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THESE

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Stomoxys calcitrans in northeastern and central Algeria: relative abundance, effect of abiotic factors and potential vector role.

Option : Entomologie, Protozoologie et Maladies vectorielles

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Abstract

Stomoxys calcitrans, commonly known as “the Stable fly,” “the dog fly,” or “the anthrax fly,” is a significant pest that affects humans and livestock because of its painful bites and its ability to transmit a variety of pathogens, both zoonotic and non-zoonotic. The current study focuses on the abundance, the dynamics, the factors influencing the distribution, as well as the potential vector role of *S. calcitrans* in the Northeastern and central regions of Algeria by daily and monthly monitoring during a year of study using Vavoua traps, statistical analysis and modeling, and molecular tools. It also aims to determine any particular host preference for *S. calcitrans*.

Six farms have been involved in the present study, two from the central region and four in the northeastern part of Algeria. The entomological survey resulted in 1251 *S. calcitrans*/ 3246 other flying species, with an overall density recorded in two farms monitored by the order of 20.06 *S. calcitrans*/trap/day. Significantly more males than females have been trapped (1107 ♂/ 137♀) in the eastern region.

The seasonal activity of *S. calcitrans* in Northeastern Algeria was characterized by an unimodal activity pattern extended from August to December, corresponding to the summer end and fall seasons, while no activity was observed during the rest of the year. The daily dynamics differed monthly; during the high activity period, *S. calcitrans* activity was bimodal, whereas during the remaining months, it was unimodal.

The present study has revealed a preference for stomoxes toward cattle hosts over other animal species.

Climatic conditions are the main driver of the stable flies' distribution, especially temperature. We identified a strong positive correlation between the *S. calcitrans* counts and temperature and rainfall. On the other hand, statistical modeling revealed that all the weather parameters significantly influence the stable fly count.

A molecular survey was conducted to identify a potential vector role of *S. calcitrans* in Algeria. One hundred five pools of *S.calcitrans* have been analyzed using conventional and nested Polymerase chain reactions; they were screened for *Bartonella* sp., Anaplasmatacae, *Habronema microstoma*, and *Habronema musca* DNA. The results revealed the absence of any significant pathogenic DNA. However, out of the 105 pools tested for Anaplasmatace, 21 (20%) were positive, and sequencing showed it to be *Wolbachia* sp. endosymbiotic bacterium.

The current study constitutes the first one in Algeria investigating *S. calcitrans* and the first molecular investigation of this pest in North Africa. Thus, it provides a baseline and initial data on the ecology and the potential vector role of the stable fly in Algeria. Consequently, it clarifies and supplies entomological information, enhancing the understanding of the vector system. Finally, this study marks a key step in establishing an appropriate control strategy to prevent *Stomoxys*-borne diseases, improve livestock breeding, and promote the country's economy.

Résumé

Stomoxys calcitrans, communément appelé « mouche piquante », « mouche du chien » ou « mouche charbonneuse », est un nuisible important qui affecte les humains et le bétail en raison de ses piqûres douloureuses et de sa capacité à transmettre divers agents pathogènes, tant zoonotiques que non zoonotiques. La présente étude s'intéresse à l'abondance, la dynamique, les facteurs influençant la distribution, ainsi que le rôle potentiel de vecteur de *S. calcitrans* dans les régions nord-est et centre de l'Algérie, par un suivi journalier et mensuel pendant une année d'étude en utilisant les pièges Vavoua, les analyses statistiques, la modélisations, et les outils moléculaires. Elle vise également à déterminer toute préférence particulière de *S. calcitrans* pour un hôte particulier.

Six fermes ont été exploitées durant la présente étude, deux dans la région centrale et quatre dans le nord-est de l'Algérie. L'enquête entomologique a permis de collecter 1251 *S. calcitrans*/3246 autres espèces volantes, avec une densité apparente globale dans les deux fermes suivies à l'ordre de 20,06 *S. calcitrans*/piège/jour. Le nombre de mâles piégés était significativement supérieur à celui des femelles (1 107 ♂/ 137♀) dans la région du Nord-est.

L'activité saisonnière de *S. calcitrans* dans le nord-est de l'Algérie était caractérisée par un schéma d'activité unimodal s'étendant d'août à décembre, correspondant à la fin de l'été et à l'automne, tandis qu'aucune activité n'était observée pendant le reste de l'année. La dynamique quotidienne variait d'un mois à l'autre ; pendant la période de forte activité, l'activité de S. *calcitrans* était bimodale, tandis que pendant les autres mois, elle était unimodale.

La présente étude a révélé une préférence des stomoxes pour les bovins comme hôtes par rapport aux autres espèces animales.

Les conditions climatiques sont le principal facteur déterminant de la répartition des mouches piquantes, en particulier la température. Nous avons identifié une forte corrélation positive entre le nombre de *S. calcitrans* et la température et les précipitations. D'autre part, la modélisation

statistique a révélé que tous les paramètres météorologiques influencent de manière significative le nombre de mouches piqueuse des étables.

Une étude moléculaire a été menée afin d'identifier le rôle potentiel de *S. calcitrans* en tant que vecteur en Algérie. 105 pools de *S. calcitrans* ont été analysés à l'aide de réactions en chaîne par polymérase conventionnelles et nichées ; ils ont été testés pour détecter la présence d'ADN de *Bartonella* sp., Anaplasmatacae, *Habronema microstoma* et *Habronema musca*. Les résultats ont révélé l'absence de tout ADN pathogène significatif. Cependant, sur les 105 pools testés pour Anaplasmatace, 21 (20 %) étaient positifs, et le séquençage a montré qu'il s'agissait de la bactérie endosymbiotique *Wolbachia* sp.

La présente étude est la première en Algérie à porter sur *S. calcitrans* et la première étude moléculaire de ce nuisible en Afrique du Nord. Elle fournit ainsi une base de référence et des données initiales sur l'écologie et le rôle potentiel de vecteur de la mouche piqueuse des étables en Algérie. Elle clarifie et fournit des informations entomologiques, améliorant ainsi la compréhension du système vectoriel. Enfin, cette étude marque une étape clé dans l'établissement d'une stratégie de contrôle appropriée pour prévenir les maladies transmises par *Stomoxys*, améliorer l'élevage du bétail et promouvoir l'économie du pays.

ملخص

Stomoxys calcitrans

، المعروفة باسم "الذبابة اللادغة"، أو "ذبابة الكلاب"، أو "ذبابة الجمرة الخبيثة"،تُعرف بأنها آفة خطيرة تؤثر على الإنسان والماشية بسبب لسعاتها المؤلمة وقدرتها على نقل العديد من الأمراض، سواء كانت حيوانية المنشأ أو غير ذلك. تهدف هذه الدراسة إلى دراسة وفرة هذه الذبابة وديناميكيتها والعوامل المؤثرة في توزيعها، بالإضافة إلى دورها المحتمل كناقل في مناطق الشمال الشرقي والوسط من الجزائر، من خلال متابعة يومية وشهرية على مدار عام باستخدام مصائد، والتحليلات الإحصائية، والنمذجة، والأدوات الجزيئية. كما تهدف إلى تحديد ما إذا كانت تمت الدراسة في ست مزارع، اثنتان في المنطقة الوسطى وأربع في الشمال الشرقي من الجزائر. وقد أسفرت الدراسة الحشرية عن جمع 1251 ذبابة *S. calcitrans*من أصل 3246 نوعًا آخر من الحشرات الطائرة، بكثافة ظاهرية إجمالية على مستوى مزرعتين بلغت 20.06 ذبابة لكل مصيدة في اليوم. وكان عدد الذكور المصطادة أعلى بكثير من عدد الإناث (1107 ♂ / 137 ♀) في منطقة الشمال الشرقي.

تميز النشاط الموسمي لـ *S. calcitrans*في الشمال الشرقي للجزائر بنمط أحادي الذروة يمتد من أغسطس إلى ديسمبر، أي في نهاية الصيف والخريف، بينما لم يُلاحظ أي نشاط خلال بقية السنة. أما الديناميكية اليومية فقد اختلفت من شهر لآخر؛ ففي فترة النشاط المرتفع كان النشاط ثنائي الذروة، بينما كان أحادي الذروة في الأشهر الأخرى.

كشفت الدراسة عن تفضيل واضح للذبابة للبقر كمضيف مقارنة بباقي الحيوانات. وتُعد الظروف المناخية، وخصوصًا درجة الحرارة، العامل الرئيسي في توزيع هذه الذبابة. وقد تم تحديد علاقة ارتباط إيجابية قوية بين عدد الذباب ودرجات الحرارة وكميات الأمطار. كما أظهرت النمذجة الإحصائية أن جميع العوامل المناخية تؤثر بشكل كبير على عدد الذباب في الحظائر.

تم إجراء دراسة جزيئية لتحديد الدور المحتمل لـ *S. calcitrans*كناقل في الجزائر. حيث تم تحليل 105 عينات باستخدام تفاعلات سلسلة البوليميراز التقليدية والمتداخلة، وتم اختبارها للكشف عن وجود الحمض النووي لـ *Bartonella sp.*، و *Anaplasmataceae*، و *Habronema microstoma*، و *Habronema musca.*لم تُظهر النتائج وجود أي حمض نووي ممرض مهم. ومع ذلك، من بين 105 عينات تم اختبارها لـ *Anaplasmataceae*، كانت 21 (20٪) إيجابية، وأظهر التسلسل أنها تعود للبكتيريا التكافلية *Wolbachia sp.*

تُعد هذه الدراسة الأولى من نوعها في الجزائر التي تتناول*S. calcitrans*

، وأول دراسة جزيئية لهذا النوع من الذباب في شمال إفريقيا.

وتوفر هذه الدراسة قاعدة بيانات مرجعية ومعلومات أولية حول بيئة الذبابة ودورها المحتمل كناقل، مما يعزز الفهم العلمي للنظام الناقل. وأخيرًا، تمثل هذه الدراسة خطوة مهمة نحو وضع استراتيجية فعالة للسيطرة على الأمراض المنقولة بواسطة *Stomoxys*، وتحسين تربية الماشية، وتعزيز الاقتصاد الوطني.

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ANOVA: Analysis of variance.

DNA: Deoxyribonucleic acid.

IPCC: Intergovernmental Panel on Climate Change.

P: Precipitation.

PCR: Polymerase Chain Reaction.

RH: Relative Humidity.

Sc*f*: *Stomoxys calcitrans* females.

Scm: *Stomoxys calcitrans* males.

Sct: *Stomoxys calcitrans* males.

T°C: Temperature (°C).

Tf: Total flies.

WS: Wind speed.

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Introduction

Over the past few decades, the alarming public and veterinary health situation has become increasingly acute, especially with the emergence of vector-borne diseases. There have been a growing number of calls to draw attention to this worrying situation and call on medical and veterinary entomologists, whose role is to optimize strategies and methods for combating human and animal vector-borne diseases. Interest must focus primarily on controlling the vector populations involved, especially with outbreaks occurring in new areas and burdens rising in endemic nations. For example, the first lumpy skin disease outbreaks are appearing in Algeria, which constitutes a real threat to the country's cattle herd **(FAO, 2024)**, given that cattle breeding in Algeria contributes enormously to the national economy, and this subsidiary provides the country's largest meat production share **(Sadoud et al., 2022)**.

Hematophagous arthropods implicated in the transmission of severe diseases for both humans and animals are Diptera from Culicidae, Ceratopogonidae, Muscidae, and Tabanidae families which are responsible for the transmission of zoonotic and non-zoonotic diseases such as West Nile fever, Dengue fever, Bluetongue disease, Lumpy skin disease, and the African Trypanosomiasis. Among the hematophagous Diptera belonging to the Muscidae family, the flies of the *Stomoxyni* tribe, called "Biting flies," are often underestimated by entomologists in Algeria **(Duvallet et al., 2017)**.

The *Stomoxys* genus comprises 18 species distributed in tropical Africa and Asia, with only one being cosmopolitan: *Stomoxys calcitrans,* commonly known as "the stable fly," "the dog fly," "the biting house fly," or " anthrax fly." The latter is attributed to this fly because of its involvement in anthrax transmission **(Kaufman et al., 2023; Turell & Knudson, 1987)**.

The Stable fly has a considerable pathogenic impact on domestic animals through their painful bites, hematophagous action, the negative correlation between their abundance and the quantity

of milk produced, and the average daily weight gain **(Zumpt, 1973)**. In the US alone, every year, the economic expenses of cattle weight loss linked to stable flies and their management have been estimated at around $100 million; this amount exceeded 1 billion US$ as a result of the economic impact of this pest on cattle industry **(Campbell et al., 1987; Campbell et al., 2001; Taylor & Berkebile, 2006)**. However, it is their role as vectors that is particularly important since many pathogens are almost mechanically transmitted by these insects, including viruses, bacteria, protozoa, and helminths, for example, the Lumpy skin disease virus, *Bacillus anthracis, Anaplasma marginale, Trypanosoma* spp. , and *Habronema microstoma* **(Baldacchino et al., 2013)**.

Stomoxys calcitrans is the most extensively studied species worldwide of all species in the *Stomoxys* genus; the main focus of investigations into this pest has been on the dynamics and the factors influencing its development, and also its associated pathogens **(Gilles, 2005; Gilles et al., 2005; González, Ruiz-Arrondo, et al., 2024; Makhahlela et al., 2022)**. However, in Algeria, studies on important veterinary vectors and their associated pathogens mainly concern Sandflies, biting midges, and ticks (Acari) **(Benallal et al., 2022; Boucheikhchoukh et al., 2018; Kadjoudj et al., 2022; Lafri & Bitam, 2021)**, very few studies have been focused on brachyceran flies and they were interested in the diversity and vector role **(Boucheikhchoukh et al., 2019; Ourrad et al., 2022)**.

The poorly documented situation of *Stomoxys calcitrans* in Algeria led us to undertake this study to fill the gap and provide initial data regarding this fly species. In addition, knowledge and understanding of the biology and ecology of these pests will enable us to establish an appropriate and effective control strategy against them, consequently improving the country's livestock and preventing diseases transmitted by these vectors.

Objectives and Issues

To gain a deeper understanding of the biology and ecology of stomoxes in northeastern and central Algeria, particularly the factors governing population dynamics, it is essential to set up an effective integrated pest management program to limit the nuisance of these insects.

The objectives of our study are listed below:

1- Population count, identification of captured flies, and comparison of sex distribution in the *S. calcitrans* population.

2- Relative abundance of *S. calcitrans* determination in the caught fly population.

3- Studying the seasonal dynamics of this fly and measuring the importance of climatic factors in the different study regions.

4- Studying the daily activity and measuring the seasonal variation.

5- Determine any trophic preference of *S. calcitrans* towards particular animal species.

6- Research and detection of vector-borne pathogens carried by *S. calcitrans*.

By achieving these objectives, we will be able to provide clear answers to the following questions:

- What are the daily and seasonal peak periods for the *S. calcitrans* fly?
- What are the main climatic factors influencing this distribution?
- Which animal species are most attractive to *S. calcitrans*?
- Which pathogens can be vectorized by *S. calcitrans* in Algeria?

Chapter I: *Stomoxys calcitrans*: the stable fly

I.1.Overview

Stomoxys Geoffroy, 1762 genus originated from the Old World **(Showler & Osbrink, 2015)**; it is distinguished from the other Stomoxyni genera by arista antennae with bristles only on the dorsal surface and maxillary palps that measure about one-third of the proboscis **(Zumpt, 1973)**.

This genus includes 18 species, of which the only cosmopolitan species is *S. calcitrans*. The remaining species have an Afro-tropical or Asian distribution. Four species are exclusively Asian, twelve are exclusively African, and one is Afro-Asian **(Sharif, 2018)**.

S. calcitrans is a hematophagous fly harmful to both humans and livestock; it is known as the stable fly or the charcoal fly because of its implication in the mechanical transmission of *Bacillus anthracis*, the anthrax pathogen **(Dsouli-Aymes, 2009)**. The "biting house fly" is another common name given to *S. calcitrans* because of its similarity in appearance with the house fly *Musca domestica* **(Patra et al., 2018) (Figure 1)**.

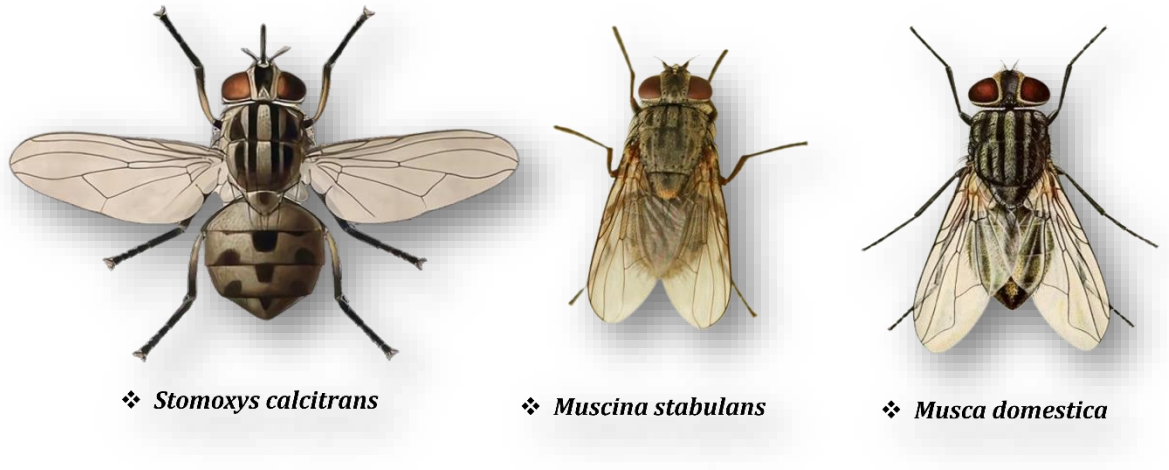

Figure 1: Similarity in appearance between the stable fly, false stable fly, and the house fly

Their piercing mouthparts make them a real nuisance, causing several persistent skin lesions in livestock in addition to stress **(González et al., 2022; Vitela-Mendoza et al., 2016)**. This stress leads to loss of milk production and weight gain, especially in cattle **(Taylor et al., 2012)**. In addition, *S. calcitrans* can mechanically transmit various pathogens, including viruses, bacteria, and protozoa. However, it is known to be a cyclic vector of a helminth: *Habronema microstoma,* an equine parasite **(Baldacchino et al., 2013)**.

I.2.Taxonomy of the stable fly

Stomoxys calcitrans is an:

- Animal (Reign: Animalia)

Eukaryote metazoan heterotroph.

- Arthropod (Branch: Arthropoda)

Metazoan invertebrates with bilateral symmetry and a chitinous exoskeleton.

From the Greek “arthron”, meaning "joint", and “podos”, meaning "foot": organisms with articulated appendices. Arthropods comprise about two million species, or 80-85% of the animal kingdom **(Dajoz, 2010; Duvallet et al., 2017; Roth, 1974)**.

- Mandibulata (Sub-branch: Mandibulata)

Have at least a pair of antennae, mandibles, and a pair of maxillae **(Duvallet et al., 2017)**.

- Insect (Class: Insecta)

The Body consists of three distinct parts: head, thorax, and abdomen, with three pairs of locomotor attachments and a pair of antennas. Adults have two pairs of wings. The respiratory system is tracheal **(Dajoz, 2010; Duvallet et al., 2017)**.

- Pterygote (Sub-class: Pterygota)

Insects that can be wingless (Siphonaptera, Phtiraptera), or have two pairs of wings carried by the mesothorax and metathorax (Diptera), the second pair being atrophied, acting as a balancer. Holometabolous or heterometabolous development **(Engel, 2015)**.

- Dipteran (Order: Diptera)

Holometabolous species (complete metamorphosis), with mouthparts of various types: biting, licking, and sometimes absent, and with a single pair of well-developed mesothoracic wings, the metathoracic wings transformed into pendulums. Females are usually oviparous, sometimes larviparous or pupiparous **(Dajoz, 2010; Duvallet et al., 2017; Roth, 1974)**.

- Brachycera cyclorrhapha (Sub-Order: Brachycera, Section: cyclorrhapha)

From the Greek: "brachy" meaning short, and "ceros" meaning horn, made up of three articles, the last of which can be subdivided. Smooth, fly-like body with short legs. Anterior circular rupture of nymphal integument. Headless larvae have three larval stages **(Duvallet et al., 2017; Roth, 1974)**.

- Muscidae (Family: Muscidae)

This family includes over 5,000 species and 200 genera. Adults are dull-colored species with biting or licking mouthparts and no mandibles or jaws. The labium and hypopharynx are housed in the labium's concavity. Antennae with hairy arista along its entire length or naked. Well-developed calypters (spoons that hide the pendulums). Vein A1 does not reach the wing margin. L3 larvae with posterior stigmatic plates with three sinuous slits. Maggot mouthparts form hooks **(Haseyama et al., 2015)**.

- Muscinae (Sub-family: Muscinae)

Oviparous species. Wings form an acute angle at rest **(Salem, 2012)**.

- Stomoxyni (Tribe: Stomoxyni)

Biting proboscis in both males and females, powerful, rigid, extending in front of the head, formed by a perforated, corny labium, containing two stylets (labrum and hypopharynx), laterally a pair of maxillary palps. The maxillary palps can be about the same length as the proboscis or much shorter **(Duvallet et al., 2017; Salem, 2012) (Figure 2)**.

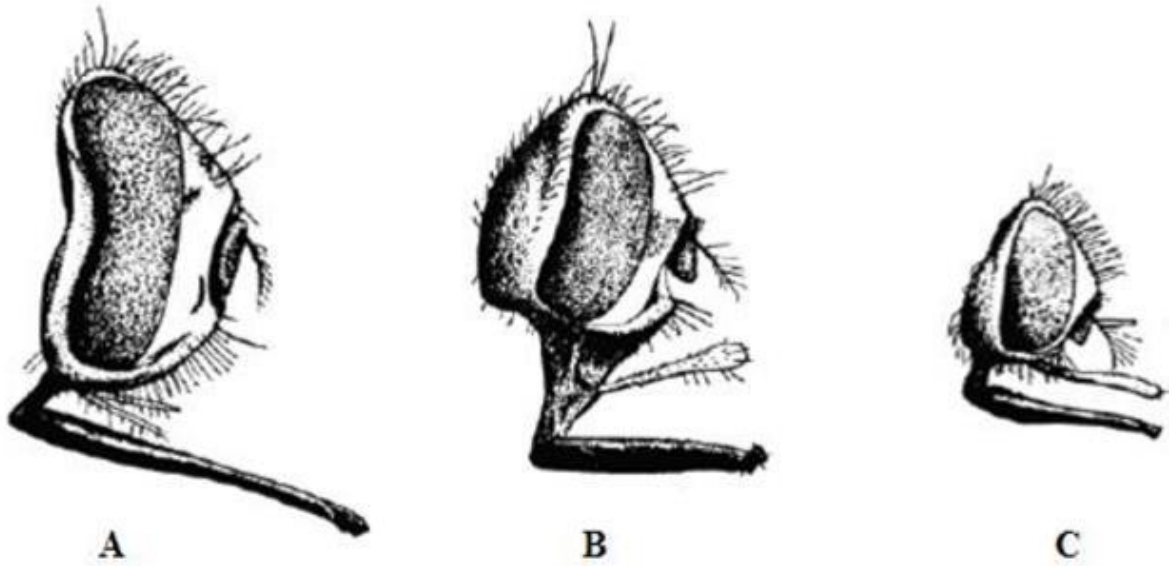

Figure 2: Stomoxyini heads différenciation *:* A*: Stomoxys calcitrans* (Linnaeus, 1758), B*: Heamatobia stimulans* (Meigen, 1824), C*: Haematobia irritans* (Linnaeus, 1758) (Zumpt, 1973).

