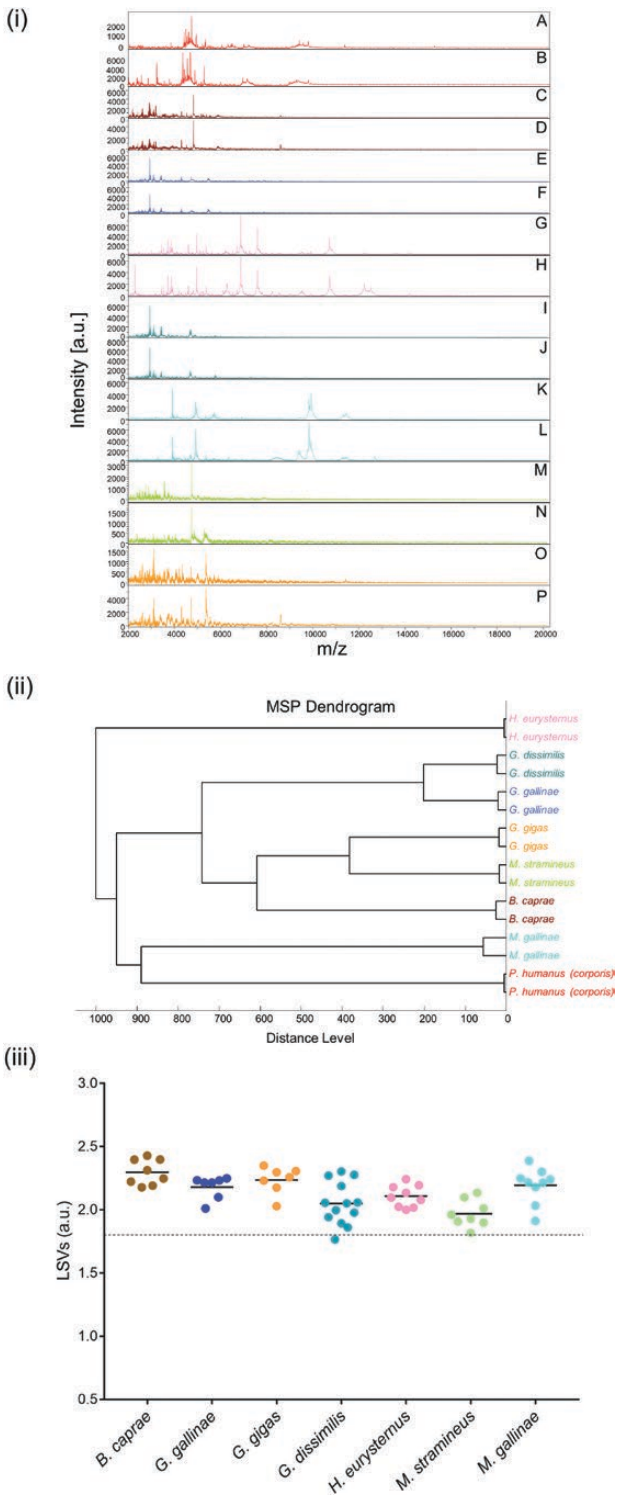


Fig. 2. Specific MALDI-TOF MS spectra of eigth lice species stored in alcohol and assessment of their MS identification. (i) Representative lice MS spectra of cephalo-thorax from *P. humanus corporis* (A, B), *B. caprae* (C, D), *G. gallinae* (E, F), *H. eurysternus* (G, H), *G. dissimilis* (I, J), *M. gallinae* (K, L), *M. stramineus* (M, N), and *G. gigas* (O, P) stored in alcohol. (ii) MSP dendrogram constructed using two representative MS spectra from the eight distinct lice species stored in alcohol. (iii) Comparison of LSVs obtained for 7 to 13 specimens per lice species stored in alcohol against the upgraded homemade MS reference database with MS spectra from respective species stored in alcohol. Dashed lines represent the threshold value for reliable identification (LSV > 1.8). a.u., arbitrary units; m/z, mass-to-charge ratio.

Discussion

For several years, the MALDI-TOF MS technique has been established for the routine diagnosis of micro-organisms such as the identification of bacteria (Seng et al. 2009, Bizzini and Greub 2010), archaea (Dridi et al. 2012), yeasts, and filamentous fungi (Seng et al. 2009, Dridi et al. 2012). This innovative proteomic tool was also successfully applied for the detection of other pathogens such as *helminths* and intestinal *protozoa* of medical and veterinary importance (Feucherolles et al. 2019). Most of these studies were based primarily on the identification of specific proteins rather than entire pathogens. However, others works reported the use of MALDI-TOF MS for species-specific identification of nematode species, *Trichinella* spp. and *Entamoeba* spp. (Calderaro et al. 2015, Mayer-Scholl et al. 2016, Bredtmann et al. 2017). Since then, the MALDI-TOF MS process has been successfully tested in medical entomology to identify different arthropod families including culicoides biting midges (Dieme et al. 2014), mosquitoes (Müller et al. 2013, Yssouf et al. 2013, Yssouf et al. 2014), phlebotomines sand flies (Mathis et al. 2015, Lafri et al. 2016), fleas (Yssouf, et al. 2014), Tsetse flies (Hoppenheit et al. 2013, 2014), and ticks (Yssouf et al. 2013a). These studies concerned mainly fresh or frozen stored arthropods. However, due to the location of the place of collection (far from the laboratory, lack of infrastructure, and transport time), the resources available in the field mean that other means of sample conservation are required. One of the most frequently method used for arthropods is storage in alcohol (Zurita et al. 2019). Previous work has shown that storing arthropods in alcohol modifies MS profiles compared to fresh specimens of the same species, hampering direct identification by MALDI-TOF MS against the reference MS spectra database which only includes counter species from fresh or frozen specimens (Kumsa et al. 2016, Diarra et al. 2017).

This study revealed that the MALDI-TOF MS profiles of *P. humanus corporis* stored in alcohol were different from the fresh ones of the same species. The differences in MS profiles according to storage for specimens from the same species have already been reported (Diarra et al. 2017, Zurita et al. 2019). Nevertheless, despite the variation of MS profiles due to storage in alcohol, it was underlined that the MS spectra remained reproducible for specimens of the same species stored for varying periods in alcohol. The intraspecies stability of the MS spectra is a key factor for suitable specimen identification using the MALDI-TOF MS tool (Diarra et al. 2017). The inclusion of MS spectra from specimens stored in alcohol is therefore essential for the reliable identification of counter species stored in the same organic buffer (Nebbak et al. 2017).

This strategy was successfully applied to *Ixodid* ticks from Ethiopia which were conserved in alcohol 70% (v/v) (Kumsa et al. 2016). It was shown that, despite storing the ticks for a long time (2 yr or more), the MS spectra were reproducible for specimens of the same species. These results were confirmed more recently, underlining the reproducibility and stability of tick and flea MS spectra from specimens stored in alcohol for periods of time (Nebbak et al. 2017). In addition, the sample preparation for MS analysis was standardized, which should facilitate sharing and exchanging results between research teams (Nebbak et al. 2017). Here, the homogeneity and specificity of the MS spectra from specimens stored in alcohol were validated by the clustering and intertwining of the MS spectra from *P. humanus corporis* lice samples stored in 70% alcohol whatever the period of conservation compared to the fresh one. The query against the upgraded MS spectra reference database

with *P. humanus corporis* samples stored in alcohol enabled relevant identification (LSV > 1.8) for nearly 94% of them.

A recent study reported on the application of MALDI-TOF MS profiling for the identification of 13 lice species of veterinary importance (Ouarti et al. 2020). In this previous study, the cephalo thorax appeared as the best body part for MS submission. All these specimens, collected from three distinct Algerian sites, Souk-Ahras, Guelma, and El-Taref, were stored at –20°C prior to MS analysis. Although six lice species (*Bovicola caprae*, *Goniocotes gallinae*, *Goniodes gigas*, *Haematopinus eurysternus*, *Menacanthus stramineus*, and *Menopon gallinae*) were shared with the present study, the MS spectra are not super imposable due to the different storage method. Here, the conservation of the lice in 70% ethanol buffer generated modifications to MS profiles compared to fresh or frozen stored counterpart species, as observed for *P. humanus corporis*. This phenomenon was previously repeatedly reported for ticks (Diarra et al. 2017, Nebbak et al. 2017), culicoides (Sambou et al. 2015), and fleas (Zurita et al. 2019).

It is interesting to note that 18S RNA and COI target gene sequences were successfully amplified using DNA extracts from *P. humanus corporis* specimens freshly collected or stored in alcohol during 6–12 mo. The failing of gene amplifications in the samples from the seven other lice species was not attributed to impairments of DNA extract or PCR experiments but rather likely to an inappropriate storing of lice samples. All the lice were stored in 70% ethanol during 4–6 yr at room temperature. Previous works reported that the preservation of sample in 70% ethanol is less efficient than 96–100% ethanol notably concerning DNA integrity (Barnes et al. 2000, Doorenweerd and Beentjes 2012). Effectively, 70% ethanol could induce incomplete fixation of the sample responsible for the degradation of the DNA, but also it low quality could participate to DNA hydrolysis (Spigelman et al. 2001).

The body part selected for MS analysis was the cephalo-thorax, which presented reproducible and specific MS profiles. The volume of homogenization buffer was adjusted to optimize the quality of the MALDI-TOF MS profiles. The main limitations are the small size of the lice, generating low protein quantities and MS spectra of lower intensity, but also the difficulty of sample grinding which made it necessary to increase the number of homogenization cycles. Compared to the standardized protocols on ticks (Diarra et al. 2017) and mosquitoes (Yssouf et al. 2013b), the grinding time was doubled and the homogenization buffer was decreased. These modifications made it possible to obtain reproducible and specific profiles for lice but different from fresh counter species, as mentioned previously for other arthropod families (Yssouf et al. 2013a). Despite these optimizations, the MS profiles continue to be low in intensity. This low intensity is mainly linked to the small size of the cephalo-thorax of lice. MS profiles with low intensity had already been reported, for instance when the early developmental stages of mosquito larvae were analyzed by MALDI-TOF MS (Dieme et al. 2014), and confirmed in a larger study aiming to monitor mosquito larvae in Marseille (Nebbak et al. 2018). Nevertheless, in this study, repeatable and specific MS spectra were obtained between seven species of lice kept in alcohol for several years. The query of the upgraded MS reference database with MS profiles from lice species stored in alcohol showed that 100% of the specimens were relevantly identified, regardless of the length of time they were stored in alcohol, confirming the use of this proteomic tool for the identification of lice stored in alcohol. The intraspecies reproducibility of the MS spectra from specimens stored in alcohol is a key factor for their reliable identification with this proteomic tool. The preservation of lice in alcohol is less restrictive than the freezing method and also makes it easier to transport or transfer samples. The enrichment of the MS spectra DB in the near future with additional lice species stored in alcohol will be essential if this tool is to be used for lice identification.

This innovative tool is to propose to physicians, researchers, or lab technicians an alternative strategy to morphological identification which requires entomologist competence or to molecular assays for which the price remains relatively high compared to MS analysis for entomological studies. The high throughput, low cost, rapidity, and accuracy of this approach could become a new tool to monitor arthropod vectors collected in the field or on mammalian hosts including humans. A rapid identification of arthropods collected on human could be helpful for physician to propose preventive treatment of pathogenic agents. We are convinced of the high potential of the MALDI-TOF MS profiling for entomological diagnosis and more globally for medical entomology.

Acknowledgments

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Chapitre III: Épidemiologie moléculaire des microorganismes associés aux animaux et aux arthropodes

Préambule

La faune sauvage et/ou des animaux domestiques sont responsables d'environ 75% des maladies humaines. Certains d'entre eux sont connus pour être réservoir de beaucoup de microorganismes infectieux tels que les bactéries (*Rickettsia*, *Bartonella*, *Borrelia*, *yersinia* ou *coxiella*), virus (le virus de West Nile, de chikungunya ou la dengue), parasites (*Plasmodium* ou *Leishmania*). La transmission de ces microorganismes entre les mammifères y compris l'homme se réalise soit avec le contact direct avec l'animal ou indirect via des ectoparasites vecteurs qui sont impliqués dans la dissémination de plusieurs pathogènes.

Afin d'anticiper les ravages qui peuvent être engendrer par ces arthropodes. Une grande évolution dans le dépistage des pathologies a transmission vectorielle a été noté et cela grâce à l'émergence de la biologie moléculaire qui s'est imposée au fil des années comme un outil d'épidémio-surveillance aussi bien chez l'hôte qu'à celle du vecteur.

Dans ce troisième chapitre de notre manuscrit « Épidemiologie moléculaire des microorganismes associés aux animaux et aux arthropodes » l'objectif été de compléter le répertoire des microorganismes associés aux arthropodes et aux animaux à l'aides des méthodes moléculaires (qPCR, PCR standard, séquençage et la phylogénie).

Notre premier travail (Article 5) a porté sur la détection des microorganismes chez 300 poux d'animaux. Pour la première fois en Algérie, nous avons détecté chez ces arthropodes la présence de *Coxiella burnetii*, *Anaplasma ovis* et une potentielle nouvelle Anaplasmataceae sp. Cette étude a été publiée sur le journal Comparative Immunology, Microbiology & Infectious Diseases (CIMID).

Notre deuxième étude (Article 6) a porté sur le dépistage des bactéries chez des tiques dures du genre *Dermacentor* et *Haemaphysalis* originaire de la Slovaquie et collectées à partir des végétations. Au niveau de ces deux genres de tiques nous avons signalé la présence de *Rickettsia raoultii*. Cette étude est en révision au journal Biologia.

Notre dernière étude a concerné la recherche des bactéries dans des petits mammifères (rongeurs) et des tiques molles des terriers dans la région du Sénégal (Article 7). Ainsi nous avons détecté la présence de *Borrelia crocidurae* et *Candidatus Ehrlichia* sp. dans les petits mammifères. De plus chez les tiques molles *Ornithodoros sonrai* ont a détecté pour la première fois au Sénégal la présence de potentielles nouvelles espèces dans la famille des Anaplasmataceae.

