veterinary sciences

MDPI

Article

Molecular Evidence of Bacterial Pathogens in *Stomoxys calcitrans* and *Haematobia irritans* Flies from Northeastern Algeria

Chaimaa Azzouzi [1], Mehdi Boucheikhchoukh [2], Scherazed Sedraoui [2], Fabrizio Bertelloni [3] and Valentina Virginia Ebani [3,4,*]

[1] Department of Veterinary Sciences, Faculty of science, Mohammed Boudiaf University of M'sila, M'sila 28000, Algeria; chaimaa.azzouzi@univ-msila.dz
[2] Department of Veterinary Sciences, Chadli Bendjedid University, El Tarf 36000, Algeria; m.boucheikhchoukh@univ-eltarf.dz (M.B.); s.sedraoui@univ-eltarf.dz (S.S.)
[3] Department of Veterinary Sciences, University of Pisa, Viale delle Piagge, 2, 56124 Pisa, Italy; fabrizio.bertelloni@unipi.it
[4] Centre for Climate Change Impact, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
* Correspondence: valentina.virginia.ebani@unipi.it

Simple Summary

Stomoxys calcitrans and *Haematobia irritans* can cause substantial stress to ruminants due to their high abundance and frequent blood-feeding behavior. Scientific research on hematophagous arthropods in Algeria has primarily focused on ticks, fleas, and lice, while Stomoxyinae flies remain largely neglected by Algerian livestock breeders and understudied by researchers. In the present study, bacterial pathogens of veterinary and public health importance were detected in *S. calcitrans* and *H. irritans*, highlighting the potential role of these flies in the dissemination of bacteria that can compromise the health and productivity of cattle and small ruminants, potentially resulting in substantial economic losses.

Abstract

The stable fly (*Stomoxys calcitrans*) and the horn fly (*Haematobia irritans*) are hematophagous flies that pose a potential threat to human and animal health, as their feeding behavior may facilitate the transmission of pathogens of medical and veterinary importance. Given the limited information available on this potential threat in Algeria, the present study investigated the occurrence of bacterial pathogens in *S. calcitrans* and *H. irritans* collected from cattle and small ruminant farms in Northeastern Algeria. A total of 145 flies, organized in 29 pools (5 flies/pool), were screened through molecular analyses (PCR and sequencing) for the presence of *Salmonella* spp., *Brucella* spp., *Listeria* spp., Shiga toxin-producing *Escherichia coli* (STEC), *Staphylococcus* spp., *Anaplasma* spp., *Ehrlichia ruminantium*, *Coxiella burnetii*, and hemotropic *Mycoplasma*. Twenty-six (89.65%) pools were positive for at least one pathogen. *Salmonella* spp. DNA was found in 68.97% (20/29) of the samples examined. DNA of staphylococci (*S. aureus*, *S. gallinarum*, *S. saprophyticus*, *Staphylococcus* spp.) was detected in flies, with an overall pool positivity rate of 51.72%. Three (10.34%) and 14 (48.27%) pools were PCR-positive for *E. ruminantium* and hemotropic *Mycoplasma*, respectively. Remaining pathogens investigated were not detected. Although bacterial DNA was detected, bacterial viability and transmission were not assessed. Nevertheless, the findings suggest that *S. calcitrans* and *H. irritans* may act as potential vectors of bacterial pathogens of human and veterinary importance in Algeria.

Keywords: Algeria; *Ehrlichia* sp.; *Haematobia irritans*; livestock; hemotropic *Mycoplasma*; *Salmonella*; staphylococci; *Stomoxys calcitrans*

Check for updates

Academic Editor: Tao Qin

Received: 20 October 2026
Revised: 15 November 2026
Accepted: 19 November 2026
Published: 20 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

In animal production systems, hematophagous fly infestations represent a significant challenge, as they can adversely affect animal health and productivity while imposing substantial economic losses. Stomoxyinae flies (Diptera: Muscidae), along with tabanids (Diptera: Tabanidae) and hippoboscids (Diptera: Hippoboscidae), are parasitic flies of medical and veterinary significance that disturb livestock [1].

With ten genera and forty-nine species, the subfamily Stomoxyinae was recognized as a distinct and monophyletic taxon. Among the representatives of this subfamily are the stable fly *Stomoxys calcitrans* and the horn fly *Haematobia irritans* [2].

In Algeria, cattle breeding plays a crucial role in the national economy, as it constitutes the country's primary sector for meat production [3]. Although the Algerian cattle population comprised 1,844,000 heads (52% were dairy cows) [4], Algeria remains a significant global milk importer [5]. On the other hand, the Algerian cattle industry is threatened, particularly since the introduction of lumpy skin disease in the country [6]. This pathology is a vector-borne disease transmitted by hematophagous arthropods, mainly *Stomoxys* flies [7]. Furthermore, horn flies can cause substantial stress to cattle due to their high abundance and frequent blood-feeding behavior, with individual flies feeding up to 38 times per day. In the United States, the resulting productivity losses have been estimated at approximately USD 2.3 billion annually [8]. Consequently, there is growing interest in integrated pest management (IPM) strategies, which can provide more environmentally sustainable approaches to horn fly control [9]. Scientific research on hematophagous arthropods in Algeria has primarily focused on ticks, fleas, and lice [10–13]. Among Diptera, research has largely concentrated on mosquitoes and sand flies [14–17]. Conversely, Stomoxyinae flies remain largely neglected by Algerian livestock breeders and understudied by researchers [18]. Recent studies in Algeria investigating brachyceran Diptera have examined the diversity and population dynamics of these pests [19,20]. However, their potential role as vectors of pathogens remains poorly documented.

In view of this knowledge gap, the present study was conducted as a preliminary screening to investigate, through molecular analyses, the occurrence of bacterial pathogens of veterinary and public health importance in *S. calcitrans* and *H. irritans* collected from farms in northeastern Algeria. In particular, the study focused on the detection of *Salmonella* spp., *Brucella* spp., *Listeria* spp., Shiga toxin-producing *Escherichia coli* (STEC), *Staphylococcus* spp., *Anaplasma* spp., *Ehrlichia ruminantium*, *Coxiella burnetii*, and hemotropic *Mycoplasma*.

2. Materials and Methods

2.1. Study Sites and Fly Collection

Flies were collected from three municipalities in the Batna region of northeastern Algeria: Oued Taga, Timgad, and Djerma. Briefly, candidate farms were initially identified from the local veterinary inspection register of the Batna Agricultural Services Division based on livestock density and farmer willingness to participate; final selection was determined by logistical accessibility for periodic sampling. Farm C was selected in early 2025 specifically for *Haematobia irritans* sampling following reports of high seasonal horn fly infestations on dairy cattle in the Djerma area, combined with farm owner consent and operational feasibility. Farm selection was based on host availability, fly abundance, and operational logistics rather than prior knowledge of active bacterial infections or herd diagnostic histories. Specific diagnostic records and individual health statuses regarding the investigated pathogens (e.g., *Brucella* spp., *Coxiella burnetii*, or STEC) were not systematically available for the enrolled herds during the sampling periods.

Stomoxys calcitrans samples were obtained from a previous study conducted between July 2022 and July 2023, where flies were captured using Vavoua traps [19]. To ensure

adequate representative sampling, specimens selected for molecular screening were pooled from catches obtained during the main activity period, including the population peaks previously documented on these farms (September for the dairy cattle farm in Timgad and October for the small-ruminant farm in Oued Taga). *H. irritans* specimens were collected in September 2025 from a dairy cattle farm located in Djerma province. Because the local seasonal population dynamics of *H. irritans* in the Batna region remain unstudied, sampling was conducted opportunistically upon observing active horn fly infestations on host animals (Figure 1).