- *Stomoxys* (Genus: *Stomoxys* sp.)

Arista is hairy only on the dorsal side, and maxillary palps are much shorter than the proboscis. Thorax has four dark longitudinal stripes; the wings are transparent, longer than the abdomen, and not crossed at the resting position **(Zumpt, 1973)**.

Stomoxys calcitrans (Linnaeus, 1758).

Species 6-8 mm long with maxillary palps shorter than the proboscis (measure about a third of the proboscis length). Thorax has four black dorsal stripes and a checkerboard of brown and grey spots on the dorsal side of the abdomen, which is wider than long **(Figure 3)**.

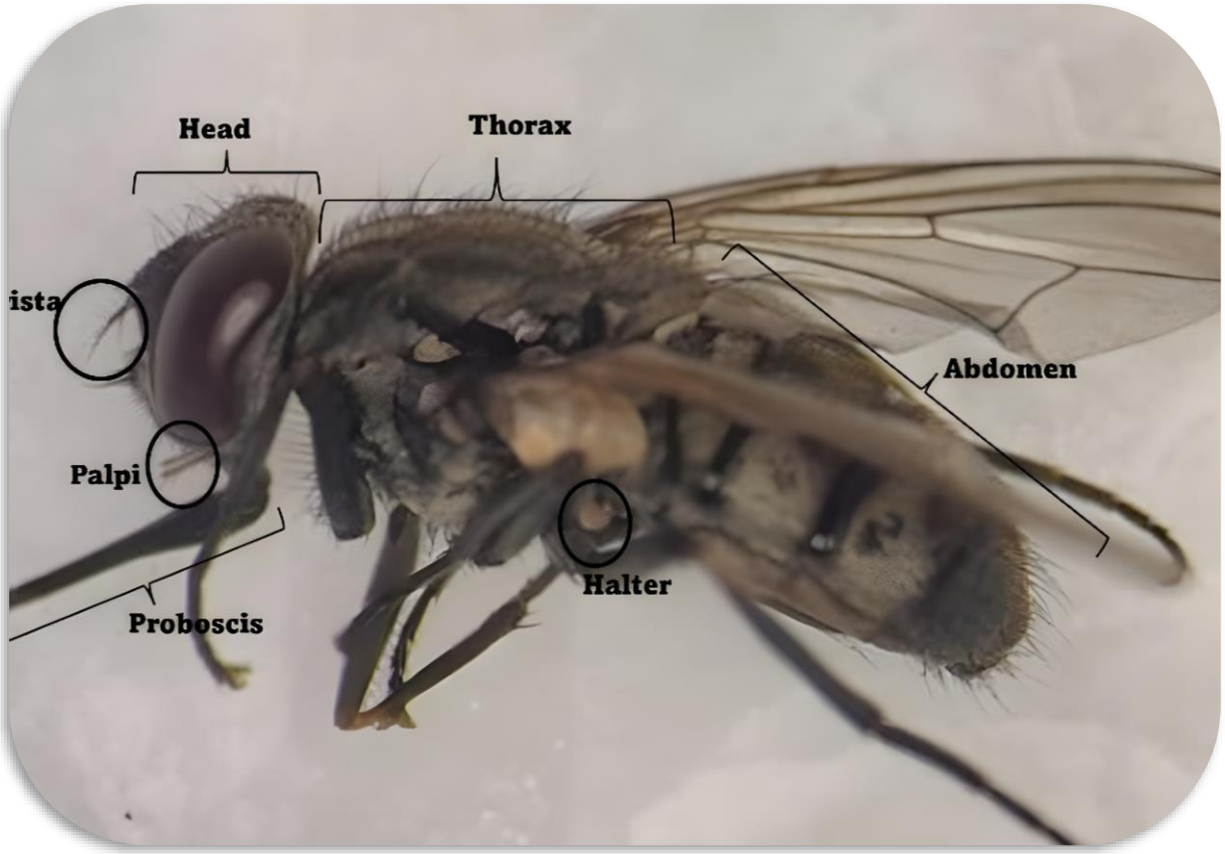

Figure 3: Morphology of an adult stable fly *Stomoxys calcitrans* (Original illustration).

I.3. Morphology of the stable fly

As indicated above, *S. calcitrans* has an insect morphology with a body divided into three segments: the head, the thorax, and the abdomen. Each segment is briefly described below.

I.3.1.The head

The head contains the sensory organs corresponding to the compound eyes, ocelli, antennae, and biting mouthparts. *Stomoxys calcitrans* has both kinds of insect eyes: three dorsal ocelli on

the forehead and crown and two large compound eyes on the front portion of each side of the head **(Peterson, 1916; Ranade, 1970; Salem, 2012) (Figure 4)**.

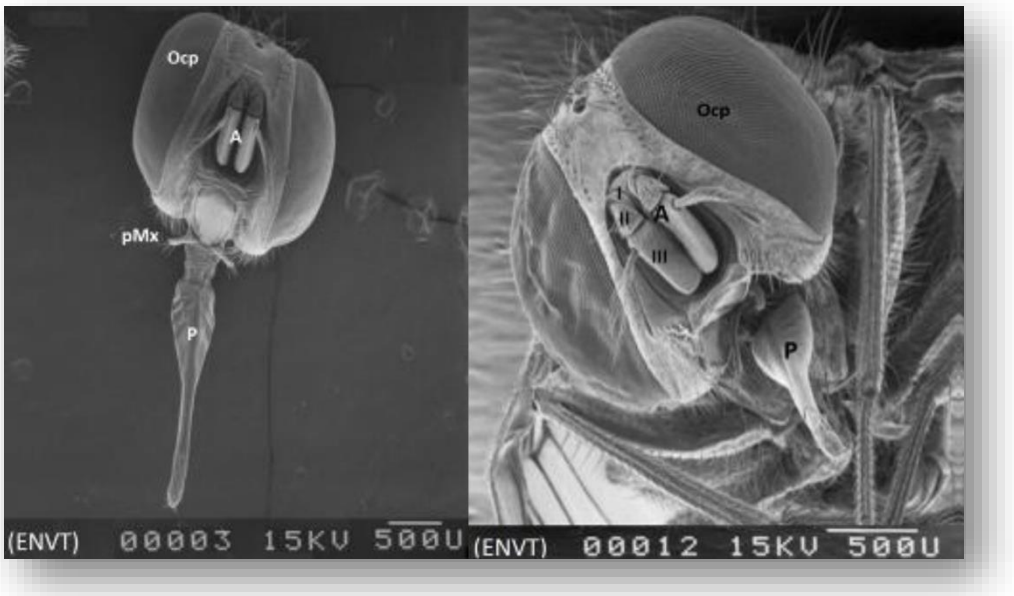

Figure 4: Head of *S. calcitrans* **(Salem, 2012)**

A: antenna, (I,II,III: antennal segments). P: proboscis, Ocp: compound eyes, pMx: maxillar palpi.

Both sexes have the same, basically black or brownish-black antennae. Each antenna comprises three segments, just like all other Brachyceran Diptera: the scape, the pedicel, and the flagellum with the arista **(Sukontason et al., 2004)**. The arista possesses lengthy dorsal setae; the third segment is almost 2.5 times longer than the second **(Roungthip Masmeatathip et al., 2006; Zumpt, 1973) (Figure 5)**.

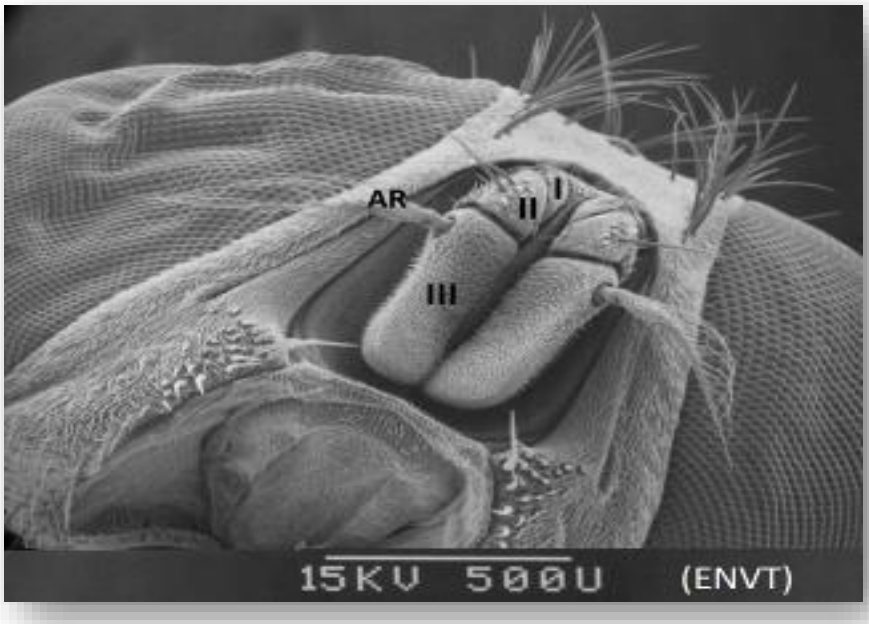

Figure 5: *S. calcitrans* antennae **(Salem, 2012)**.

AR: Arista, **(I,II, III)**: antennal segments.

The proboscis is rigid and non-retractable. It is carried horizontally forward in line with the body and is black. It comprises three long, strongly clarified, non-retractable parts: the labium or lower lip, which ends in a short labellum with developed peristomal teeth; the labrum or upper lip; and the hypopharynx. The tubular hypopharynx contains the salivary duct **(Smith, 1890; Veer et al., 2002) (Figure 6)**.

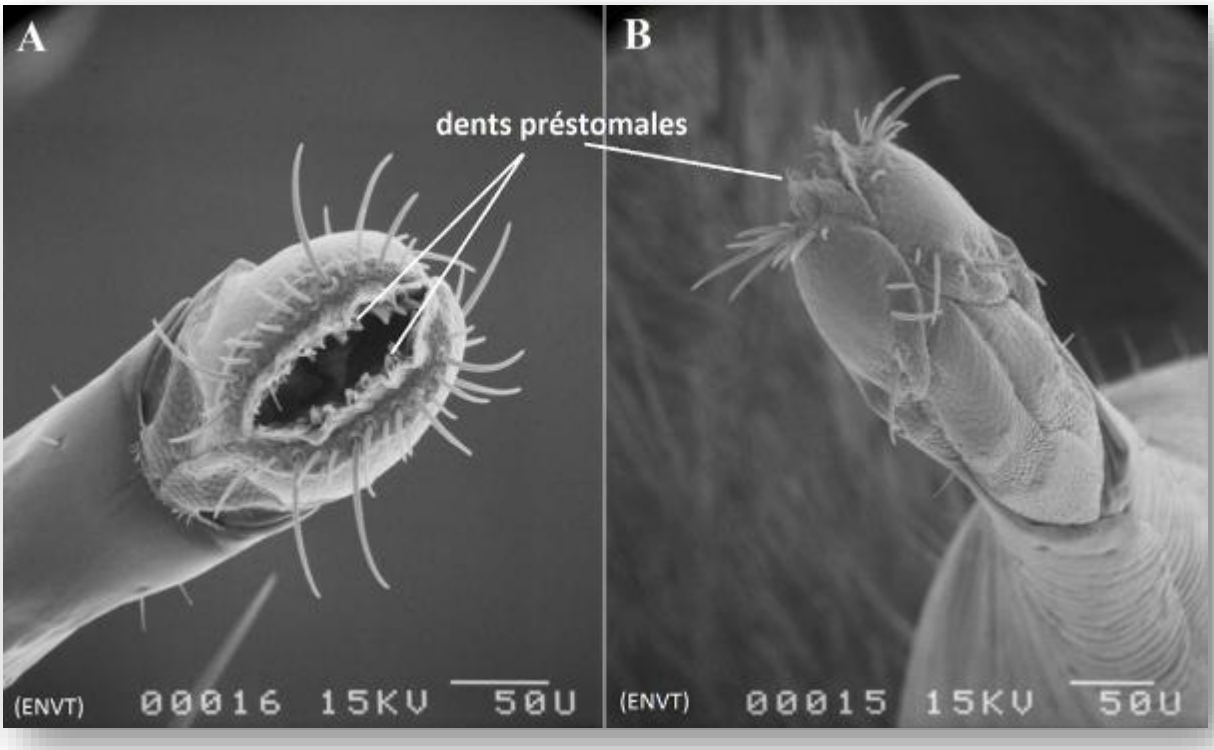

Figure 6: Mouthparts of *S. calcitrans* under electron microscopy **(Salem, 2012)**.

A: ventral view, **B:** Dorsal view.

I.3.2. Thorax

As with all insects, the thorax of *Stomoxys calcitrans* is divided into three segments from anterior to posterior: the prothorax, the mesothorax, and the metathorax. Each segment carries a pair of legs and consists of several cuticular plates called sclerites, which form the exoskeleton: the sternite or sternum ventrally, the tergite or tergum dorsally, and the pleurites laterally **(Chapman, 1998)**. The mesothorax is overdeveloped compared to the other two segments, as it carries the only pair of wings needed for flight; like all members of the Muscidae family, the stable flies' wings are hyaline, and their venation organization is one of the criteria used to the species diagnose **(Roungthip Masmeatathip et al., 2006; Roth, 1974) (Figure 7)**.

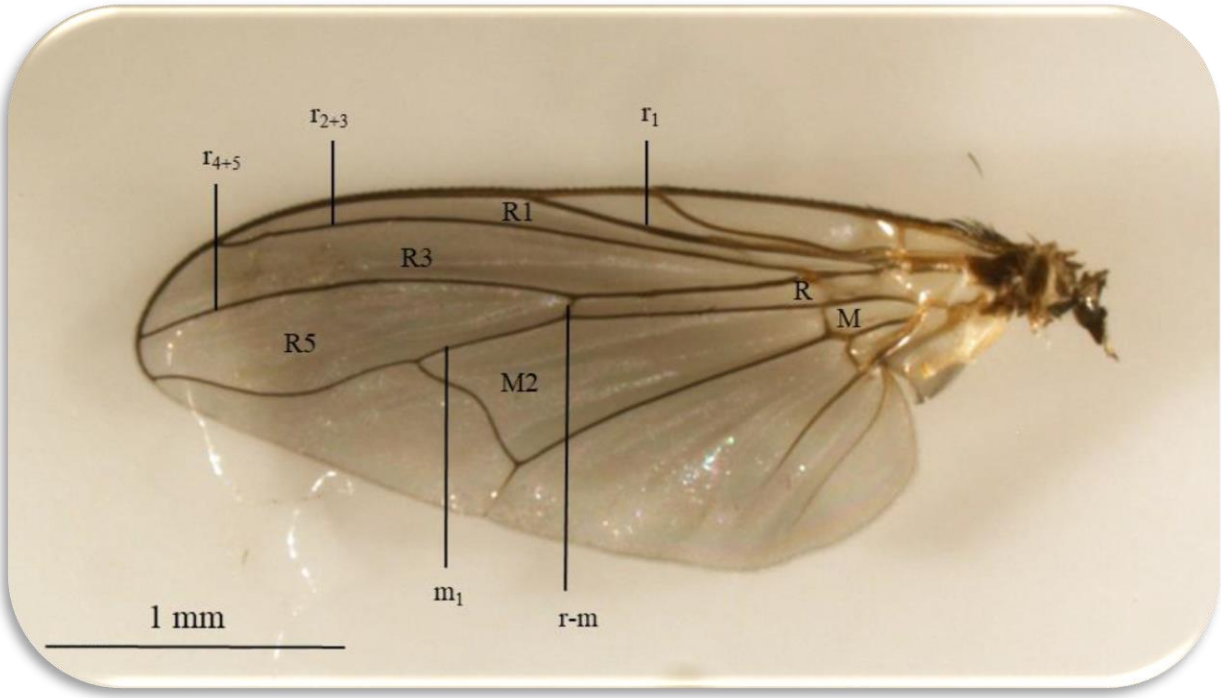

Figure 7: Left-wing venation of *S. calcitrans* **(Huyart, 2020)**.

I.3.3.Abdomen

The abdomen in insects is broader than it is long and is divided into two parts: the pre-abdomen and the post-abdomen. The pre-abdomen consists of four segments, with tergites I and II fused into a single tergite called tergite I+II. However, the corresponding sternites are separate, resulting in four segments with four tergites and five sternites. The post-abdomen carries the reproductive organs. The abdomen is composed of tergites and sternites but lacks a pleurite. Instead, a thin tissue membrane connects the dorsal and ventral cuticular plates, allowing the abdomen to expand significantly, particularly ventrally, during a blood meal. The second and third segments of the abdomen have a dark spot in the middle and two dark spots on each side, and This checkerboard pattern distinguishes *S. calcitrans* from other *Stomoxynae* species **(Roungthip Masmeatathip et al., 2006; Zumpt, 1973)**.

I.3.4. Legs

There are five articles on the dark-colored legs of *S. calcitrans*: the coxa, trochanter, femur, tibia, and tarsus, composed of five articles that may be lighter or even yellowish at their base **(Figure 8)**. Two lateral claws outline a pair with adhesive bristles at the extremities of the legs, allowing the *Stomoxys* flies to attach to any surface and in any direction. An independent sensory bristle known as “the empodium” is located at the center of this complex **(Roungthip Masmeatathip et al., 2006)**.

Figure 8: Leg of *S. calcitrtans*: 1: Coxa, 2: Trochanter, 3: Femur, 4: Tibia, 4: Tarsis **(Huyart, 2020)**.

I.2.Bio-ecology of Stomoxys calcitrans

I.2.1.Feeding behavior

Stable flies are diurnal, spending most of their time resting on supports. They can fly long distances to feed and migrate to more favorable conditions; it has been noted that *S. calcitrans* can migrate 5 km or more in search of a blood meal source, and some marked flies have even been found up to 100 km from their marking site **(Bailey et al., 1973; Hogsette et al., 1987)**.

S. calcitrans flies are blood and nectar feeders; blood is necessary for oogenesis and spermatogenesis, while nectar improves reproductive capacities when added to the blood diet, especially larval emergence **(Müller et al., 2012; Tawich et al., 2021)**.

The first stable flies' meal may occur within a few hours of emergence. They can take one or several blood meals per day, Cattle, horses, dogs, and humans are among the majority of the hosts they feed on **(Hafez & Gamal-Eddin, 1959; Mavoungou et al., 2008; Skidmore, 1985)**.

Like all insect vectors, *S. calcitrans* employ a variety of multimodal cues at various points in space, including host fragrance, colour, morphological, auditory, gustatory, and mechanosensory signals. They use these multimodal cues to reduce errors and make nearly perfect decisions while finding their blood meal source, nectar, and mating among them Fragrance, which is crucial in helping flies find suitable hosts and avoid unsuitable environments **(Birkett et al., 2004; Getahun et al., 2024)**. Carbon dioxide is the main attractant for host-seeking stable flies; however, Visual and thermal host-like features are the primary draws for the host-foraging stable flies **(Hung et al., 2024)**. Moreover, host skin microbiota attracts stable flies; this microbiota changes from host to host; this is why we can find an attractivity of these flies to certain species; for example, *Staphylococcus* bacteria in the cattle skin attract host-seeking *S. calcitrans* **(Nayani, 2024; Nayani et al., 2023)**.

Once on their host, *S. calcitrans* feed on the lower limbs, including human feet and the forelegs of horses and cattle; in dogs, however, the flies prefer to bite the ears **(Dougherty et**

al., 1995; Yeruham & Braverman, 1995), their bites are extremely painful due to the absence of anaesthetic substances, however, an important signaling biomolecule: lysophosphatidylcholine is present in their salivary glands and plays a role in a variety of metabolic activities, such as host immunomodulation and pathogen proliferation and differentiation **(Florencio et al., 2024)**.

I.2.2. Life cycle

S. calcitrans females are oviparous species. Their development cycle comprises six stages: eggs, three larval stages (L1, L2, L3), pupae, and adults **(Figure 9)**. Several stages are strongly affected by temperature and humidity, which impact the overall cycle time **(Foil & Hogsette, 1994; Gilles et al., 2005; Lysyk, 1998)**.

A male *Stomoxys* can fertilize several females, while the female can only mate with a single male. The male must have ingested at least one blood meal before being able to fertilize, while the female needs at least three blood meals to reach maturation for the first egg-laying **(Anderson, 1978)**. Mating can occur both in flight and at rest; it lasts an average of 5 minutes and it is usually more frequent when both sexes are 4 to 5 days old **(Duvallet et al., 2017; Salem et al., 2012)**.

Gravid females *S. calcitrans* oviposit in various organic substrates such as animal excrement; they are directed by cues generated from potential oviposition sites carried by airborne microbes **(Nayani et al., 2024)**. Livestock manure is preferable for stable fly development **(Khwanket et al., 2024)**.

Egg-laying takes approximately 20 days, peaking at eight days; a female may lay a total of 700-800 eggs during her lifetime in batches of 25 to 50 **(Foil & Hogsette, 1994; Lysyk, 1998)**. Eggs take between 20 and 80 hours to hatch into L1 instar larvae, which then grow into L2 and L3 stages **(Skidmore, 1985)**.

During their growth phase, larvae are active and particularly attracted by the odors of horse and cattle droppings and various products (ammonia, ethylamine, trimethylamine, and acetone). The molt from L1 to L2 takes less than 24 hours, while the molt from L2 to L3 takes one day under optimal conditions. The L3 stage has the most extended lifetime: 8 days at 26°C and 80% humidity in summer or several months in winter **(Berry & Campbell, 1985; Hafez & Gamal-Eddin, 1959; Parr, 1959)**.

The larvae's tropism changes as they approach pupation; they prefer environments with humidity between 75 and 83% and temperatures between 15 and 25°C. L3 instar larvae become immobilized under decomposing organic matter in moist soil and pack in on themselves. The integument hardens, forming a protective envelope known as the "puparium"**(Hafez & Gamal-Eddin, 1959)**. This puparium houses the nymph, which evolves into an imago for several days before emerging through a circular opening, like all members of the Cyclorrhapha section. The average duration of this stage varies between 6 and 26 days, depending on temperature **(Meyer et al., 1991)**.

Stomoxys calcitrans doesn't exhibit winter diapause, They can overwinter in all stages, their cycle lengthens, and the various forms seek environments that allow them to escape the low temperatures **(Berry & Campbell, 1985; Lysyk, 1998)**.