Nous avons aussi participé à un travail en collaboration qui a rapporté sur deux cas humains infestés et mordus par *Archaeopsylla erinacei*, la puce d'hérisson dans la région de Strasbourg (Article 8). Ce travail ne sera pas discuté ici il sera présenté en annexe chapitre 6.

Article N°5:

Molecular detection of microorganisms in lice collected from farm animals in northeastern Algeria.

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Résumé

Les poux (Phthiraptera) sont des insectes très spécifiques organisés en quatre sous-ordres (Anoplura, amblycera, ischnocera et rhynchophthirina). Les poux peuvent affecter la santé humaine et animale. Notre objectif était d'étudier la communauté bactérienne des poux collectés en Algérie. En utilisant des outils moléculaires, nous avons pu identifier par PCR en temps réel la présence d'ADN de *Coxiella burnetii* dans 1% (3/300) de *Linognathus africanus* et dans 0,3% (1/300) de *Linognathus vituli* collectés respectivement chez des chèvres et des bovins.

Nous avons également détecté la présence de bactéries Anaplasmataceae dans *Bovicola bovis*, *Linognathus vituli* provenant de bovins et dans *Linognathus africanus* provenant de chèvres. Par PCR standard et séquençage, nous avons pu identifier *Anaplasma ovis* dans *L. africanus* ainsi qu'un nouveau génotype d'Anaplasmataceae sp. correspondant probablement à un nouveau genre au sein de cette famille.

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, Linognathus vituli* from cattle and in *Linognathus 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.

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

1. Introduction

Lice are ectoparasites insects known for their high host-specificity [1,2]. There are nearly 4500 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 $\leq$ 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

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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 1000 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 2 and 3) 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, 1–3% of infectious endocarditis is

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.

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]

Table 2
BLAST analysis of *Anaplasma* spp. *23S* rRNA and *Ehrlichia* (*groEL*) sequences obtained from tested lice.

Host	Primers	Species lice	Molecular identification by BLAST	Percent Identity	Query Cover	Accession Number
Goat	Anaplasmataceae *23S* rRNA gene	*Linognathus africanus*	*Anaplasma ovis*	100 %	100 %	CP015994.2
Cattle	Anaplasmataceae *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

Table 3
Collection, morphological identification of mammalian and poultry lice and molecular detection of bacteria in lice using real-time PCR.

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	/	/	/

Note: All real time PCR tests were negative for the detection of *Borrelia* spp., *Rickettsia* spp., and *Bartonella* spp.
[a] Anoplura.
[b] Mallophaga.

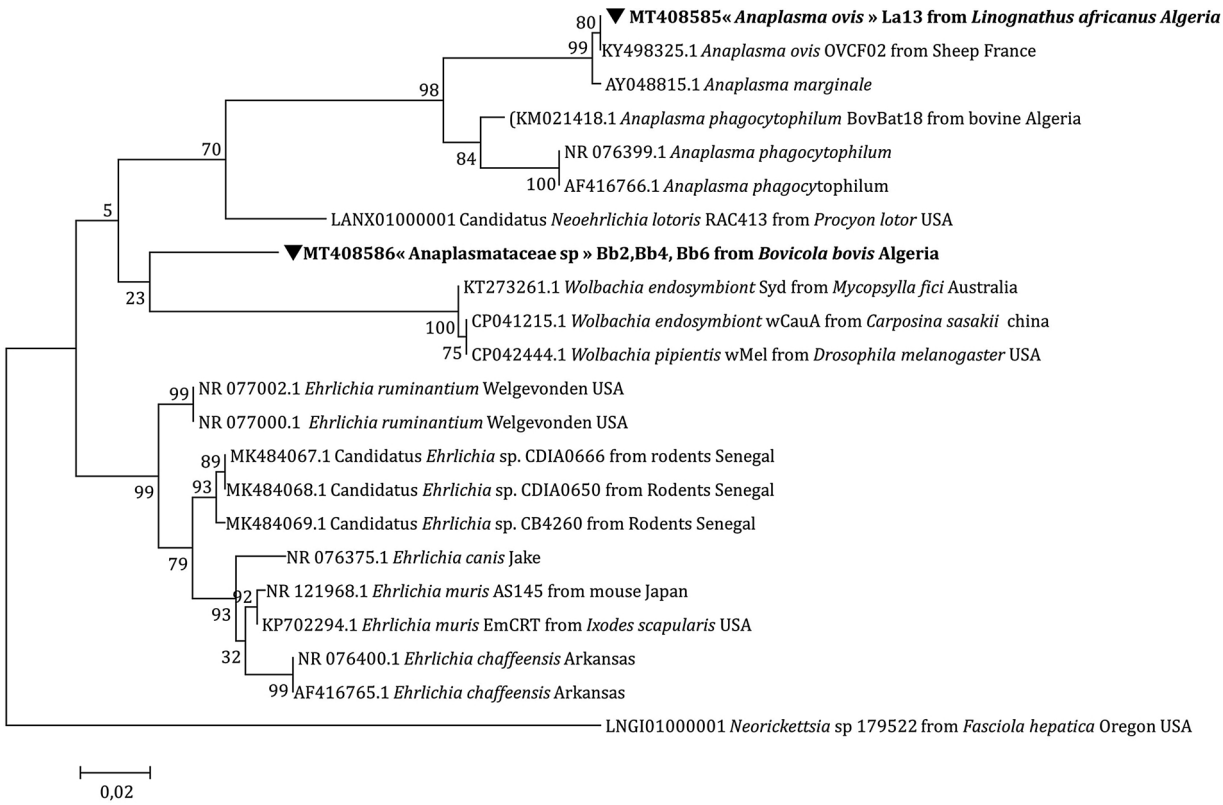

Fig. 1. Maximum-likelihood phylogenetic tree of Anaplasmataceae, based on the partial 513-bp *23S* gene.

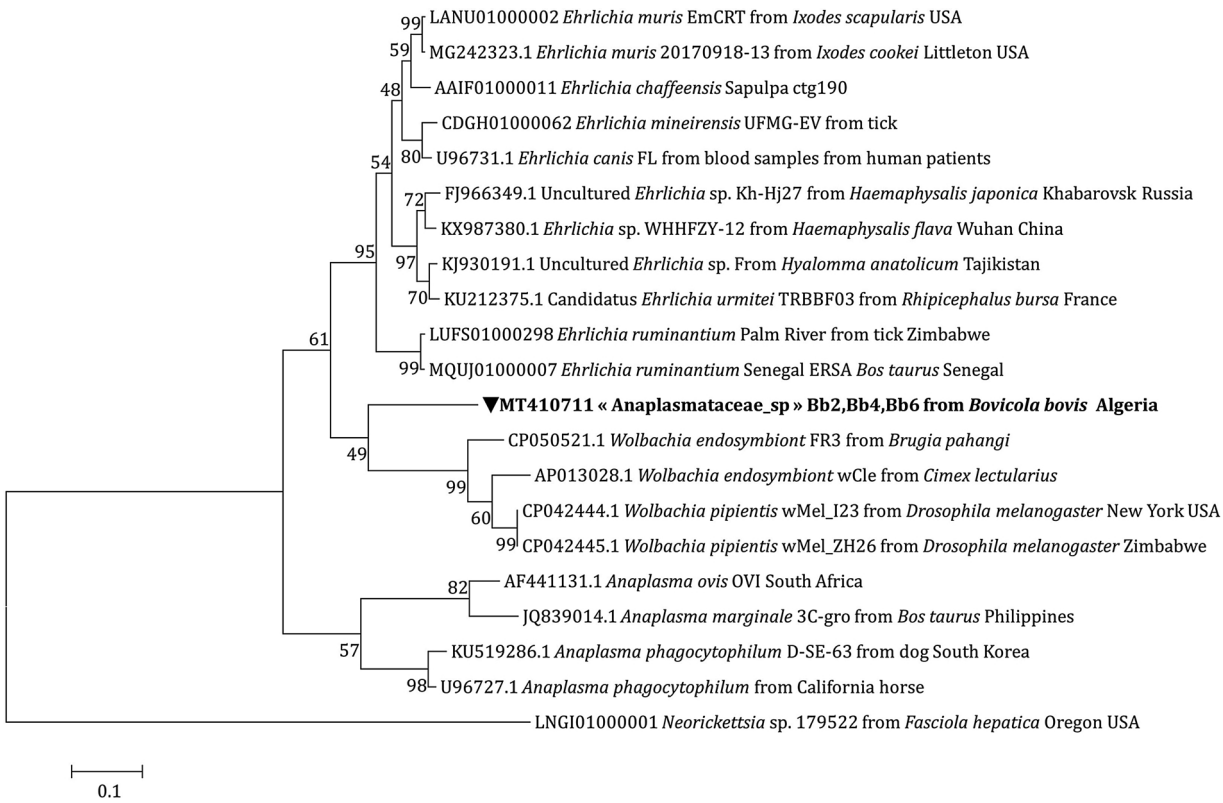

Fig. 2. Maximum-likelihood phylogenetic tree of *Ehrlichia* spp, based on the partial 633-bp *groEL* gene.

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.

5. 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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Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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Article N°6:

Detection of Rickettsia raoultii. in Dermacentor reticulatus and Haemaphysalis inermis ticks in Slovakia.

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Résumé

Les tiques sont un arthropode vecteur responsable de la transmission de plusieurs agents pathogènes qui affectent la santé humaine et animale dans le monde entier. Dans cette étude, notre objectif était d'analyser, en utilisant des outils moléculaires, la communauté bactérienne des tiques *Dermacentor reticulatus* et *Haemaphysalis inermis* collectées dans le sud-est de la Slovaquie. En utilisant la PCR en temps réel, nous avons pu identifier la présence d'ADN de *Rickettsia* spp. au niveaux de 14/59 (23,72%) et 29/173 (16,76%) dans ces deux espèces de tiques (*D. reticulatus* et *H. inermis*) respectivement. Ensuite, en utilisant la PCR standard et le séquençage, nous avons pu identifier la présence de l'ADN de *Rickettsia raoultii* dans 13 tiques appartenant aux deux genres *Dermacentor* et *Haemaphysalis*.

Les résultats du blast *Rickettsia raoultii* ont révélé un pourcentage d'identification moyen de 99,62%.

Suite aux résultats de cette étude moléculaire, il est possible que *D. reticulatus* et *H. inermis* jouent un rôle potentiel dans la transmission de *R. raoultii*.

Pour prouver la possibilité de validité de cette hypothèse, nous suggérons de réaliser des modèles expérimentaux dans de futures études. Nos résultats peuvent servir de données préliminaires pour de futurs modèles de transmission.

Biologia

Detection of Rickettsia raoultii in Dermacentor reticulatus and Haemaphysalis inermis ticks in Slovakia

--Manuscript Draft--

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Abstract:	Ticks are a vector arthropod responsible for the transmission of several pathogenic agents that affect both human and animal health worldwide. In this study our objective was to analyse, using molecular tools, the bacterial community of Dermacentor reticulatus and Haemaphysalis inermis ticks collected in south-eastern Slovakia. Using real-time PCR, we were able to identify the presence of Rickettsia spp. DNA at levels of 14/59 (23.72%) and 29/173 (16.76%) in these two species of ticks (D. reticulatus and H. inermis) respectively. Then, using standard PCR and sequencing, we were able to identify the presence of Rickettsia raoultii DNA in 13 ticks belonging to the two genera Dermacentor and Haemaphysalis . Rickettsia raoultii blast results revealed an average identification percentage of 99.62%. Following the results of this molecular study there is a possibility that D. reticulatus and H. inermis play a potential role in the transmission of R. raoultii . To prove the possibility of validity of this hypothesis, we suggest performing experimental models in future studies. Our results can serve as preliminary data for future transmission models.
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Detection of *Rickettsia raoultii* in *Dermacentor reticulatus* and *Haemaphysalis inermis* ticks in Slovakia

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Abstract

Ticks are vector arthropods responsible for the transmission of several pathogenic agents that affect both human and animal health worldwide. In this study our objective was to analyse, using molecular tools, the bacterial community of *Dermacentor reticulatus* and *Haemaphysalis inermis* ticks collected in south-eastern Slovakia. Using real-time PCR, we identified the presence of *Rickettsia* spp. DNA at levels of 14/59 (23.72%) and 29/173 (16.76%) in *D. reticulatus* and *H. inermis*, respectively. In addition, using standard PCR and sequencing, we identified the presence of *Rickettsia raoultii* DNA in 13 ticks belonging to the two investigated species. *Rickettsia raoultii* blast results revealed an average identification percentage of 99.62%. Following the results of this molecular study there is a possibility that *D. reticulatus* and *H. inermis* play a potential role in the transmission of *R. raoultii*. To prove the possibility of validity of this hypothesis, we suggest performing experimental models in future studies. Our results can serve as preliminary data for future transmission models.

Keywords:

Ticks; *Dermacentor reticulatus*; *Haemaphysalis inermis;* Slovakia.

Highlights

- *Dermacentor reticulatus* and *Haemaphysalis inermis* ticks collected in south-eastern Slovakia are infected by *Rickettsia raoultii*.
- These preliminary data could be used in a future transmission model.

Introduction

Bacteria belonging to the genus *Rickettsia* are known as causative agents of various vector-borne zoonotic diseases. They are responsible for mild to severe diseases in humans (Parola et al. 2013; Eldin and Parola 2018). The diversity of pathogens in ecosystems also depends on the species diversity of vectors and hosts and the diversity of habitats (de la Fuente et al. 2008). Many ticks in Europe, including *Dermacentor reticulatus* Fabricius, 1794 are known to act as carriers, reservoirs and/or vectors of different pathogenic *Rickettsia* species e.g., *Rickettsia slovaca* (Sekeyová et al, 1998; Garcia-Vozmediano et al. 2020) and *Rickettsia raoultii* (Boldiš et al. 2008; Földvári et al. 2013).

Revising the role of another tick species, *Haemaphysalis inermis* Birula, 1895 in the spread of rickettsiae continues to be challenging as few data have been previously published (Portillo et al. 2008; Špitalská et al. 2018). In a study carried out in Hungary, the authors detected the presence of *R. helvetica* in *H. inermis* and suggested that this tick species could be a potential vector of this pathogen (Hornok et al. 2010).