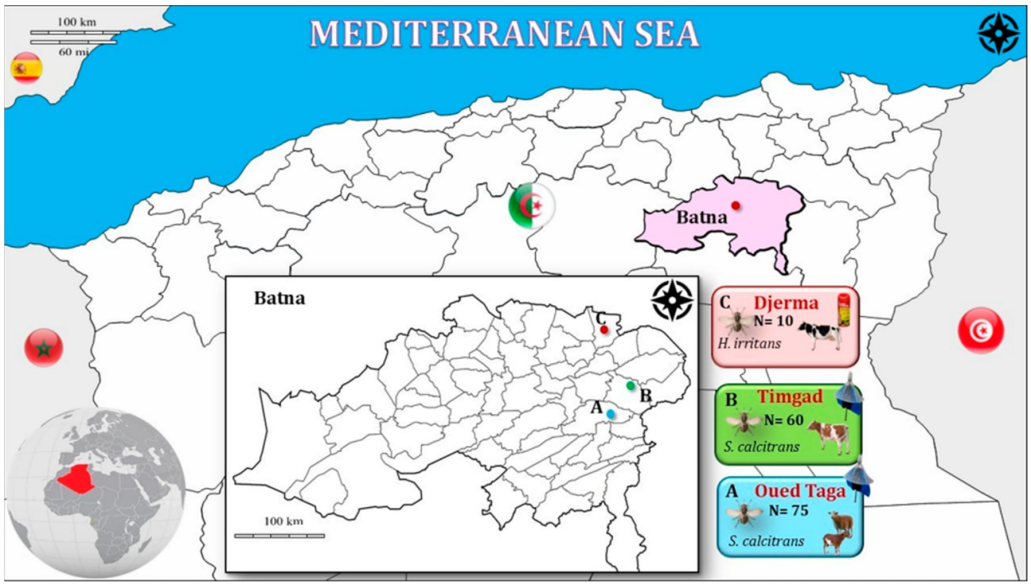

Figure 1. Geographic location of the fly sampling sites in the Batna region, Northeastern Algeria. The map indicates the three sampling farms (Farm A: Oued Taga; Farm B: Timgad; Farm C: Djerma), host types, collected fly species (*Stomoxys calcitrans* and *Haematobia irritans*), scale bar, directional orientation, and detailed municipality boundaries.

Detailed geographical coordinates, sampling dates, host farming types, and sample numbers for each site are summarized in Table 1. Horn flies were collected directly off cattle using a commercial permethrin-based insecticide spray (Stop®) sprayed in close proximity to infested animals to knock down flies, which were immediately gathered into sterile collection tubes. All collected specimens were stored in 70% ethanol at 4 °C until molecular analysis.

Table 1. Description of the fly species and the number of flies analyzed in the current study.

Farm	Location	Farming Type	Geographical Coordinates	Sampling Period	Fly Species Collected	Number of Flies Collected
A	Oued Taga	Small ruminants	35.43653227, 6.4470824	October 2022	*Stomoxys calcitrans*	75
B	Timgad	Dairy cattle	35.5601469, 6.5169649	September 2022	*Stomoxys calcitrans*	60
C	Djerma	Dairy cattle	35.710079, 5.878495	September 2025	*Haematobia irritans*	10

The subsampling of *S. calcitrans* specimens from the larger collection reported previously was guided by two primary criteria: morphological preservation quality and the objective of achieving approximately balanced sample sizes between the two farm types

for molecular analysis, rather than by proportional random sampling based on total catch sizes. High-quality, intact specimens stored in ethanol were prioritized to minimize DNA degradation. From Farm A (Oued Taga), 75 out of 82 available specimens (91.5%) were suitable for extraction. From Farm B (Timgad), a representative subset of 60 high-quality specimens spanning the main capture months was selected to match the sample scale of Farm A.

2.2. Fly Identification and Sample Preparation

Collected flies were identified under a binocular lens using the Zumpt identification key [2], then preserved in 70% ethanol until molecular analysis. Whole flies were pooled without prior surface washing or external chemical decontamination to preserve total sample yield. A total of 145 flies, 10 *H. irritans* from one cattle farm and 135 *S. calcitrans* from one cattle farm and one small ruminant farm, were obtained and used for successive molecular analysis. All collected flies were grouped into pools of five specimens each, yielding 27 pools of *S. calcitrans* (15 pools from Farm A [Oued Taga, small ruminants] and 12 pools from Farm B [Timgad, dairy cattle]) and 2 pools of *H. irritans* (from Farm C [Djerma, dairy cattle]) (Table 1). Pooling was employed to optimize the efficiency of molecular screening. It was performed strictly by farm location, fly species, and collection date/sampling event; individual pools contained only flies collected on the same date from the same farm, avoiding the mixing of specimens across different collection dates. Therefore, the results represent pool-level positivity rather than individual fly prevalence. Homogenization of whole pooled flies was performed directly prior to DNA extraction using the Quick-DNA Tissue/Insect Miniprep Kit according to the manufacturer's protocol.

Because this investigation was designed as a preliminary molecular screening of available archived and field-collected specimens, sample size was determined by specimen availability and preservation quality rather than formal a priori statistical power calculations. Consequently, the dataset of 145 flies (29 pools) serves as an exploratory baseline to detect pathogen DNA presence in these synanthropic fly populations.

2.3. Molecular Analysis

2.3.1. DNA Extraction

DNA was extracted from each pool using the Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Whole pooled flies were homogenized and subjected to DNA extraction in October 2025. At the end of the protocol, DNA was eluted in 100 µL of DNA Elution Buffer and stored at 4 °C until PCR analyses. A blank sample including all the standard extraction reagents was used for the DNA extraction negative control. DNA was quantified by measuring the optical density (OD) at 260 nm. Meanwhile, DNA purity was determined by calculating the ratio of absorbance at 260 and 280 nm; DNA extracts with an A260/A230 ratio greater than 2.0 were considered of good quality and used in PCR assays. OD measurements were performed using a Nanodrop ND–1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.3.2. Detection of Pathogens

Extracted DNA was used in conventional PCR for the detection of the following bacterial pathogens: *Salmonella* spp., *Brucella* spp., *Listeria* spp., Shiga toxin-producing *Escherichia coli* (STEC), *Staphylococcus aureus*, other *Staphylococcus* spp., *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma ovis*, *Anaplasma capra*, *Ehrlichia ruminantium*, *Coxiella burnetii*, and hemotropic *Mycoplasma*.

Each PCR reaction was carried out in a 25 µL final volume, containing 12.5 µL of DreamTaq Hot Start Green Master Mix (Life Technologies Italia, Milan, Italy), 0.3 µM of each primer, 3 µL of extracted DNA, and ultrapure water to reach the final volume. All PCR amplifications were carried out in an automated thermal cycler (SimpliAmp™ Thermal Cycler, Applied Biosystems, Waltham, MA, USA) with the following conditions: 95 °C for 5 min of initial denaturation followed by 40 cycles at 95 °C for 1 min, annealing for 1 min, and 72 °C for 2 min; the reaction was completed by a final step of 10 min at 72 °C. Target genes, primers used, annealing temperatures, and size of the expected amplicons of each protocol are reported in Table 2.

Table 2. Target genes, primers, annealing temperatures, and size of the expected amplicons of each PCR protocol.

