Surveying Midge and Mosquito Populations

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Introduction

- Globalisation and climate change means insect borne diseases are increasingly likely to threaten livestock, including horses. Midges can spread African horse sickness and mosquitoes can spread other viruses which affect horses.

- Data about midge and mosquito populations can help inform mathematical models to predict how these diseases would spread through the UK.

- The aim of this initial study was to provide information about research methods to collect these data and determine how practical/realistic they are to carry out.

Materials and Methods

- To collect the insects we used battery powered BG-Sentinel traps. An octenol bait and a rag rubbed on a sweaty horse were used. We also added green LED lights in order to attract the most *Culicoides* (1).

- Traps were placed in three different locations:

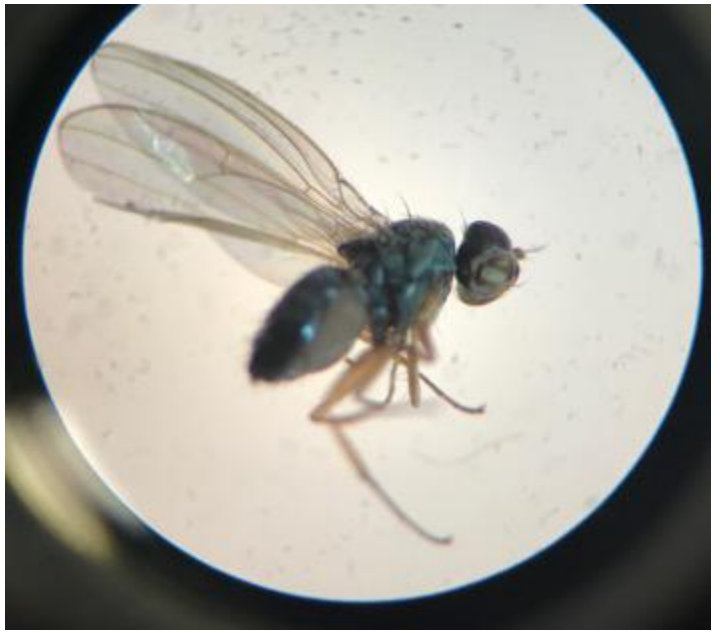
Location 1: Acton Hall Equestrian Centre, ST5 4EF

Location 2: Whitmore Riding School, ST5 4DS

Location 3: Sutton Bonington Campus, LE12 5RD

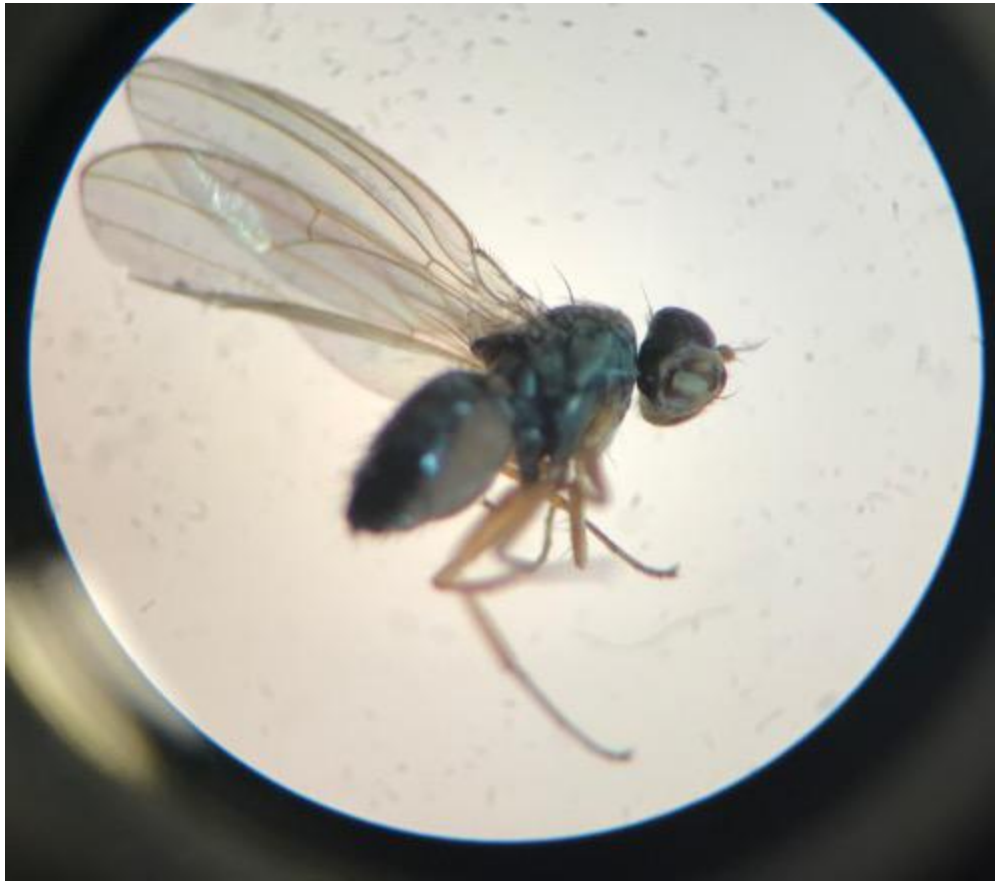
Traps were originally placed in four sites; feed room, stable, field and next to muck heap.