Recent epidemiological studies of *Rickettsia* in Slovakia were completed in suburban, natural and rural habitats (Minichová et al. 2017), or in an urban temperate forest (Chvostáč et al. 2018) in the western part of the country. A dry forest-steppe biotope is rarely referenced in literature except, in the past century, by Řeháček et al. (1976). The authors indicated that: "The biotopes of the spotted fever group rickettsiae occurring in east Slovakia-namely, cultivated and meadow steppes with sparse forests-share some of the characteristics of the biotopes of *Rickettsia sibirica*. Thus, besides the new species of spotted fever group rickettsiae, the circulation of *R. sibirica* and perhaps of *Rickettsia conorii* in east Slovakia should not be excluded". However, none of them were ever confirmed in south-eastern Slovakia afterwards (Řeháček et al. 1976).

Our objective was to investigate the possibility of detecting rickettsiae in *D. reticulatus* and *H. inermis* two relatively abundant tick species in the steppe habitats of south-eastern Slovakia.

Materials and methods

Tick collection and sampling site

The tick collection was carried out on October 23, 2015 by flagging grass and shrubs using a white cotton flag passed over the vegetation at the level of three microhabitats (forest, ecotone and meadow) along a line of 100 meters in the vegetation of the protected zone of Slovak Karst National Park in south-eastern Slovakia (Central Europe) near the village of Hrhov (200–220 m above sea level, 48° 34.899 N, 20° 46.743 E) (Fig. 1).

DNA extraction and molecular detection of bacteria in ticks using real time PCR

Half of the tick body without legs was selected for DNA extraction using EZ1 DNA tissue kit (Qiagen, Hilden, Germany) following a protocol previously elaborated in our laboratory (Diarra et al. 2017).

After extraction, the DNA of each sample was screened with the CFX96 real-time PCR (Bio -Rad, Marnes-la-Coquette, France) and the LightCyclerR 480 Probes Master Mix (Roche Diagnostics, Indianapolis, USA) for the presence of the following bacterial microorganisms (*Rickettsia* spp., *Bartonella* spp., *Borrelia* spp., *Coxiella burnetii* and *Anaplasma* spp.) using specific primers and probes listed in Diarra et al. (2020) and Ouarti et al. (2020).

The qPCR reaction mixture for each plate has been used according to the manufacturer's protocol. Negative controls were used in each qPCR and consisted of DNA extracted from uninfected *Rhipicephalus*

sanguineus ticks from the laboratory colony of IHU - VITROME (Marseille, France). Positive controls included DNA extracted from a dilution of cultured strains of *Borrelia crocidurae*, *Bartonella henselae*, *Rickettsia montanensis*, *Ehrlichia canis* and *Coxiella burnetii* (Socolovschi et al. 2012b; Aouadi et al. 2017). Results were deemed positive if the cycle threshold (Ct) value obtained by CFX96 was lower than ≤ 35 (Ouarti et al. 2020).

Bacterial species identification using sequencing

All ticks tested positive for *Anaplasma* spp., *Borrelia* spp. and *Rickettsia* spp. in qPCR were subjected to amplification using standard PCR prior to sequencing for identification of the bacterial species (Dahmani et al. 2015b; Diarra et al. 2017). In standard PCR the primers used to amplify the *ompA* gene were (f-70 and r-701). For the sequencing we used the following primers (f-70, f-180 and r-701) which amplify (629 to 632 bp) of protein A (Table 1) (Socolovschi et al. 2012a).

Primers targeting the Anaplasmataceae 23S, *Borrelia 16S* rRNA and *Rickettsia ompA* genes (Table 1), were used as described previously (Socolovschi et al. 2012a; Dahmani et al. 2015a; Ouarti et al. 2020).

The purified DNA products were sequenced using a Big Dye Terminator kit and a genetic analyzer ABI PRISM 3130 (Applied BioSystems, Courtaboeuf, France). The sequences obtained were analyzed with the software ChromasPro, version 1.34 (Technelysium Pty, Ltd., Tewantin, Queensland, Australie) in order to compare them to the GenBank database using BLAST (http: //blast.ncbi.nlm. Nih.gov/Blast.cgi) (Diarra et al. 2020).

Results

A total of 232 adult ticks (63 *H. inermis* males and 110 females, 31 *D. reticulatus* males and 28 females) were collected from vegetation in the protected zone of the Slovak Karst National Park. The proportion of ticks examined are detailed in (Table 3).

Using qPCR, 14/59 (23.72%) *D. reticulatus* ticks tested positive for *Rickettsia* spp., 2/173 (1.15%) *H. inermis* ticks tested positive for Anaplasmataceae bacteria, 1/173 (0.57%) for *Borrelia* spp., and 29/173 (16.76%) for *Rickettsia* spp.

The 43 ticks positive for *Rickettsia* in qPCR were then subjected to standard PCR amplification.

A total of 13/43 (30.23%) ticks were successfully sequenced. BLAST analysis of these 13 sequences obtained showed an identity percentage ranging from 97.98% to 100% with the *R. raoultii* sequence. Details are given in Table 2. No *Borrelia* spp. or Anaplasmataceae sequences could be amplified.

Discussion

The investigated locality (Slovak karst near the village of Hrhov) is specific and unique due to the joint occurrence of seven tick species, *Ixodes ricinus* (Linnaeus, 1758), *I. trianguliceps* Birula, 1895, *I. frontalis* (Panzer, 1795), *Dermacentor marginatus* Sulzer, 1776, *D. reticulatus*, *Haemaphysalis concinna* C. L. Koch, 1844 and *H. inermis* (Černý 1972; Nosek 1972; Bona and Stanko 2013; Heglasová et al. 2020), which is an exceptional phenomenon in the conditions of Central Europe. *Haemaphysalis punctata*, Canestrini & Fanzago, 1878 which was the dominant species in tick communities in the area of Slovak karst in the last century, has not been confirmed there in recent decades. The high concentration of potential vectors (seven tick species) and hosts (cattle, free-living ungulates, several species of rodents and shrews) in the studied locality makes the Slovak karst a unique model

for studying the circulation of several species of pathogens in their natural habitat (Nosek 1971; Heglasová et al. 2020). Uniqueness of the site (hereby presented) is from the point of view of the common occurrence of several species of ticks (see above). In terms of uniqueness or differentiation of pathogens in ticks compared to other sites, confirmation of a variety of bacteria in *Haemaphysalis* spp. which live on a common site with both species of ticks of the genus *Dermacentor* is exclusive. In Slovakia, the presence of *D. reticulatus* has been identified along the rivers in south-western and south-eastern Slovakia (Nosek 1972). Ticks of the genus *Haemaphysalis* (*H. inermis*, *H. concinna*, *H. punctata*) are known to have a more focal distribution (Černý 1972; Nosek 1973).

In previously published studies from the Slovak karst area, we confirmed several species of rickettsiae (*R. slovaca*, *R. helvetica*, *Rickettsia felis*, *Rickettsia* sp.) and *Borrelia miyamotoi* in small mammals (especially in rodents), from rodent-attached ticks, as well as from questing ticks sampled from vegetation (Radzijevskaja et al. 2015; Heglasová et al. 2018). There is a characteristically high concentration of deer in the area, as well as cattle grazing on the pastures and both vertebrate groups are hosts for adult ticks. These factors increase the significance of the study area from an epidemiological point of view (Černý 1972).

Here, we demonstrated the circulation of *R. raoultii* in the studied area. Although *R. raoultii* has been already detected in *D. reticulatus* and in patients bitten by *D. reticulatus* (Parola et al. 2009), the vector competence of *D. marginatus* and *H. inermis* to transmit *R. raoultii* cannot be confirmed, because the detection of this bacterium in the latter could be the consequence of a bacterial blood meal of these ticks. Therefore, additional epidemiological studies and experimental models will be needed.

Both *Dermacentor* and *Haemaphysalis* ticks from which confirmed positive cases of *Rickettsia* originate are typical species for forest-steppe zones (*D. marginatus*, *H. inermis*), alluvial forests and wet meadows, which are commonly seen biotopes in eastern Slovakia (*D. reticulatus*, *H. concinna*). The joint occurrence of steppe landscape and xerothermic tick species together with ticks that prefer humid habitats in the studied area is probably caused by the presence of ponds and canals in the karst area as well as by the high density of hosts (birds, small mammals, wild ungulates, cattle) for all life stages of ticks. *Dermacentor reticulatus* was described as harbouring more bacteria than *D. marginatus* (Zhang et al. 2019). *Rickettsia raoultii* is usually associated with *Dermacentor* spp. (Špitalská et al. 2012; Švehlová et al. 2014). Sequencing partial *ompB* genes revealed the presence of *R. raoultii* in the larvae and nymphs of *D. reticulatus* ticks in Germany (Schmuck et al. 2020). In recent decades, the spread of the *D. reticulatus* to new territories has been confirmed, having been reported in, for example, in Poland (Kiewra and Czulowska 2013; Mierzejewska et al. 2016), Germany (Dautel et al. 2006) as well as other European countries (Földvári et al. 2016; Kjær et al. 2019; Capligina et al. 2020). One study carried out in Latvia detected the presence of *R. raoultii* in *D. reticulatus*, their results corroborate with our study. Experimental models will therefore be necessary to understand the role of *D. reticulatus* in the appearance of *R. raoultii* (Capligina et al. 2020).

The indication of *H. inermis* harbouring rickettsiae was noted (Řeháček et al. 1976). However, the role of *Haemaphysalis* spp. as vectors of *Rickettsia* spp. is unknown (Minichová et al. 2017). There is little data on bacterial associations with *H. inermis*. *Rickettsia aeschlimannii* was detected in La Rioja, Spain (Portillo et al., 2008). *Rickettsia slovaca* (Ibarra et al. 2006) and *R. raoultii* are responsible for tick-borne lymphadenopathy/ Dermacentor-borne necrosis erythema and lymphadenopathy/scalp eschar and neck lymphadenopathy (TIBOLA/-DEBONEL/SENLAT) (Oteo et al. 2004; Selmi et al. 2008; Parola et al. 2013). This illness commonly occurs in Slovakia (Sekeyová et al. 2013) and was recently identified as the causative agent of tick-borne lymphadenopathy

in Belgium (Lernout et al. 2019). *Rickettsia helvetica* is considered less pathogenic than the two aforementioned rickettsial pathogens (Sprong et al. 2009; Boulanger et al. 2019).

Conclusion

This molecular study supports the data that *R. raoultii* could be a source of lymphadenopathy in Slovak populations. Further studies, including experimental models, will be needed to assess the role of *D. reticulatus* and *H. inermis* in the transmission of *R. raoultii*. Nevertheless, based on the positive detection of *R. raoultii*, we assume that *Haemaphysalis* and *Dermacentor* should be considered as potential reservoirs of *R. raoultii* in Slovakia.

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Author Contributions

ZS**,** PP and OM conceived and designed the research. BO, BE and ML conducted the experiments. MS, and ZS contributed analytical tools. BO and BE analysed the data. BO and ZS wrote the manuscript. All authors read and approved the manuscript.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Table 1 Standard PCR primers used for the detection of microorganisms in ticks.

Table 2 BLAST analysis of *Rickettsia* obtained from tested ticks.

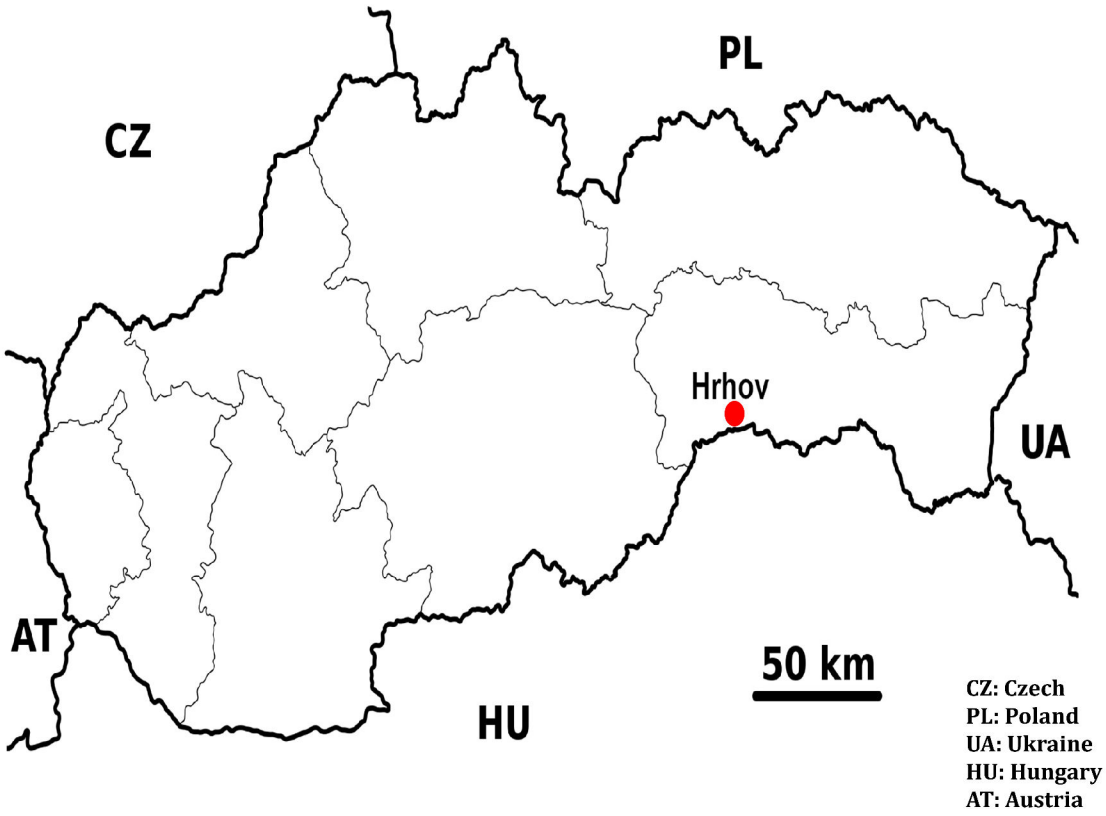
Table 3 The proportion of ticks examined according to microhabitats (forest: ecotone: meadow).