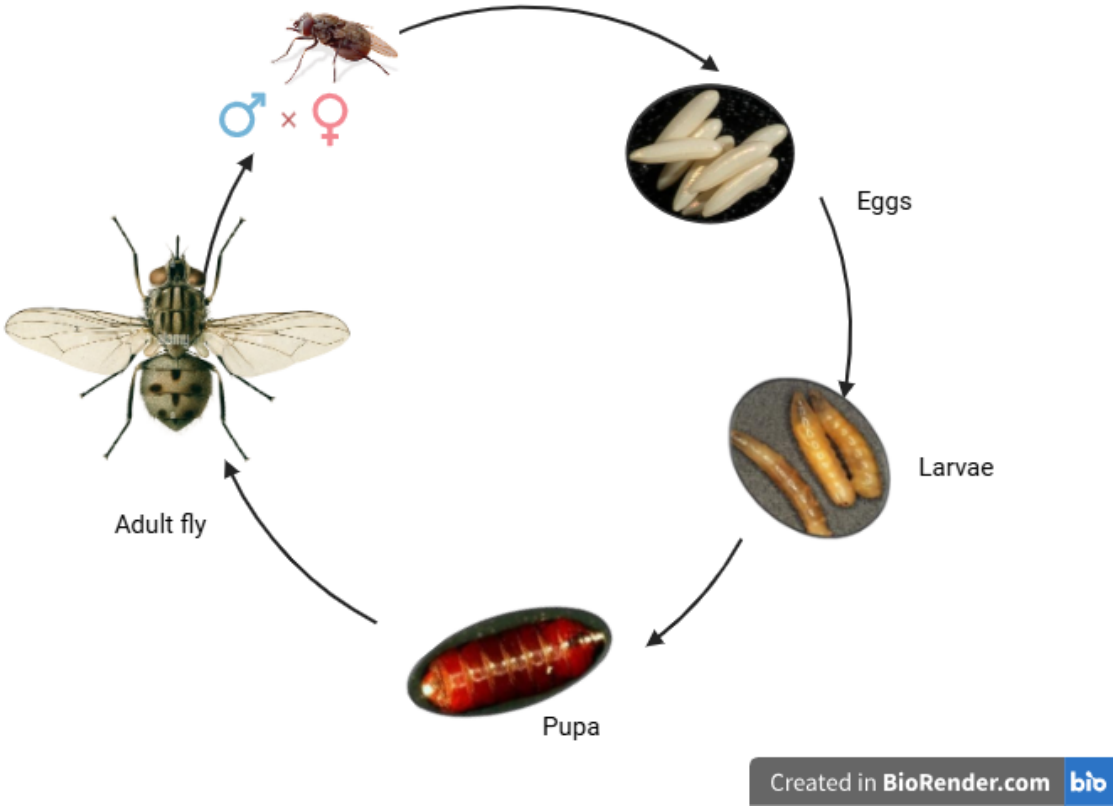

Figure 9: *S. calcitrans* lifecycle (Salem, 2012; Sharif, 2018)- Reconstructed.

I.2.3. Population dynamics

Stable flies are highly mobile. With minimal genetic differentiation, they spread locally between hosts and oviposition locations, regionally via weather fronts, and globally, most likely due to human livestock operations **(Showler & Osbrink, 2015)**. For instance, in Nebraska, equitable distribution of stable flies occurred in all directions up to 5.1 km from larval sites; 50% of the population stays within 1.6 km, affecting livestock within a 5 km diameter **(Taylor et al., 2010)**.

Their population dynamics exhibit distinct seasonal patterns varying depending on the meteorological conditions in different areas; for instance, in temperate regions, they have a bimodal seasonal activity pattern, i.e., they have two activity peaks during the year, these peaks

are observed in different months according to the region, for example, in Nebraska, their activity peaks were observed between June and July, and at mid-September **(Taylor et al., 2007)**, while in Tunisia, they are more active from March to July, and between November and January **(Khalifa et al., 2022)**.

In Northern and tropical regions, stable flies adopt an unimodal activity pattern; the alternation of the cold and warm seasons and dry and humid seasons is the primary driver of *S. calcitrans* seasonality in these regions respectively **(Semelbauer et al., 2018)**.

In southwest England, stable fly populations gradually increased starting in June and peaked in late August and September **(Parravani et al., 2019)**. In Denmark, their abundance peak was observed in July **(Skovgård & Nachman, 2012)**. Brazilian *S. calcitrans* population dynamics exhibit seasonal behavior, peaking in November and December **(Rodríguez-Batista et al., 2005)**. In all the examples cited above, temperature and rainfall are key factors influencing the stable flies' dynamics.

The influence of different climatic parameters, mainly temperature and rainfall, on the dynamics of stable flies results from their influence on the fly's different life stages, from eggs to pupae, which will be further detailed and discussed in the Discussion section of the thesis.

I.3.Geographical distribution :

We describe in this section the geographical distribution of all the species of the genus *Stomoxys* Geoffory 1762, and provide some available pictures of some species **(Figure 13)**:

- ***S. bengalensis*** Picard, 1908: Oriental region.
- ***S. boueti*** Roubaud, 1911: Congo and Benin.
- ***S. indicus*** Picard, 1908: Eastern regions and Palearctic territories.
- ***S. inornatus*** Grunberg, 1906: tropical Africa.
- ***S. luteolus*** Villeneuve, 1934: central and western Africa.
- ***S. niger niger*** Macquart, 1851: Ethiopia **(Figure 10)**.

Figure 10: *S. n. niger* (by Lendzele).

- ***S. niger bilineatus*** Grunberg, 1906: prevalent across the Afro-tropical areas, except for arid or semi-arid zones, in both dense rainforest and savannah environments.
- ***S. ochrosoma*** Speiser, 1910: Central and western Africa.
- ***S. omega*** Newstead, 1907: Ethiopian region **(Figure 11)**.

Figure 11: *S. omega* (by Lendzele).

- ***S. pallidus*** Rounaud, 1911: Tropical Africa.
- ***S. pullus*** Austen, 1909: India.
- ***S. sitiens*** Rondani, 1873: Afro-tropical and eastern region.
- ***S. stigma*** Van Emden, 1939: Congo and Uganda.
- ***S. taeniatus*** Bigot, 1888: Ethiopian region.
- ***S. transvittatus*** Villeneuve, 1916: Southern and central Africa.
- ***S. uruma*** Shinonaga et Kano, 1966: Eastern region.
- ***S. varipes*** Bezzi, 1907: Eastern and central Africa.
- ***S. xanthomelas*** Roubaud, 1937 **(Figure12)**: Congo, Tanzania and Uganda.

Figure 12: *S. xanthomelas* (by Lendzele).

S. calcitrans Linnaeus, 1758: Worldwide distributed, *S. calcitrans* feeds on cattle, horses, donkeys, pigs, goats, sheep, and dogs. Old World Originating has spread worldwide due to the mobility of people and animals **(Marquez et al., 2007)**. An oriental origin has been suggested for *S. calcitrans* **(Zumpt, 1973)**; however, a recent study deduced that the Ethiopian region would be the most likely origin of *S. calcitrans* **(Tsai et al., 2023)**.

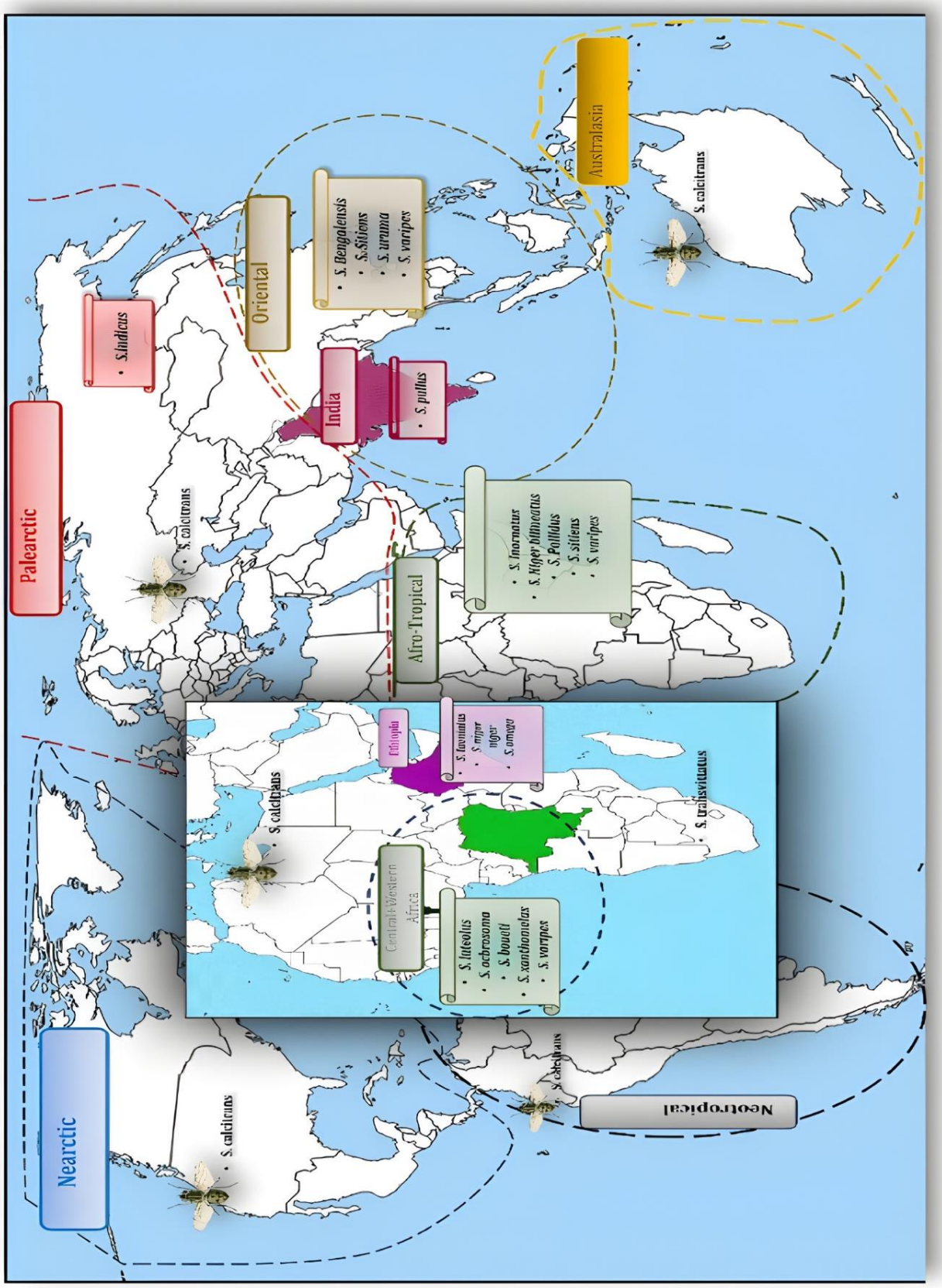

Figure 13: Geographical Distribution of the *Stomoxys* species (Original illustration).

I.4. Importance of the stable fly

I.4.1.Direct effects

Stable flies are persistent and aggressive blood-feeders, both male and female. When their favorite hosts are unavailable, they can even harm and feed on humans **(Rochon et al., 2021)**. They can imbibe 11–15 μL of blood on average per meal; females ingest more blood than males; however, the hosts' defensive movements of the head, ears, skin, legs, and tails do not allow them to obtain a whole blood meal in a single meal. Therefore, they usually result in host energy loss, stress, or decreased time spent eating and consuming feed **(Holloway & Phelps, 1991; Schowalter & Klowden, 1979)**.

Bites from stable flies damage more than other biting flies **(Elzinga & Broce, 1986)**, The lack of anesthetic components in *S. calcitrans'* saliva makes their bites extremely painful and stressful **(Cortinas & Jones, 2006)**. During their high population activity periods, blood loss and feeding disruptions may cause a 10-15% reduction in body weight. Moreover, compared to animals treated with pesticides, untreated animals showed an average body weight reduction of 0.2 kg **(Campbell et al., 2001; Patra et al., 2018)**.

Estimating the loss in milk production revealed that a single stable fly can cause an average loss of 0.7% per cow, A total of 8.51 $ was estimated to have been lost by the producer for each animal, with five stable flies per front leg, while most of the loss (88%) was attributed to lower feed efficiency **(Bruce & Decker, 1958; Campbell et al., 1987)**.

In addition, *S. calcitrans* bites cause severe persistent lesions due to their piercing mouthparts which perforate the host skin; these lesions encourage the development of myiasis agents **(González et al., 2022)**.

Dogs, horses, and calves had skin sores due to stable fly bites; in dogs, cutaneous lesions varying from large, ulcerated areas to blade spots were identified **(Braverman, 1995; Santos et al., 2018)**.

Stable flies are also a major annoyance for beef calves because they are implicated in a decrease in food intake and efficiency, which lowers weight gain **(Talley, 2008)**.

I.4.2.Indirect effects: vector role

Pathogen transmission mechanisms in *S. calcitrans*

Stable flies are vectors of several pathogens, including bacteria, viruses, protozoa, and worms (table 1). They can mechanically transmit this variety of pathogens because they interrupt the blood meal due to the host's defensive movement. Consequently, they may inject some of the contaminated blood that's still in their mouthparts **(Baldacchino et al., 2013)**. Furthermore, experimental evidence has demonstrated that stable flies are able to regurgitate a portion of their previous blood meal before consuming a new one **(Butler et al., 1977)**.
In addition, a portion of the blood ingested by *S. calcitrans* can reach its crop, where disease agents may be able to persist longer due to the more suitable conditions and the lack of digestive secretions **(Coronado et al., 2004)**.

The identification of *Habronema microstoma* DNA in different segments of *S. calcitrans,* including heads, thoraces, and abdomens of both field-collected and lab-reared flies, proved that stable flies are intermediate hosts of this nematode **(Traversa et al., 2008)**.

On the other hand, although Stable flies transmitted mechanically *Trypanosoma* sp. under experimental conditions **(Mihok et al., 1995; Mohammed et al., 2010; Sumba et al., 1998)**, this transmission would not be accomplished without the presence of a biological vector of these parasites **(Heller et al., 2024)**.

Pathogens transmitted by *S. calcitrans*

Recently, the eradication of lumpy skin disease remains difficult; stable flies are found to be able to transmit the disease from even subclinically infected cattle, which makes disease control more challenging (Haegeman et al., 2023). Consequently, the key to controlling vector-borne diseases, especially those transmitted by stable flies, is managing these pests, which will be reviewed in the next section.

The table below reviews pathogens from several groups, including bacteria, viruses, protozoa, and helminths associated with stable flies. The association could be a proven mechanical or biological transmission under experimental or natural conditions or a possible transmission; we mean by a possible transmission that the pathogen was only detected in the fly without an experimental demonstration for its transmission.

Table: Pathogens associated with the stable fly *S. calcitrans*

Table 1: Pathogens associated with *S. calcitrans*

Pathogen	Disease	Geographic incidence	Host	Transmission	Reference
Bacteria					
Bacillus anthracis	Anthrax	Worldwide	Mammals	Mechanical	(Turell & Knudson, 1987)
***Brucella* spp.**		Worldwide	Cattle	Mechanical (Possible)	(Krinsky, 1976)
Dermatophilus congolensis	Cutaneous streptothricosis	Worldwide		Mechanical	(Richard & Pier, 1966)
Erysipelothrix rhusiopathiae	Erysipelas	Worldwide	Swine, mammals, and birds	Mechanical	(Wellmann, 1950)
Enterobacter sakazakii	Meningitis, enterocolitis, sepsis	Worldwide	Immunocompromised infants	Biological Mechanical	(Mramba et al., 2007)
Francisella tularensis	Tularemia	North America, Europe, Northern Africa, the Middle East, Asia	Invertebrates, mammals, and birds	Mechanical	(Krinsky, 1976; Olsufiev, 1940)
Pasteurella multocida	Hemorrhagic Septicemia	Worldwide	Buffalo	Mechanical	(Nieschulz & Kraneveld, 1929)
Anaplasma marginale	Anaplasmosis	Worldwide	Cattle	Mechanical	(Araújo et al., 2021; Scoles et al., 2005; Tian et al., 2023)
Mycoplasma wenyonii	Mycoplasmosis		Cattle	Possible	(Thongmeesee et al., 2022)
Viruses					
AFSV	African swine fever	Africa, Sardinia (Italy)	Suidae	Mechanical	(Balmos et al.; Mellor et al., 1987; Schwarz et al., 2020)
LSDV	Lumpy skin disease	Africa, Middle East	Cattle	Mechanical	(Haegeman et al., 2023; Sohier et al., 2019)
EIAV	Equine infectious anemia	Worldwide	Equids	Mechanical	(Foil et al., 1983; Hawkins et al., 1973)
WNFV	West Nile fever	Worldwide	Avian, Human	Mechanical	(Doyle et al., 2011; Johnson et al., 2014)
RVFV	Rift valley fever	Africa, Middle East	Ruminants, camels, Humans	Mechanical	(Hoch et al., 1985)
BHV	Infectious bovine rhinotracheitis	Worldwide	Cattle	Mechanical	(Gums et al., 1973)
BLV	Bovine leukosis	Worldwide	Cattle	Mechanical	(Krutko et al., 2022; Weber et al., 1988)

BDV	Viral bovine diarrhea	Worldwide	Calves	Possible	(Carlson et al., 2018)
VSV	Vesicular stomatitis	America	Cattle, horses, and swine	Mechanical	(Ferris et al., 1955)
BPV	Papillomatosis	Worldwide	Cattle	Possible	(Haspeslagh et al., 2018)
Poliomyelitis virus	Poliomyelitis		Monkeys	Mechanical	(Sawyer & Herms, 1913)
Protozoa					
Besnoitia besnoiti	Besnoisiosis	Africa, Asia, Europe, Middle East, South America	Cattle	Mechanical	(Liénard et al., 2013)
Leishmania tropica	Cutaneous Leishmaniasis	Asia, North Africa, Middle East.	Humans	Mechanical	(Berberian, 1938)
Trypanosoma brucei	Sleeping sickness	Sub-Saharan Africa	Humans	Mechanical	(Mihok et al., 1995)
	Nagana disease		Livestock		
Trypanosoma congolense	Trypanosomiasis	Africa	Livestock	Mechanical	(Sumba et al., 1998)
Trypanosoma evansi	Surra disease	Africa, Asia, Europe, South America	Livestock	Mechanical	(Sumba et al., 1998)
Trypanosoma vivax	Animal trypanosomiasis	Africa, South America	Livestock	Mechanical	(Mihok et al., 1995)
Theileria orientalis	Oriental Theileriosis	Worldwide	Livestock	Possible	(Hornok et al., 2020)
Theileria equi	Theileriosis	Worldwide	Livestock	Possible	(Hornok et al., 2020)
Theileria capreoli	Theileriosis	Worldwide	Livestock	Possible	(Hornok et al., 2020)
Theileria sinensis		Asia	Livestock	Possible	(Phetkarl et al., 2023)
Helminths					
Habronema microstoma	Habronemosis	Worldwide	Horses	Biological	(Traversa et al., 2008)
Dirofilaria repens	Subcutaneous nodules		Cats & dogs	Suspected	(Krinsky, 1976)
Dirofilaria roemeri			Wallabies and kangaroos	Suspected	
Onchocerca gibsoni	Subcutaneous filaria			Suspected	

I.5. Control methods

Multiple reasons make stable fly control challenging: first, variable assemblages of decaying vegetation are frequently their habitats during development; second, In many rural and urban settings, they can take advantage of cultural tradition; and finally, Adults are significantly mobilized **(Rochon et al., 2021)**.

Several control strategies have been used against stable flies to reduce their nuisance and improve breeding quality; these management options include multiple tools, such as chemicals, physicals, and even cultural options, to fight this pest **(Cook, 2020)**.

I.5.1. Physical management

Trapping, farm hygiene, organized sanitation, physical barriers to fly emergence, livestock protection, animal bedding, and manure improvements are physical methods for consistent fly control **(Cook, 2020)**.

Multiple trapping systems have been used to remove stable flies from regions where they seriously affected livestock; blue fabric tissue traps like Vavoua, Nzi, and the biconical trap were found to be more practical and less expensive for biting flies' trapping, especially the *Stomoxys* flies **(Djiteye et al., 1998; Gilles et al., 2007)**.

To enhance these traps, attempts have been made to capture more stable flies using a variety of components, among them dry ice, used separately or in combination with octenol, was found effective for trapping more stable flies when added to both Vavoua and Alsynite cylinder traps **(Cilek, 1999; Phasuk et al., 2016)**.

Black-white contrast tissues have also proven their effectiveness in capturing stable flies due to their attractivity to these colors **(Murchie et al., 2018)**. Furthermore, polyethylene terephthalate-made traps were most effective in capturing stable flies **(Taylor & Berkebile,**

2006). Other physical alternatives for stable fly trapping are the Knight Stick trap and Knight Stick Sticky wraps; they are effective for management because they use modified light waves and sticky wraps to draw in and collect stable flies **(Hogsette & Ose, 2017)**.

However, the trapping method is limited; traps attract other non-targeted species, which could threaten the ecological balance **(Gilles et al., 2007)**.

I.5.2. Chemical management

Various chemical options are involved in *S. calcitrans* management, each possessing processes and effectiveness. Different levels of susceptibility to pyrethroid insecticides, such as permethrin, deltamethrin, and cypermethrin, have been seen in *Stomoxys calcitrans* populations, indicating their efficiency in managing and suppressing these pests **(Lorn et al., 2022)**. Conventional pesticides remain the major tool for consistent fly control. Nevertheless, resistance development frequently reduces their efficacy **(Mramba, 2006)**.

Biopesticides, which include plant-derived compounds, showed a significant repellency against stable flies and other hematophagous Diptera, for example, catnip oil (*Nepeta cataria*); in field testing, they are more than 95% protective for up to six hours, making them helpful chemical choices for stable fly control. They also function as larval growth inhibitors and oviposition deteriorators **(Zhu et al., 2012; Zhu et al., 2014)**. Other essential oils have been proven their efficiency against *S. calcitrans*, among them clove bud, clove leaf, geranium, theme red, and oregano, and adulticidal activities of the tea tree; Melaleuca *alternifolia* oil has been reported, the same was reported for *Citrus aurantium* **(Changbunjong et al., 2022; Dillmann et al., 2020; Hieu et al., 2010)**.

Other findings demonstrated the potential of the commonly known Indian borage or *Plectranthus amboinicus* essential oil, which can be a substitute control agent since it exhibits fumigant and contact toxicities against the target species **(Leesombun et al., 2022)**.

I.5.3. Cultural management

Managing insect pests using cultural options remains usually the earliest method, they are frequently the most economical methods to control stable flies and extremely important everywhere stable flies continue to harm cattle; these options include, for example, the removal of larval development locations and stable fly oviposition substrates, adding substances to animal litter, to stop volatilizing ammonia, as examples of these substances: Calcium cyanamide, an extremely nitrogenous fertilizer and sodium bisulfate **(Cook, 2020)**.