Pathogen	Target Gene	Primer	Sequence	Amplicon (bp)	Annealing Temp. (°C)	Ref.
Staphylococcus spp.	*16S rRNA*	Staph756F	AACTCTGTTATTAGGGAAGAACA	756	61	[21]
		Staph750R	CCACCTTCCTCCGGTTTGTCACC			
Staphylococcus aureus	*nuc*	Nuc1	GCGATTGATGGTGATACGGTT	279	61	
		Nuc2	AGCCAAGCCTTGACGAACTAAAGC			
Salmonella spp.	*invA*	invAF	GTTGTACCGTGGCATGTCTG	930	50	[22]
		invAR	GCCATGGTATGGATTTGTCC			
Listeria spp.	*prs*	PRS_1	GCTGAAGAGATTGCGAAAGAAG	370	53	[23]
		PRS_2	CAAAGAAACCTTGGATTTGCGG			
Brucella spp.	*bcsp31*	B4	TGGCTCGGTTGCCAATATCAA	223	60	[24]
		B5	CGCGCTTGCCTTTCAAGGTCTG			
STEC	*stx1*	stx1F	ATAAATCGCCATTCGTTGACTAC	180	60	[25]
		stx1R	GAACGCCCACTGAGATCATC			
	stx2	stx2F	GGCACTGTCTGAAACTGCTCC	255	60	
		stx2R	TCGCCAGTTATCTGACATTCTG			
Anaplasma capra	*gltA* *	Outer-f	GCGATTTTAGAGTGYGGAGATTG	1031	55	[26]
		Outer-r	TACAATACCGGAGTAAAAGTCAA			
	gltA **	Inner-f	TCATCTCCTGTTGCACGGTGCCC	594	60	
		Inner-r	CTCTGAATGAACATGCCCACCCT			
Anaplasma ovis	*msp4*	MSP45	GGGAGCTCCTATGAATTACAGA-GAATTGTTTAC	852	60	[27]
		MSP43	CCGGATCCTTAGCTGAACAGGAATCTTGC			
Anaplasma bovis	*16S rRNA*	Forward	TAGCTTGCTATGGGGACAATTAG	547	52	[28]
		Reverse	TCTCCCGGACTCCAGTCTGGC			
Anaplasma phagocytophilum	*16S rRNA* *	ge3a	CACATGCAAGTCGAACGGATTATTC	932	55	[29]
		ge10r	TTCCGTTAAGAAGGATCTAATCTCC			
	16S rRNA **	ge9f	AACGGATTATTCTTTATAGCTTGCT	546	55	
		ge2	GGCAGTATTAAAAGCAGCTCCAGG			
Coxiella burnetii	*IS1111*	Trans-1	TATGTATCCACCGTAGCCAGT	687	64	[30]
		Trans-2	CCCAACAACACCTCCTTATTC			

Table 2. *Cont.*

Pathogen	Target Gene	Primer	Sequence	Amplicon (bp)	Annealing Temp. (°C)	Ref.
Hemotropic *Mycoplasma*	*16S rRNA*	Mfel-F	ACGAAAGTCTGATGGAGCAATA	170/193	60	[31]
		Mfel-R	ACGCCCAATAAATCCGRATAAT			
Ehrlichia ruminantium	*pCS20* •	ITM130 *	TCAATTGCTTAATGAAGCACTAACTCAC	473	62	[32]
		AB128	ACTAGTAGAAATTGCACAATCTAT	280	58	
		AB129	TGATAACTTGGTGCGGGAAATCCTT			

Legend. *: First step of nested PCR; **: second step of nested PCR; •: semi-nested PCR. The first reaction with primers ITM130 and AB129 produces an expected amplicon of 473 bp, whereas the second reaction with the primers AB128 and AB129 produces an expected amplicon of 280 bp.

Sterile distilled ultrapure water was used as a negative control, whereas DNA extracted from the following ATCC strains was used as a positive control: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Salmonella enterica* sub. *enterica* serovar Typhimurium (ATCC 14028), *Listeria monocytogenes* ATCC 7644, *Brucella ovis* strain 63/290 ATCC 25840, and Quantitative Genomic DNA from O157:H7 *E. coli* ATCC®43895DQ TM. In addition, DNA extracted from IFAT slides (Fuller Laboratories, Fullerton, CA, USA) for *A. phagocytophilum* and *C. burnetii* were used as positive controls. No positive controls were available for the remaining pathogens investigated.

When nested PCR and semi-nested PCR protocols were used, master mixes of the first round and the second round were assessed in different spaces and using separate sets of pipettes and related materials to avoid cross-contamination.

A 1.5% agarose gel stained with ethidium bromide was used to confirm the success of the PCR amplification. The gel was run at 100 V for 40 min. Amplicons were visualized under ultraviolet illumination (Vilber Lourmat Compact UV Transilluminator ECX-F15.M, Marne La Vallee, France) using 100 bp DNA Ladder Ready to Load (Solis BioDyne, Tartu, Estonia) as a DNA marker. Positive samples were purified using the NucleoSpin Gel and PCR Clean-up Mini Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The purified amplicons were then submitted to the external laboratory BMR Genomics (Padova, Italy) for bidirectional sequencing. The resulting sequences, assembled and corrected through visual inspection of the electropherogram using BioEdit v.7.0.2, were compared with sequences available in GenBank using the BLAST program 2.15.0 (http://www.ncbi.nlm.nih.gov/BLAST, accessed on 21 July 2026).

3. Results

Among the 29 pools examined, 26 (89.65%) were positive for at least one pathogen. Simultaneous detection of multiple bacterial DNA targets occurred in 19 pools (13 pools with two pathogens, 5 with three pathogens, and 1 with four pathogens), while only one pathogen was detected in 7 pools (Tables 3 and 4). Because each pool comprised five individual flies, these co-detections reflect the presence of multiple bacterial taxa within the pool rather than confirmed coinfections within individual flies.

Table 3. Results in relation to the examined pool and farm provenance.

Pool ID	Farm	Fly Species	Pathogens Detected
1	C	*Haematobia irritans*	*Staphylococcus* spp., *Salmonella enterica*
2	B	*Stomoxys calcitrans*	*Staphylococcus* spp., *Salmonella enterica*
3	C	*Haematobia irritans*	*Staphylococcus saprophyticus*, *Salmonella enterica Ehrlichia* sp., hemotropic *Mycoplasma* sp.

Table 3. *Cont.*

Pool ID	Farm	Fly Species	Pathogens Detected
4	A	*Stomoxys calcitrans*	*Staphylococcus* spp., *Salmonella enterica,* hemotropic *Mycoplasma* sp.
5	A	*Stomoxys calcitrans*	*Salmonella enterica*, hemotropic *Mycoplasma* sp.
6	A	*Stomoxys calcitrans*	*Staphylococcus* spp., *Salmonella enterica*, hemotropic *Mycoplasma* sp.
7	B	*Stomoxys calcitrans*	*Salmonella enterica, Ehrlichia* sp., hemotropic *Mycoplasma* sp.
8	A	*Stomoxys calcitrans*	*Staphylococcus* spp., hemotropic *Mycoplasma* sp.
9	A	*Stomoxys calcitrans*	*Staphylococcus gallinarum*, *Salmonella enterica*
10	A	*Stomoxys calcitrans*	*Salmonella enterica*
11	A	*Stomoxys calcitrans*	*Salmonella enterica*, hemotropic *Mycoplasma* sp.
12	B	*Stomoxys calcitrans*	*Salmonella enterica*, hemotropic *Mycoplasma* sp.
13	A	*Stomoxys calcitrans*	*Salmonella enterica*, hemotropic *Mycoplasma* sp.
14	A	*Stomoxys calcitrans*	*Salmonella enterica*, hemotropic *Mycoplasma* sp.
15	A	*Stomoxys calcitrans*	*Salmonella enterica*
16	B	*Stomoxys calcitrans*	*Staphylococcus aureus*, *Salmonella enterica,* hemotropic *Mycoplasma* sp.
17	A	*Stomoxys calcitrans*	*Salmonella enterica*, *Staphylococcus* spp.
18	A	*Stomoxys calcitrans*	negative
19	B	*Stomoxys calcitrans*	negative
20	B	*Stomoxys calcitrans*	hemotropic *Mycoplasma* sp.
21	B	*Stomoxys calcitrans*	negative
22	B	*Stomoxys calcitrans*	*Salmonella enterica*, *Staphylococcus saprophyticus*
23	A	*Stomoxys calcitrans*	*Staphylococcus* spp., *Ehrlichia* sp.
24	B	*Stomoxys calcitrans*	*Staphylococcus saprophyticus*, *Salmonella enterica*, hemotropic *Mycoplasma* sp.
25	B	*Stomoxys calcitrans*	*Staphylococcus* spp., hemotropic *Mycoplasma* sp.
26	B	*Stomoxys calcitrans*	*Staphylococcus gallinarum*
27	B	*Stomoxys calcitrans*	*Salmonella enterica*
28	A	*Stomoxys calcitrans*	*Staphylococcus* spp.
29	A	*Stomoxys calcitrans*	*Salmonella enterica*

Legend. ID: Identification number; A: small ruminants in Oued Taga; B: dairy cattle in Timgad; C: dairy cattle in Djerma.

