# Temporal population dynamics and host-specific blood meals of *Culicoides* midges at Onderstepoort, South Africa

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# ABBREVIATIONS

| | |
|---|---|
| AHS | African horse sickness |
| AHSV | African horse sickness virus |
| cAHSV | Canine African horse sickness virus |
| BTV | Bluetongue virus |
| $CO_2$ | Carbon dioxide |
| EEV | Equine encephalosis virus |
| EHDV | Epizootic haemorrhagic disease virus |
| IFAT | Indirect fluorescent antibody tests |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PK Mix | Protein kinase mix |

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# SUMMARY

*Culicoides* Latreille (Diptera: Ceratopogonoidae) biting midges are found throughout most of the world. As proven vectors of viruses, nematodes and protozoa of medical and veterinary importance they have a negative economic impact wherever they are prevalent. This is especially true in the equine and livestock industries. For this reason, *Culicoides* midges have been studied fairly extensively. Despite this, there are still areas in which more information is required to allow us to better understand the role of these tiny insects in the epidemiology of high impact arbovirus infections.

This dissertation describes a prospective study in which weekly collections of *Culicoides* midges at Onderstepoort were performed over the period of a year. The objectives were to determine to what extent inter-seasonal activity of midges is present in the Onderstepoort area in South Africa. By making use of carbon dioxide ($CO_2$) traps it was possible to determine potential daytime activity of *Culicoides* midges. A special effort was made to determine the role of domestic dogs in the epidemiology of African horse sickness (AHS) in South Africa. Blood meal analysis of freshly blood fed females was performed to shed light on the host preferences of livestock associated *Culicoides* species in South Africa.

The study showed both inter-seasonal and daytime activity of *Culicoides* midges in the Onderstepoort area of South Africa. This may have an impact on the potential overwintering of *Culicoides* transmitted diseases in the area and the control measures implemented to curb the spread of diseases such as AHS. The study showed that *Culicoides* midges will feed on dogs and are thereby likely able to transmit African horse sickness virus (AHSV) to canines. However, the small numbers of positive canine blood meals compared to those taken from livestock suggests that dogs are only incidental hosts and therefore only play a minor role in the epidemiology of AHSV.

# CHAPTER 1: GENERAL INTRODUCTION

*Culicoides* Latreille (Diptera: Ceratopogonoidae) biting midges are of great veterinary and economic importance worldwide. *Culicoides* biting midges were first described by the reverend W Derham in 1731. In 1944 they were shown to play an important role in the transmission of viruses such as African horse sickness virus (AHSV) and bluetongue virus (BTV) (Du Toit, 1944). Although extensive studies have been conducted to allow us to better understand their role as disease vectors, there is yet much to learn. This lack of knowledge was emphasised by the unexpected northwards transmission of *Culicoides* transmitted diseases in Europe and South America (Rodríguez-Sánchez et al., 2008; Conraths et al., 2009; Allen et al., 2019; Pascall et al., 2020). They are proven vectors of orbiviruses such as AHSV, epizootic haemorrhagic disease virus (EHDV), equine encephalosis virus (EEV) and BTV (Meiswinkel et al., 2004; Purse et al., 2015).

Traditionally, adult *Culicoides* midges were believed to be mainly nocturnal and to be inactive during the colder winter months (Meiswinkel et al., 2004; Purse et al., 2015). A study done in the USA from August 2012 to August 2013 using carbon dioxide ($CO_2$) traps shows daytime as well as inter-seasonal activity for *Culicoides sonorensis* Wirth and Jones, a vector for BTV in the USA (Mayo et al., 2014). This study was possible as $CO_2$ traps are functional during the day whereas traditional light traps are only functional at night. In the Palearctic region, particularly during the summer months, it has become apparent that *Culicoides* can be active and abundant well before sunset (Meiswinkel and Elbers, 2016). Numbers of *Culicoides chiopterus* (Meigen) collected by sweep netting have been found to peak up to two hours before sunset which may lead to an under-estimation of their total numbers and role in virus transmission when using light traps for collection (Meiswinkel and Elbers, 2016). Similarly, in Europe it was found that the peak in *Culicoides obsoletus* (Meigen) activity changes from after sunset in summer, to before sunset in spring and autumn (Viennet et al., 2012). Few studies have been done in South Africa using $CO_2$ traps without an additional light source. This has limited the ability to ascertain to what extent stock associated *Culicoides* in South Africa are active during the day.