I.5.4. Integrated pest management

In addition to cultural norms like animal protection and hygienic measures, the integrated pest management strategy for stable fly control contributes to environmentally beneficial and sustainable pest management **(Cook, 2020)**. The problem of sustaining stable fly populations has been recently addressed using a comprehensive and eco-friendly strategy **(González, Duvallet, et al., 2024)**. It involves combining all the above-cited options to minimize the annoyance of stable flies and keep the ecological balance. In this strategy, all stages of stable flies are targeted: an adult-specific trapping technique, immature stages are pretended using natural enemies like mites and wasps, in addition to animal protection and sanitary-cultural processes. Employing such an approach has a variety of implications: firstly, it encourages the use of control agents that can be obtained commercially. Secondly, it promotes the natural predators and beneficial insects of stable fly populations; and finally, it decreases the threat of selection favoring pesticide resistance **(Cook, 2020)**.

Chapter II: Materials & methods

II.1. Description of the trapping sites

The present study was conducted in two different geographical regions of Algeria: North-East and Centre. The Wilaya of Batna represents the northeastern region, while the Wilayas of Algiers and Boumerdes represent the central region.

The farm selection was made based on their proximity and owners' acceptance after being oriented by a regional veterinary colleague.

Four farms were selected in the Northeastern region and two in the center. The trapping sites are described below:

II.1.1. Northeastern region

- **Timgad cattle farm**

It is a large dairy cattle farm with a capacity of 1,400 cows and a herd of 400 cows. The farm is located in the commune of Timgad, in a village called "Ain Abderrahmane", and covers an area of 3800,000 m2.

- **Boumia cattle farm**

This farm is not quite as large as the first one, containing around a hundred heads of dairy and fattening cattle, sheep, dogs, and poultry. The farm is located in the commune of Boumia, in an agricultural region with corn and sorghum fields.

- **Small ruminant breeding**

This is a traditional small ruminant farm with dogs and poultry. The farm is located in the Oued Taga commune, in an agricultural region.

- **Ouyoun Elasafir Equestrian Center**

Located at Ayoun Laasafer, with around twenty horses **(Figure 14)**.

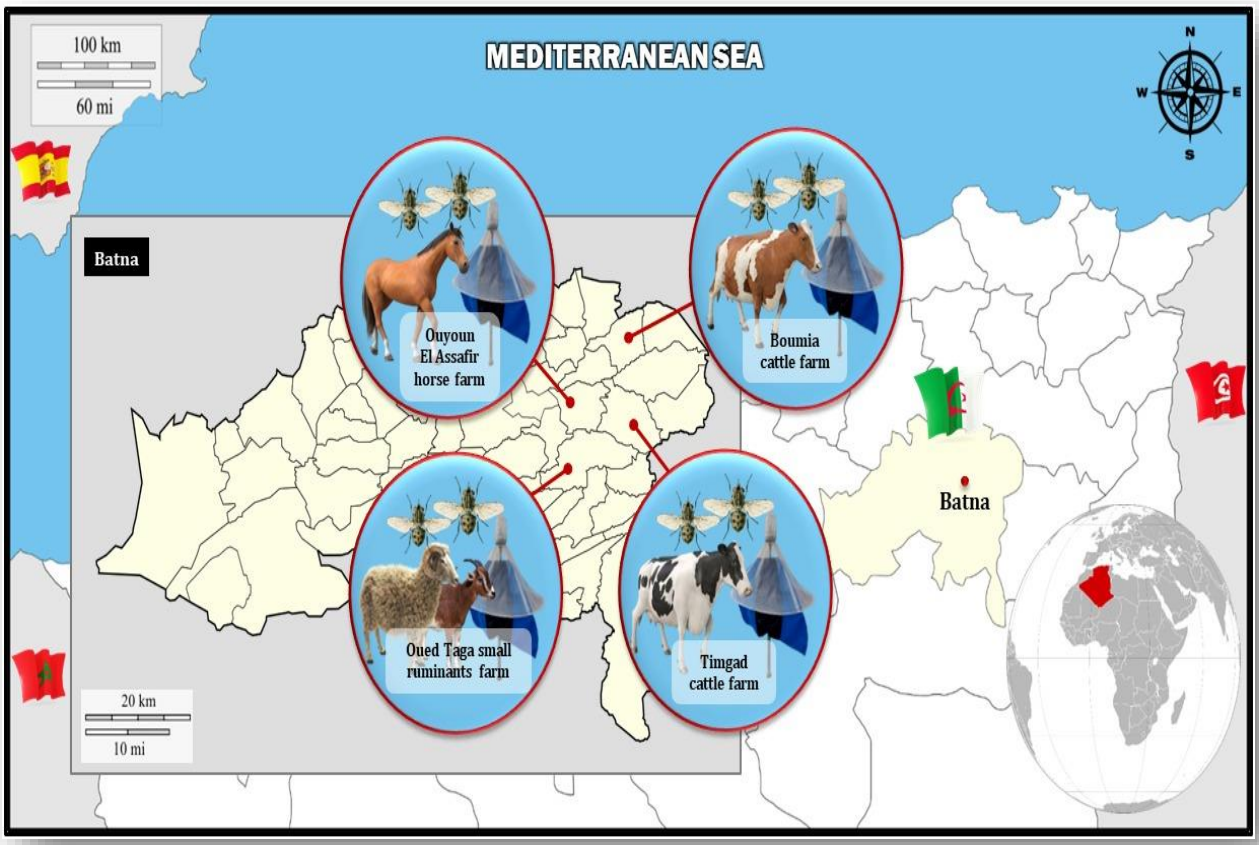

Figure 14: Localisation of the Prospected farms in the Eastern Region.

II.1.2. Central region

- **Boumerdes cattle farm**

A dairy cattle farm between Algiers and Boumerdes (Khemis El Khechna) consists of 16 cows.

- **Hraoua equestrian center**

This riding center contains around thirty horses, with a family area and a racecourse. It is located in an agricultural area in Hraoua **(Figure 15)**.

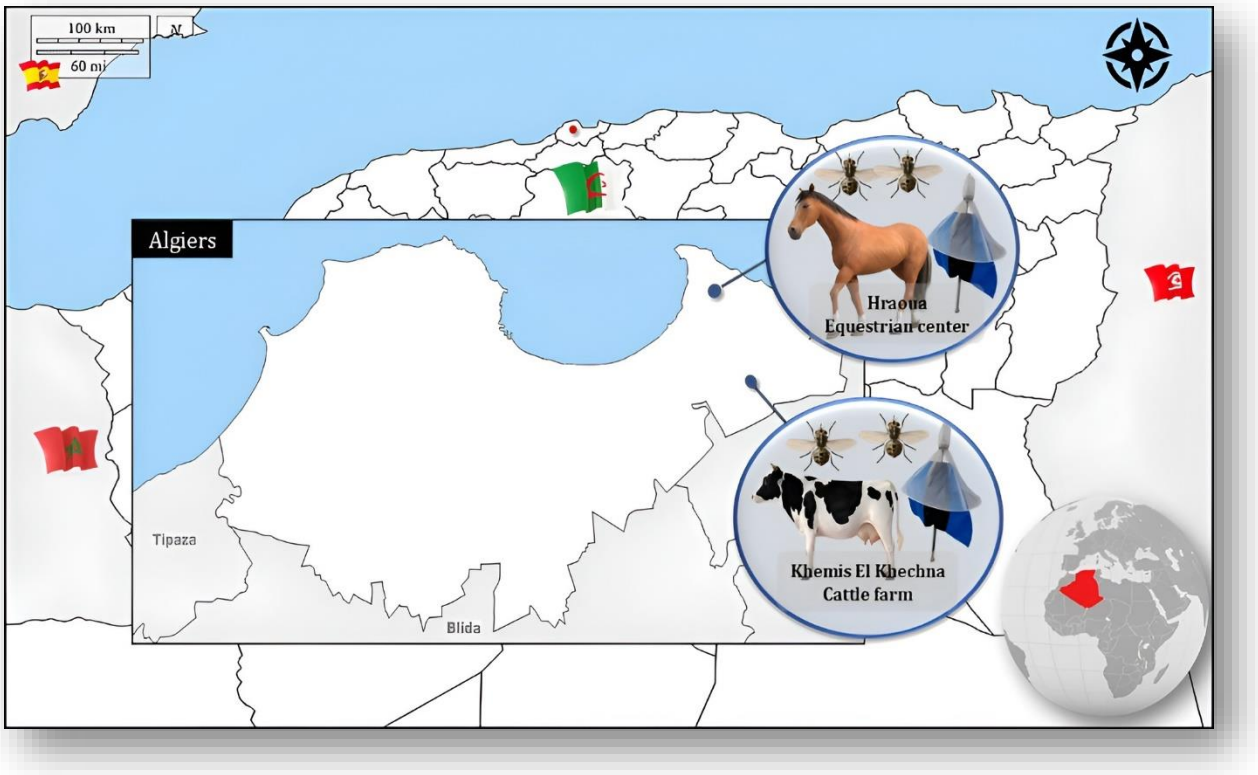

Figure 15: Localization of the Prospected farms in the Central region.

II.2. Vavoua trap

The Vavoua trap was first designed in Côte d'Ivoire, in the Vavoua region from which it took its name, as part of a control program against tsetse fly vectors of African Trypanosomiasis. This trap comprises three screens designed at 120°, blue called "Azur blue" and black; the external part is blue, and the internal part is black. These screens are surmounted by a cone of mosquito netting tulle, which, in turn, is surmounted by a plastic box called a "capture box"; the latter is filled with a liquid containing water, soap, and sugar to facilitate the capture of trapped flies. **(Laveissière, 1988)**.

The Vavoua trap used in our study is homemade **(Figure 16)**.

Figure 16: Vavoua trap (Mono-conical) used in the current study (Original picture).

II.3.3. Trapping procedure

Starting from July 2022, every month, the Vavoua trap was placed on each farm, 10 meters from the animal stabling area, distant from their circulation area, and 50 cm above the ground. During the trapping day, traps were set from 7 a.m. to 6 p.m. and every two hours; the trapped flies were collected and preserved in jars containing 70° alcohol and identified by the date of trapping, the interval hour of capture, and the farm.

We carried out annual monitoring at the Timgad cattle farm and the small ruminant farm. Continuous trapping every month for one year (2022-2023) allowed us to study stable flies' seasonal and daily dynamics. However, we could not continue the annual monitoring of the remaining farms. The period and frequency of trapping on each farm are shown in **Table 2**.

Table 2: Trapping period and frequency at each farm.

Farm	Trapping period	Number of trapping days
Timgad cattle	August 2022- July 2023	16
Small ruminants	July 2022- June 2023	13
Boumia cattle	July - August 2022	4
Ayoun Laasafer Equestrian Center	July - August 2022	3
H'raoua Equestrian center	August -September 2022	2
Boumerdes Cattle	August 2022	1

II.4. Count and identification of trapped flies

After every trapping trip, the flies caught in each hour are counted, and the stomoxes are identified in the parasitology laboratory using a binocular lens based on the Stomoxyinae identification keys. **(Zumpt, 1973)**.

For each two-hour fly collection, we noted:

- The total number of all the trapped flies (All combined species) (Mt).
- The total number of *Stomoxys calcitrans* (*Sc*T).
- The number of males *Stomoxys calcitrans* (*Sc*M).
- The number of females *Stomoxys calcitrans* (*Sc*F).

II.5. *Stomoxys* abundance calculation

Stable fly abundance is expressed by the fly density per trap and per day (FDT) and calculated using the following formula :

$$\boldsymbol{FDT} = \frac{Number\ of\ trapped\ S.calcitrans}{(Number\ of\ traps \times Number\ of\ trapping\ days)}$$

FDT: Fly density per trap **(Lendzele et al., 2019)**.

II.6. Meteorological data collection

Temperature, relative humidity, precipitation, and wind speed for every two hours of collection on each trapping day were first obtained online from the Prediction of Worldwide Energy Resource (POWER) project at the Langley Research Center (LaRC) of the National Aeronautics and Space Administration (NASA), funded by the NASA Earth Science/Applied Science Program **(Power, 2022)**.

The average measurement was taken for each parameter over two hours. In addition to this data, mini climate stations were installed on each farm to measure temperature and humidity in situ.

II.7. Statistical analysis

All data (T*f*, *Sc*T, *Sc*F, *Sc*M, T°C, P, WS, RH) were recorded in an Excel database. They were then processed and analyzed using the SPSS statistical software v.21.0 (IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp). The nonparametric Kruskal-Wallis test (ANOVA) has been used to compare the *S. calcitrans* distribution between the two farms.

Spearman correlation was used to correlate *S. calcitrans* abundance with each climatic parameter.

II.7.1. Statistical modeling

This section aims to evaluate how different climatological factors affect the prevalence of *S. calcitrans* flies. Since the dependent variable has many zeros and shows over-dispersion, it was impossible to establish linear regression and predict the number of stable flies as a function of various climatic variables.

We performed statistical modeling using the same database based on the results obtained from the nonparametric tests (ANOVA, correlation). Multiple regression analysis models were initially performed using the Huber-White-Hinkley method to fill the heteroscedasticity gap. Then, negative binomial regression was used to model the factors influencing *S. calcitrans* count by entering a factor each time. Then, the AICs are compared. The best model is the one with the lowest AIC.

All statistical analyses were performed using SPSS software v.21.0 statistical software (IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp).

II.8. Molecular analysis of *S. calcitrans* flies

This section was conducted in the molecular biology laboratory at the University of Agricultural Sciences and Veterinary Medicine (USAMV Cluj-Napoca, Roumania).

II.8.1. Pool preparation

Each two-hour collection of stomoxes was stored in plastic jars containing 70° Alcohol and identified by their location, date, and interval hour. At the laboratory, each *S. calcitrans* fly was cut longitudinally; the first half was subjected to molecular analysis, while the second one was stored in 70° Alcohol for further analysis.

Each pool contains one to five half-stomoxes, depending on every two-hour collection. One hundred five (105) pools were obtained: 86 from the Timgad Cattle farm, 18 from the small ruminant farm (Batna), and a single pool from the equine farm in Algiers.

II.8.2. DNA extraction

Pools were left over a night to be dried from Alcohol, then heated at 56°C for three hours to be sure that they were dried entirely from Alcohol. After that, they were processed for a DNA extraction using an Isolate II genomic DNA extraction kit (meridian, BIOSCIENCE®) following the manufacturer recommendations **(Figure 17)**:

- ✓ **The pre-lysis process**

180 µL of Lysis Buffer GL and 25 µL of proteinase K solution were added to each pool. The sample was completely covered and mixed by vortex. Incubate at 56°C for 3 hours (until complete lysis) with shaking from time to time.

- ✓ **The sample lysis**

Samples were well shaken, then 200 µl of lysis buffer was added, vortexed, and incubated at 70°C for 10 minutes.

- ✓ **Adjust DNA fixation conditions**

Vortex rapidly and add 210 µL of 96% ethanol to the pool. Shake well, then centrifuge for 90 seconds.

- ✓ **DNA fixation**

Place the kit's spin column in a 2-ml sample tube.

Introduce the sample into the column and centrifuge for one minute.

Discard the liquid and reuse the collection tube.

- ✓ **Silica membrane wash**

Add 500 µL washing buffer.

Centrifuge for one minute.

Discard the flow tube and reuse the collection tube.

Add 500 µL of wash buffer.

Centrifuge for a minute, then discard the flow tube and reuse the collection tube.

- ✓ **Drying the silica membrane**

Centrifuge for one minute to remove residual ethanol.

Place the collection column in a 1.5 ml tube.

- ✓ **DNA elution**

Add 100 µL of elution buffer pre-warmed to 70°C to the center of the silica membrane.

Let the mixture stand for one minute, then centrifuge for one minute.

Figure 17: DNA extraction (original illustration).

II.8.3. Polymerase chain reaction (PCR)

Before proceeding with the PCR, we targeted the pathogens to be investigated, the choice being made according to the availability of the material required for this technique: primers and positive controls are essential.

The pathogens targeted are the following:

- Anaplasmatacae family.
- *Bartonella* sp.
- *Habronema microstoma*
- *Habronema muscae*

Figure 18: PCR mix praparation (Original illustration).

The primers used for the detection of each pathogen are detailed in the table :

Table 3: Primers used for pathogen detection in the current study.

	Target gene	Product size	Forward Primer	Reverse Primer	Reference
Bartonella spp.	*gltA*	380–400	bart781: GGG GAC CAG CTC ATG GTG G	bart1137: AAT GCA AAA AGA ACA GTA AAC A	(Norman et al., 1995)
Anaplamataceae	16S		EHR16SD: 5′-GGT ACCYACAGAAGAAGT CC-3′	EHR12SR: 5′-TAGCACTCATCGTTTACA GC-3′	(Parola et al., 2000)
Habronema-first amplification			D (5k-GAGTCGATGAAGAAC GCAG-3k)	B1 (5k-GAATCCTGGTTAGTTTCT TTTCCT-3k)	(Traversa et al., 2004)
Habronema microstoma	ITS2		Hmi (5k-GATCGCAATATGTGT AACAC-3k)	B1 (5k-GAATCCTGGTTAGTTTCT TTTCCT-3k)	(Traversa et al., 2004)
Habronema muscae	ITS2		Hmu (5-CTGGTAAAGCATCAA TGCATCAG-GTATG-3)	B1 (5k-GAATCCTGGTTAGTTTCT TTTCCT-3k)	(Traversa et al., 2004)

II.8.4. Detection protocol for each pathogen

Preparation was carried out under sterile conditions in a safety hood. A specific detection protocol was used for each pathogen:

Anaplasmataceae :

The 16s gene was amplified in a mix of 8.5 µL H2O, 12.5 µL Master Mix (Rovalab), one microliter of each primer: EHR16SD and EHR12SR, and 2 µL DNA from each pool.

Each PCR tube contains 23µL of mix + 2 µL of DNA.

The cycler was programmed as follows :

Denaturation : 95°C 30s

94° C 30s

Hybridization: 55°C 30s

Elongation: 72°C 90s

***Bartonella* spp.**

A conventional PCR protocol has been used for *Bartonella* detection, targeting the *GltA* mitochondrial gen.

A mix of 6.5 µL water, 12.5 µL of Master Mix (Rovalab), one microliter of each primer (Bar 781et Bar 1137), and 4 µL of DNA from each pool.

PCR Program:

94°C during 5 min.

35 cycles :

- 94°C 30 s
- 49°C 30 s
- 72°C 1 min

72°C during 5 min.

Habronema microstoma* and *Habronema muscae

To detect the two species of *Habronema*, we used a semi-nested PCR-specific protocol amplifying ITS2 gen from DNA samples of *S. calcitrans* in a 1500 µl mix of Ampli Taq, 1020 µl of distilled water, 120µl of each primer. The set of primers D (5'-GAGTCGATGAAGAACGCAG-3') - B1 (5'-GAATCCTGGTTAGTTTCTTTTCCT-3') has been used in a first PCR step, which was followed by a second round using the primer set Hmi (5k-GATCGCAATATGTGTAACAC-3k)-B1 and Hmu (5-CTGGTAAAGCATCAATGCATCAG-GTATG-3)-B1.

In the first PCR step, 2µL of DNA was included in the reaction. In the second step, 1µL of each amplicon was subjected to the same conditions.

The cycles were processed as follows:

- Initial denaturation at 94°C for 12 min
- Denaturation: 30 cycles of 94°C for 30 s in the first PCR step and 35 cycles in the second.
- Hybridization: 58°C at 45 s.
- Elongation: 72°C at 45 s. followed by a final elongation step at 72°C during 7 min.

Positive and negative controls are added to each reaction. Positive DNA is added to the mix for the positive control, while no DNA is added for the negative control.

Figure 19: Polymerase chain reaction (PCR): Cyclers programming (Original illustration).

II.8.5. Gel Electrophoresis

Agarose gel electrophoresis is used to visualize PCR results. The gel must be prepared to be able to read the PCR results.

Gel preparation

Weight 2.1 g of Agar.

Pour into a 140mL of TBA solution.

Mix and heat in a microwave until boiling.

Add 7 µL of SYBER SAFE.

Once the gel is ready, pour it into a mold containing combs, remove the combs once the gel is cooled, then place 5µL of amplicons (PCR products) into each comb tooth. In the last point, we put a molecular weight marker. Then, electrophoresis is started. (**Figure 20**)

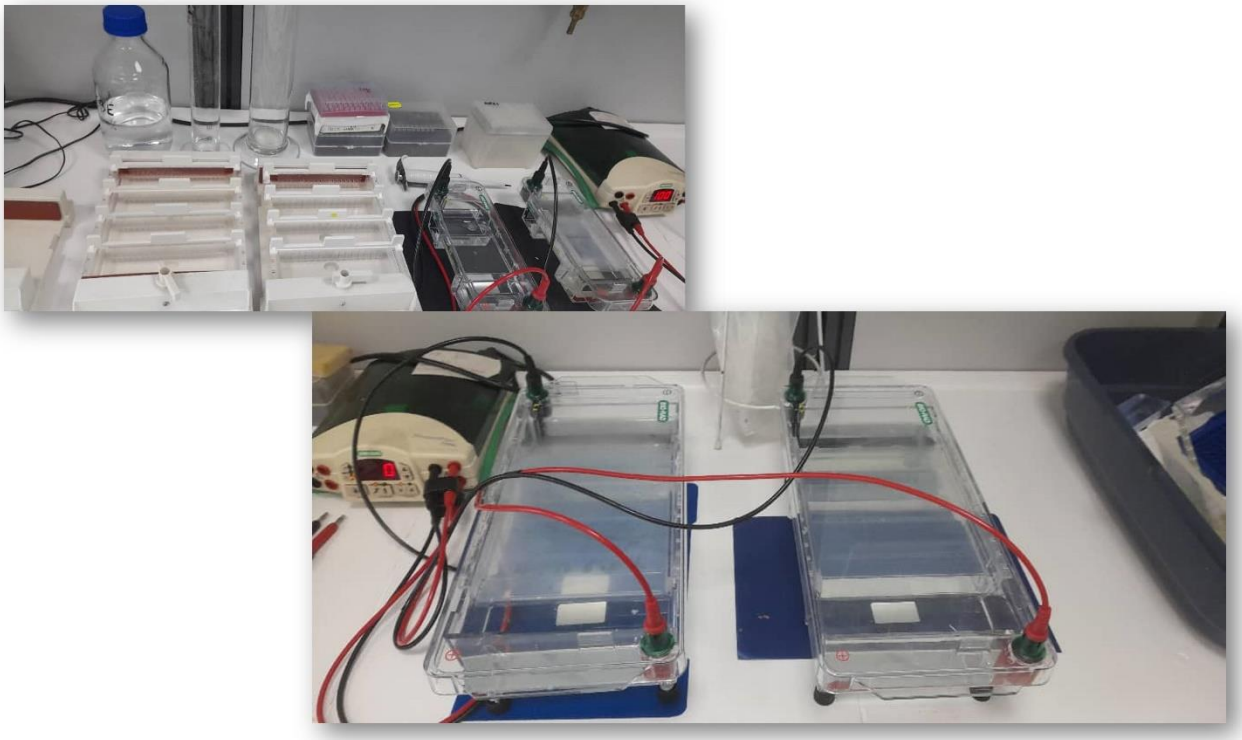

Figure 20: Electrophoresis and migration (Original illustration).