Table 4. Pool positivity rate for each detected pathogen.

Farm ID (Location)	Host Type	Fly Species	Total Pools *	Positive Pools (%)			
				Salmonella enterica	***Staphylococcus* spp.**	**Hemotropic *Mycoplasma* sp.**	***Ehrlichia* sp.**
A (OuedTaga)	Small ruminants	*S. calcitrans*	15	11 (73.3%)	7 (46.7%)	7 (46.7%)	1 (6.7%)
B (Timgad)	Dairy cattle	*S. calcitrans*	12	7 (58.3%)	6 (50.0%)	6 (50.0%)	1 (8.3%)

Table 4. *Cont.*

Farm ID (Location)	Host Type	Fly Species	Total Pools *	Positive Pools (%)			
				Salmonella enterica	***Staphylococcus* spp.**	**Hemotropic *Mycoplasma* sp.**	***Ehrlichia* sp.**
C (Djerma)	Dairy cattle	*H. irritans*	2	2 (100%)	2 (100%)	1 (50.0%)	1 (50.0%)
Total *				20 (68.97%)	15 (51.72%)	14 (48.27%)	3 (10.34%)

Legend. *: Total of positive pools among the 29 tested.

3.1. Detection of *Salmonella* spp.

Twenty (68.97%) pools (2 *H. irritans* and 18 *S. calcitrans*) had *Salmonella* spp. DNA. The sequencing analysis identified *Salmonella enterica* for all amplicons, since 99% homology with *S. enterica* sequences present in the database was observed. One sequence was deposited in GenBank under accession number QB170548.

3.2. Detection of *Staphylococcus* spp.

Staphylococcus spp. DNA was detected in 15 (51.72%) pools (2 *H. irritans* and 13 *S. calcitrans* pools). Among these, one *S. calcitrans* pool (3.44%) tested positive for the *nuc* gene, and sequencing analysis confirmed its identification as *S. aureus* (registered in GenBank under accession number QB170547). The remaining 14 positive samples were analyzed by sequencing: *S. saprophyticus* was identified in 3 (10.34%) samples (2 *S. calcitrans* and 1 *H. irritans* pools), and *S. gallinarum* was identified in 2 (6.89%) *S. calcitrans* pools. One sequence of *S. saprophyticus* and one sequence of *S. gallinarum* were deposited in GenBank under accession numbers QB160009 and QB160008, respectively. The remaining 9/29 (31.03%) positive samples were confirmed as *Staphylococcus* spp. by sequencing, but the species could not be identified.

3.3. Detection of *E. ruminantium*

Three (10.34%) pools (1 *H. irritans* and 2 *S. calcitrans*) were PCR-positive for *E. ruminantium*. Sequencing analysis identified a 98.26% homology with *E. ruminantium* sequences present in GenBank (such as PV560932; CP063045, AY236061, MK371028, OQ181416). Since the three sequences of this study were identical, only one was deposited under accession number QB170546.

3.4. Detection of Hemotropic *Mycoplasma*

Fourteen (48.27%) pools (1 *H. irritans* and 13 *S. calcitrans*) were positive for hemotropic *Mycoplasma*. Sequencing analysis of the amplicons identified, in all cases, a 98.09% homology with several sequences of *Mycoplasma wenyonii* reported in GenBank (such as PZ575308, PZ575296, OZ482342, OZ405538, PP544183); only one sequence, selected at random, was deposited in the GenBank database under accession number QB191966.

Table 5 reports data obtained from BLAST related to the sequencing analyses of *E. ruminantium* and hemotropic *Mycoplasma* amplicons.

Table 5. Results of sequencing analyses related to BLAST findings.

Suspected Pathogen	Gene (Accession Number of This Study)	Length of Compared Sequence (bp)	Accession Length	Closest Identity (Accession Number of Reference Sequence)	Query Coverage
Ehrlichia ruminantium	*pCS20* (QB170546)	230	272	98.26% (PV560932, *E. ruminantium*)	100%
			1,516,356	98.26% (CP063045, *E. ruminantium*)	100%
			993	98.26% (AY236061, *E. ruminantium*)	100%
			279	98.26% (MK371028, *E. ruminantium*)	100%
			278	98.26% (OQ181416, *E. ruminantium*)	100%
Hemotropic *Mycoplasma* sp.	16S *rRNA* (QB191966)	157	587	98.09% (PZ575308, *M. wenyonii*)	100%
			598	98.09% (PZ575296, *M. wenyonii*)	100%
			588	98.09% (OZ482342, *M. wenyonii*)	100%
			588	98.09% (OZ405538, *M. wenyonii*)	100%
			629	98.09% (PP544183, *M. wenyonii*)	100%

3.5. Detection of Listeria, Brucella, STEC, C. burnetii, and Anaplasma spp.

DNA of these pathogens was not detected in any of the samples analyzed.

4. Discussion

The present study examined the potential role of *S. calcitrans* and *H. irritans* as vectors of bacterial pathogens of veterinary and public health importance in Northeastern Algeria. Research on biting muscid flies in Algeria remains limited, with most published data focusing on dipteran diversity and the population dynamics of *S. calcitrans* [19]. Recently, Hafsa et al. (2026) [33] provided the first confirmed evidence of *H. irritans* collected from cattle during 2021 and 2022 in northeastern Algeria. Globally, vector-borne pathogens are becoming increasingly known as significant contributors to morbidity and mortality in both domestic animals and humans [34]. A recent study has suggested that flies on dairy farms pose a significant zoonotic threat, highlighting the potential transfer of pathogenic microorganisms from manure to flies [35].

In our survey, *Salmonella* spp. were detected in 68.97% of the examined pools. This high detection rate highlights the potential epidemiological relevance of *S. calcitrans* as a carrier of this zoonotic pathogen, particularly because *Salmonella* has previously been detected in this fly species [36]. Due to their feeding behavior and close association with animal production environments, flies such as *Musca domestica* can readily become contaminated with *Salmonella*, particularly in livestock and breeding facilities, and have been considered potential mechanical vectors of this pathogen [37]. Similarly, *Salmonella* has been detected on the body surface of *S. calcitrans* [36]. The high pool positivity rate observed in the present study further suggests that Stomoxyinae flies can harbor *Salmonella* and may contribute to its dissemination within livestock environments. However, the detection of bacterial DNA alone does not demonstrate vector competence or confirm active trans-

mission. Further studies are therefore needed to determine whether *S. calcitrans* and *H. irritans* can acquire, maintain, and mechanically transmit viable *Salmonella* under natural or experimental conditions. Our findings are consistent with previous reports indicating that various insect species, particularly synanthropic flies, can carry *Salmonella* and may contribute to the dissemination of foodborne pathogens [38]. Further research is warranted to clarify the interactions between insects and bacterial pathogens and to better understand the epidemiological role of flies in the persistence and spread of zoonotic bacteria.

More than 50% of the pools analyzed were positive for staphylococci, and three distinct species were identified. The detection of several staphylococci in *S. calcitrans* and *H. irritans* may confirm previous reports, suggesting a common opportunistic presence of these bacteria and their possible colonization of the endogenous microbial flora of biting flies [39]. *Staphylococcus aureus*, detected in *S. calcitrans* in the present study, is an important pathogen capable of causing severe infections in both humans and animals. In cattle, *S. aureus* is a major causative agent of bovine mastitis and is associated with substantial economic losses, estimated at approximately USD 177 per infected cow annually in Northwestern Europe and Canada. These losses are primarily attributable to reduced milk production and discarded milk, as well as additional expenses related to veterinary care, medication, labor, and the culling of affected cows [40]. Recent studies have investigated the attraction of *S. calcitrans* to *Staphylococcus* spp. associated with bovine mastitis and the potential involvement of these flies in the transmission of the pathogen. In particular, *S. calcitrans* has been shown to be attracted to ammonia produced by *Staphylococcus* bacteria [41,42]. Our findings are consistent with previous reports describing an association between *Stomoxys* flies and bacterial pathogens implicated in bovine mastitis [39,43], further supporting the need for effective strategies to control both *S. aureus* and potential arthropod-mediated pathways of pathogen dissemination.