Fig. 1 Geographical position of the tick collection locality in the south-eastern region of Slovakia.

Bacterial species	Targeted sequences	Primers (5′–3′)	Reference
Anaplasmataceae	*23S* rRNA	f_ATAAGCTGCGGGGAATTGTC r_TGCAAAAGGTACGCTGTCAC	(Dahmani *et al*., 2015a)
Rickettsia spp.	*ompA*	f_70_ATGGCGAATATTTCTCCAAAA r_701_GTTCCGTTAATGGCAGCATCT f_180_GCAGCGATAATGCTGAGTA	(Socolovschi *et al*., 2012a)
Borrelia spp.	*16S* rRNA	f_GCTGGCAGTGCGTCTTAAGC r_GCTTCGGGTATCCTCAACTC	(Socolovschi *et al*., 2012a)

Ticks species	Query Cover (%)	Percent Identity (%)	Accession Number	Molecular identification by BLAST
Dermacentor reticulatus	100	97.98	HM161789.1	*Rickettsia raoultii*
Dermacentor reticulatus	100	100	KX506737.1	*Rickettsia raoultii*
Dermacentor reticulatus	100	99.84	HM161789.1	*Rickettsia raoultii*
Dermacentor reticulatus	100	100	KX506737.1	*Rickettsia raoultii*
Dermacentor reticulatus	100	99.68	HM161792.1	*Rickettsia raoultii*
Haemaphysalis inermis	99	99.84	CP003426.1	*Rickettsia raoultii*
Haemaphysalis inermis	99	98.63	CP003426.1	*Rickettsia raoultii*
Haemaphysalis inermis	100	99.41	CP003426.1	*Rickettsia raoultii*
Haemaphysalis inermis	99	99.84	HM161789.1	*Rickettsia raoultii*
Dermacentor reticulatus	100	100	KX506737.1	*Rickettsia raoultii*
Dermacentor reticulatus	100	99.84	HM161789.1	*Rickettsia raoultii*
Haemaphysalis inermis	100	100	KX506737.1	*Rickettsia raoultii*
Dermacentor reticulatus	99	100	HM161792.1	*Rickettsia raoultii*

Species of ticks	Forest	Ecotone	Meadow	Total
Dermacentor reticulatus	2 (3.4%)	20 (33.9%)	37 (62.7%)	59
Haemaphysalis inermis	27 (15.61%)	121(69.94%)	25 (14.45%)	173


PL
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AT
HU
50 km
CZ: Czech
PL: Poland
UA: Ukraine
HU: Hungary
AT: Austria

Article N°7:

The detection of zoonotic bacteria in the brain, spleen of rodents and burrows ticks in in the region of Senegal.

Basma Ouarti[a,b], El Hadji Ibrahima Ndiaye[a,b], Adama Zan Diarra[a,b], Jean Michel Berenger[a,b], Georges Diatta[c], Laurent Granjon[d], Jean Le Fur[e] and Philippe Parola[a,b]

Préparation pour soumission dans CIMID
(2021)

Résumé

Les petits mammifères et les tiques sont des réservoirs et des vecteurs naturels de plusieurs bactéries zoonotiques.

L'objectif de notre étude était d'élargir le répertoire des agents infectieux présents chez les rongeurs et/ou les tiques molles du nord du Sénégal en utilisant des outils moléculaires, à savoir la PCR en temps réel et la PCR standard. Ces analyses ont été effectuées sur 59 rates, 59 cerveaux de rongeurs (58 *Mus musculus* et un *Arvicanthis niloticus*) et 92 tiques molles *Ornithodoros sonrai*. Nous avons identifié *Borrelia crocidurae* dans 13 échantillons et un Candidatus *Ehrlichia* sp. dans l'espèce *Mus musculus*.

De plus, nous avons pu détecter pour la première fois dans le nord du Sénégal la présence de nouvelles espèces potentielles appartenant à la famille des Anaplasmataceae chez les tiques *O. sonrai*. Une plus grande attention devrait être accordée aux rongeurs et aux tiques molles, car ils peuvent être des vecteurs potentiels de nouvelles espèces de bactéries.

Detection of zoonotic bacteria in the brain, spleen of rodent and burrows ticks in Senegal

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Abstract

Small mammals and ticks are natural reservoirs and vectors for several zoonotic bacteria. The objective of our study was to broaden the repertoire of infectious agents present in rodents and/or soft ticks from northern Senegal using molecular tools, namely real-time PCR and standard PCR. These analyses were performed on 59 spleens, 59 brains of rodents (58 *Mus musculus* and one *Arvicanthis niloticus*) and 92 *Ornithodoros sonrai* soft ticks. We identified *Borrelia crocidurae* in 13 samples and one Candidatus *Ehrlichia* sp. in species *Mus musculus*. Also, we were able to detect for the first time in northern Senegal the presence of potential new

species belonging to the Anaplasmataceae family in *O. sonrai* tick. More attention should be granted to rodents and soft ticks as they can be potential vectors for new species of bacteria.

Keywords:

Anaplasmataceae, *Borrelia crocidurae*, *Ornithodoros sonrai*, *Mus musculus*, Senegal.

1. Introduction

Ticks are strict blood-sucking arthropods [1] included into two families, the *Ixodidae* (around 700 hard ticks) and *Argasidae* (around 200 soft ticks species) [2]. Ticks can affect animals and human health, being the second vectors of infectious agents after mosquitoes [3]. Tick-borne relapsing fever (TBRF) are caused by spirochetes of the genus *Borrelia* (*Borrelia crocidurae* (*B. crocidurae*), *Borrelia duttonii* and *Borrelia hispanica*) transmitted by soft ticks of the genus *Ornithodoros* [4–6]. In West Africa transmission of *B. crocidurae* occurs through the tick species *Ornithodoros sonrai* (*O. sonrai*) and rodents are the main reservoirs [5,7].

In Senegal several studies have reported the presence of *B. crocidurae* in ticks and/or rodents [5,8,9]. Other research has shown the presence of , *Coxiella burnetii* (*C. burnetii*), *Tropheryma whipplei* and *Rickettsia* spp. in febrile patients and environmental collections [10]. In northern Senegal no study has reported the presence of Anaplasmataceae in *O. sonrai*. In general, knowledge about tick-borne diseases in Senegal is often limited due to the lack of specialized diagnostic services. Hence the objective of this study is to detect by molecular biology the presence of tick-associated bacteria in *O. sonrai* and /or rodents in northern Senegal to expand the infectious repertoire in order to gain more sensitization concerning diseases transmitted by soft ticks and /or rodents that may affect human and animal health.

2. Materials and methods

2.1. Study area and collection of ticks and rodents

Ticks and rodents were collected in 2018 in intra-domiciliary dwellings in the rural community of Dodel in the Saint-Louis region located in the northern of Senegal at 379 km from the capital (16° 29' 15.58572" N, 14° 25' 33.46176" E). Ticks were collected from rodent burrows using a modified leaf vacuum [5]. The rodents were captured in the houses using lattice traps [7].

2.2. Morphological identification of ticks

The morphological identification of soft ticks was carried out based on a tick identification guide [11]. The observation was carried out using an optical microscope Zeiss Axio Zoom V16 (Zeiss, Marly-le-Roi, France) and an electron microscope SEM scanning electron microscope (Hitachi High-Technologies, Tokyo, Japan).

2.3. DNA extraction and molecular identification of ticks

DNA extraction was performed on a small part of the spleen, brain in rodents and the abdomen in ticks using an EZ1 DNA tissue extraction kit (Qiagen, Hilden, Germany) following a protocol described previously [1,9,12]. All ticks were subjected to standard PCR (PCR's) and sequencing to confirm morphological identification using the *16S tick* gene [12].

2.4. Molecular screening for pathogens in the brain, spleen, rodents and burrow ticks

Pathogen screening was performed using a Real-Time PCR (qPCR) CFX96 system (Bio-Rad, Marnes-la-Coquette, France). Bacteria detection was applied on each sample to be able to detect the presence of the following bacteria: *Anaplasma* spp., *Borrelia* spp., *Bartonella* spp., *C. burnetii* and *Rickettsia* spp. using specific primers and probes [13]. Positive and negative controls were used in each molecular reaction as described previously [13]. Samples with cycle threshold Ct ≤ 35 were considered positive [13,14].

DNA from samples considered positive in qPCR were amplified using PCR's and sequencing to identify the bacterial species [13,15]. Concerning Anaplasmataceae and *Borrelia* spp. detected in rodents and /or ticks were targeted by the following genes: Anaplasmataceae *23S* rRNA [13] and *Borrelia 16S* rRNA [16]. The positive samples with the Anaplasmataceae *23S* rRNA gene system were confirmed with additional PCR specific to the genus *Ehrlichia groEL* [17] and Anaplasmataceae *16S* rRNA gene [18]. Once the PCR products were amplified, an electrophoretic migration was performed for each PCR reaction in a 1.5% agarose gel stained with SYBR SafeTM and visualized using the ultraviolet imager ChemiDocTM MP (Bio-Rad, Marnes-la-Coquette, France). Then the PCR products were purified and sequenced using a Big Dye Terminator and a genetic analyzer. ABI PRISM 3130 (Applied BioSystems, Courtaboeuf, France) [13]. Using the ChromasPro software, version 1.34 (Technelysium Pty, Ltd., Tewantin, Queensland, Australie). The sequences obtained were analyzed and assembled for comparison with the GenBank database using the BLAST (http: //blast.ncbi.nlm. Nih. Gov / Blast.cgi) as previously described [9,13].

The phylogenetic analyses were established using the software MEGA version 7.0.21 with 100 bootstrap replications [19].

3. Results

3.1. Ticks

In total, 59 brains and 59 spleens preserved in 96 ° ethanol from 58 rodents *Mus musculus* and one *Arvicanthis niloticus*, plus 92 soft ticks kept under the same conditions. Morphologically

all ticks belong to the species *O. sonrai*. A random selection of 18 ticks specimens was made for possible molecular confirmation using ticks *16S* gene.

Using PCR's, we were able to amplify the DNA of 6/18 (33.33%) ticks. These 6 specimens were successfully sequenced. The results of the Blast confirmed the identity of our species *O. sonrai* with identification percentages ranging from 98.39% to 99. 75% (Table 1).

3.3. Detection of *Borrelia* spp. and *Anaplasma* spp. by molecular biology in rodents

3.3.1. *Borrelia* spp.

We detected by qPCR 3/59 (5.08%) and 10/59 (16.94%) spleens and brains positive for *Borrelia* spp. respectively with average CT of 28.06. Then by means of PCR's and sequencing we were able to identify 9/13 (69.23%) species of *B. crocidurae.* Details are on the (Table 2).

3.3.2. *Anaplasma* spp.

The qPCR results revealed 6/59 (10.16%) and 4 /59 (6.77%) positive samples for *Anaplasma* spp. collected from spleens and brains respectively. Then a PCR's and sequencing were performed on the positive samples obtained from the qPCR. Blast results showed that 1/10 (10%) was identified as Candidatus *Ehrlichia* sp. with a percent identity of 100% (Table 3).

All rodents tested negative for *Bartonella* spp., *C. burnetii* and *Rickettsia* spp.

3.4. Detection of Anaplasmataceae in *O. sonrai*

The results of the qPCR and the PCR's of *Anaplasma* spp. detected in *O. sonrai* are detailed on the (Table 4).

All ticks were negative for *Borrelia* spp., *Bartonella* spp., *C. burnetii* and *Rickettsia* spp.

3.5. Phylogenetic analysis

Phylogenetic analysis shows that our *Anaplasma* sp. is situated between *Anaplasma platys* (M021425.1) and *Anaplasma ovis* (MT408585.1) on the phylogenetic tree with Anaplasmataceae *23S* rRNA gene (Fig.1). Using the Anaplasmataceae *16S* rRNA gene it forms a separate clade of Uncultured *Anaplasma* sp. (MK041546.1) (Fig.2).

Concerning *Ehrlichia* sp. its position on the tree was identical with Candidatus *Ehrlichia* sp. (MK484067.1) (Fig.1) and Uncultured *Ehrlichia* sp. (MK041545.1) by targeting the Anaplasmataceae *23S* rRNA and Anaplasmataceae *16 S* rRNA genes respectively (Fig. 2).

And finally, with the *Ehrlichia groEL* gene it formed a line distinguished between Uncultured *Ehrlichia* sp. (MG385128.1) and *Ehrlichia ruminantium* (U13638.1) (Fig. 2). The accession numbers assigned by Genbank for all our sequences are listed as follows:
With the Anaplasmataceae *23S* rRNA gene (*Anaplasma* sp. (MW790939)) and (*Ehrlichia* sp. (MW790940)). With the Anaplasmataceae *16S* rRNA gene (*Anaplasma* sp (MW790941) and *Ehrlichia* sp. (MW790942)) and finally the gene *Ehrlichia groEL* (*Ehrlichia* sp.(MW791746)).

4. Discussion

The TBRF as its name indicates it is manifested mainly by periodic fever and other additional symptoms, in severe cases the infection can reach the nervous and cardiac system [6]. In West Africa the incidence of TBRF is important due to the high number of investigations carried out in this region [5]. Humans are infected by bites from infected ticks that lived in rodent burrows [9]. In our study we amplified the DNA of 9/59 (15.25%) cases of *B. crocidurae* and 1/59 (1.69%) potential new species of Candidatus *Ehrlichia* sp in spleens and brains of rodents from northern Senegal (Table 2 and 3). Our results corroborate with a study by Dahmana *et al.*, which detected in the spleens of rodents the presence of several parasites and bacteria among them *B. crocidurae* and a potential new Candidatus "*Ehrlichia senegalensis*"[9]. From these results rodents can be reservoir hosts for several zoonotic diseases which are capable of transmitting them in multiple ways such as arthropods [7,9]. In our study all ticks were negative for *Borrelia* spp. this may be explained by the low number of ticks in the area, so the prevalence was probably not sufficient at the time of collection in this location to be infected. Or due to the presence of PCR inhibitors potentially originating from the blood in the ticks abdomen which prevented the amplification of the DNA [22].