II.8.6. Purification & Sequencing

To identify pathogens at the species level, we need to proceed with sequencing, for which we must first purify the amplicons obtained. Purification was carried out using a specific kit and following the steps of the gel extraction protocol for sequencing as indicated by the manufacturer:

- **Gel dissociation**
    - Extract the slice of Agarose gel containing the DNA fragments concerned and remove excess agarose.
    - Place 300 mg of the fragment in a 1.5-ml microcentrifuge tube.
    - Add 500µL of DF buffer and vortex.
    - Incubate at 55-60°C for 10-15 min, inverting tubes every 2-3 min to ensure the gel fragment has completely dissolved.
    - Cool the dissolved samples at room temperature.
- **Step1: DNA fixation**
    - Place the DF column in a 2 ml collection tube.
    - Transfer 800 ml of the sample mixture to the DF column.
    - Centrifuge at 14-16,000 x g for 30 seconds.
    - Discard the liquid and replace the DF column in a 2ml collection tube.
- **Step 2: Washing**
    - Add 400µL of buffer W1 to the DF column and centrifuge at 14-16,000 x g for 30 seconds.
    - Discard the liquid and replace the DF column in the 2ml collection tube.
    - Add 600µL of wash buffer (after adding ethanol) to the DF column.
    - Keep for 1 minute at room temperature, then centrifuge at 14-16,000 x g for 30 seconds.

- Discard the liquid and replace the DF column in the collection tube.
- Centrifuge at 14-16,000 x g for 3 minutes to dry the column membrane.

➢ **Step 3: DNA elution**

- Transfer the dried DF column to a new 1.5 ml microcentrifuge tube.
- Add 20-50 µl of elution buffer pre-warmed to 60°C to the **center** of the column matrix.
- Stand for at least 2 minutes to ensure the elution buffer is completely absorbed.
- Centrifuge for 2 minutes at 14-16,000 x g to elute purified DNA.

After purification, the samples were sent for sequencing (Macrogen Europe, Netherlands).

Chapter III: Results

III.1. Abundance of *S. calcitrans* in northeastern Algeria

A total of six farms were used for fly trapping in Algeria: four farms in the northeastern and two in the central region; among them, two farms in the eastern region were surveyed for a whole year.

A total of 3246 species individuals were captured during the study in both regions. However, 1251 *S. calcitrans* were captured, including 1106 males and 136 females. The abundance of stable flies in the Northeastern region was 20.06 (±30.85 *S. calcitrans*/trap/day).

Trapping allowed us to capture species belonging to several dipteran families, most of which were Muscidae of the genus *Musca* sp., *Haematobia* sp. others such as Calliphoridae, Tabanidae, (*Tabanus* sp.). The Vavoua trap was also able to capture other species of flying insects besides Diptera, such as bees (*Apis melifera*), wasps (the mason wasp and the cuckoo wasp), bee flies (Bombilyiidae), and even cicadas (*Cicada ornis*) and butterflies.

III.2. Comparison of the distribution of *S. calcitrans* on the two monitored farms

The number of *S. calcitrans* trapped from the Timgad cattle farm was significantly higher than in the small ruminant farm ($p < 0.05$). Consequently, their abundance was higher in cattle than in small ruminants **(Figure 21, Table 4)**.

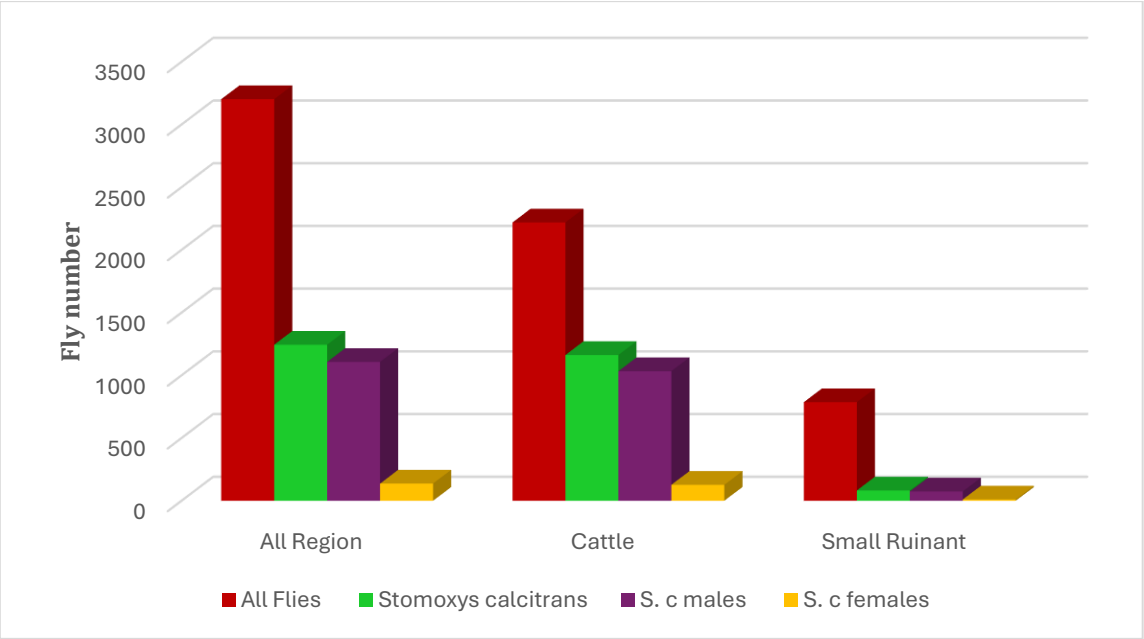

Figure 21: The fly number at the farms surveyed is Boumia Cattle and Small Ruminants (Original illustration).

Table 4: Number and abundance of *S. calcitrans* trapped on the two surveyed farms.

	Cattle		**Small ruminants**	
	N	FDT	N	FDT
Stomoxys calcitrans	1162	68.35	84	5.85
***S. calcitrans* males**	1030	60.58	74	5.29
***S. calcitrans* females**	132	7.76	8	0.57

III.3. Dynamics of *S. calcitrans* in Northeastern Algeria

III.3.1. Seasonal and monthly dynamics

A single peak activity characterized the stable fly activity during the study period. This activity is extended between August and December in northeastern Algeria, with an abundance peak recorded in September, corresponding to the end of the fall. However, starting from winter (December), their density decreases and disappears until spring begins, when stable flies start appearing with very few numbers (**Figure 22**).

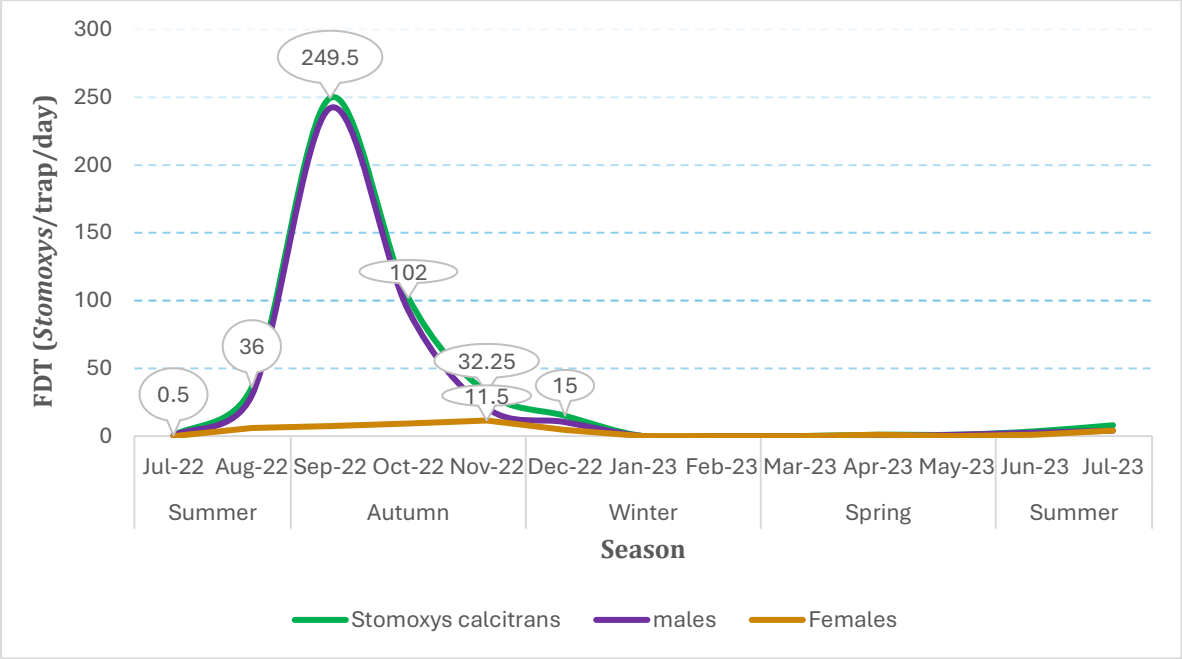

Figure 22: Seasonal dynamics of *S. calcitrans* in Northeastern Algeria.

III.3.2. Daily dynamics

In the surveyed area, the daily dynamics of stable flies *S. calcitrans* varied from month to month during their high activity period. No significant activity was observed at the start of trapping (July and August). *Stomoxys* activity was highest in September; they seem to have two daily peak activities during this month, the first being large, extending from 8 AM to midday, then decreasing in abundance at the interval hour between 12–2 PM. It gradually increases again to peak towards the end of the day. In October, stable flies' activity starts at 8 AM, and their daily abundance gradually increases to peak between 10 AM and 12 PM, then decreases until around 4 PM, remaining constant for the rest of the day.

In November and December, the density of stable flies decreased, and their activity was characterized by an unimodal peak between 12 and 2 PM but with a low density (ADT< 20 Stomoxys/trap/day).

No stable fly activity was observed from January to June **(Figure 23)**.

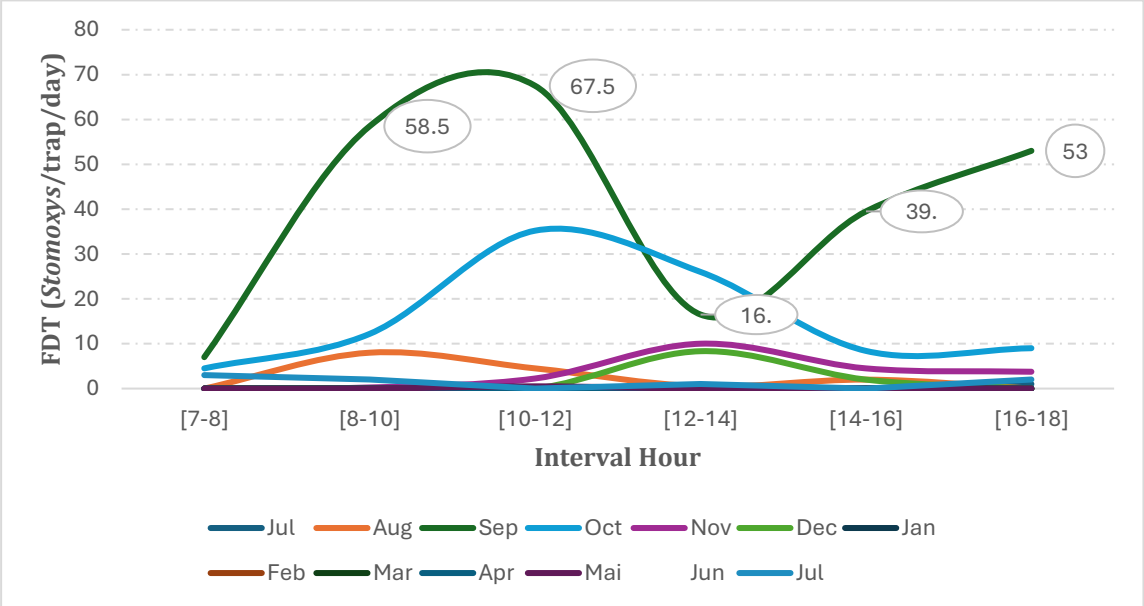

Figure 23: Daily dynamics of *S. calcitrans* in northeastern Algeria.

III.3.3. Daily dynamics of *S. calcitrans* by sex

The daily activity of males and females *S. calcitrans* was different. Males were more active in September. Their daily dynamics varied from month to month. During their high activity, they were characterized by a bimodal daily pattern with a large peak in the morning between 8 a.m. and 12 and a second peak in the afternoon. This daily pattern becomes unimodal in the remaining months, peaking between 10 and 12 in October and between 12 and 14 in November **(Figure 24)**.

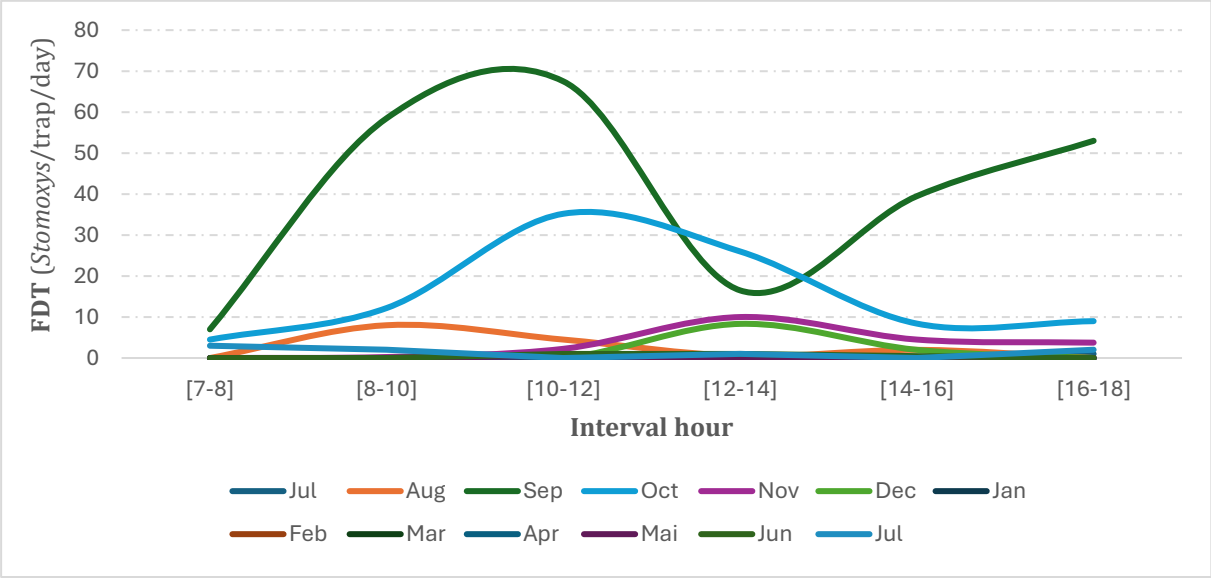

Figure 24: Daily dynamics of males *S. calcitrans*.

Unlike males, females' activity was slightly different; first, their number was significantly lower than that of males (FDT < 6 Stomoxys/trap/day). When they were active, their activity varied from month to month. In November, we observed a peak in female activity between 12 and 2 p.m., even in December. During the remaining months, no significant activity was observed for stable flies females (FDT <3 *Stomoxys*/trap/day) **(Figure 25)**.

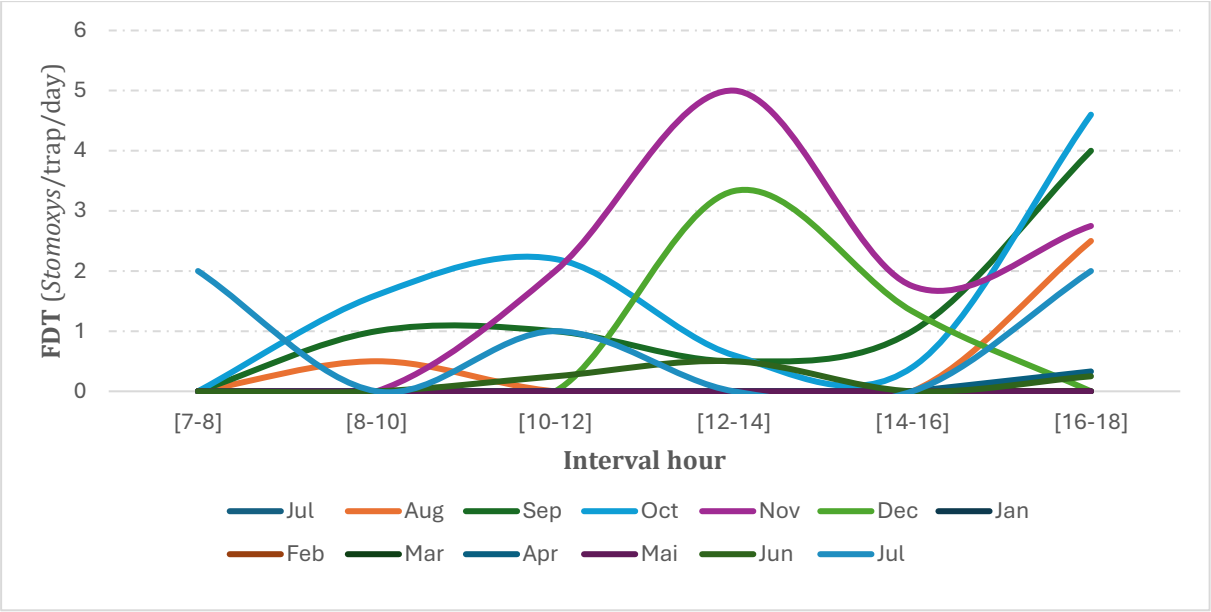

Figure 25: Daily dynamics of females *S. calcitrans*.

III.3.4. Dynamics of *S. calcitrans* in the cattle farm

III.3.4.1. Seasonal dynamics

Stomoxys flies were active in the cattle farm between August and December, with a peak abundance in September, corresponding to the autumn season (FDT = 500 *Stomoxys*/trap/day). Starting from September, the density of stable flies gradually decreases until it disappears in winter.

No stable flies were trapped from winter until summer (**Figure 26**)

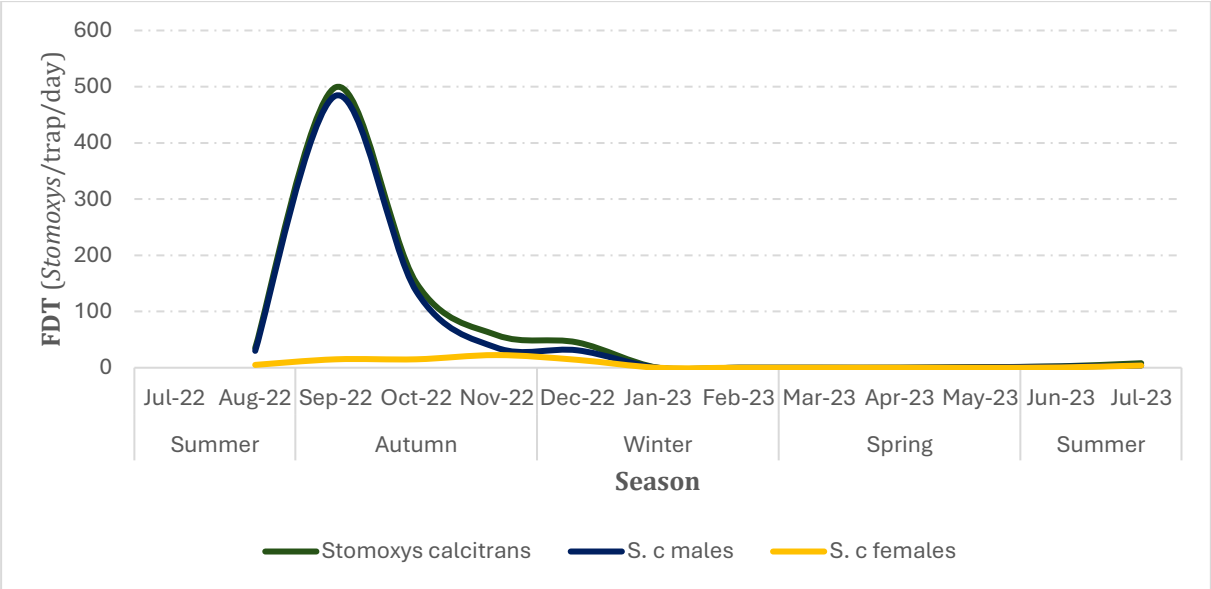

Figure 26: Seasonal dynamics of *S. calcitrans* in the cattle farm.

III.3.4.2. Daily dynamics

During their high activity (September), Daily male *Stomoxys* activity was characterized by a bimodal peak, with a prominent peak between 8 a.m. and 12 and a second peak at 6 p.m. However, in October, flies are most active between 10 a.m. and 12 p.m., after which their density gradually decreases until the evening. This peak shifts to between 12 and 2 pm in November and December. No significant activity was observed in the remaining months (figure). However, females were active at the end of the day in September and October, and in November and December (their seasonal peak period), their daily peak abundance in these months was recorded between 12 and 2 p.m. (FDT= 10 *Stomoxys*/trap/day). In the remaining months, the stable fly density was very low or even non-existent (< 2 *Stomoxys*/trap/day) **(Figure 27)**.

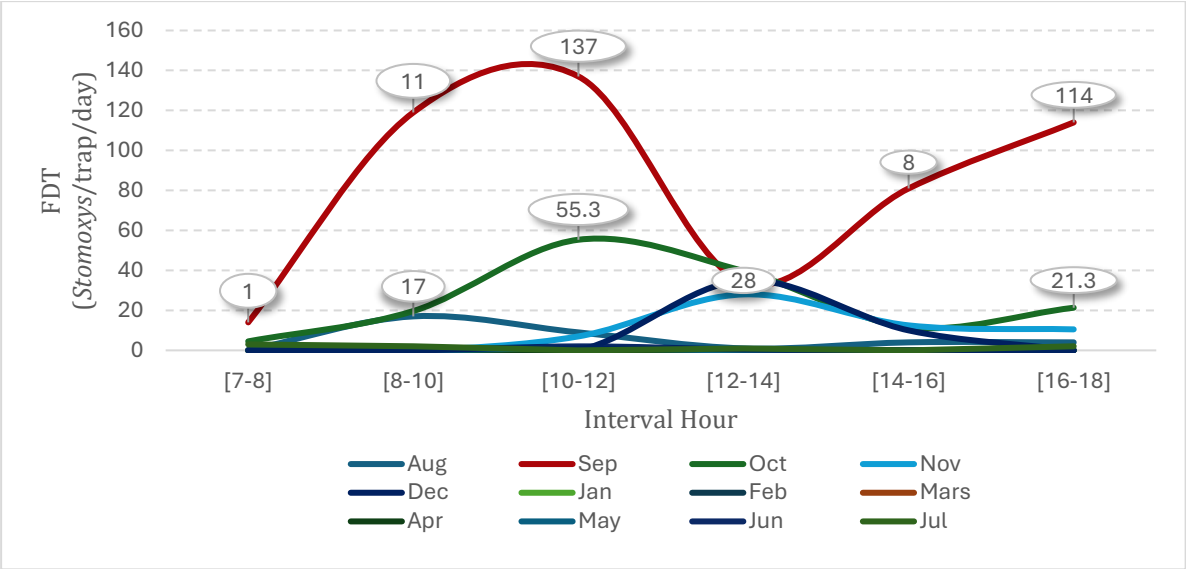

Figure 27: Daily dynamics of *S. calcitrans* in the cattle farm.