Staphylococcus saprophyticus was detected in *H. irritans* and *S. calcitrans* collected from cattle farms. These findings are consistent with a recent study reporting an association between *S. saprophyticus* and *S. calcitrans* [44]. To the best of our knowledge, however, our study reports the first molecular detection of *S. saprophyticus* in Stomoxyinae flies in Algeria. *Staphylococcus saprophyticus* is an opportunistic pathogen that can colonize the gastrointestinal tract and skin of humans and animals. Flies may acquire this bacterium externally through contamination of the proboscis, cuticle, or legs, or internally through ingestion during feeding [45]. Although *S. saprophyticus* is not considered a classical vector-borne pathogen, its detection in horn flies and stable flies suggests that these insects may contribute to the mechanical dissemination of the bacterium, also at human–animal interfaces. Nevertheless, further studies are required to determine whether these flies can acquire, maintain, and mechanically transmit viable *S. saprophyticus*.

In our study, *Staphylococcus gallinarum* was also detected in *S. calcitrans* flies collected from small ruminants and cattle farms. This finding aligns with a recent study that associates this bacterium with *S. calcitrans* [44]. *Staphylococcus gallinarum* is a commensal bacterium frequently found in poultry [46]; it is an opportunistic human pathogen and could also be implicated in mastitis in cattle [47]. In our case, the association of *S. gallinarum* with *S. calcitrans* could be related to the co-occurrence of poultry on the farms where the flies were trapped, as observed during trapping. Furthermore, *Staphylococcus* spp. were detected in several samples, although species-level identification by sequencing was not possible. This finding provides further evidence of the presence of staphylococci in Stomoxyinae flies and is consistent with their potential role as carriers of opportunistic bacteria. However, the inability to identify these bacteria to the species level limits the interpretation of their potential epidemiological significance. Several factors may have contributed to this

limitation, including low DNA yield, the presence of mixed bacterial populations within the samples, or insufficient sequencing coverage.

The present study also investigated the presence of *C. burnetii*, *Brucella*, *Listeria*, and STEC; however, none of these pathogens were detected in the tested flies. Although brucellosis and Q fever are endemic in domestic ruminants in Algeria, our negative molecular findings must be interpreted with caution. Because farm selection was driven by vector availability and logistical feasibility rather than documented clinical outbreaks or herd-level seroprevalence data, the absence of pathogen DNA in fly samples may reflect either low pathogen circulation on these specific farms during the sampling window or limited sample size, rather than a lack of vector competence.

These pathogens are frequently found in cattle and small ruminants, which may act as asymptomatic carriers or develop clinical disease [48,49]. The role of Stomoxyinae flies as mechanical vectors of *Brucella* spp. and *Listeria* spp. has been suspected but has not yet been demonstrated [50]. *Brucella* was not detected in the current study, despite Brucellosis being considered a major endemic zoonosis in Algeria [51]. At the same time, STEC was previously isolated from *S. calcitrans*, albeit with low detection rates [36,52]. In addition, *E. coli* and other Gram-negative bacteria were found associated with Phlebotominae sand flies in Northeastern Algeria [53]. The negative results obtained in this study may be due to the low circulation of these pathogens in the investigated farms or to the limited capacity of *S. calcitrans* to serve as a carrier.

In our survey, a high percentage of samples were positive for hemotropic mycoplasmas. In particular, sequencing analysis identified a pathogen closely related to *M. wenyonii*. The obtained amplicons showed 98.09% sequence identity with several *M. wenyonii* sequences available in GenBank. However, this level of sequence similarity was insufficient to reliably identify the *Mycoplasma* species, and therefore its taxonomic assignment remains uncertain. Bovine hemoplasmosis is usually caused by *M. wenyonii* and *Candidatus* Mycoplasma hemobos, which were identified in asymptomatic cattle in Algeria for the first time by Boularias et al. (2020) [54]. *Mycoplasma wenyonii*, initially classified as *Eperythrozoon wenyonii*, is known as a bovine pathogen. Recent studies have detected *M. wenyonii* in both clinically ill and apparently healthy cattle, with infections characterized by a range of symptoms, including anemia, abortion, infertility, delayed estrus, icterus with hemoglobinuria, and decreased milk production [55]. Moreover, chronically infected cows may exhibit subclinical productivity losses such as reduced weight of calves at birth and reduced milk production [55]. Transmission of hemotropic mycoplasmas has been suspected to occur via blood-sucking insects and ticks, which are proposed as potential vectors [56]. Moreover, it was found in *S. calcitrans* in Thailand [57]. However, transmission to cattle via arthropods for *M. wenyonii* remains lacking [56].

Anaplasmataceae were investigated in this survey. *Anaplasma phagocytophilum*, *A. bovis*, *A. capra*, and *A. ovis* were not detected, whereas three pools were PCR-positive for *E. ruminantium*. The obtained amplicons showed 98.26% sequence identity with several *E. ruminantium* sequences available in GenBank; however, this level of sequence similarity was insufficient to reliably identify this species. *Ehrlichia ruminantium*, previously named *Cowdria ruminantium*, is the etiologic agent of heartwater, a fatal disease of ruminants in tropical areas [58]. Some human cases have also been reported [59]. This globally important pathogen is primarily transmitted by ticks of the *Amblyomma* genus [58]. The geographical distribution of *E. ruminantium* is extensive, encompassing most of sub-Saharan Africa, Madagascar, and the French West Indies in the Caribbean [60]. To the best of our knowledge, however, no previous reports of this pathogen have been documented in Algeria. A previous study detected *E. ruminantium* DNA in tsetse flies (*Glossina pallidipes*)

in Ethiopia [61], but no reports of this arthropod-borne pathogen in *S. calcitrans* and *H. irritans* are available.

The present study also screened pools for *C. burnetii*, *Brucella* spp., *Listeria* spp., STEC, and several *Anaplasma* species; however, none of these targets were detected in the tested samples. These negative molecular findings must be interpreted strictly within the context of the specific pools examined and should not be construed as evidence that these pathogens are absent from the participating farms or that *S. calcitrans* and *H. irritans* are inherently incapable of harboring or transmitting them. Given the limited sample size and exploratory pooling design, localized pathogen presence below the threshold of detection or low infection intensity within unsampled fly cohorts cannot be ruled out. Larger, systematic epidemiological studies combining host animal diagnostics with expanded entomological sampling are necessary to evaluate the true occurrence and vector role of these flies.

Several methodological limitations must be considered when interpreting these findings. First, the study design relied on pooled samples of five flies per pool. While pooling is a practical strategy for initial pathogen screening, pool-level positivity rates cannot be directly extrapolated to individual fly infection positivity rates. Second, the sampling scheme was highly asymmetric: *S. calcitrans* was sampled across two farms ($n = 27$ pools; 135 flies), whereas *H. irritans* was retrieved from a single farm in very low numbers ($n = 2$ pools; 10 flies). This uneven sampling structure precludes robust statistical comparisons between the two fly species regarding their pathogen rates or vector capacities. Furthermore, sub-sampling of archived *S. calcitrans* flies was structured to achieve balanced sample representation across farms rather than reflecting the proportional field abundance recorded in prior ecological surveys. Finally, the simultaneous PCR detection of multiple pathogen species within a single pool (e.g., Pool 3 containing four bacterial targets) indicates the collective microbial burden of five pooled insects; it does not demonstrate true coinfection within individual flies. Future studies employing larger, balanced sample sizes and individual-level screening (or lower pool sizes) are needed to establish individual-level prevalence.

Additional methodological factors regarding sample collection, storage duration, and potential cross-contamination warrant consideration. For *S. calcitrans*, flies remained in Vavoua trap collection chambers containing a sugar–water solution two hours prior to ethanol immersion, and specimens were initially stored bulk-by-catch in 70% ethanol. Because flies were not rinsed prior to bulk storage or pooling, cross-contamination of bacterial cells or exogenous DNA among specimens within the same collection vessel—or via shared feeding on the trap's sugar solution—cannot be completely ruled out.