The order *Rickettsiales* currently includes the family of Anaplasmataceae [13]. The latter includes the following genres: *Wolbachia*, *Ehrlichia*, *Anaplasma*, *Neorickettsia* and *Aegyptianella* [9]. The *Anaplasma* genus are intracellular bacteria with negative Gram stain [23]. Causes Anaplasmosis which affects human and animal health often transmissible by ticks [2,13,24]. In our study, for the first time in northern Senegal, the presence of Anaplasmataceae was detected in *O. sonrai*. Our results show that we were able to amplify the presence of two potential new species, namely: *Anaplasma* sp. and *Ehrlichia* sp. in *O. sonrai.* On the phylogenetic trees, our potential new species of *Anaplasma* sp. forms a separate cluster from other *Anaplasma* spp. (Fig.1.2) which means that it does not conform to any previously recognized species this may be due to the fact that it is a potential new species. Concerning our amplicon *Ehrlichia* sp. based on the two genes: Anaplasmataceae l'ARNr *23S* and

Anaplasmataceae l'ARNr *16S* our sequence was 100% identical with Candidatus *Ehrlichia* sp. and Uncultured *Ehrlichia* sp. Respectively. These last are potential new species that have not been described (Fig.1.2) [25]. With the *Ehrlichia groEL* gene our amplicon forms a distinct cluster on the tree which could not be attributed to an already recognized species (Fig.2). The detection of Anaplasmataceae in *O. sonari* could be the consequence of a bacterial engorgement or following a mixed infestation after being engorged with infected invertebrates this hypothesis has been described in other tick species [26,27]. The vector competence of *O. sonrai* to transmit Anaplasmataceae cannot yet be confirmed, but there is a risk that it could be potentially a vector of this bacteria. Further studies and experimentation are needed to better understand the epidemiological relationship between Anaplasmataceae and these soft ticks.

5. Conclusion

This study contributed to expand the repertoire of zoonotic bacteria in rodents and ticks in northern Senegal. *Ornithodoros sonrai* ticks could be a potential vector of Anaplasmataceae. Further investigations and experimentation should be conducted in depth to understand the potential epidemiological role of these ticks and their impact on human and animal health.

Author Contributions

P.P, G.D, L.G and J.F developed the research; G.D, L.G and J.F collected the samples, B.O carried out the scientific experiments; B.O. and E.N, A.Z and J.B analyzed the data; B.O. wrote the manuscript. All authors have 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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294 **Table 1**.

295 Blast analysis results of molecular identification of *Ornithodoros sonrai* ticks

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Soft ticks	PCR Standard ticks *16S*	Cover	Per. ident	Accession Number	Identification
Ornithodoros sonrai	(+)	99%	99.75%	KP644213.1	*Ornithodoros sonrai*
Ornithodoros sonrai	(+)	99%	99.75%	KP644213.1	*Ornithodoros sonrai*
Ornithodoros sonrai	(+)	100%	98.87%	KP644213.1	*Ornithodoros sonrai*
A Ornithodoros sonrai	(+)	95%	99.30%	KP644213.1	*Ornithodoros sonrai*
Ornithodoros sonrai	(+)	95 %	98.39%	KP644213.1	*Ornithodoros sonrai*
Ornithodoros sonrai	(+)	95%	99.53%	KP644213.1	*Ornithodoros sonrai*
Total 6	6/6	/	/	/	*Ornithodoros sonrai*

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303 **Table 2.**

304 The results of the detection of *Borrelia* spp. in spleens and brains rodent using qPCR and

305 PCR's.

Hosts	**CT (qPCR)**	**PCR's**	**Cover**	**Per. ident**	**Accession Number**	**Identification**
Mus musculus[b]	30.29	(+)	99%	97.92%	CP003426.1	*B. crocidurae*
Mus musculus[b]	33.55	/	/	/	/	/
Mus musculus[b]	10.35	/	/	/	/	/
Mus musculus[b]	23.13	(+)	100%	99.93%	CP003426.1	*B. crocidurae*
Mus musculus[b]	28	(+)	100%	95.35%	JX292897.1	*B. crocidurae*
Mus musculus[b]	28.11	(+)	99%	98.95%	CP003426.1	*B. crocidurae*
Mus musculus[b]	27.16	(+)	99%	98.63	CP003426.1	*B. crocidurae*
Mus musculus[b]	27.96	(+)	100%	99.41%	CP003426.1	*B. crocidurae*
Mus musculus[b]	29.28	(+)	99%	98.44%	CP003426.1	*B. crocidurae*
Mus musculus[b]	24.74	(+)	99%	99.40%	CP003426.1	*B. crocidurae*
Mus musculus[s]	35.81	/	/	/	/	/
Mus musculus[s]	36.34	/	/	/	/	/
Mus musculus[s]	30.14	(+)	96%	99,85%	CP003426.1	*B. crocidurae*
Total 13	28.06	9/13 (69.23%)	/	/	/	/

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307 [s]= spleen

308 [b]=brain

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Table 3.

The results of the detection of *Anaplasma* spp. in spleens and brains rodent using qPCR and PCR's.

Hosts	**CT(qPCR)**	**PCR's**	**Cover**	**Per. ident**	**Accession Number**	**Identification**
Mus musculus[s]	25.47	/	/	/	/	/
Mus musculus[s]	26.13	/	/	/	/	/
Mus musculus[b]	29.21	/	/	/	/	/
Mus musculus[s]	17.95	/	/	/	/	/
Mus musculus[b]	20.18	/	/	/	/	/
Mus musculus[s]	24.78	/	/	/	/	/
Mus musculus[s]	22.49	(+)	92%	100	MK484067.1	*Candidatus Ehrlichia* sp.
Mus musculus[s]	27.57	/	/	/	/	/
Mus musculus[b]	18.42	/	/	/	/	/
Mus musculus[b]	20.45	/	/	/	/	/
Total 10	23.26	1/10 (10%)	/	/	/	/

[s]= spleen

[b]=brain

Table 4.

The results of the qPCR and the PCR's with the BLAST results obtained from the *O. sonrai* tested by targeting the following primers: *Anaplasma* spp. *23S* rRNA, Anaplasmataceae *16S* rRNA and *Ehrlichia groEL*.

Soft ticks	**Real-time PCR Primer**	**Real-time PCR (CT)**	**Results of bacteria detected in ticks using Real-time PCR**	**PCR standard Primer**	**Molecular identification by Blast**	**Percent Identity %**	**Query Cover %**	**Accession Number**
O. sonrai	Anaplasmataceae *23S* rRNA gene	7.23	Anaplasmataceae spp.	Anaplasmataceae *23S* rRNA gene	Candidatus E*hrlichia* sp.	100	95	MK484067.1
		/	Anaplasmataceae spp.	Anaplasmataceae *16S* rRNA gene	Uncultured E*hrlichia* sp.	100	100	MK041545.1
		/	Anaplasmataceae spp.	*Ehrlichia groEL*	*Ehrlichia* sp.	90.50	100	HQ697591.1
O. sonrai		20.68	Anaplasmataceae spp.	Anaplasmataceae *23S* rRNA gene	*Anaplasma ovis*	94.97	94	MT408585.1
		/	Anaplasmataceae spp.	Anaplasmataceae *23S* rRNA gene	Uncultured A*naplasma* sp.	98.77	100	MK041546.1
O. sonrai		9.75	Anaplasmataceae spp.	/	/	/	/	/
O. sonrai		11.12	Anaplasmataceae spp.	/	/	/	/	/
O. sonrai		29.93	Anaplasmataceae spp.	/	/	/	/	/
O. sonrai		14.07	Anaplasmataceae spp.	/	/	/	/	/
O. sonrai		7.05	Anaplasmataceae spp.	/	/	/	/	/
O. sonrai		21.78	Anaplasmataceae spp.	/	/	/	/	/
O. sonrai		20.69	Anaplasmataceae spp.	/	/	/	/	/
Total 9 specimens		CT m= 15.81		/	/	/	/	/

CT m = The threshold value of the average cycle.

Fig. 1. Maximum-likelihood phylogenetic tree of Anaplasmataceae, targeting the partial 513-bp *23S* gene.

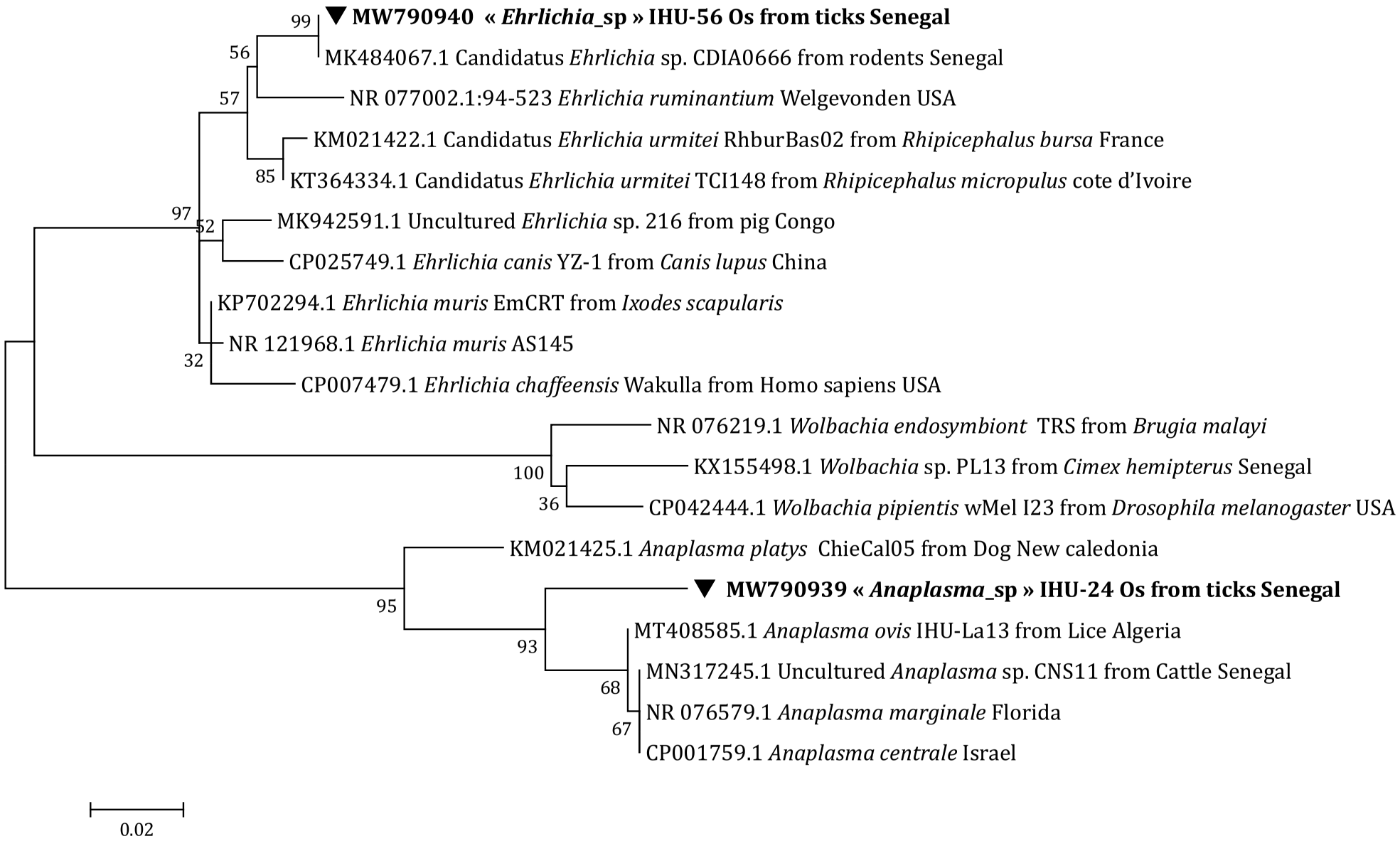


Fig. 2. Maximum-likelihood phylogenetic tree of Anaplasmataceae, targeting the partial 345 bp *16S* gene.