III.3.5. Stable fly dynamics in the small ruminant farm

III.3.5.1. Seasonal dynamics

The stable fly density in the small ruminant farm was significantly lower than that observed on the cattle farm. Their activity was also characterized by a peak observed in October since their abundance gradually decreased until it disappeared in December. Females were trapped in deficient numbers, and their apparent density didn't reach 5 *Stomoxys*/trap/day **(Figure 28)**.

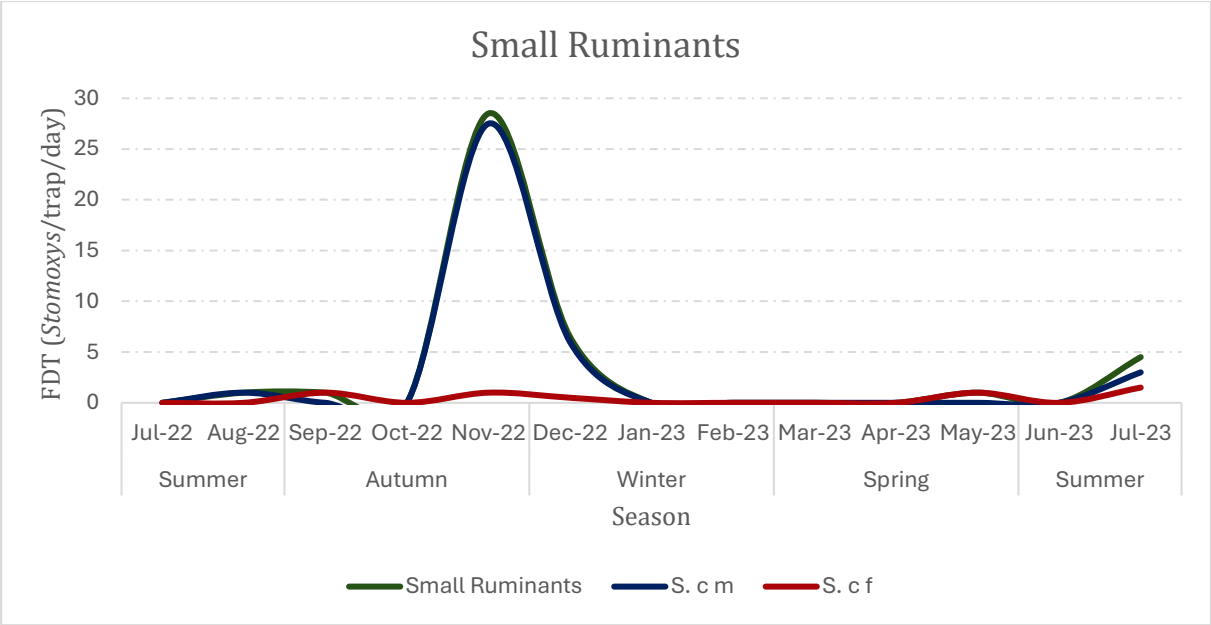

Figure 28: Seasonal dynamics of *S. calcitrans* in the small ruminants breeding.

III.3.5.2. Daily dynamics

It was in October that we observed a relatively significant *Stomoxys* activity in the small ruminant farm. Their daily activity peaked between 10 a.m. and midday. During the remaining months, the stable fly activity was lower and nonsignificant (<4 *Stomoxys*/trap/day) **(Figure 29)**.

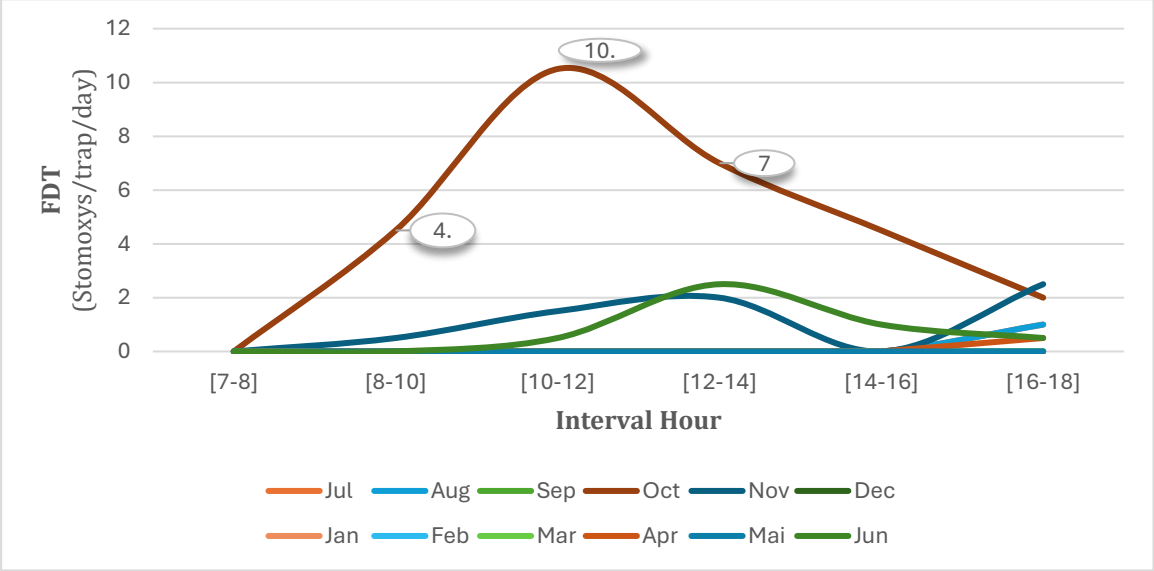

Figure 29: Daily dynamics of S. calcitrans in the small ruminant farm.

III.4. Influence of abiotic factors

III.4.1. Correlation of *S. calcitrans* abundance with different climatic parameters

Spearman's non-parametric correlation test revealed a strong correlation between *S. calcitrans* density (*S. calcitrans*, males and females) and temperature and rainfall ($p<0.05$). However, a non-significant negative correlation was observed between relative humidity and *S. calcitrans* abundance and males, while no significant correlation was observed between the latter's density and wind speed. On the other hand, the test revealed a negative but non-significant correlation between the latter and the density of *Stomoxys* females **(Table 5)**.

Table 5: Correlation matrix of different climatic parameters with *S. calcitrans* counts.

		Temperature	Precipitation	Wind speed	Relative humidity
Stomoxys calcitrans	Correlation coefficient	0.290	0.153	0.034	-0.035
	Spearman's P value	0.000	0.034	0. 637	0.625
Males *S. calcitrans*	Correlation coefficient	0.250	0.153	0.047	-0.022
	Spearman's P value	0.000	0.034	0.520	0.760
Females *S. calcitrans*	Correlation coefficient	0.165	0.144	-0.052	0.015
	Spearman's P value	0.022	0.046	0.469	0.837

III.4.2. Temperature influence

We observed a significant correlation between the apparent density of stomoxes and temperature ($p<0.05$).

- In July-August, a temperature maximum was observed (> 30°C). The apparent density of stable flies in these two months was relatively low, but this density increased progressively with temperature.
- The temperature dropped to 28.8°C in September, and the peak in Stomoxys density was recorded during this month.
- From October to January, the temperature gradually fell to its lowest values, while the stable flies' apparent density gradually decreased until it disappeared in January. Since then, no *Stomoxys* have been captured until April, when a very low density began to appear **(Figure 30)**.

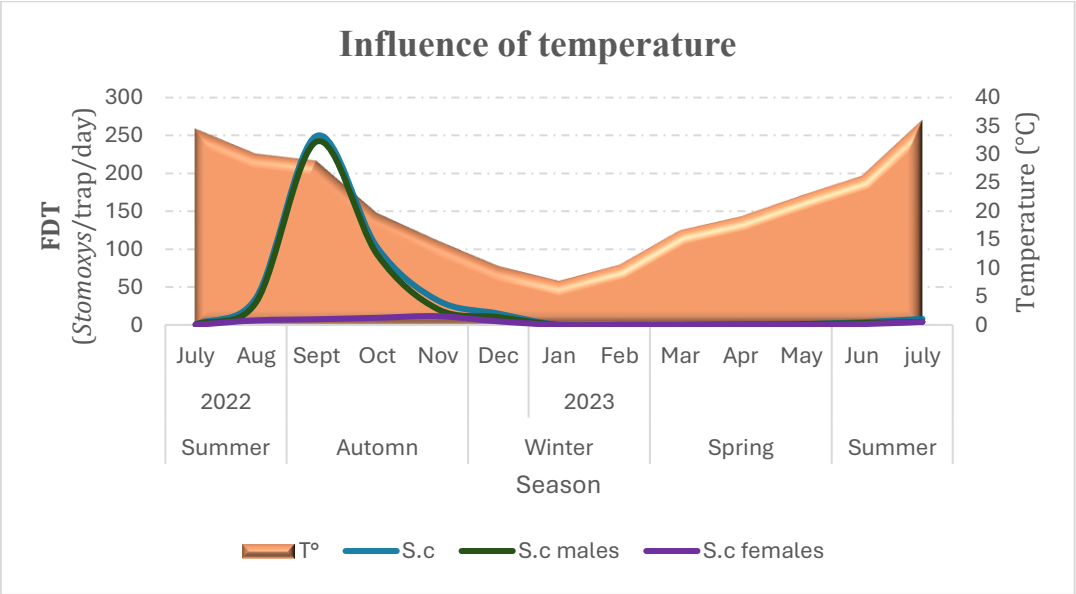

Figure 30: Seasonal dynamics of *S. calcitrans* and temperature.

III.4.3. Rainfall influence

There is an influence of precipitation on the apparent density of *S. calcitrans* in our study ($p<0.05$). The period of *Stomoxys* activity corresponded to the rainy period throughout our research. The *S. calcitrans'* apparent density was correlated with rainfall; however, the density peak was recorded when rainfall decreased in September. Despite the low rainfall recorded, the latter favored stable fly activity.

According to the results we obtained:

- July-August: as rainfall increases (even with low values), so does the apparent density of *Stomoxys*.
- September: rainfall decreases, then gradually increases again, reaching its peak in October. However, stable flies' abundance peaks in September, then gradually decreases with rainfall: a positive correlation.

Our year can be divided into two parts: dry and rainy periods. *S. calcitrans* activity was only observed during the rainy period. **(Figure 31)**.

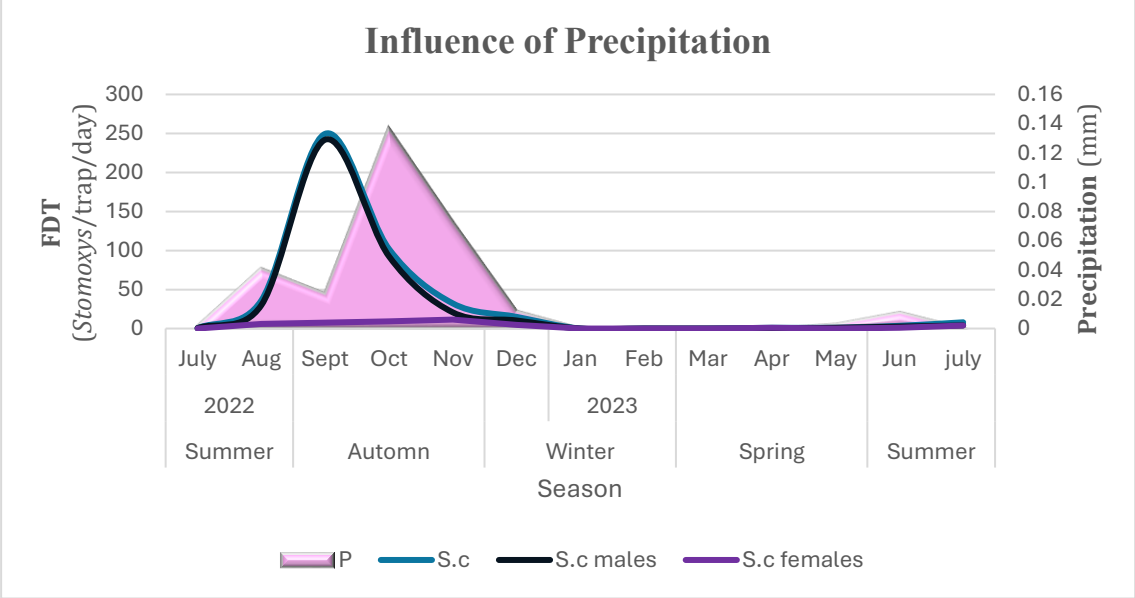

Figure 31: Seasonal dynamics of *S. calcitrans* and rainfall.

III.4.4. Impact of relative humidity

According to Spearman's non-parametric correlation test, a non-significant negative correlation was observed between the number of *S. calcitrans*, male *S. calcitrans*, and relative humidity **(Table 5)**; no significant correlation was observed between female' density and hygrometry. However, the multiple regression statistical model revealed a significant positive correlation between relative humidity and *S. calcitrans* density **(Table 6)**. From the figure:

- July-August: stable flies' density starts to increase slightly at a low density (<50 *S. calcitrans*/trap/day); similarly, the relative humidity recorded was low and gradually increased (between 20 and 40%).
- September: the peak in Stomoxys density was recorded during the increasing hygrometry phase.
- September-December: this is the phase of decreasing *Stomoxys* density, although the percentage of relative humidity continues to rise until it reaches 60% in December, when stable flies' density falls and disappears until July (Figure 32).

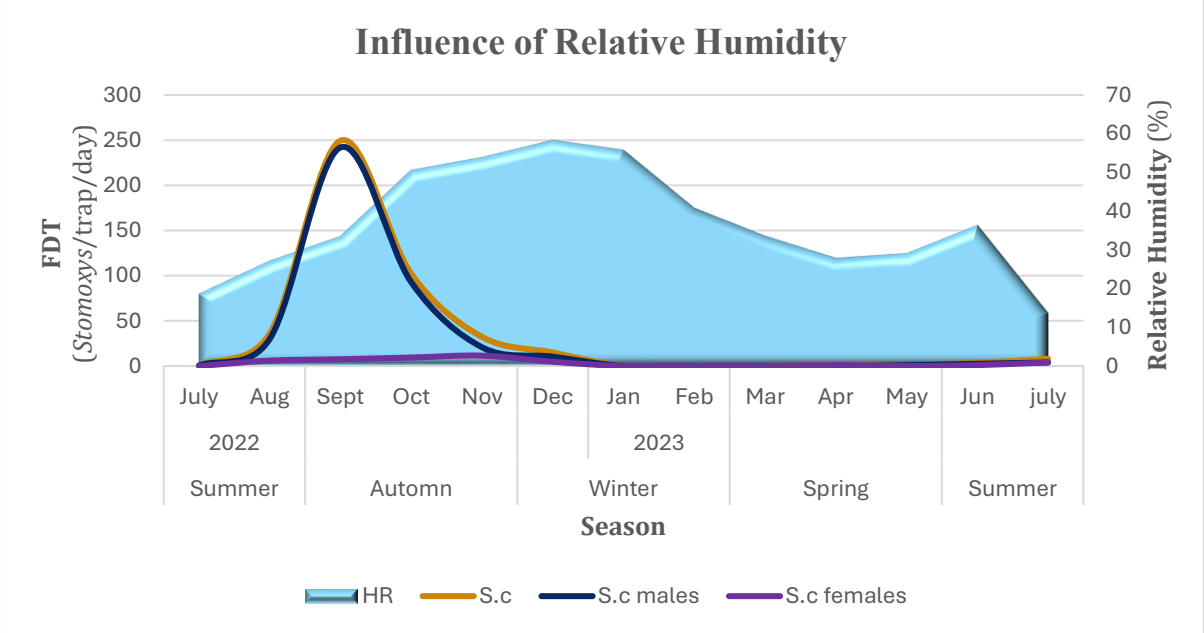

Figure 32: Seasonal dynamics of *S. calcitrans* and relative humidity.

III.4.5. Wind influence

Wind speed does not influence *Stomoxys* density, as no significant correlation was observed between *S. calcitrans* and wind speed. However, the multiple regression model revealed a negative correlation (see section below).

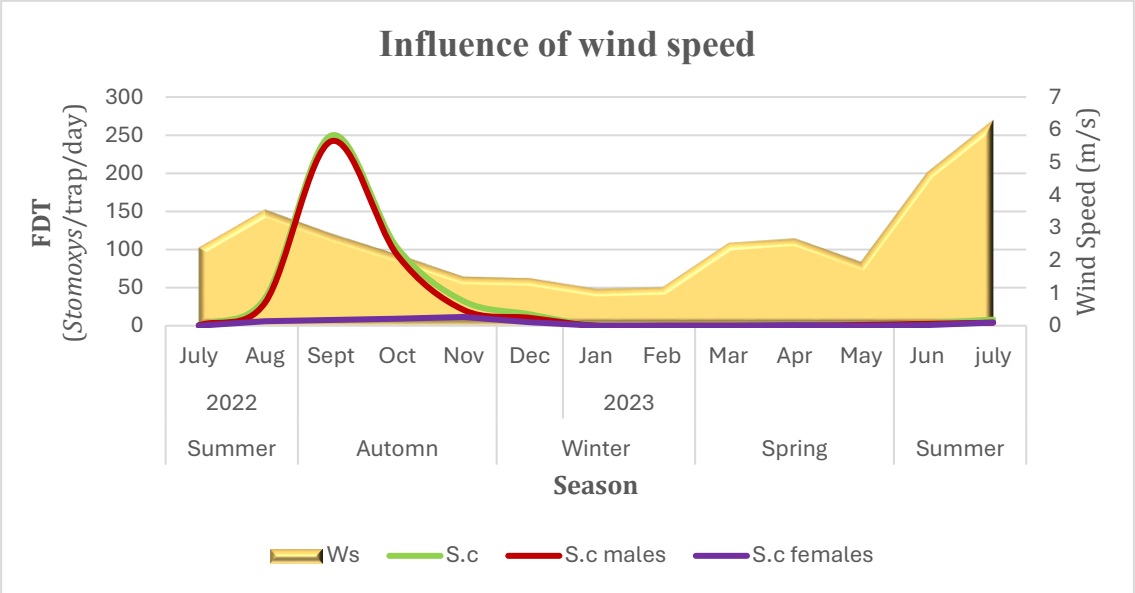

Figure 33: Seasonal dynamics of *S. calcitrans* and wind speed.

III.5. Statistical modeling of the abiotic factors influencing *S. calcitrans* density

With the data obtained during the survey, using the Huber-White-Hinkley method and negative binomial regression, we could generate statistically significant multiple regression models ($p \leq 0.05$) with significant coefficients **(Tables 6,7,8,9)**.

III.5.1. Multiple regression analysis

- When the temperature rises by one unit (1°C), the number of stable flies increases by 0.90, which is highly significant ($p< 0.05$).
- When rainfall rises by one unit, the stable fly number increases by 1.55; although it remains insignificant ($P>0.05$), it has contributed to the model.
- When relative humidity increases by 1%, stable flies increase by 0.2.
- wind speed has a negative effect. When it rises by one unit (1 m/s), the number of stable flies decreases by approximately 2.

Table 6: Temperature, wind speed, precipitation, and relative humidity effects on the *S. calcitrans* density.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	-15.20348	6.950542	-2.187381	0.0299
Température	0.904021	0.289807	3.119393	0.0021
WS	-1.804751	0.757545	-2.382367	0.0182
PRECIPITATION	1.550208	1.174572	1.319807	0.1885
HR%	0.213074	0.084632	2.517654	0.0126
R-squared	0.065393	Mean dependent var		6.445596
Adjusted R-squared	0.045508	S.D. dependent var		19.89107
S.E. of regression	19.43320	Akaike info criterion		8.797408
Sum squared resid	70998.07	Schwarz criterion		8.881934
Log likelihood	-843.9499	Hannan-Quinn criter.		8.831638
F-statistic	3.288509	Durbin-Watson stat		1.050979
Prob(F-statistic)	0.012395	Wald F-statistic		2.717454
Prob(Wald F-statistic)	0.031144			

By incorporating another variable into the model (Host), it becomes more significant and the AIC decreases **(Table 7)**:

- the stable flies number increases by 0.84 when the temperature increases by one degree
- this number decreases by ~ 2 when wind speed increases by one unit (1 m/s)
- Particularly, the influence of rainfall was highly significant ($p<0.05$): when it rises by one unit, the stable fly number increases by 4.
- Regarding the Host, this factor was highly significant: when stable flies moved from cattle to sheep, their number decreased by 11.

Table 7: Influence of the climatic parameters and the host on the *S. calcitrans* density.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	3.107639	6.034252	0.515000	0.6072
Température	0.847768	0.268644	3.155733	0.0019
WS	-1.778486	0.775664	-2.292855	0.0230
PRECIPITATION	4.051257	1.514336	2.675270	0.0081
HR%	0.206556	0.080854	2.554680	0.0114
HOST	-11.36821	2.658426	-4.276293	0.0000
R-squared	0.144386	Mean dependent var		6.445596
Adjusted R-squared	0.121509	S.D. dependent var		19.89107
S.E. of regression	18.64348	Akaike info criterion		8.719464
Sum squared resid	64997.31	Schwarz criterion		8.820895
Log likelihood	-835.4283	Hannan-Quinn criteria.		8.760540
F-statistic	6.311294	Durbin-Watson stat		1.124811
Prob(F-statistic)	0.000019	Wald F-statistic		3.986107
Prob(Wald F-statistic)	0.001851			

Combining temperature and wind speed and fitting them to the previous model allow us to obtain a new, more significant model with the lowest AIC and a higher R^2 **(Table 8)**.

Table 8: The previous model was fitted with the combined effect of temperature and wind speed.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	-0.608850	5.922450	-0.102804	0.9182
Température	1.086545	0.321350	3.381183	0.0009
Température*WS	-0.087542	0.031908	-2.743566	0.0067
HR%	0.203749	0.079473	2.563762	0.0111
HOST	-11.59381	2.694981	-4.302000	0.0000
PRECIPITATION	3.835194	1.443876	2.656179	0.0086
R-squared	0.152876	Mean dependent var		6.445596
Adjusted R-squared	0.130226	S.D. dependent var		19.89107
S.E. of regression	18.55075	Akaike info criterion		8.709492
Sum squared resid	64352.34	Schwarz criterion		8.810922
Log likelihood	-834.4659	Hannan-Quinn criter.		8.750568
F-statistic	6.749385	Durbin-Watson stat		1.133503
Prob(F-statistic)	0.000008	Wald F-statistic		4.062523
Prob(Wald F-statistic)	0.001594			

The combined effect of (temperature, wind and speed) and (temperature + relative humidity) was fitted to the previous model. This has given a new, more significant model: a lower AIC and a higher R^2 **(Table 9)**.