Another key methodological aspect of the present study concerns sample processing and the anatomical origin of the detected bacterial DNA. Because molecular analysis was performed on whole, unwashed flies, the assay detected cumulative bacterial DNA associated with the entire insect host. Consequently, our findings cannot differentiate whether the identified bacterial DNA originated from external surface contamination (cuticle/legs/proboscis), internal gut microbiota, or ingested blood meals from host animals. Additionally, *S. calcitrans* and *H. irritans* specimens were collected using different sampling methodologies (historical trap collections vs. direct permethrin knockdown in 2025) and stored for different durations. Chemical knockdown and prolonged ethanol storage may affect DNA integrity or recovery; successful PCR amplification and high-quality sequence identification across target genes indicate that the detected targets were amplifiable, but do not exclude degradation or inhibition affecting negative assays. Nevertheless, future studies incorporating surface-washing protocols (to separate cuticular from internal microbiota) and standardized collection methods will help delineate the precise anatomical localization and acquisition pathways of these bacterial pathogens. Furthermore, the absence of base-

line epidemiological and diagnostic health data on the host animals from the participating farms represents an additional limitation. Without concurrent diagnostic screening of host blood, milk, or fecal samples, it is not possible to confirm whether negative findings in flies reflect true herd freedom from infection or localized non-circulation during fly capture. Integrated vector–host surveillance studies incorporating simultaneous host diagnostics are recommended to fully evaluate vector transmission dynamics.

Finally, the molecular analyses conducted in this survey should be considered preliminary. Positive controls for *E. ruminantium* and *M. wenyonii* were not available, and although sequencing analyses revealed high sequence homology with the target pathogens, additional molecular targets should be investigated to further confirm these findings. Future studies should therefore include appropriate positive controls and complementary molecular assays to strengthen the reliability of pathogen identification.

5. Conclusions

The present study provides preliminary molecular evidence for the presence of bacterial pathogens in fly pools collected from livestock farms in northeastern Algeria. The detection of these pathogens is of particular relevance to animal health; they can contribute to significant economic losses, mainly through reduced milk production and reproductive disorders. Moreover, staphylococci and salmonellae are of major importance to human health; therefore, flies carrying these pathogens may represent a potential risk to public health. Given the preliminary nature of this screening and the limited sample size examined, negative findings apply exclusively to the evaluated specimens and do not imply pathogen absence at the herd level.

Although this study detected bacterial DNA without assessing bacterial viability or transmission, the obtained results suggest that Stomoxyinae flies may contribute to the transmission of bacterial pathogens through both blood-feeding and external contamination. Blood-feeding may facilitate the acquisition of pathogens such as *E. ruminantium* and hemotropic mycoplasmas, which circulate in the blood of infected animals, whereas other bacteria may be acquired through contamination of the flies' body surface and mouthparts. Considering the limited information available on the role of Stomoxyinae flies in Algeria in the transmission of the investigated pathogens, further epidemiological investigations are warranted to better elucidate their potential vector and mechanical transmission roles. In addition, awareness and educational programs addressing the potential role of flies in pathogen dissemination should be developed for farmers, veterinarians, and the general public. Furthermore, integrated fly-control programs combining the various available control methods should be implemented within a One Health framework, taking into account the interconnectedness of animal, human, and environmental health.

Author Contributions: Conceptualization, C.A., M.B. and V.V.E.; methodology, C.A.; formal analysis, C.A. and F.B.; investigation, C.A. and F.B.; writing—original draft preparation, C.A.; writing—review and editing, M.B. and V.V.E.; supervision, S.S., M.B., F.B. and V.V.E.; funding acquisition, F.B. and V.V.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Pisa, Funds "Fondi di Ateneo 2023 Ebani, Fondi di Ateneo 2023 Bertelloni".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank the farm owners for their generosity, as well as the veterinarian from the Batna Agricultural Services Division for providing support during the fly sampling process.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Halos, L.; Jamal, T.; Maillard, R.; Girard, B.; Guillot, J.; Chomel, B.; Vayssier-Taussat, M.; Boulouis, H.J. Role of Hippoboscidae flies as potential vectors of *Bartonella* spp. infecting wild and domestic ruminants. *Appl. Env. Microbiol.* **2004**, *70*, 6302–6305. [CrossRef]
2. Zumpt, F. *The Stomoxyine biting flies of the world. Diptera: Muscidae. Taxonomy, Biology, Economic Importance and Control Measures*, 1st ed.; Pub. Gustav Fischer: Vienna, Austria, 1973.
3. Sadoud, M.; Hocquette, J.F. La filière viande bovine en Algérie. *Viandes Prod. Carnés* **2022**, VPC-2022-3835. Available online: https://hal.inrae.fr/hal-04661119v1/document (accessed on 31 August 2026).
4. Sadoud, M. Analyse des contraintes pesant sur la compétitivité de la filière viande bovine en Algérie. *Viandes Prod. Carnés* **2017**, VPC-2017-33-4-5.
5. Mamine, F.; Fares, M.; Duteurtre, G.; Madani, T. Régulation du secteur laitier en Algérie entre sécurité alimentaire et développement d'une production locale: Synthèse. *Rev. Elev. Med. Vet. Pays Trop.* **2021**, *74*, 73–81. [CrossRef]
6. Food and Agriculture Organization of the United Nations. FAO Alerts Countries in Near East, North Africa and Southern Europe to Enhance Preparedness for Lumpy Sskin Disease. 2024. Available online: https://openknowledge.fao.org/server/api/core/bitstreams/9fbc74c0-392b-4753-800e-453d13dc211d/content (accessed on 5 September 2026).
7. Haegeman, A.; Sohier, C.; Mostin, L.; De Leeuw, I.; Van Campe, W.; Philips, W.; De Regge, N.; De Clercq, K. Evidence of lumpy skin disease virus transmission from subclinically infected cattle by *Stomoxys calcitrans*. *Viruses* **2023**, *15*, 1285. [CrossRef]
8. Brewer, G.J.; Boxler, D.J.; Domingues, L.N.; Trout Fryxell, R.T.; Holderman, C.; Loftin, K.M.; Machtinger, E.; Smythe, B.; Talley, J.L.; Watson, W. Horn Fly (Diptera: Muscidae)—Biology, Management, and Future Research Directions. *J. Integr. Pest. Manag.* **2021**, *12*, 42. [CrossRef]
9. González, M.A.; Duvallet, G.; Morel, D.; de Blas, I.; Barrio, E.; Ruiz-Arrondo, I. An Integrated Pest Management Strategy Approach for the Management of the Stable Fly *Stomoxys calcitrans* (Diptera: Muscidae). *Insects* **2024**, *15*, 222. [CrossRef]
10. Hamza, A.; Mokhtaria, K.; Ammar, S.S.M.; Taha, B. Identification, seasonal prevalence, and preferred attachment sites of ixodid tick species infesting cattle in four municipalities of the province of Tiaret, northwest Algeria. *Exp. Appl. Acarol.* **2025**, *94*, 24. [CrossRef]
11. Boucheikhchoukh, M.; Leulmi, H.; Dib, L.; Mechouk, N.; Khalfallah, M.; Benakhla, A. Lice (Phthiraptera) diversity in ruminants and domestic birds in northeastern Algeria. *Int. J. Trop. Insect Sci.* **2023**, *43*, 2233–2244. [CrossRef]
12. Boucheikhchoukh, M.; Mechouk, N.; Leulmi, H.; Aouadi, A.; Benakhla, A. Fleas (Siphonaptera) of domestic and wild animals in extreme northeastern Algeria: First inventory, hosts, and medical and veterinary importance. *J. Vector Ecol.* **2022**, *47*, 81–87. [CrossRef]
13. Zeroual, F.; Leulmi, H.; Saidani, K.; Simion, V.E.; Benakhla, A.; Parola, P.; Bitam, I. Detection of *Rickettsia* species in ticks feeding on wild boar (*Sus scrofa algira*) in Algeria. *Vet. J.* **2025**, *314*, 106468. [CrossRef]
14. Boumaza, M.; Kihel, I.; Merabti, B.; Zemouli, C.; Ouakid, M.L. Diversity, abundance and distribution of mosquitoes (Diptera, Culicidae) in Northeast Algeria. *J. Insect Biodivers. Syst.* **2025**, *11*, 417–435. [CrossRef]
15. Boulares, M.; Nassima, R.; Houhamdi, I.; Baaloudj, A.; Soltani, N.; Houhamdi, M. Systematic and ecological study of mosquitoes (Diptera: Culicidae) at lake Fetzara (Annaba, Northeast Algeria). *Ukr. J. Ecol.* **2023**, *13*, 1–7.
16. Messahel, N.E.; Benallal, K.E.; Halada, P.; Lafri, I.; Manseur, H.; Hakem, A.; Houali, K.; Harrat, Z.; Volf, P.; Dvorak, V. Identification of blood source preferences and *Leishmania* infection in sand flies (Diptera: Psychodidae) in north-eastern Algeria. *Vet. Parasitol. Reg. Stud. Rep.* **2022**, *31*, 100729. [CrossRef]
17. Benallal, K.E.; Mefissel, M.; Dib, Y.; Depaquit, J.; Kavan, D.; Harrat, Z.; Dvořák, V.; Volf, P.; Halada, P. Phlebotomine sand fly survey, blood meal source identification, and description of *Sergentomyia imihra* n. sp. in the central Sahara of Algeria. *Parasit. Vectors* **2024**, *17*, 449. [CrossRef]
18. Azzouzi, C.; Rabah-Sidhoum, N.; Boucheikhchoukh, M.; Mechouk, N.; Sedraoui, S.; Benakhla, A. Checklist of Medico-Veterinary Important Biting Flies (Ceratopogonidae, Hippoboscidae, Phlebotominae, Simuliidae, Stomoxyini, and Tabanidae) and Their Associated Pathogens and Hosts in Maghreb. *Parasitologia* **2025**, *5*, 1. [CrossRef]
19. Azzouzi, C.; Boucheikhchoukh, M.; Mechouk, N.; Sedraoui, S.; Zenia, S. Monthly and Daily Dynamics of *Stomoxys calcitrans* (Linnaeus, 1758) (Diptera: Muscidae) in Livestock Farms of the Batna Region (Northeastern Algeria). *Parasitologia* **2025**, *5*, 52. [CrossRef]