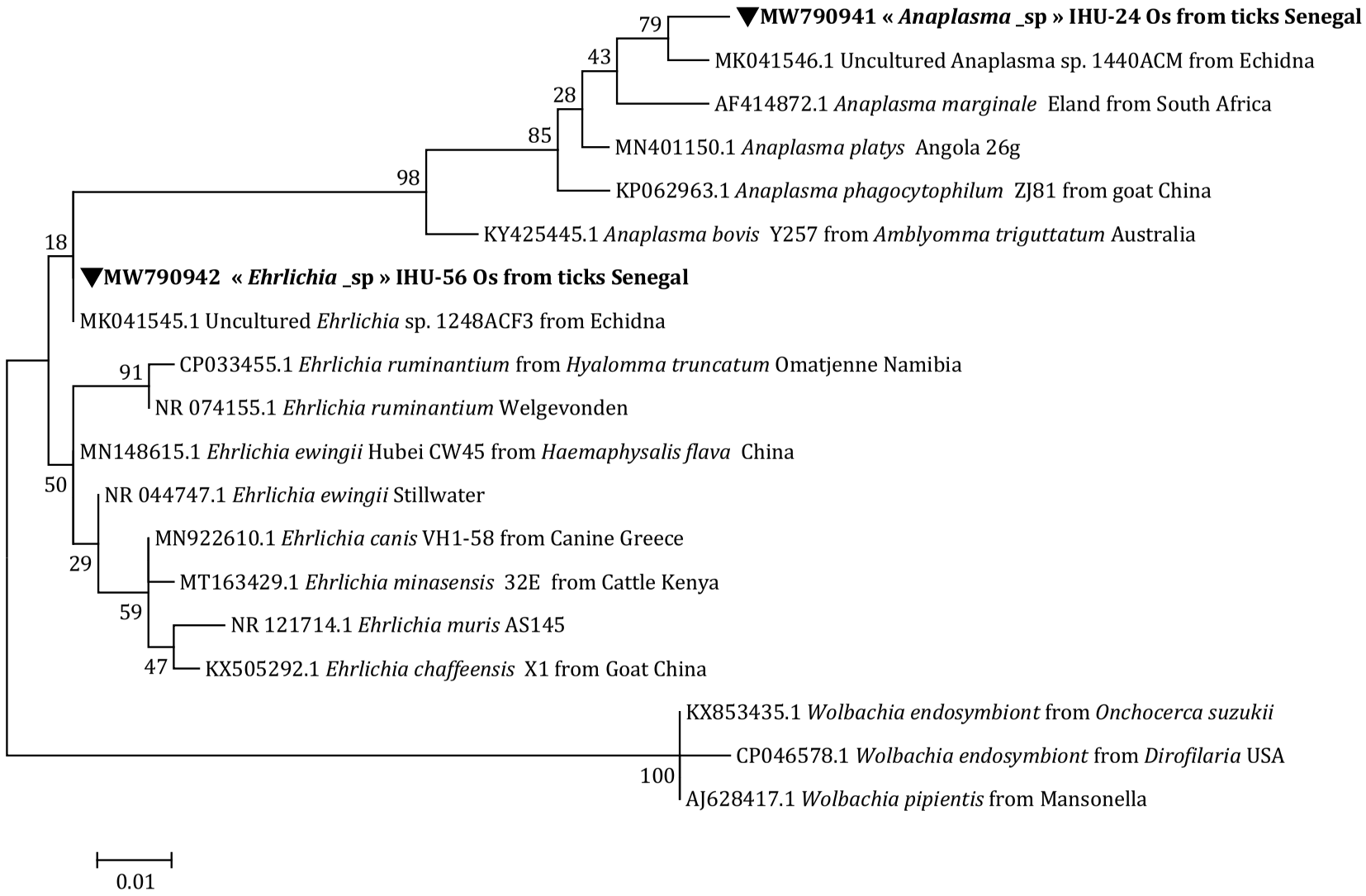
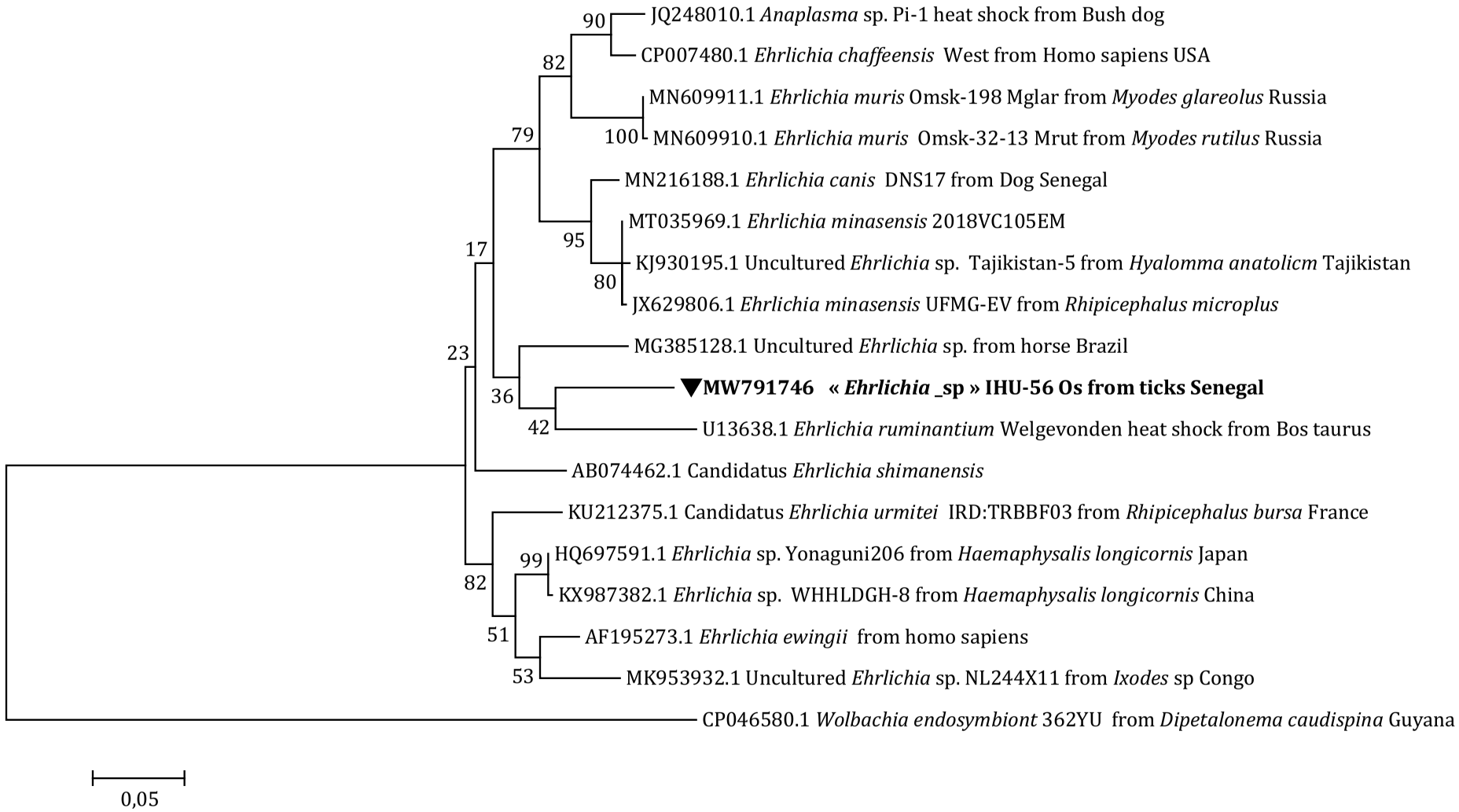


Fig. 3. Maximum-likelihood phylogenetic tree of *Ehrlichia* spp, targeting the partial 633-bp *groEL* gene.



Chapitre IV: Conclusion générale et perspectives

L'ensemble des projets réalisés dans cette thèse ont permis d'optimiser et de développer de nouveaux outils pour assurer et faciliter l'épidémio-surveillance des maladies vectorielles. La première partie de cette thèse présentée sous forme de revue bibliographique a consisté à dresser et à actualiser une liste exhaustive des pathogènes d'importance médicale détectés chez des poux humains en Afrique. D'après l'analyse des données, beaucoup de bactéries ont été détectées chez les poux de corps et ainsi de tête. Nous avons constaté qu'en Afrique il n'y a que peu d'études sur les maladies transmises par les poux humains. Les experts devraient s'intéresser davantage aux poux, car ils peuvent être des vecteurs potentiels de nouveaux types de pathogènes (Article 1).

Chez les animaux, suite à notre enquête sur les ectoparasites des mammifères effectués au nord-est de l'Algérie, nous avons noté que peu de travaux ont été réalisés en Algérie sur les poux qui continuent d'affecter les troupeaux du pays. Ce travail est donc une étape très importante vers la compréhension de la distribution des poux dans la région nord-est de l'Algérie (Article 2).

La deuxième partie de cette thèse qui nous a permis d'améliorer nos connaissances sur les poux d'animaux en Algérie, en utilisant comme outil de diagnose la spectrométrie de masse MALDI-TOF MS. Dans un premier temps nous avons démontré que la partie céphalothorax des poux est la partie idéale à utiliser pour générer des spectres de bonne qualité reproductible et spécifique pour chaque espèce de poux et par conséquent établir une bonne identification des poux, en utilisant cet outil protéomique, nous avons pu identifier au total 14 espèces de poux.

Nous avons aussi contribué à alimenter et à diversifier la base de données de référence du laboratoire par l'introduction de 57 spectres de référence appartenant aux 14 espèces de poux. Dans un deuxième temps et étant donné que l'alcool est une méthode de conservation peu coûteuse, et fréquemment utilisée et à usage facile pour le transfert des échantillons du terrain

vers les laboratoires de recherche nous avons optimisé un protocole de préparation efficace et simple pour le MALDI-TOF MS afin d'établir une identification correcte chez les poux du terrain ou d'élevage en laboratoire conservés dans de l'alcool 70% pendant des périodes variables. Ce nouveau protocole est actuellement utilisé pour l'analyse des poux conservés en alcool dans notre laboratoire. En outre une base de données de spectres de référence de sept espèces de poux conservées dans l'alcool a été créée dans notre base de données.

Le troisième volet de cette thèse a porté sur l'épidémiologie moléculaire des microorganismes associés aux animaux et aux ectoparasites. Ces travaux nous ont permis d'identifier chez les poux pour la première fois en Algérie la présence de *Coxiella burnetii, Anaplasma ovis* et une potentielle nouvelle espèce *Anaplasma* sp.

Ensuite, les investigations sur des tiques dure de la Slovaquie ont permis de détecter la présence de *Rickettsia raoultii* chez deux espèces de tiques *Dermacentor reticulatus* et *Haemaphysalis inermis*, et sur des tiques molles du Sénégal on a signalé la présence d'une potentiel nouvelle espèce chez la famille des Anaplasmataceae.

Aussi, Chez les petits mammifères du Sénégal nous avons identifié *Borrelia crocidurae* l'agent causale de la maladie de la fièvre récurrente et une *Candidatus Ehrlichia* sp.

Ainsi, les séquences de bactérie obtenues à travers ces travaux ont été introduites à la base de données NCBI sous ces numéros d'accession, chez les poux (MT48585, MT408586 et MT410711) et chez les tiques MW790940, MW79039, MW790941, MW790942 et MW791746).

Ainsi, l'ensemble des travaux réalisés dans le cadre de cette partie a permis d'enrichir et d'améliorer le répertoire des microorganismes associés aux arthropodes (poux et tique et petits mammifères) originaire de plusieurs régions telles que l'Algérie, la Slovaquie et le Sénégal.

Enfin, sur la base des résultats obtenus dans cette thèse, et en vue d'une surveillance vectorielle il serait très intéressant d'envisager de tester la capacité du MALDI-TOF MS à différencier entre des poux infectés ou non par des microorganismes.

Cet outil protéomique très avantageux pourrait devenir un nouvel outil pour surveiller en temps réel les vecteurs arthropodes collectés sur le terrain ou sur des hôtes mammifères et par conséquent prévenir le danger et le risque d'épidémie.

Durant cette thèse, et à partir des travaux effectués, j'ai eu l'opportunité d'acquérir des compétences variées. Ainsi, j'ai eu l'occasion d'approfondir mes connaissances et de développer mes compétences en matière d'identification morphologique et de la taxonomie de plusieurs arthropodes (poux, tiques, punaises de lit, moustiques), monter leurs élevages dans le laboratoire et aussi faire leurs collectes sur le terrain.

J'ai eu aussi le grand privilège d'apprendre à maitriser beaucoup d'outils comme le MALDI-TOF MS, la culture cellulaire, la microscopie, la biologie moléculaire (conception d'amorces et de sondes, des PCR standard, PCR en temps réel, séquençage, la phylogénie). Par ailleurs, j'ai eu également l'occasion de faire une formation en expérimentation animale, en entomologie médicale et en biostatistique et méthodologies de base pour la recherche en biologie et en médecine.

Au terme de cette thèse et des différents travaux accomplis, je souhaiterai vivement donc appliquer ces connaissances dans le cadre des projets en perspective.

Chapitre V: Références bibliographiques

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Chapitre VI: Annexes

Article N°8:

The Trick of the Hedgehog: Case Report and Short Review About Archaeopsylla erinacei (Siphonaptera: Pulicidae) in Human Health.

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Résumé

Les puces sont des ectoparasites de divers animaux, dont Homo sapiens Linnaeus, 1758 (Primates : Hominidae). Parmi les espèces pertinentes pour le domaine de la santé humaine, soit en raison de leur potentiel dermatopathologique, soit en raison de leur rôle de vecteur de micro-organismes responsables de maladies infectieuses, telles que la peste ou le typhus murin, figurent la puce humaine, la puce du rat oriental, les puces étroitement apparentées du chat et du chien, et la puce chique. Cependant, d'autres espèces peuvent infester accidentellement les humains. Nous avons rapporté ici deux cas inhabituels d'humains infestés et mordus par *Archaeopsylla erinacei*, la puce du hérisson. Cette espèce a été identifiée par stéréomicroscopie, sur la base de ses principales caractéristiques. En outre, une brève revue de la littérature a révélé que les puces du hérisson pouvaient être porteuses d'agents infectieux pour l'homme, tels que *Rickettsia felis* Bouyer et al. 2001 (Rickettsiales : Rickettsiaceae) ou *Bartonella henselae* Regnery et al.1992 (Rhizobia les : Bartonellaceae).

En utilisant la biologie moléculaire, nous avons donc testé neuf spécimens d'*A. erinacei* prélevés chez ces patients, pour plusieurs espèces de bactéries communément associées aux arthropodes hématophages, impliquées dans la pathologie humaine. Le rôle d'*A. erinacei* dans l'épidémiologie humaine n'a jamais été évalué à ce jour. Ce rapport visait à rappeler que ces puces peuvent être des parasites accidentels chez l'homme. En outre, les découvertes récentes concernant les bactéries d'intérêt médical présentes dans ces insectes devraient être mises en avant, étant donné que la question de leur rôle de vecteur dans les infections humaines reste sans réponse et mérite d'être approfondie.

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Short Communication

Short Communication

The Trick of the Hedgehog: Case Report and Short Review About *Archaeopsylla erinacei* (Siphonaptera: Pulicidae) in Human Health

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Abstract

Fleas are ectoparasites of various animals, including *Homo sapiens* Linnaeus, 1758 (Primates: Hominidae). Among the species relevant to the human health field, either due to their dermatopathological potential or because of their role as vectors of microorganisms responsible for infectious diseases, such as plague or murine typhus, are the human flea, oriental rat flea, closely related cat and dog fleas, and chigoe flea. However, other species can accidentally infest humans. We have herein reported two unusual cases of humans infested and bitten by *Archaeopsylla erinacei*, the hedgehog flea. This species has been identified using stereomicroscopy, on the base of key characteristics. Furthermore, a brief literature review has revealed that hedgehog fleas could carry human-infectious agents, such as *Rickettsia felis* Bouyer et al. 2001 (Rickettsiales: Rickettsiaceae) or *Bartonella henselae* Regnery et al.1992 (Rhizobiales: Bartonellaceae). Using molecular biology, we thus tested nine *A. erinacei* specimens taken from these patients, for several bacteria species commonly associated with hematophagous arthropods, implicated in human pathology. However, all our samples were proven negative. The role of *A. erinacei* in human epidemiology has never been evaluated to date. This report sought to remind us that these fleas can be accidental parasites in humans. In addition, recent findings pertaining to bacteria of medical interest that are present in these insects should be brought to the fore, given that the question of their role as vectors in human infections remains unanswered and deserves further investigation.