Table 9: The previous model was fitted with the combined effect of temperature and relative humidity.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	10.42915	3.533757	2.951292	0.0036
Température	0.504813	0.256009	1.971856	0.0501
Température*WS	-0.066391	0.032301	-2.055378	0.0412
HOST	-11.31028	2.666662	-4.241363	0.0000
PRECIPITATION	3.418498	1.448152	2.360594	0.0193
Température*HR_	0.010583	0.004738	2.233730	0.0267
R-squared	0.152589	Mean dependent var		6.445596
Adjusted R-squared	0.129931	S.D. dependent var		19.89107
S.E. of regression	18.55389	Akaike info criterion		8.709831
Sum squared resid	64374.16	Schwarz criterion		8.811261
Log likelihood	-834.4987	Hannan-Quinn criter.		8.750907
F-statistic	6.734423	Durbin-Watson stat		1.136514
Prob(F-statistic)	0.000009	Wald F-statistic		4.255567
Prob(Wald F-statistic)	0.001092			

Despite the low R^2 and adjusted R^2 values obtained, the multiple regression analysis confirmed the significant positive correlation between the stable fly numbers and temperature, rainfall, and relative humidity and a negative correlation between stable fly numbers and wind speed. These findings suggest that other environmental factors such as light and its intensity, quality of stable bedding, presence of other animals, hygiene, and ventilation may also influence fly abundance. Further models could be generated to identify the key factors influencing stable fly populations.

III.5.2. Negative Binomial regression

Model overview:

The tables below describe the statistical model used: the probability distribution used is a negative binomial, the link function was a log, and all 193 cases obtained after the survey were used in the analysis **(Tables 10, 11)**.

Table 10: Model overview.

Dependent variable	*S c*T (Total *Stomoxys calcitrans*)
Probability distribution	Negative Binomial
Link function	Log

Table 11: Observation processing summary.

	N	**Percentage**
Included	193	100,0%
Excluded	0	0,0%
Total	193	100,0%

Information on the distribution of the categorical predictor variable, the continuous predictor variable, and the dependent variable are then provided **(Tables 12, 13)**.

Table 12: Details regarding the category predictor variable's distribution.

Host	**N**	**Mean**	**Variance**
Cattle	93	12,49	745,905
Small Ruminants	100	,82	7,826
Total	193		

Table 13: Distribution of the continuous predictor variable overview.

Variable		**N**	**Min**	**Max**	**Mean**	**Deviance**
Dependent Variable	***Sct***	193	0	137	6,45	19,891
Covariable	**Time**	193	7,5	17,5	12,189	3,2575
	T°C	193	-,45	37,94	18,7941	9,22557
	HR%	193	9,50	92,54	43,0834	20,29586
	WS	193	,26	8,64	2,5799	1,60930
	Precipitation	193	,000	3,190	,08703	,372351

The table below provides data about the goodness of fit of the model:

Table 14: Goodness of fit of the model

	Value	df	Valeur/ddl
Déviance	127,717	187	0,683
Déviance mise à l'échelle	127,717	187	
Khi-carré de Pearson	184,519	187	0,987
Khi-carré de Pearson mis à l'échelle	184,519	187	
Log de vraisemblance[b]	-328,127		
Critère d'information d'Akaike (AIC)	668,254		
Corrigé pour petits échantillons (AICC)	668,706		
Critère d'informations bayésien (BIC)	687,830		
AIC cohérent (CAIC)	693,830		
Variable dépendante : Sct Modèle : (Constante), Time, T°C, HR%, Host			

Comparing the AIC criteria of the multiple regression models and that of NBR, we find that the NBR model is the best to consider as it has the lowest AIC.

After applying NBR, residual over-dispersion does not appear to be a major concern, as the dispersion estimate is much closer to 1(0.987).

Omnibus test:

The Omnibus test showed that our model remains significant ($p<0.0001$). Compared to a null model that just includes the intercept, we can observe that our complete model indicates a statistically significant improvement in fit **(Table 15)**.

Table 15: Omnibus test.

Khi-carré de rapport de vraisemblance	df	Sig.
56,029	4	,000
Variable dépendante : Sct		

Modèle : (Constante), Time, T°C, HR%, Host
a. Compare le modèle ajusté et le modèle constitué uniquement de constantes.

The likelihood ratio chi-square tests the overall model, which compares it to a model with no predictors (a "null" model). By examining the p-value of this test, we may determine that our model is substantially better than such a model.

We can observe that every predictor (except time) is statistically significant in the Test of Model Effects **(Table 16)**. According to the table, *Sc*T is significantly predicted by the variables: Temperature, time, HR%, and Host.

Table 16: Model effects test.

Source	Type III		
	Khi-carré de Wald	df	Sig.
(Constante)	15,303	1	,000
Time	1,636	1	,201
T°C	22,241	1	,000
HR%	13,709	1	,000
Host	45,747	1	,000
Dependent variable: *Sc*t Model: (Constante), Time, T°C, HR%, Host			

McFadden's pseudo-R-square was 0.079. This indicates our full NBR model represents a 7.9% improvement in fit relative to a null (intercept-only) NBR model.

$$McFadden's = 1 - \frac{-2LL_{full}}{-2LL_{null}} = 1 - \frac{deviance_{full}}{deviance_{null}} = 1 - \frac{656254}{712.282} = 0.079 = .0304$$

Estimates of parameters

Table 17 provides each predictor variable's negative binomial regression coefficient, standard errors, Wald chi-square values, p-values, and 95% confidence ranges for the coefficient.

Table 17: Parameter estimates

Paramètre	B	Erreur standard	IC(95 %) Wald		Test d'hypothèse			Exp(B)	Intervalle de confiance de Wald à 95 % pour Exp(B)	
			Inférieur	Supérieur	Khi-carré de Wald	df	Sig.		Inférieur	Supérieur
(Constante)	-7,950	1,7246	-11,330	-4,570	21,250	1	<0,0001	0,000	1,201E-5	0,010
Time	0,098	0,0765	-0,052	0,248	1,636	1	0,201	1,103	0,949	1,281
T°C	0,206	0,0436	0,120	0,291	22,241	1	<0,0001	1,228	1,128	1,338
HR%	0,051	0,0136	0,024	0,077	13,709	1	<0,0001	1,052	1,024	1,080
[Host =Bovin]	2,717	,4017	1,930	3,504	45,747	1	<0,0001	15,136	6,887	33,261
[Host =Ovin]	0[a]	.	.	.	.	.	.	1	.	.
(Echelle)	1[b]									
(Binomiale négative)	5,904	0,9357	4,328	8,055						
a. Set to 0, as this parameter is redundant.										
b. Fixed display value.										

The model equation for Poisson regression and negative binomial regression has the same form.

Log(Sct)=Intercept+β_1(Host=Bovin)+ β_2Time+β_3T°C+β_4HR%

This implies: Sct=exp(Intercept+b1(Host=Bovin)+b2Time+b3T°C+b4HR%)=

exp (Intercept)*exp(b1(Host=Bovin))*exp(b2Time)*exp(b3T°C)* exp(b4HR%)

- The host remains a substantial and positive predictor of the log *Sc*t (b=2.717, s.e.=0.4017, p<0.0001). Keeping all other variables equal, the IRR shows that cattle were exposed 15.136 times more than sheep. Accordingly, cattle are estimated to catch 1414.5% [100%(15.136-1)=1414.5%] more flies than sheep.

- With a coefficient of 0.098, the variable "Time" is a statistically non-significant predictor of the log count of *S. calcitrans* flies (b = 0.098, s.e = 0.0765, p-value = 0.201). However, According to the IRR, the expected number of stable flies increased by a

factor of 1.103 for each unit increase in the Time variable. This implies that the number of flies was expected to rise by 10.3% for every unit increase.

- The statistical significance of the variable "Temperature" is demonstrated by its coefficient of 0.206 (p-value <0.0001). This indicates that there will be 0.206 more *S. calcitrans* flies than before for every unit increase in temperature. The projected number of S. calcitrans flies increased by 1.228, according to the IRR, with each unit increase in the temperature variable. Accordingly, it was estimated that the number of flies would rise by 22.8% for every unit increase.
- Relative humidity (HR) is a statistically significant predictor with a coefficient of 0.051 (p<0.0001). This indicates that the estimated log count of the total number of *S. calcitrans* flies rises by 0.051 flies for every unit increase in HR. According to the IRR, the expected number of stable flies rises by a factor of 1.052 for each unit increase in the HR% variable. This implies that the number of flies was expected to rise by 5.2% for every unit increase.

III.6. Potential vector role of *Stomoxys calcitrans* in Northeastern and Center Algeria

III.6.1. *Bartonella* sp.

The test of *Bartonella* sp. in the *S. calcitrans* DNA was negative; the following figure shows the results of gel electrophoresis **(Figure 34)**.

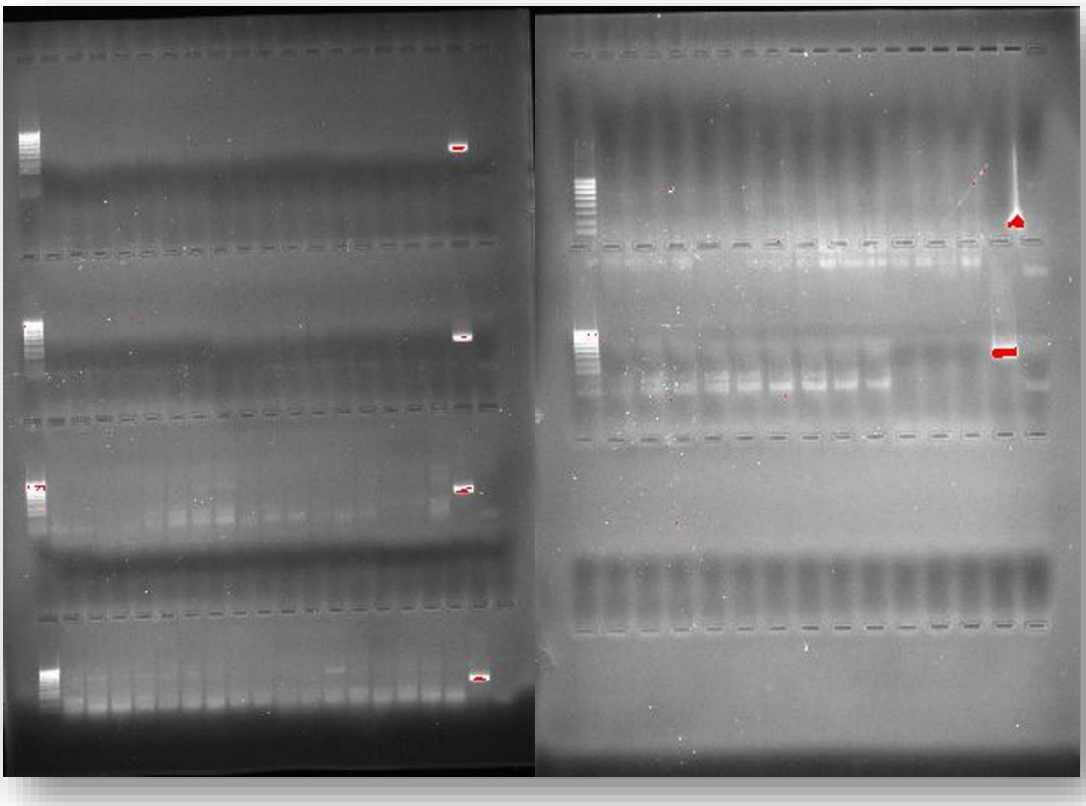

Figure 34: Negative results of *S. calcitrans* DNA screening for *Bartonella* spp.

III.6.2. *Habronema* sp.

The screening of *S. calcitrans* DNA for *Habronema microstoma* and *Habronema musca* revealed the absence of the two pathogenic DNA species **(Figure 35)**.

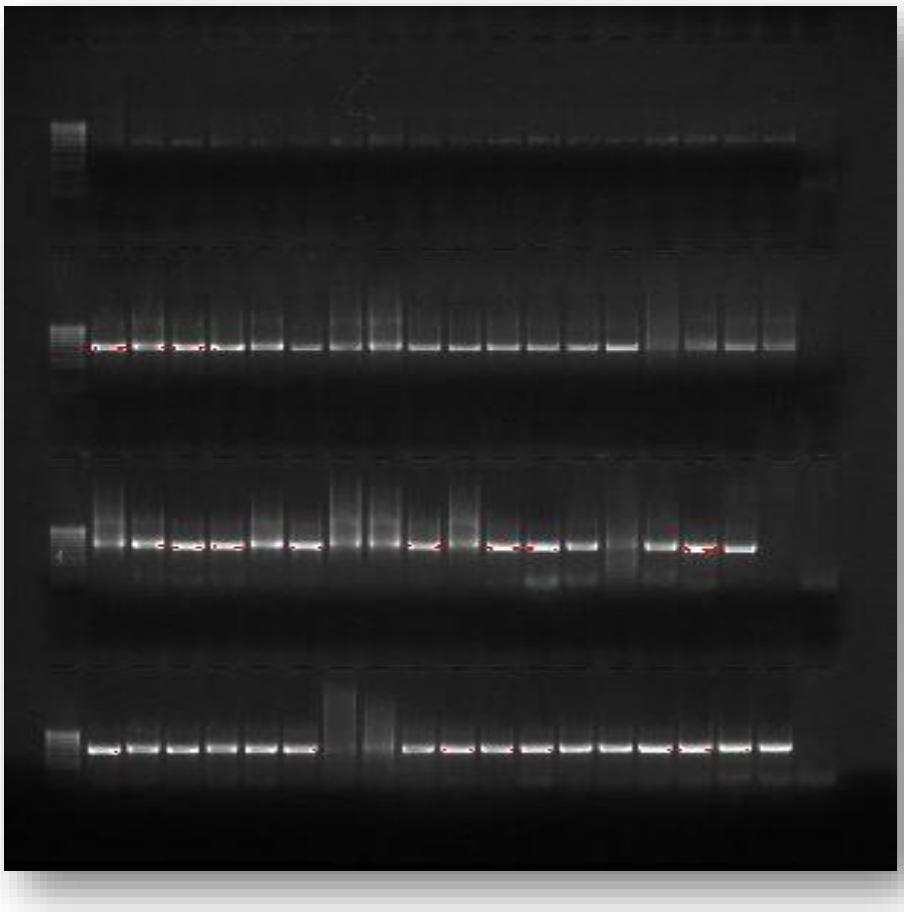

Figure 35: Negative results of *S. calcitrans* DNA screening for *Habronema microstoma* and *H. musca.*

III.6.3. Anaplasmataceae

Out of the 106 pools tested for the Anaplasmataceae family, 21 were revealed to be positive (22.26%) **(Figure 36)**.

Sequencing showed that they were *Wolbachia* sp. endosymbiontic bacterium.

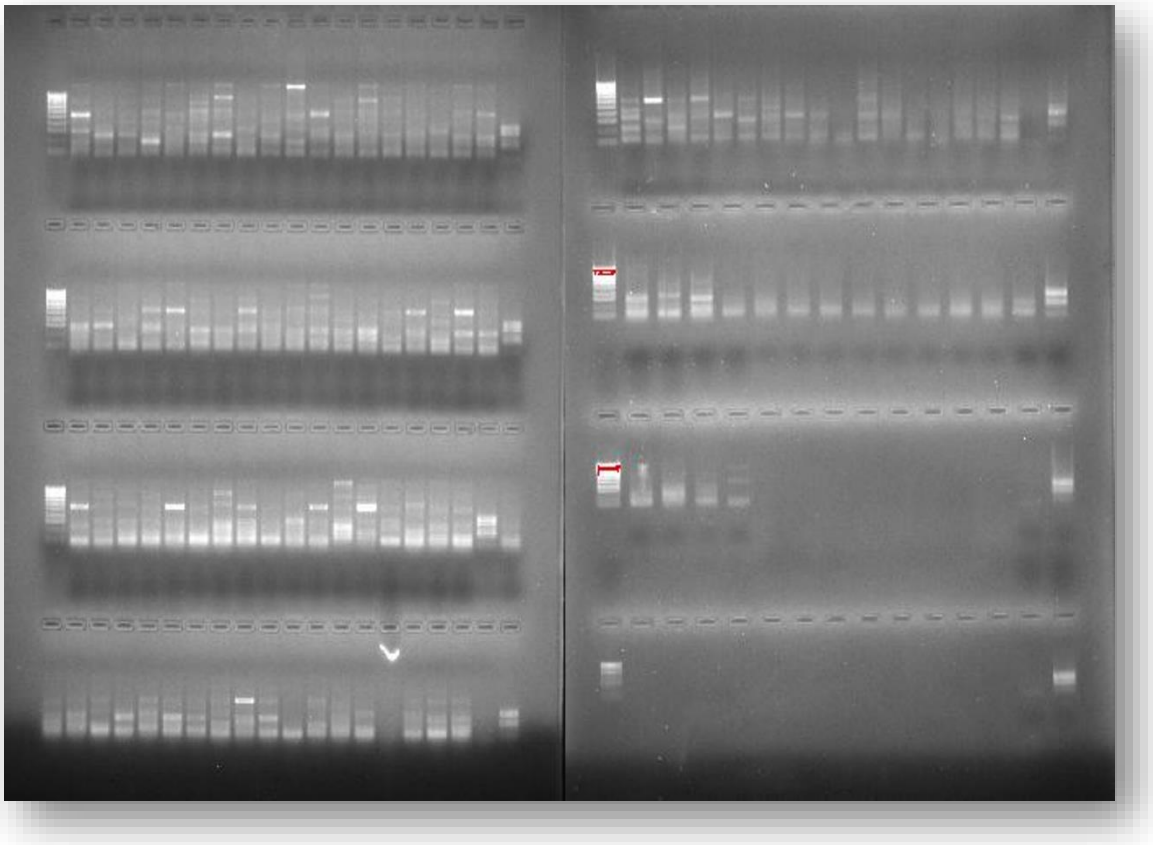

Figure 36: Anaplasmatacae DNA bands on gel.

Chapter IV: Discussion

IV.1. Abundance of *S. calcitrans*

A single species belonging to the genus Stomoxys Geoffroy 1762 was identified during the current study. This result is consistent with those of **(Zumpt, 1973),** that *S. calcitrans* is the only cosmopolitan species.

Three thousand two hundred forty-six (3246) flies of different species were trapped in the six prospected farms in the northeastern and the center of Algeria, with 1251 *S. calcitrans* (38.54%). In the northeast region, 3205 flies were trapped, and the percentage of the stable flies was 38.81%, with an overall abundance of FDT = 20.06 (±30.85 *S. calcitrans*/trap/day).

This result differs from that observed in other similar studies in Africa using the same trap (Vavoua): a higher number was recorded in Tunisia, while in Cameroon, the number was lower than our findings **(Khalifa et al., 2022; Lendzele et al., 2019)**. The difference could result from the variation in sample procedures between these studies. The number of traps used was not the same; in Tunisia, nine were used monthly. This number is higher than we used in our study: we used a single Vavoua trap. Consequently, the number of flies trapped will differ; a higher number was recorded in Tunisia. In Cameroon, different trap types in addition to the Vavoua were used, and the survey resulted in a lower number of *S. calcitrans* than our findings.

We can also explain these differences in findings by the difference in altitudes between the study regions, in addition to the variation between the climatic conditions and the localization of each study: Cameroon is located in central Africa, and its weather is influenced by equatorial and tropical air masses, which is different from our study region, the weather in Algeria is approximately similar to Tunisia and characterized by a Mediterranean semiarid climate **(Côte, 1998; Molua, 2006)**.

Although the Vavoua trap has been proven to be the ideal trap for trapping *Stomoxys* flies, a greater number of Stable flies was recorded in a similar study using sticky traps in Slovakia.

Indeed, sticky traps are also attractive for the Stomoxys flies and can be an effective method for their control **(Murchie et al., 2018; Semelbauer et al., 2018)**.

In the current study, the number of *S. calcitrans* males is significantly greater than females. This result is similar to other results found in the Réunion island and Thailand, which indicates that the Vavoua trap attracts more males than females; in contrast, the reverse was observed when the trap is located close to the host **(Clero, 2003; R Masmeatathip et al., 2006)**. Blood is necessary for stable flies for oogenesis and egg maturation; each stage of follicular development requires a blood meal. Consequently, females take blood more frequently, so they stay closer to their hosts. Five to six ovarian follicles develop continuously, but at different stages: when the first follicle has completed its development at stage V, the second is at stage III and the third at stage II. Each blood meal thus increases the development of the first series of eggs and follicles by one degree, which explains why the female must consume more meals than the male **(Foil & Hogsette, 1994)**.

In contrast, in males, blood is required to mature the accessory gland essential for sperm transfer and insemination of the female. Without mating, no egg-laying occurs **(Venkatesh & Morrison, 1980)**. Moreover, as opportunistic sugar feeders, stable flies are also attracted to fruits and flowers **(Müller et al., 2012; Taylor & Berkebile, 2014)**. On the other hand, **(Lendzele et al., 2021)** trapped more females than males in riverfront pastures from where livestock take their drink; this ensures the host availability for female stable flies and confirms the previous theory that females are extremely attached to the vertebrate hosts **(Clero, 2003; Lendzele et al., 2021)**.

The abundance of *S. calcitrans* was significantly higher in the Timgad cattle farm than in the small ruminant farm. No stable fly was trapped in the equestrian farm or the other cattle farm

(Boumia). This result suggests that stable flies are more attracted to cattle than other host species.

A similar finding was reported in Thailand, where more stable flies were caught in cattle farms than in zoos **(Malaithong et al., 2021)**. Indeed, cow manure has been proven recently as a preferable substrate for both gravid females and *S. calcitrans* larvae **(Khwanket et al., 2024)**. Moreover, livestock breathes attract host-foraging stable flies. Thus, their attractiveness is influenced by bacteria on the cow's skin **(Kovacs et al., 2024; Nayani et al., 2023)**. We may also explain this difference by the host number; the Timgad cattle farm has the highest number of cattle among all the prospected farms, with 400 heads of cattle, and could associate the high number of stable flies with dairy production. It was recently found that the cow number in a stable is among the greatest factors influencing the distribution of stable flies **(Semelbauer et al., 2018)**, more recently, a greater number of *S. calcitrans* flies was found to be associated with older cows than the younger ones **(Hansen et al., 2023)**.

On the other hand, despite the low number of hosts in the other prospected farms, we observed a great diversity in the species trapped in the small ruminant farm, the equestrian farm, and the Boumia cattle farm compared with the Timgad cattle farm, where the stable fly was the most abundant species. Even though they preferred sheep and donkey dung as a substrate for oviposition, a characteristic behavior was recently observed in gravid females *S. calcitrans*; they avoid laying their eggs in substrates containing conspecific larvae such as *Musca domestica,* the house fly. This behavior is based on sensory cues of the stable flies and enhances their progeny fitness **(Baleba et al., 2020; Baleba et al., 2019)**.

In the Boumia cattle farm, most of the species trapped were identified as Hymenoptera (wasps). They are considered natural enemies for the *Stomoxys* flies and could be used for biological control of these pests **(Cook, 2020)**.