20. Dekkiche, K.; Benatallah, A.; Marniche, F. First comprehensive assessment of dipteran diversity and vectorial potential in northeastern Algeria: Ecological, veterinary, and one health perspectives. *Comp. Immunol. Microbiol. Infect. Dis.* **2026**, *126*, 102438. [CrossRef]
21. Zhang, K.; Sparling, J.; Chow, B.L.; Elsayed, S.; Hussain, Z.; Church, D.L.; Gregson, D.B.; Louie, T.; Conly, J.M. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *J. Clin. Microbiol.* **2004**, *42*, 4947–4955. [CrossRef]
22. Bhowmick, P.P.; Devegowda, D.; Karunasagar, I. Virulotyping of seafood associated *Salmonella enterica* subsp. *enterica* isolated from Southwest coast of India. *Biotechnol. Bioinf Bioeng.* **2011**, *1*, 63–69.
23. Doumith, M.; Buchrieser, C.; Glaser, P.; Jacquet, C.; Martin, P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* **2004**, *42*, 3819–3822. [CrossRef]
24. Dos Santos, L.S.; Sá, J.C.; Dos Santos Ribeiro, D.L.; Chaves, N.P.; da Silva Mol, J.P.; Santos, R.L.; da Paixão, T.A.; de Carvalho Neta, A.V. Detection of *Brucella* sp. infection through serological, microbiological, and molecular methods applied to buffaloes in Maranhão State, Brazil. *Trop. Anim. Health Prod.* **2017**, *49*, 675–679. [CrossRef]
25. Paton, A.W.; Paton, J.C. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx 1, stx 2, eaeA, enterohemorrhagic *E. coli* hlyA, rfb O111, and rfb O157. *J. Clin. Microbiol.* **1998**, *36*, 598–602. [CrossRef]
26. Yang, J.; Liu, Z.; Niu, Q.; Liu, J.; Han, R.; Guan, G.; Hassan, M.A.; Liu, G.; Luo, J.; Yin, H. A novel zoonotic *Anaplasma* species is prevalent in small ruminants: Potential public health implications. *Parasit. Vectors* **2017**, *10*, 264. [CrossRef]
27. de la Fuente, J.; Atkinson, M.W.; Naranjo, V.; Fernández de Mera, I.G.; Mangold, A.J.; Keating, K.A.; Kocan, K.M. Sequence analysis of the msp4 gene of *Anaplasma ovis* strains. *Vet. Microbiol.* **2007**, *119*, 375–381. [CrossRef]
28. Teja, M.M.S.; Mamatha, G.S.; Lakkundi, J.N.; Chandranaik, B.M.; Murthy, C.M.K.; Gomes, A.R. Multiplex PCR for detection of *Anaplasma marginale, A. bovis* and *A. platys* in cattle. *J. Parasit. Dis.* **2023**, *47*, 659–663. [CrossRef]
29. Massung, R.F.; Slater, K.; Owens, J.H.; Nicholson, W.L.; Mather, T.N.; Solberg, V.B.; Olson, J.G. Nested PCR assay for detection of granulocytic ehrlichiae. *J. Clin. Microbiol.* **1998**, *36*, 1090–1095. [CrossRef]
30. Berri, M.; Rekiki, A.; Boumedine, K.S.; Rodolakis, A. Simultaneous differential detection of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. *BMC Microbiol.* **2009**, *9*, 130. [CrossRef]
31. Jensen, W.A.; Lappin, M.R.; Kamkar, S.; Reagan, W.J. Use of a polymerase chain reaction assay to detect and differentiate two strains of *Haemobartonella felis* in naturally infected cats. *AM. J. Vet. Res.* **2001**, *62*, 604–608. [CrossRef]
32. Faburay, B.; Geysen, D.; Munstermann, S.; Taoufik, A.; Postigo, M.; Jongejan, F. Molecular detection of *Ehrlichia ruminantium* infection in *Amblyomma variegatum* ticks in The Gambia. *Exp. Appl. Acarol.* **2007**, *42*, 61–74. [CrossRef]
33. Hafsa, B.; Karima, K.; Lotfi, D.; Pont, A.C. First record of *Haematobia irritans* (L.) (Diptera: Muscidae) infesting cattle in Northeastern Algeria. *Parasitol. Res.* **2026**, *125*, 23. [CrossRef]
34. Kernif, T.; Leulmi, H.; Raoult, D.; Parola, P. Emerging Tick-Borne Bacterial Pathogens. *Microbiol. Spectr.* **2016**, *4*. [CrossRef]
35. Sommer, A.J.; Skarlupka, J.H.; Teseo, S.; Otani, S.; Suen, G.; Coon, K.L.; Sapountzis, P. Genomic evidence for flies as carriers of zoonotic pathogens on dairy farms. *npj Biofilms Microbiomes* **2025**, *11*, 111. [CrossRef]
36. Castro, B.G.; Souza, M.M.; Régua-Mangia, A.H.; Bittencourt, A.J. Enterobacterial microbiota on *Stomoxys calcitrans* external surface. *Transbound. Emerg. Dis.* **2010**, *57*, 22–24. [CrossRef]
37. Bertelloni, F.; Bresciani, F.; Cagnoli, G.; Scotti, B.; Lazzerini, L.; Marcucci, M.; Colombani, G.; Bilei, S.; Bossù, T.; De Marchis, M.L.; et al. House Flies (*Musca domestica*) from Swine and Poultry Farms Carrying Antimicrobial Resistant Enterobacteriaceae and *Salmonella*. *Vet. Sci.* **2023**, *10*, 118. [CrossRef]
38. Blazar, J.M.; Lienau, E.K.; Allard, M.W. Insects as vectors of foodborne pathogenic bacteria. *Terr. Arthropod Rev.* **2011**, *4*, 5–16. [CrossRef]
39. Sommer, A.J.; Deblois, C.L.; Tu, A.D.J.; Suen, G.; Coon, K.L. Opportunistic pathogens are prevalent across the culturable exogenous and endogenous microbiota of stable flies captured at a dairy facility. *Vet. Res.* **2025**, *56*, 40. [CrossRef]
40. Hogeveen, H.; Steeneveld, W.; Wolf, C.A. Production diseases reduce the efficiency of dairy production: A review of the results, methods, and approaches regarding the economics of mastitis. *Annu. Rev. Resour. Econ.* **2019**, *11*, 289–312. [CrossRef]
41. Nayani, S.A.; Meraj, S.; Mohr, E.; Gries, R.; Kovacs, E.; Devireddy, A.; Gries, G. *Staphylococcus* microbes in the bovine skin microbiome attract blood-feeding stable flies. *Front. Ecol. Evol.* **2023**, *11*, 1212222. [CrossRef]
42. Nayani, S.; Meraj, S.; Renyard, A.; Gries, G. Blood-feeding stable flies, *Stomoxys calcitrans* (Diptera: Muscidae), are attracted to, and transmit *Staphylococcus aureus* (Bacillales: Staphylococcaceae), a causal agent of bovine mastitis: A laboratory pilot study. *J. Med. Entomol.* **2024**, *61*, 1470–1477. [CrossRef]
43. Bertolini, A.B.; Prado, A.M.; Thyssen, P.J.; de Souza Ribeiro Mioni, M.; de Gouvea, F.L.R.; da Silva Leite, D.; Langoni, H.; de Figueiredo Pantoja, J.C.; Rall, V.M.; Guimarães, F.F.; et al. Prevalence of bovine mastitis-related pathogens, identified by mass spectrometry in flies (Insecta, Diptera) captured in the milking environment. *Lett. Appl. Microbiol.* **2022**, *75*, 1232–1245. [CrossRef]