Key words: flea, ectoparasite, hedgehog, *Rickettsia*, *Archaeopsylla erinacei*

Fleas are flightless insects belonging to the Siphonaptera order. They are ectoparasites of numerous bird and mammal species, including humans. Several species are involved in human health, among which *Pulex irritans* (human flea) Linnaeus, 1758 (Siphonaptera: Pulicidae), *Xenopsylla cheopis* (oriental rat flea) Rothschild, 1903 (Siphonaptera: Pulicidae), *Ctenocephalides felis* (cat flea) Bouché, 1835 (Siphonaptera: Pulicidae), *Ctenocephalides canis* (dog flea) Curtis, 1826 (Siphonaptera: Pulicidae), and *Tunga penetrans* (chigoe flea) Linnaeus, 1758 (Siphonaptera: Hectopsyllidae). These species are responsible for dermatological diseases such as pruritus or tungiasis, but are also vectors of infectious agents, such as *Yersinia pestis* Lehmann & Neumann, 1896 (Enterobacteriales: Yersiniaceae) or *Rickettsia typhi* Wolbach & Todd, 1920 (Rickettsiales: Rickettsiaceae), responsible for plague and murine typhus, respectively (Steen et al. 2004, Laroche et al. 2018).

We have reported herein a case of two humans infested and bitten by *Archaeopsylla erinacei*, the hedgehog flea. In 2017, a 57-yr-old woman was referred to the Parasitology Laboratory of Strasbourg University Hospital owing to fleas found on herself and her 1-yr-old granddaughter, as well as on her home carpets. Both presented bites without associated inflammatory lesions, resulting in several pruriginous and erythematous spots with a diameter of about 1 mm. Stereomicroscope examination identified adult *A. erinacei* specimens (Fig. 1). Management consisted of extensive washing and treatment of infested carpets, clothing, and household linen with acaricides sprayed in a sealed bag, along with avoiding garden areas frequented by hedgehogs. Three months later, no more hedgehog fleas were detected in the house.

Both patients lived in a small rural village of about 300 inhabitants, located about 30 km from Strasbourg, Northeastern France,

and surrounded by crop fields. To her grandmother's knowledge, neither she herself nor her granddaughter had recently had any direct contact with hedgehogs (*Erinaceus europaeus* Linnaeus, 1758 [Eulipotyphla: Erinaceidae]). However, on an evening, the grandmother had stood in the garden of her house about 30 cm away from one adult and two cubs, one of whom the patient described as 'scratching intensely'. Moreover, while hearing noises at night, the grandmother had put her hand in what turned out to be the hedgehogs' den. Concerning her granddaughter, as she often went exploring the garden, she might have been in contact with the hedgehogs or their habitat. Of note, the first fleas the grandmother found were in the child's hair.

Over the past few years, several reports have been published about bacteria of human medical interest in *A. erinacei* sampled from various animals across Europe or North Africa (Bitam et al. 2006, De Sousa et al. 2006, Gilles et al. 2008a, b, Khaldi et al. 2012, Marié et al. 2012 a, b, Hornok et al. 2014, Leulmi et al. 2016, Marciano et al. 2016). We have thus reviewed these reports and screened these fleas for bacteria associated with these arthropods, using molecular biology. Moreover, this case report has provided an opportunity for us to recall the key characteristics that enable *A. erinacei* to be differentiated from other flea species by medical biologists.

Fig. 1. Overall view of a flea found on one of the patients.

Materials and Methods

The 57-yr-old patient carried to our laboratory 12 fleas, sampled on herself, her granddaughter's head, and several carpets. As these fleas had all been collected in one place, it was impossible to say which one had been sampled at which location. First, all the fleas were mounted on temporary slides in view of stereoscopic examination for diagnosis. They were then all demounted with three chosen to be preserved on long-lasting microscopic slides, whereas nine were employed for DNA extraction.

Preparation of long-lasting microscope slides was performed as follows: the fleas were immersed in a water bath followed by a 10% KOH solution bath overnight to allow for the tissues to be enlightened. The next morning, the chemical action of KOH was neutralized by transferring the fleas into a 10% acetic acid bath followed by a water bath. Next, fleas were progressively dehydrated using ethanol solutions concentrated at 70, 90, and 100% for 1 h each, then transferred into a Euparal essence bath for several minutes prior to slide mounting using the Euparal medium.

DNA extraction was performed using the DNEasy Blood & Tissue Kit (Qiagen, Courtaboeuf, France), while following the manufacturer's instructions. Quantitative polymerase chain reactions (PCRs) were conducted to detect the presence of bacteria (*Rickettsia* spp., *Bartonella* spp., Anaplasmataceae, *Borrelia* spp., and *Coxiella burnetii* Derrick, 1939 [Legionellales: Coxiellaceae]) in the fleas. All nine samples were screened for specific bacterial microorganism sequences, as listed in Table 1. PCRs were conducted in a PCR Real-Time CFX96 thermocycler (Bio-Rad, Marnes-la-Coquette, France) using the qPCR LightCycler 480 Probes Master kit (Roche, Meylan, France), as previously described for each PCR (Table 1). Positive and negative controls were employed for each experiment. For *C. burnetii*, two PCRs were applied: first, the IS30A

Table 1. Primers and probes used for the detection of microorganisms in *Archaeopsylla erinacei*

Microorganism	Targeted sequences	Primers (5′–3′) and probes (FAM-TAMRA)	Reference
Rickettsia spp.	*glta(RKND03)*	F: GTGAATGAAAGATTACACTATTTAT R: GTATCTTAGCAATCATTCTAATAGC P: CTATTATGCTTGCGGCTGTCGGTTC	Rolain et al. (2002)
Anaplasma spp.	*23SrRNA (TtAna)*	F: TGACAGCGTACCTTTTGCAT R: TGGAGGACCGAACCTGTTAC P: GGATTAGACCCGAAACCAAG	Djiba et al. (2013)
Borrelia spp.	*(Bor ITS4)*	F: GGCTTCGGGTCTACCACATCTA R: CCGGGAGGGGAGTGAAATAG P: TGCAAAAGGCACGCCATCACC	Mediannikov et al. (2010)
Coxiella burnetii	*(IS30A)*	F: CGCTGACCTACAGAAATATGTCC R: GGGGTAAGTAAATAATACCTTCTGG P: CATGAAGCGATTTATCAATACGTGTATG	Rolain and Raoult (2005)
	(IS1111)	F: CAAGAAACGTAACGCTGTGGC R: CACAGAGCCACCGTATGAATC P: CCGAGTTCGAAACAATGAGGGCTG	Aouadi et al. (2017)
Bartonella spp.	*(Barto ITS2)*	F: GATGCCGGGGAAGGTTTTC R: GCCTGGGAGGACTTGAACCT P: GCGCGCGCTTGATAAGCGTG	Rolain et al. (2003)

C. burnetii–specific sequence was targeted after which all positive samples were tested for the IS1111 sequence for confirmation purposes (Rolain and Raoult 2005; Aouadi et al. 2017). Only samples positive for both targets were considered positive.

The photographs were created using the focus-stacking technique, which combines multiple images taken at different focus distances, resulting in a greater depth of field for the final images. A ×10 microscope objective (Mitutoyo, Roissy-en-France, France), adapted to an EOS 80D camera (Canon, Paris, France) with a BALPRO-1 bellow (Novoflex, Montigny Le Bretonneux, France), was used. This material was fixed on an automated focus-stacking macro rail (StackShot, Cognysis, Traverse City, MI) for acquiring images at different focus distances. Two LED spots diffused with paper were used for illuminating the slides. Between 10 and 19 images were merged for each final image with Zerene Stacker software (P_{max} mode). Some retouching (such as exposure and contrast) was performed using Photoshop CC 2018 and Lightroom Classic CC 2018 (Adobe, San Jose, CA).

Results

Stereoscopic examination on temporary slides allowed us to confidently identify *A. erinacei* for the 12 samples brought up by the patient. The identification was supported by the combination of the following characteristics: the presence of a clearly visible sclerotized falx of head, an asymmetrical antenna with partially welded basal segments, the presence of a pleural rod of mesothorax, hind tibia with six seta-bearing notches along the dorsal margin with a row of 6–11 little setae near to the dorsal margin, a vestigial genal comb composed of one to three spines, and a vestigial pronotal comb composed of a maximum of six spines (Fig. 2; Jordan and Rothschild 1912, Hopkins and Rothschild 1953, Beaucournu and Launay 1990, Zurita et al. 2017).

Nine *A. erinacei* samples were screened for bacteria such as *Rickettsia* spp., *Bartonella* spp., *Borrelia* spp., Anaplasmataceae, and *Coxiella burnetii*, though we did not detect bacteria in any of these fleas.

Discussion

Hedgehogs are spiny mammals of the Erinaceinae subfamily. There are 17 species of hedgehogs in five genera found in Europe, Asia, and Africa (Hutterer 2005). Hedgehogs in Western Europe are mainly represented by European hedgehogs (*E. europaeus*), also known as common hedgehogs. Described for the first time by Peter Friederich Bouché in 1835, *A. erinacei* is a flea, commonly found on European hedgehogs, thus known as the hedgehog flea (Thompson 1939, Visser et al. 2001, Gorgani-Firouzjaee et al. 2013, Hajipour et al. 2015). Two subspecies have been described: *Archaeopsylla erinacei erinacei* and *Archaeopsylla erinacei maura*. *Archaeopsylla erinacei* is able to infest other hedgehog species, such as northern

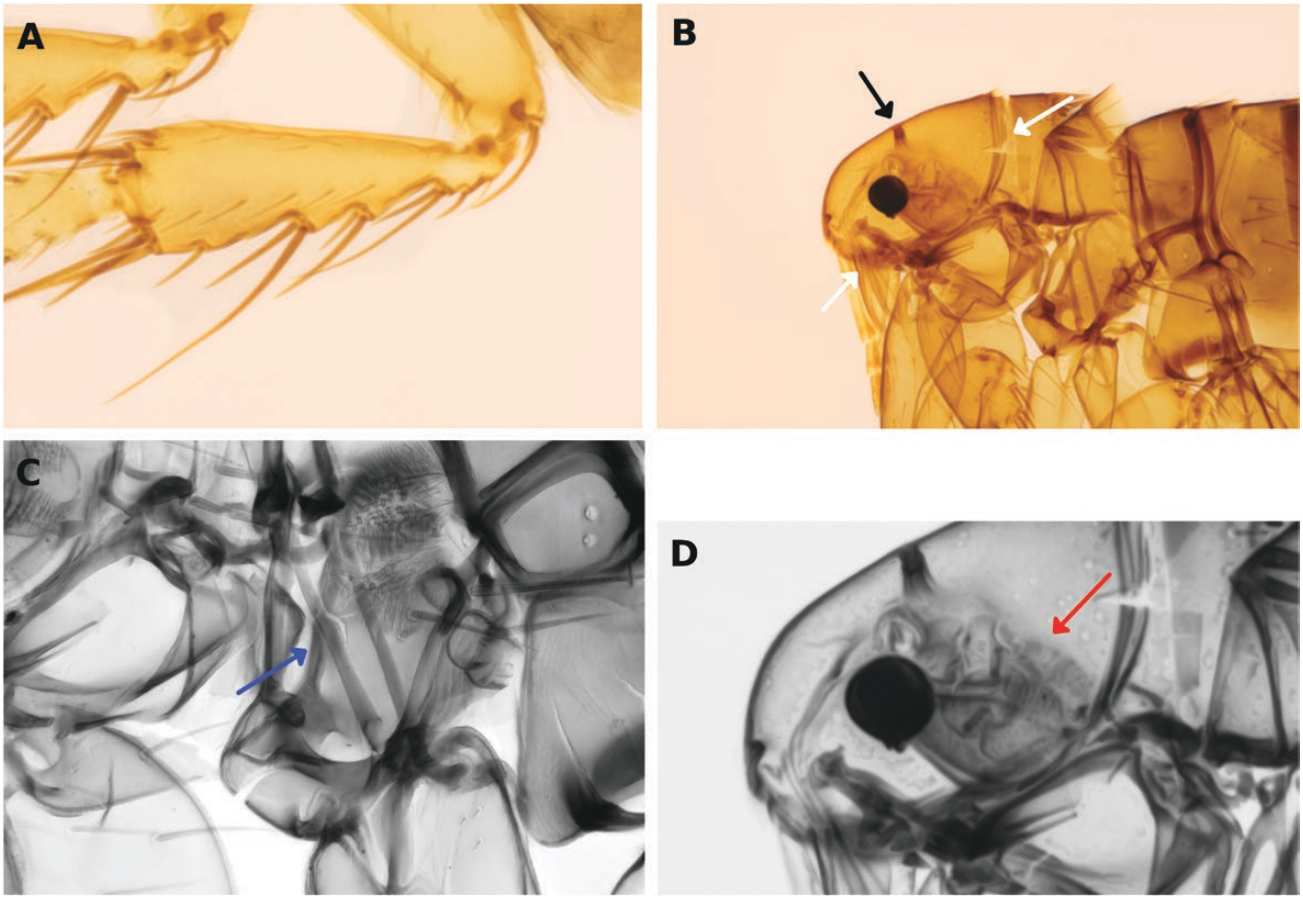

Fig. 2. Identification features of *Archaeopsylla erinacei*. (A) Hind tibia with six seta-bearing notches along dorsal margin with a row of 6–11 little setae near to dorsal margin. (B) Sclerotized falx of head (black arrow), vestigial genal comb composed of one to three spines, and vestigial pronotal comb composed of maximally 6 spines (white arrows). (C) Pleural rod (arrow). (D) Asymmetrical antenna with partially welded basal segments (arrow).

white-breasted hedgehogs (*Erinaceus roumanicus* Barrett-Hamilton, 1900 [Eulipotyphla: Erinaceidae]) found in Central and Eastern Europe, southern white-breasted hedgehogs (*Erinaceus concolor* Martin, 1838 [Eulipotyphla: Erinaceidae]) found in Anatolia and the Middle East, and North African hedgehogs (*Atelerix algirus* Lereboullet, 1842 [Eulipotyphla: Erinaceidae]), and desert hedgehogs (*Paraechinus aethiopicus* Ehrenberg, 1832 [Eulipotyphla: Erinaceidae]), both found in North Africa (Bitam et al. 2006, Földvári et al. 2011, Khaldi et al. 2012, Goz et al. 2016, Dudek et al. 2017). Furthermore, hedgehog fleas are known to infest felids (*Felis sylvestris* Schreber, 1777 [Carnivora: Felidae]), canids, such as dogs (*Canis lupus* Linnaeus, 1758 [Carnivora: Canidae]) and foxes (*Vulpes vulpes* Linnaeus, 1758 [Carnivora: Canidae]), hares (*Lepus europaeus* Pallas, 1778 [Lagomorpha: Leporidae]), and the nests of kestrels (*Falco tinnunculus* Linnaeus, 1758 [Falconiformes: Falconidae]); Aubert and Beaucournu 1976, Pomykal 1985, Beck et al. 2006, Bond et al. 2007). A prior study showed that *A. erinacei* was the most common and abundant flea found on foxes (*V. vulpes*) in Northeastern France, even more so than dog fleas (*C. canis*), despite their specific association with canids. These results have been confirmed in a more recent study (Aubert and Beaucournu 1976, Beaucournu and Launay 1990). This highly prevalent nature, compared with Western and Southern France, suggests favorable conditions for the proliferation of the hedgehog flea, and for common colocalization of foxes and hedgehogs in this area (Aubert and Beaucournu 1976, Beaucournu and Launay 1990).