In addition to the Dipteran species, other than stable flies, like the house fly (*Musca domestica*) and the horn fly (*Haematobia irritans*), The Vavoua trap succeeded in spotting other flying non-dipteran species but with low numbers: Butterflies, Bees (*Apis melifera*), wasps, bee flies (*Bombiluis* sp.), which we could explain by the following:

Some photoreceptors of some fly species like *Drosophila* and *Musca*, contain blue light filters, which are photostable carotenoid pigments **(Hardie, 1985)**. For bees, their attraction to flowers of different colors and patterns is complex. With the use of ultraviolet (UV), blue, and green photoreceptor types in their compound eyes, they can discover food sources in environments where plant cues and their perceived backdrops are constantly changing **(Rao & Ostroverkhova, 2015)**. Moreover, the ommatidia of butterflies contain photoreceptors that express several colors, including blue **(Perry et al., 2016)**.

IV.2. *S. calcitrans* dynamics

IV.2.1. Seasonal dynamics

The seasonal survey of the stable fly dynamics during 2022/2023 in the Timgad cattle farm and the Small ruminant farm representing the Batna region resulted in an unimodal peak activity. This peak was during the autumn season. The stable fly activity was observed from August to December 2022. The peak activity was recorded in September. No stable fly activity occurred in the remaining seasons.

A recent similar study conducted in Tunisia in a semi-arid Mediterranean climate reported two activity peaks of the *S. calcitrans* in a cattle farm. Flies were active during spring from March to July and in late fall- early winter between November and January **(Khalifa et al., 2022)**. Stable flies are known to have a bimodal peak of activity in temperate or semiarid regions in spring and autumn **(Cruz-Vazquez et al., 2004; Jacquiet et al., 2014; Khalifa et al., 2022; Taylor et al., 2007)** and a typical unimodal pattern in tropical and northern regions resulting from alternating rainy and dry periods, and cold and warm seasons respectively

(Lysyk, 1993; R Masmeatathip et al., 2006; Semelbauer et al., 2018; Skovgård & Nachman, 2012).

Our different findings could be explained by the difference in the altitudes of the two study regions of Algeria and Tunisia. Moreover, climatic change could be a factor. The extension of the desert climate zone to the detriment of the temperate and steppe climate zones has been forecasted by simulation models. This trend appears particularly noticeable by the end of the twenty-first century **(Zeroual et al., 2020)**. As the north-Afican region is among the most susceptible areas to climate change based on IPCC examinations **(Chourghal et al., 2016)**, In our region, we recorded an unusually wet summer in 2022, and the rest of the year was dry. This could be a reason for stable flies to adopt a tropical-like behavior and, therefore, have a single peak of activity during the year.

Similar to our findings, a single seasonal peak activity of *S. calcitrans* was recorded in Canada, Florida, Denmark, England, Thailand, Ethiopia and South Africa **(Dawit et al., 2012; Evert, 2014; Karam, 2021; Lysyk, 1993; Machtinger et al., 2016; Malaithong et al., 2021; R Masmeatathip et al., 2006; Parravani et al., 2019; Skovgård & Nachman, 2012)**. The difference between these findings was in the peak periods, for example, and similar to our results, in Alberta, Canada, peak activity of the stable flies was observed in mid-September **(Lysyk, 1993)**. In England and Ethiopia, the flies' activity peak was between August and September **(Dawit et al., 2012; Parravani et al., 2019)**, while in Manitoba and Denmark, the peak activity of the stable flies was recorded in July **(Karam, 2021; Skovgård & Nachman, 2012)**.

The activity period of the stable flies in our region can be divided into two parts: an increasing period from August to September and a decreasing period from September to December, which will be discussed with the influence of the climatic factors section.

IV.2.2. Daily dynamics

During the highly stable fly activity, their daily dynamics changed every month. In the high fly activity (September), *S. calcitrans* seems to have a bimodal activity, with a large peak between 8 a.m. and 12 p.m.. Their density decreases until 2 p.m. and re-increases until reaching another (small) peak by the end of the day. In the remaining months, the peak density was recorded only once daily, between 10 and 12 a.m. in October and from 12 to 2 p.m. during November and December, respectively.

Similar to our findings, several studies reported a bimodal daily peak activity of the stable flies; for example, in Thailand, stable flies were more active between 8 and 10 a.m. and in the afternoon **(R Masmeatathip et al., 2006; Muenworn et al., 2010)**. However, the females' activity was different; it increased during the day from the morning until the evening **(Keawrayup et al., 2012; Muenworn et al., 2010)**. In Cameroon, two daily activity peaks were recorded during the rainy season **(Lendzele et al., 2019)**. On the other hand, similar to the daily activity of the stable flies in our region during October, November, and December, unimodal diurnal peak activity of *S. calcitrans* was recorded in Tunisia. They were more active between 11 a.m. and 1 p.m. **(Khalifa et al., 2022)** and between 14 and 16 during the dry season in Cameroon **(Lendzele et al., 2019)**.

Basically, the diurnal activity of stable flies is unimodal. Under laboratory conditions, it was found to increase progressively during the first hours of the photophase, peaking after 4 hours **(Schofield & Brady, 1996)**. This could explain the shift in peak between October and November, as the days become shorter and the photophase shifts.

Under natural field circumstances, the primary diurnal stable flies' activity cycle is also unimodal. It has been suggested that this pattern is determined by starvation: with the increase of hunger, the daytime biting activity grows exponentially, however, this activity cycle could be transformed into non-unimodal under the influence of extrinsic factors **(Berry & Campbell,**

1985). Moreover, a study in Mali was carried out to describe the stable flies' feeding behavior, it concluded that these flies change their activity pattern according to their feeding behavior: when it is hematophagous, they adopt a bimodal activity pattern, this pattern changes to a single peak activity when the feeding activity of the flies becomes sugar-feeding **(Müller et al., 2012)**.

IV.2.3. Dynamics of *S. calcitrans* between the two prospected farms

Comparing the seasonal dynamics of the stable flies in the cattle and the small ruminant farm, we observed a slight difference: in the cattle farm, stable flies' activity occurred between September and December, with a peak in September, while in the small ruminant farm, the peak shifted to October. When coming to the daily fluctuations, in their high activity period, stable flies had a single daily peak activity in the small ruminant farm, between 10 and 12, while in the cattle farm, their activity was bimodal, with a large peak from 8 to 12, and a second one by the evening.

As previously found and mentioned above, stable Flies adjust their activity pattern following how they feed: while they are hematophagous, they take on a bimodal activity pattern; when they start feeding on sugar, this pattern becomes a single peak activity **(Müller et al., 2012)**, this could explain the difference in the daily pattern of stable flies between the two farms, especially during their high activity period, considering that animals in the small ruminant farm were not present on the farm all day, they went to pasture, whereas in the cattle farm, cows were in the farm during all the day. Moreover, there was very little to no vegetation in the area surrounding the cattle farm, however, some agricultural activities were present in the vicinity of the small ruminant breeding (apple and fig trees, vegetable gardens …) which offers a sugar source for the stable flies.

IV.3. Influence of abiotic factors

Our findings regarding the seasonal and daily fluctuations of the *S. calcitrans* flies and comparing them to similar worldwide studies show that climatic factors strongly influence stable fly dynamics.

IV.3.1. Influence of temperature

We found a significant positive correlation between temperature, rainfall, and stable fly density. At the same time, peak activity of *S. calcitrans* was recorded during the rainy period of the year despite the very low precipitation levels. The remaining climatic factors (Relative humidity and wind speed) showed a negative and non-significant correlation with the stable fly density.

On the other hand, predictive models generated in the current study revealed that the factors studied can significantly predict the size of the *S. calcitrans* population. According to the multiple regression models, temperature, host, precipitation, and wind speed are significant predictors of the stable flies number, while using negative binomial regression, the significant predictive variables are time, temperature, host, and relative humidity. Similarly, stable flies' proportion was influenced by all the climatic variables, among them, temperature and RF were the strongest and the most influencing factors **(Semelbauer et al., 2018)**.

Similar to our findings, recent African studies showed that temperature positively influenced the abundance of stable flies in Tunisia and Cameroon; moreover, it was also positively influenced by the monthly rainfall in Cameroon; however, this parameter negatively influenced the fly density in Tunisia **(Khalifa et al., 2022; Lendzele et al., 2019)**. In Brazil, the fly density was positively correlated with temperature and rainfall, this correlation was strong and weak with rainfall **(Rodríguez-Batista et al., 2005)**.

Temperature has always been a factor influencing all the life stages of stable flies; data from several studies focusing on the influence of temperature on stable flies at different life stages were collected and summarized: stable flies' eggs and larvae develop fast at a temperature of 35°C, between 14 and 39°C, there was a high rate of stable fly survival from egg to larvae. The viability peaks at 23°C for pupae, and the pupal stage's shortest duration occurs at around 32°C **(Skovgård & Nachman, 2017)**.

We found that the *S. calcitrans* density was characterized by an increasing period from August to September at temperatures ranging from 5°C to 35°; the density peak occurred when the temperature reached 35°C.

Stable flies survived highly at a temperature range between 20° and 25°; at the same time, There was a significant relationship between temperature and the development of each stable fly life stage rate when temperature ranges between 15-30°C, at 25°, 30°, and 35°, the time development of stable flies was significantly shorter, it decreased for each five-degree increases of temperature at an interval of [15°-30°C]. However, between 30° and 35°C, stable flies take more time to develop **(Gilles et al., 2005)**. The decreasing period of the stable flies in our study was found to occur from September to December when the temperature starts decreasing from 35°C.

In contrast, a spectacular reduction in the survival of *S. calcitrans* was observed at 35°C; at this temperature, the pupae died **(Gilles et al., 2005)**. We could assume that this temperature has triggered the decreasing phase of the stable flies in our case. On the other hand, stable flies' survival decreased significantly at 15°C: larvae mortality was observed at this temperature **(Gilles et al., 2005)**.

On the other hand, the average development time of *S. calcitrans* from egg to adult is inversely proportional to temperature: over 60 days at 15° and less than 12 days at 30° **(Lysyk, 1998)**. This could explain the absence of winter catches in our region: stable flies have no real

winter diagnosis. However, possible hibernation is made by a lengthening of the cycle in all stages **(Lysyk, 1998; Somme, 1961)**.

Temperature is a significant predictor according to multiple and negative binomial regressions; it positively affects the *S. calcitrans* density. This parameter has always been the main driver of stable fly development, from egg to adult; it also influences the stable flies' fecundity and longevity **(Lysyk, 1998)**. According to the regression analysis, we would have a 22.8% increase in the stable flies number when temperature increases by one unit. Nevertheless, the maximum temperature recorded in our study was 35°C. Considerably stable fly mortality has been observed at 30°, reaching a 100% rate when the temperature exceeded 35° **(Gilles et al., 2005; Lysyk, 1998)**.

IV.3.2. Influence of relative humidity and rainfall

Stable fly' counts were positively correlated with precipitation despite the low rainfall in our region; this positive impact has been emphasized in several worldwide studies **(Dawit et al., 2012; Lendzele et al., 2019; Taylor et al., 2007)**. Moreover, According to the generated statistical models, Precipitation and relative humidity influenced and predicted significantly the *S. calcitrans* count ($p<0.05$); for each unit increase in rainfall and relative humidity, we would have a rise of 40% and 5.2 % respectively in the stable fly number. Consequently, the predictive models confirmed the positive influence of rainfall on the stable fly density.

In Africa, results analogous to ours have been documented, especially in Ethiopia, where the peak density was recorded at the end of the long rainy season **(Dawit et al., 2012)**. Other worldwide studies also reported similar findings. In Thailand, a high stable fly density was found during the rainy season **(Malaithong et al., 2021; R Masmeatathip et al., 2006)**.

In a semi-arid climate in Mexico, a significant correlation was found between stable fly abundance and relative humidity, while there was no significant relationship has been observed with rainfall **(Cruz-Vazquez et al., 2004)**.

A possible explanation for our findings is that a peak of activity is observed if the relative humidity is high enough to humidify the oviposition locations. Humid oviposition sites attract female gravid stable flies and increase their capacity to lay eggs **(Nayani et al., 2024)**. However, Under laboratory conditions, pupating and emerging *S. calcitrans* larvae increase significantly at 70% RH, while a higher egg number hatches at 90% **(Issimov et al., 2020)**. Consequently, the low rainfall from September to December moistened the egg-laying and instar stage development sites, which explains the only peak in *S. calcitrans* activity recorded during our study.

IV.3.3. Combined effect of temperature and relative humidity

Temperature-relative humidity pair significantly influenced *S. calcitrans* density according to multiple regression analysis. It had been shown that climatic factors measured at time t had a significant impact on trapping intensity in the following weeks **(Taylor et al., 2007)**, moreover, heavy rainfall was followed by heavy trapping over the following three to six weeks **(Rouet, 2011)**. In our case, the peak density of *S. calcitrans* was observed after a slight rainfall period, despite the very low precipitations recorded and the low relative humidity. During winter, relative humidity conditions are relatively optimal for larval development (<40%). Nonetheless, average temperatures are too low to allow this, confirming that temperature is the main factor governing these variations in the *S. calcitrans* seasonality.

IV.3.4. Influence of wind speed

Wind speed negatively affected the density of stable flies, and we found a negative correlation between this factor and the *S. calcitrans* counts. Nevertheless, a recent study associated a high daily trap catch of stable flies with a high wind speed rate and recorded the peak activity of stable flies at 1.46 m/s **(Lendzele et al., 2019)**. Our study recorded a peak activity of *S. calcitrans* at 2.8 m/s wind speed.

Closer to our findings, more stable flies were collected on the trap sections that were shielded from the wind, and their proportion augmented on the lower parts of the cylindric trap when the wind was higher. Moreover, there was an inversely proportional relationship between trap height and the trapped stable flies' proportion **(Broce et al., 1991)**. This could explain our findings, as the Vavoua trap is 50 cm above the ground. Overall, our results agree with previous studies showing that wind negatively influences biting fly activity **(Service, 1980)**.

IV.3.5. Combined effect of temperature and wind speed

The associated increase in temperature and wind negatively influences Stable fly density: for each unit increase in these parameters, the number of captured *S. calcitrans* would decrease by 6%.

The combination of high temperatures and high winds provides an extra-dry environment, affecting fly development by reducing the humidity of the immature stages' habitat. Meanwhile, relative humidity below 40% combined with excessively high temperatures are highly unfavorable conditions for the activity of *S. calcitrans* **(Rouet, 2011)**.

IV.3.6. Other factors

Time

According to the predictive models, for every unit increase in time, the number of stable flies is estimated to increase by 10%, even though it remains insignificant; this prediction is reflected by the daily dynamics of *S. calcitrans* obtained in our results, it also confirms the previous findings regarding the gradual increase in stable fly population during the photophase hours **(Schofield & Brady, 1996)**.

Host

We trapped significantly more stable flies in cattle than in small ruminants, these results are confirmed by the statistical prediction model: moving from a cattle farm to a small ruminant farm, the number of stomoxes decreases by 11, this confirms the preference and attractiveness of stomoxes to cattle and is in line with the results of the literature (Hafez & Gamal-Eddin, **1959)**.

IV.4. Potential vector role of *Stomoxys calcitrans* in Algeria

The screening of *S. calcitrans* DNA for the presence of pathogenic DNA of *Bartonella* sp., *Habronema microstoma*, *Habronema musca*, and the *Anaplasmatacae* family revealed the absence of any significant pathogenic DNA. However, the DNA of the Anaplasmatacae family was found in 22.26% of the tested pools of *S. calcitrans* including 6/21 (28.57%) in the small ruminant farms and 15/21 (71.43%) in the cattle farm.

Bartonella are Gram-negative bacteria transmitted by hematophagous arthropods to vertebrate hosts including humans **(Chang et al., 2001; Chung et al., 2004; Ciervo & Ciceroni, 2004)**. Recent molecular investigations detected the DNA of *Bartonella chomelii* in forest flies *Hippobosca equina* from Algeria **(Boucheikhchoukh et al., 2019; Boularias et al., 2020)**.

However, our study revealed the absence of this pathogen in *Stomoxys* flies, although biting flies are potential vectors of *Bartonella* species **(Aguirre et al., 2012)**.

On the other side, the DNA of *Bartonella tamiae* was detected in bat flies (*Nycteribiidae*) in northeastern Algeria **(Leulmi et al., 2016)**.

In addition to the absence of *Bartonella* DNA, neither DNA of *Habronema microstoma* nor *H. musca* was detected in the present study, even though *S. calcitrans* is known to be a cyclic vector of this helminth **(Traversa et al., 2008)**. The potential explanation for our finding is that we did not have enough specimens of *S. calcitrans* from the equine farm, considering that *Habronema* species are equid parasites **(Anderson, 2000)**, and a single stable fly was collected from an equestrian farm and used for molecular assays. Moreover, the house fly *Musca domestica* is an intermediate host of *H. musca* **(Traversa et al., 2008)**, so it would be more interesting to screen these flies for the presence of *H. musca* DNA for a better understanding of the epidemiology of these parasites.

Our findings could also be explained by the fact that most of the stable flies collected and used for molecular screening were not gorged with blood, which reduces our chances of detecting pathogens.

Out of the 105 pools tested for the Anaplasmatacae family, 21 were positive. The sequencing revealed that they are *Wolbachia* spp. endosymbiotic bacterium.

To the best of our knowledge, our study constitutes the first molecular investigation of *S. calcitrans* in Algeria and North Africa. It makes the second report of *Wolbachia* spp. in biting flies in Algeria, after a recent detection of this bacterium in the sheep ked (*Melophagus ovinus*) and the forest flies (*Hippobosca equina*) **(Boucheikhchoukh et al., 2019)**. More recently, it was detected in the *Stomoxyini* flies (the horn flies *Haematobia irritans*) in Spain **(González, Ruiz-Arrondo, et al., 2024)**. In Algeria, an association of *Wolbachia* endosymbiotic bacterium

with fleas was recently reported **(Aouadi et al., 2022; Sidhoum et al., 2024)**. In addition, previous studies have found an association of this bacterium with other arthropods, such as bedbugs Cimex *lectularius* **(Hosokawa et al., 2010)**.

Wolbachia are alphaproteobacteria belonging to the Rickettsial Order. They may infect 40% of terrestrial arthropod species, and they are vertically transmitted between infected individuals but also could be horizontally transmitted interspecies **(Zug & Hammerstein, 2012)**. This bacteriome has various effects on arthropods depending on the host species; for example, in wasps, it facilitates oocyte maturation and increases fecundity, while *Wolbachia* provides B vitamins in bedbugs. On the other hand, the host reproductivity is manipulated by this bacterium to ensure its vertical transmission. Several attempts were made to use *Wolbachia* as a biological tool for the control of stable flies; however, the success of these attempts was not proven **(Madhav et al., 2020)**.

Our study reveals that stable flies are infected by *Wolbachia* spp. Endosymbiotic bacteria, it would be interesting to undertake further research on *Stomoxys* in Algeria by screening these flies for other pathogens to better understand their potential vector role. In addition, the detection of Wolbachia in *Stomoxys* flies in Algeria highlights the possible use of this bacterium for the biological control of these pests as an integrated pest management.

Conclusion, Recommendations & Perspectives

In the present study, we investigated the abundance and the dynamics of *Stomoxys calcitrans*, the abiotic factors influencing their distribution, and their potential vector role in Northeastern and central Algeria by conducting annual monitoring of two different farms using the Vavoua trap and Molecular biology tools, mainly conventional PCR and nested PCR.

A total of 1251 *S. calcitrans* has been trapped out of 3246 flies, with an apparent density of 20.06 *Stomoxys* / trap/ day in Northeastern Algeria. In this Stomoxys population trapped, the' number was higher than females; We trapped 1106 males and 136 females.

Stable flies were active during the late Summer and fall seasons, their activity peak was recorded during the fall in September. In contrast, no *Stomoxys* activity was observed in winter or in spring.

During their activity season, The daily activity of stable flies was different every month, it was characterized by a bimodal pattern in September and an unimodal in the remaining months.

The current study highlights that climatic conditions are the main drivers of *S. calcitrans* activity, especially temperature and rainfall.

Stable flies are more attracted to cattle than small ruminant hosts; the number trapped in cattle was significantly higher than that found in the small ruminant farm.

Regarding their possible vector role in Algeria, no significant pathogenic DNA has been detected in this study; however, the DNA of *Wolbachia* sp. endosymbiotic bacteria has been found in 20% of the tested flies. This detection could be an initiative to use *Wolbachia* in the integrated management of *Stomoxys calcitrans* in Algeria.

From these findings, it should be strongly recommended that more in-depth studies be conducted to better understand the interactions between host pathogens and vector-microbiota to establish an effective control measure against these pests.

It would also be advantageous to implement the following strategies as a first step for stable fly control strategies on a national scale:

Educate the Algerian breeders on using Vavoua traps to control stable flies.

Intensify the control of Stable flies during summer and autumn using Vavoua traps and insecticides.

Introduce and encourage the use of commercially available parasitoids against *S. calcitrans* for biological control.

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Abstract

Stomoxys calcitrans, commonly known as “the Stable fly,” “the dog fly,” or “the anthrax fly,” is a significant pest that affects humans and livestock because of its painful bites and its ability to transmit a variety of pathogens, both zoonotic and non-zoonotic. The current study focuses on the abundance, the dynamics, the factors influencing the distribution, as well as the potential vector role of *S. calcitrans* in the Northeastern and central regions of Algeria by daily and monthly monitoring during a year of study using Vavoua traps, statistical analysis and modeling, and molecular tools. It also aims to determine any particular host preference for *S. calcitrans*.

Six farms have been involved in the present study, two from the central region and four in the northeastern part of Algeria. The entomological survey resulted in 1251 *S. calcitrans*/ 3246 other flying species, with an overall density of 20.06 *S. calcitrans*/trap/day. Significantly more males than females have been trapped (1107 ♂/ 137♀) in the eastern region.
The seasonal activity of *S. calcitrans* in Northeastern Algeria was characterized by an unimodal activity pattern extended from August to December, corresponding to the summer end and fall seasons, while no activity was observed during the rest of the year. The daily dynamics differed monthly; during the high activity period, *S. calcitrans* activity was bimodal, whereas during the remaining months, it was unimodal.

The present study has revealed a preference for stomoxes toward cattle hosts over other animal species. Climatic conditions are the main drivers of the stable flies' distribution, especially temperature. We identified a strong positive correlation between the *S. calcitrans* counts and temperature and rainfall. On the other hand, statistical modeling revealed that all the weather parameters significantly influence the stable fly count.

A molecular survey was conducted to identify a potential vector role of *S. calcitrans* in Algeria. One hundred five pools of *S.calcitrans* have been analyzed using conventional and nested Polymerase chain reactions; they were screened for *Bartonella* sp., Anaplasmatacae, *Habronema microstoma*, and *Habronema musca* DNA. The results revealed the absence of any significant pathogenic DNA. However, out of the 105 pools tested for Anaplasmatace, 21 (20%) were positive, and sequencing showed it to be *Wolbachia* sp. endosymbiotic bacterium.
The current study constitutes the first one in Algeria investigating *S. calcitrans* and the first molecular investigation of this pest in North Africa. Thus, it provides a baseline and initial data on the ecology and the potential vector role of the stable fly in Algeria. Consequently, it clarifies and supplies entomological information, enhancing the understanding of the vector system. Finally, this study marks a key step in establishing an appropriate control strategy to prevent *Stomoxys*-borne diseases, improve livestock breeding, and promote the country's economy.