44. Sommer, A.J.; Worley, T.K.; Sapountzis, P.; Coon, K.L. Comparative genomics reveals phylogenetic intermixing of Stomoxys fly, manure, and bovine mastitis-associated bacteria in dairy settings. *bioRxiv* **2025**, 2025.01.31.635862. [CrossRef]
45. Prorok, P.; Bierowiec, K.; Skrok, M.; Karwańska, M.; Siedlecka, M.; Miszczak, M.; Książczyk, M.; Kapczyńska, K.; Rypuła, K. Characteristics of *Staphylococcus saprophyticus* Isolated from Humans and Animals. *Int. J. Mol. Sci.* **2025**, *26*, 6885. [CrossRef]
46. Sorour, H.K.; Shalaby, A.G.; Abdelmagid, M.A.; Hosny, R.A. Characterization and pathogenicity of multidrug-resistant coagulase-negative Staphylococci isolates in chickens. *Int. Microbiol.* **2023**, *26*, 989–1000. [CrossRef]
47. de Oliveira, R.P.; da Silva, J.G.; Aragão, B.B.; de Carvalho, R.G.; Juliano, M.A.; Frazzon, J.; Farias, M.P.O.; Mota, R.A. Diversity and emergence of multi-resistant *Staphylococcus* spp. isolated from subclinical mastitis in cows in of the state of Piauí, Brazil. *Braz. J. Microbiol.* **2022**, *53*, 2215–2222. [CrossRef]
48. EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union One Health 2024 Zoonoses Report. *EFSA J.* **2025**, *23*, e9759. [CrossRef]
49. Nabi, I.; Achek, R.; Karim, A.; Melzer, F.; Brangsch, H.; Elschner, M.C.; Neubauer, H.; El-Adawy, H. Serological, phenotypic and molecular characterization of brucellosis in small ruminants in northern Algeria. *Front. Microbiol.* **2025**, *15*, 1505294. [CrossRef]
50. Baldacchino, F.; Muenworn, V.; Desquesnes, M.; Desoli, F.; Charoenviriyaphap, T.; Duvallet, G. Transmission of pathogens by *Stomoxys* flies (Diptera, Muscidae): A review. *Parasite* **2013**, *20*, 26. [CrossRef]
51. Merdja, S.E.; Dahmani, A.; Khaled, H.; Bouyoucef, A. Concurrent seroprevalence of the zoonotic diseases Q fever and brucellosis and their association with abortion in small ruminants from Medea province, northern Algeria. *J. Zoonotic Dis.* **2026**, *10*, 1114–1122. [CrossRef]
52. Castro, B.G.; Souza, M.M.; Regua-Mangia, A.H.; Bittencourt, A.J. Occurrence of Shiga-toxigenic *Escherichia coli* in *Stomoxys calcitrans* (Diptera: Muscidae). *Rev. Bras. Parasitol. Vet.* **2013**, *22*, 318–321. [CrossRef]
53. Feghrour, A.; Bounamous, A. Identification of the fungal and bacterial microflora in the midgut and cuticle of phlebotomine sandflies from Northeastern Algeria. *Env. Exp. Biol.* **2025**, *23*, 193–200. [CrossRef]
54. Boularias, G.; Azzag, N.; Gandoin, C.; Bouillin, C.; Chomel, B.; Haddad, N.; Boulouis, H.J. Bovines Harbor a Diverse Array of Vector-Borne Pathogens in Northeast Algeria. *Pathogens* **2020**, *9*, 883. [CrossRef]
55. Saada, C.; Maillard, R.; Pelletier, C.; Hygonenq, M.C.; Robert, H.; Citti, C.; Nouvel, L.X. Investigating *Mycoplasma wenyonii* and *Candidatus* Mycoplasma haematobovis coinfection patterns in cattle from southwestern France reveals strain-specific traits. *Vet. Res.* **2026**, *57*, 143. [CrossRef]
56. Hofmann-Lehmann, R.; Meli, M.L.; Dreher, U.M.; Gönczi, E.; Deplazes, P.; Braun, U.; Engels, M.; Schüpbach, J.; Jörger, K.; Thoma, R.; et al. Concurrent infections with vector-borne pathogens associated with fatal hemolytic anemia in a cattle herd in Switzerland. *J. Clin. Microbiol.* **2004**, *42*, 3775–3780. [CrossRef]
57. Thongmeesee, K.; Chonglomkrod, B.; Srisakdi, C.; Saributr, M.; Suksai, P.; Kamkong, P.; Tiawsirisup, S. Molecular detection of *Mycoplasma wenyonii* and its closely related hemotropic *Mycoplasma* sp. in blood-sucking flies from a buffalo farm in Chachoengsao province, Thailand. *Acta Trop.* **2022**, *235*, 106647. [CrossRef]
58. Meyer, D.F.; Moumène, A.; Rodrigues, V. Microbe Profile: *Ehrlichia ruminantium*—Stealthy as it goes. *Microbiology* **2023**, *169*, 001415. [CrossRef]
59. Esemu, S.N.; Ndip, L.M.; Ndip, R.N. Ehrlichia species, probable emerging human pathogens in sub-Saharan Africa: Environmental exacerbation. *Rev. Env. Health* **2011**, *26*, 269–279. [CrossRef]
60. Smit, A.; Mulandane, F.C.; Wójcik, S.H.; Malabwa, C.; Sili, G.; Mandara, S.; Pineau, K.P.; Lecollinet, S.; Exbrayat, A.; Rodrigues, V.; et al. *Ehrlichia ruminantium* (Ehrlichiaceae) infection rates and genotyping in *Amblyomma* species from southern Africa. *Ticks Tick. Borne Dis.* **2025**, *16*, 102462. [CrossRef]
61. Hornok, S.; Abichu, G.; Takács, N.; Gyuranecz, M.; Farkas, R.; Fernández De Mera, I.G.; De La Fuente, J. Molecular screening for Anaplasmataceae in ticks and tsetse flies from Ethiopia. *Acta Vet. Hung.* **2016**, *64*, 65–70. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.