Identification of *A. erinacei* relies on stereoscopic examination based on the key characteristics as described earlier (Figs. 1 and 2). We hereby do not present the key characteristics allowing for males and females to be differentiated (as well as subspecies *A. e. erinacei* and *A. e. maura*) in this study. These features have already been extensively described in previous papers and are less relevant from a human health perspective (Jordan and Rothschild 1912, Hopkins and Rothschild 1953, Beaucournu and Launay 1990, Zurita et al. 2017). The relevant combination of morphological characteristics, enabling medical parasitologists to differentiate *A. erinacei* from other fleas of common medical relevance, namely *P. irritans*, *X. cheopis*, or *Ctenocephalides* spp., has been summarized in Table 2. The molecular characterization of *A. erinacei* has recently been conducted using PCR, targeting nuclear and mitochondrial markers, such as Internal Transcribed Spacers (ITS) 1 and 2, partial cytochrome c-oxidase 1 (*cox1*), and cytochrome b (*cytb*), to permit the genetic discrimination of two genetic lineages corresponding to the two subspecies described, *A. e. erinacei* and *A. e. maura* (Zurita et al. 2017).

In 2014, a team developed a method using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry designed to identify flea species; the authors created a preliminary database including the spectra of several species, including *A. erinacei* (Yssouf et al. 2014). As these new techniques do not require expertise in flea morphology to identify key characteristics, they could be suitable alternatives to time-consuming stereoscopic examination for large-scale epidemiological studies.

In the literature, there have been only little data about *A. erinacei* biting humans (Pomykal 1985, Bork et al. 1987). In these published reports, bites were associated with limited local reactions, without any further complications. We observed the same lesions on our two patients, consisting of limited *purpura pulicosa*, located on the child's scalp and at the grandmother's waistline, all of which spontaneously resolved within 2–3 d. There were no signs of complications associated with these flea bites.

The first reports of the presence of *Rickettsia felis* Bouyer et al. 2001 (Rickettsiales: Rickettsiaceae), the agent of the flea-borne spotted fever, in *A. erinacei* sampled from European hedgehogs and North African hedgehogs in Portugal and Algeria, were published in 2006 (Bitam et al. 2006, De Sousa et al. 2006). Since then, numerous studies have identified the presence of *R. felis* in *A. erinacei* sampled from a range of mammals, including hedgehogs (Gilles et al. 2009, Khaldi et al. 2012, Marié et al. 2012b, Leulmi et al. 2016), cats (Gilles et al. 2008a,b), dogs (Gilles et al. 2008b), and foxes (Marié et al. 2012a), as shown in Table 3. In these studies, the proportion of infected fleas proved to be very high in all cases, ranging from 72.7 to 100%, with the exception of fleas sampled from foxes, which displayed 10.5% prevalence. A few other bacteria of medical interest have been identified in *A. erinacei* sampled from hedgehogs (*E. roumanicus* and other species not detailed), belonging to the *Rickettsia* and *Bartonella* genera: *R. helvetica* Beati, Peter, Burgdorfer, Aeschlirnami & Raoult, 1993 (Rickettsiales: Ric kettsiaceae), *B. henselae*, *B. clarridgeiae* Lawson & Collins, 1996 (Rhizobiales: Bartonellaceae), and *B. elizabethae* Daly et al. 1993 (R hizobiales: Bartonellaceae) (Bitam et al. 2012, Hornok et al. 2014, Marciano et al. 2016). These bacteria were found at much lower prevalence rates than *R. felis*, estimated at 1.5, 0.7, 9.1, and 25%, respectively. One article reported uncultured *Bartonella* species to be present in fleas sampled from southern white-breasted hedgehogs (*E. concolor*) in Israel (Marciano et al. 2016). Finally, one article reported hemotropic *Mycoplasma* spp. (hemoplasma) from the hemofelis group, the causative agent of feline infectious anemia, to be present in hedgehog fleas sampled in Algeria (Hornok et al. 2014).

Table 2. Comparison among flea species of human health interest (Hopkins and Rothschild 1953, Holland 1985, Beaucournu and Launay 1990)

Identification key	*Archaeopsylla erinacei*	*Ctenocephalides* spp.	*Pulex irritans*	*Xenopsylla cheopis*
Family	Pulicidae	Pulicidae	Pulicidae	Pulicidae
Subfamily	Archaeopsyllinae	Archaeopsyllinae	Pulicinae	Xenopsyllinae
Falx of head	Sclerotized	Sclerotized	Absent	Absent
Antenna	Asymmetrical	Asymmetrical	Asymmetrical	Asymmetrical
Pleural rod	Present	Present	Absent	Present
Pronotal ctenidium	Vestigial	Present	Absent	Absent
Genal ctenidium	Vestigial	Present	Absent or a single tooth	Absent
Vector for microorganisms of human medical interest	No record	Yes: *Rickettsia felis* *Dipylidum caninum* *Bartonella henselae*	Yes: *Yersinia pestis* *Rickettsia typhi* *Dipylidum caninum* *Bartonella* sp.	Yes: *Yersinia pestis* *Rickettsia typhi*

Table 3. Bacteria isolated from *Archaeopsylla erinacei* samples in previously published studies

Bacteria	Host	Prevalence of infected *A. erinacei*	Location	References
Rickettsia felis	European hedgehog (*Erinaceus europaeus*)	100% (1/1)	Mértola, Portugal	De Sousa et al. (2006)
		96% (144/150)	Bavaria, Germany	Gilles et al. (2008b)
		99.2% (128/129)	Marseille, France	Marié et al. (2012b)
	North African hedgehog (*Atelerix algirus*)	100% (4/4)	Oran, Algeria	Bitam et al. (2012)
	Dog (*Canis lupus*) Cat (*Felis sylvestris*)	100% (34/34)	Germany	Gilles et al. (2008b)
	Cat (*Felis sylvestris*)	100% (1/1)	Limoges, France	Gilles et al. (2008a)
	Fox (*Vulpes vulpes*)	10.5% (2/19)	Southeastern France	Marié et al. (2012a)
	North African hedgehog (*Atelerix algirus*) Desert hedgehogs (*Paraechinus aethiopicus*)	100% (316/316)	Algeria	Khaldi et al. (2012)
	Domestic and wild mammals (including *Atelerix algirus*)	72.7% (80/110)	Algeria	Leulmi et al. (2016)
Rickettsia felis-like	European hedgehog (*Erinaceus europaeus*)	96% (144/150)	Southern Germany	Gilles et al. (2009)
	Northern white-breasted hedgehog (*Erinaceus roumanicus*)	98.5% (132/134)	Budapest, Hungary	Hornok et al. (2014)
Rickettsia helvetica	sNorthern white-breasted hedgehog (*Erinaceus roumanicus*)	1.5% (2/134)	Budapest, Hungary	Hornok et al. (2014)
Bartonella henselae	Northern white-breasted hedgehog (*Erinaceus roumanicus*)	0.7% (1/134)	Budapest, Hungary	Hornok et al. (2014)
Bartonella clarridgeiae	Hedgehogs (no detail)	9.1% (4/44)	Algeria	Bitam et al. (2012)
Bartonella elizabethae	Hedgehogs (no detail)	25% (11/44)	Algeria	Bitam et al. (2012)
Uncultured *Bartonella* sp.	Southern white-breasted hedgehogs (*Erinaceus concolor*)	33.3% (1/3)	Israel	Marciano et al. (2016)
Hemoplasma (haemofelis group)	Northern white-breasted hedgehog (*Erinaceus roumanicus*)	5.2% (7/134)	Budapest, Hungary	Hornok et al. (2014)

In addition to the obvious veterinary interest, this could similarly be interesting from a human medical point of view because human infections with hemoplasma belonging to the hemofelis group have already been described in HIV-infected patients (Dos Santos et al. 2008, Steer et al. 2011).

Despite these numerous reports, we did not find any bacteria belonging to the *Rickettsia* or *Bartonella* genera. Without knowing the reservoir of most of these bacteria, these negative results are difficult to explain. One hypothesis is that these fleas had not previously been in contact with infected hosts. This might reflect a form of geographical insulation of the hedgehogs from which these fleas were issued. In any case, there has been no report, so far, dealing with human infections subsequent to *A. erinacei* bites.

Conclusions

These two unusual cases of hedgehog flea bites in humans should remind us that these insects can inadvertently feed on humans. In these cases, the determination of key characteristics allows for identifying the respective species. In spite of numerous bacteria of human medical interest described in *A. erinacei*, human infection has never been reported so far as resulting from a bite by such an insect. Given the wide distribution of this flea species across potential hosts in Europe and North Africa, along with the prevalence of zoonotic organisms, the potential for these arthropods to act as vectors should not be underestimated. Further research is needed to better define the role *A. erinacei* plays to apprehend its contribution to arthropod-borne conditions in humans.

Acknowledgments

The authors declare that they have no competing interests.

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Communications et Mérite

MÉDITERRANÉE INFECTION

Marseille, le 26 juillet 2018

ATTESTATION DE PRESENCE

A qui de droit,

Je soussignée, Melle Micheline Pitaccolo, Gestionnaire Administrative de la Fondation Méditerranée Infection atteste que Melle Basma OUARTI a participé à la journée d'information de l'Infectiopôle Sud – Méditerranée Infection le 6 juillet 2018.

Melle Basma OUARTI a présenté un poster.

Cordialement,

Fondation de Coopération Scientifique
IHU
MÉDITERRANÉE INFECTION
19-21 bd Jean Moulin
13005 Marseille - France
N° Siret : 501 9[illegible]
Tél. : 04 13 73 22 11

Micheline Pitaccolo
Gestionnaire Administrative
Méditerranée Infection

MÉDITERRANÉE INFECTION

Marseille, le 12 juillet 2019

ATTESTATION DE PRESENCE

A qui de droit,

Je soussignée, Melle Micheline Pitaccolo, Gestionnaire Administrative de la Fondation Méditerranée Infection atteste que Melle Basma OUARTI a participé à la journée d'information de l'Infectiopôle Sud – Méditerranée Infection le 5 juillet 2019.

Melle Basma OUARTI a présenté un poster.

Cordialement,

Fondation de Coopération Scientifique
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MÉDITERRANÉE INFECTION
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Tél. : 04 13 73 22 11

Micheline Pitaccolo
Gestionnaire Administrative
Méditerranée Infection

MÉDITERRANÉE INFECTION

Marseille, le 10 Août 2020

ATTESTATION DE PRESENCE

A qui de droit,

Je soussignée, Melle Micheline Pitaccolo, Gestionnaire Administrative & financière de la Fondation Méditerranée Infection atteste que Mlle OUARTI Basma a participé à la journée d'information de l'Infectiopôle Sud – Méditerranée Infection le 03 juillet 2020 de 8h00 à 16h00 et a présenté un poster.

Cordialement,

Fondation de Coopération Scientifique
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Micheline Pitaccolo
Gestionnaire Administrative & financière
Méditerranée Infection

RÉPUBLIQUE ALGÉRIENNE DÉMOCRATIQUE ET POPULAIRE

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et de la recherche scientifique
Université Chadli Bendjedid
El Tarf
Faculté des Sciences de la Nature et de la Vie
Département des sciences Vétérinaires

جامعة الشاذلي بن جديد
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وزارة التعليم العالي والبحث العلمي
جامعة الشاذلي بن جديد
الطارف
كلية علوم الطبيعة والحياة
قسم العلوم البيطرية

ATTESTATION DE COMMUNICATION

2ème Journées Internationales Vétérinaires
Arthropodes hématophages Et Maladies Vectorielles
2ème Edition – 16-17 Octobre 2018

Le comité d'organisation de la 2ème Edition des Journées Internationales Vétérinaires organisée à l'Université Chadli Bendjedid – El Tarf le 16 et 17 Octobre 2018 certifie que :

Mme. OUARTI Basma

A participé activement à la journée et a présenté une communication **Orale** intitulée :
Development of MALDI-TOF MS for the identification of animal lice

Le Doyen de la FSNV

عميد الكلية علوم الطبيعة والحياة
الأستاذ الدكتور: هشام نصري

Le Président
رئيس الملتقى الدكتور:
فيصل زروال

4798-B

La Fondation Méditerranée Infection

19-21 Boulevard Jean Moulin, 13 005 Marseille

Décerne à :

Basma OUARTI

La Médaille COVID-19

Avec les remerciements de la Présidente et du Directeur de la Fondation,

Yolande Obadia **Didier Raoult**

MÉDITERRANÉE INFECTION