Once caught, the insects were frozen in order to kill and preserve them. They were separated into morphological groups using an inverted microscope.

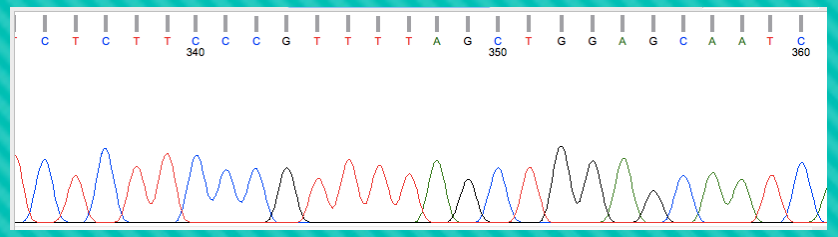


Morphological identification:

- Hairs on wing
- Vein patterns on wing
- Colour
- Eye type
- Mouthpiece
- Antenna
- Legs



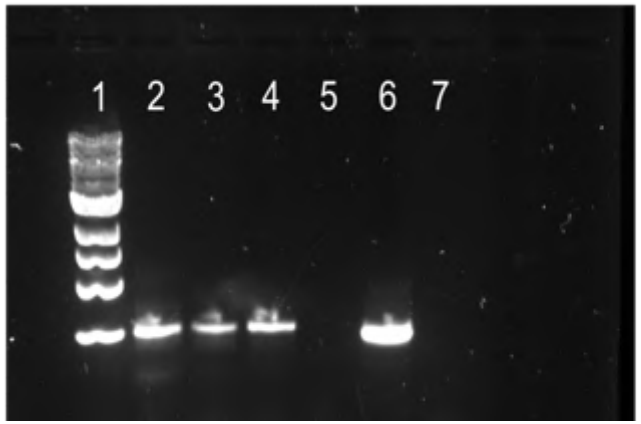
DNA Extraction/Sequencing



- DNA was extracted from the insects suspected to bite (i.e. midges or mosquitos) and therefore transmit viruses.
- Initially, we used a protocol that had been tested by a previous student. However, after several failed attempts we changed method and used a QIAGEN DNEasy Extraction kit. Due to only having small amounts of DNA, we reduced the volume of elution buffer to 55µl to increase the concentration of DNA.
- Following PCR, the samples were run through an agarose gel and pictured using a UV transilluminator.
- If the result of electrophoresis was positive, the DNA was purified using a QIAGEN QIAquick PCR purification kit. The samples were then sent to be sequenced.
- The sequenced data was viewed using FinchTV, as shown above.

Results

- A BLAST search was performed on the sequences to give the species of the insects.
- The results of sequencing were used to confirm identification of morphologically identical insects without needing to sequence all samples.



Lane 1 - Hyperladder
Lane 2 – Sample A
Lane 3 – Sample B
Lane 4 – Sample C
Lane 5 – Sample D
Lane 6 – Positive control
Lane 7 – Negative control

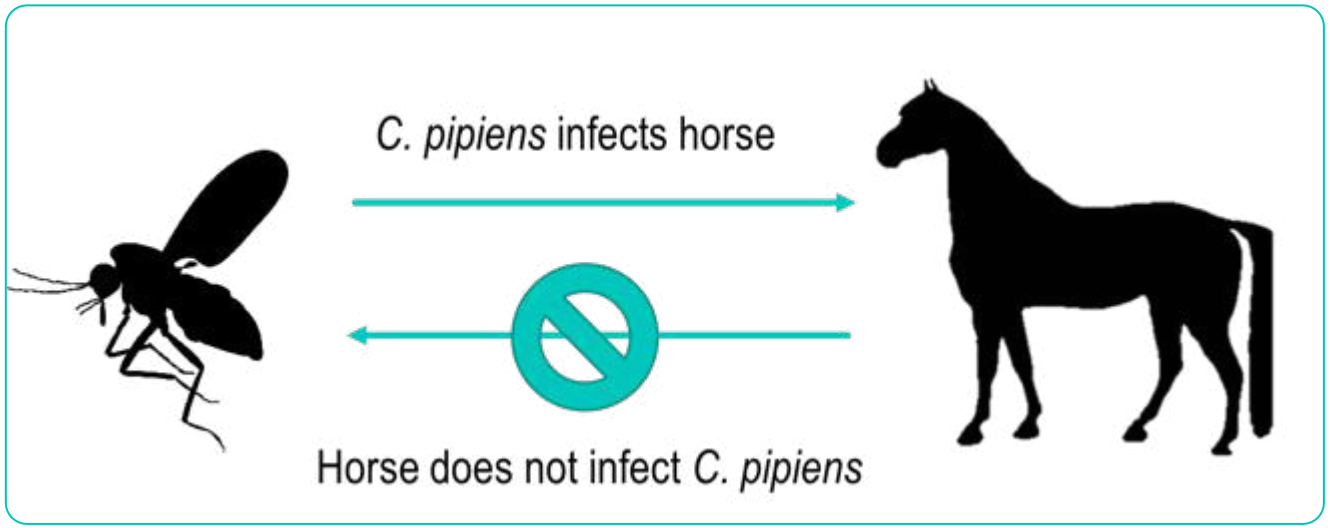
- DNA was extracted from a total of 11 groups of insects. Of these, 9 gave positive results after PCR using universal (Diptera plus other Insecta) primers. The result of four DNA extractions on separate species is shown in the image.
- In the image, DNA extraction was successful for Samples A, B and C but unsuccessful for Sample D.

Species Identified

Species	Description
Cecidomyiidae sp.	Non blood feeders
Chironmidae	Non blood feeders
Culex pipiens	Blood feeders
Hydrellia notata	Non blood feeders
Oscinella	Non blood feeders
Psychoda	Non blood feeders

Culex pipiens

- Only one species of blood feeder was found (*C. pipiens*). *C. pipiens* is also known as the common house mosquito. They are known to be vectors of West Nile virus and Japanese encephalitis virus.
- Horses and humans are dead end hosts, meaning they can become infected from a mosquito bite but cannot infect a mosquito that bites them.



Discussion and Future Work

Having demonstrated that this approach can be used to identify the presence of blood feeding insects, the following should be noted for future work.

- The QIAGEN DNEasy Extraction kit was more reliable than ethanol precipitation
- No midges were caught. It is possible that no biting midges (*Culicoides*) were present in the three areas we surveyed. However, in the future the traps could be modified to be make them more attractive to biting midges:

    1. The user manual of the BG-Sentinel traps suggests the addition of CO_2 in order to attract *Ceratopognidae* – a species in the genus *Culicoides*.

    1. The octenol bait we used is a chemical found in human breath/sweat. As we were trapping at stables, the midges present may have been more attracted to a horse specific bait.

- In the future, it should not always be necessary to do the DNA extraction/sequencing for identification. For example, as we have now successfully identified *C. pipiens*, when found in the future we can confidently identify *C. pipens* based on morphological features alone. This will make work in the future quicker and easier.

References

(1) González M *et al.* (2019) Comparison of different light sources for trapping *Culicoides* biting midges, mosquitoes and other dipterans. Vet Parasitol;226:44-9.

(2) Dolan DV *et al.* (2007) Rapid diagnostic PCR assays for members of the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes, implicated vectors of bluetongue virus in Europe. Vet Microbiol;124:82-94