Numerous studies have been done throughout the world to clarify vector-host relationships by studying *Culicoides* host preferences via blood meal analysis (Pettersson et al., 2013; Martínez-de la Puente et al., 2015). Studies have concentrated on *Culicoides* as a vector for livestock associated diseases. These studies indicated that *Culicoides* can be broadly divided into ornithophilic and mammophilic species, with little to no species-specific preferences (Pettersson et al., 2013; Martínez-de la Puente et al., 2015).

Since 1904, AHSV has been seen in our canine population and until recently it was believed that dogs only become infected by ingesting infected meat. In 2012, AHSV was detected in a dog with no history of eating infected meat (van Sittert et al., 2013). Although AHS antibodies had been reported in dogs, there was no evidence of horse sickness in dogs being caused by insect-borne virus, (McIntosh, 1955) and until recently, little emphasis has been placed on determining whether *Culicoides* will feed on dogs and thereby transmit AHSV to dogs naturally.

After reviewing the literature, two trapping methods were identified for use in this study. By collecting *Culicoides* midges concurrently with both $CO_2$ and light traps we were able to investigate the seasonal and inter-seasonal population dynamics of *Culicoides* at Onderstepoort. The use of $CO_2$ traps allowed the evaluation of daytime activity of midges as well as assessing the potential efficiency of $CO_2$ traps for collection of stock associated *Culicoides* in South Africa. Seasonal abundance and species composition of *Culicoides* midges collected near dogs at Onderstepoort was compared to *Culicoides* collected near livestock and blood meal analysis allowed us to determine to what extent they feed on dogs. The comparison of the proportion of positive dog blood meals to blood meals obtained from livestock provided an indication of possible host preferences.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 *CULICOIDES*

### 2.1.1 Introduction

*Culicoides* Latreille is a genus of biting midges in the order Diptera, family Ceratopogonidae. They were first described by the reverend W. Derham in 1731 and have an almost cosmopolitan distribution occurring throughout the globe with the exception of Antarctica, New Zealand and Hawaii (Mellor et al., 2000). Common names include no-see-ums, punkies, sandflies, moose flies and five-o's. Of the more than 1 400 species of *Culicoides* described worldwide (Borkent, 2017), at least 120 have been recorded in South Africa, with several species still awaiting formal description (Labuschagne, 2016).

### 2.1.2 Identification

Adults are commonly 1 mm to 2.5 mm in length (Purse et al., 2015; Borkent, 2017) making them one of the smallest species of blood feeding flies. The wing patterns, consisting of hairy grey and hairless white spots, are species specific and can be used for identification to species level using stereo microscopy (Meiswinkel et al., 2004). Approximately 10% of African species, however, do not have wing patterns and need to be dissected and mounted onto glass slides and examined by light microscopy for identification (Meiswinkel et al., 2004; Labuschagne, 2016). In this case the features used to identify species include the shape and number of spermathecae, the shape of the third palpal segment and the distribution of the sensillae on the antennae (Labuschagne, 2016). Molecular methods, e.g. DNA barcoding can also be used to identify *Culicoides* species (Harrup et al., 2016).

### 2.1.3 Life cycle

*Culicoides* females are hematophagous and a blood meal is required to complete the gonotrophic cycle. Although some *Culicoides* species will feed on blood fed mosquitoes, e.g. *Culicoides anophelis* Edwards (Ma et al., 2013) most species can be broadly classified as being either mammal- or bird feeders (Martínez-de la Puente et al., 2015).

Once a blood meal has been taken, egg maturation takes 2 to 4 days depending on environmental temperatures (Veronesi et al., 2009). There are four larval stages, and the pupal stage is usually reached within 10 to 20 days after blood feeding. In temperate regions, *Culicoides* can over-winter as larvae and the larval stage can last for several months (Kettle, 1977; Blanton and Wirth, 1979). If temperatures are between 4 °C to 6 °C, the larval stages will not mature and development will only resume once environmental temperatures increase (Nevill, 1970; Hunt and Tabachnick, 1995; Bishop et al., 1996). The pupal stage lasts approximately four days (Purse et al., 2006). A single generation (egg to egg) takes a minimum of 25 days (Purse et al., 2006).

Females may be classified as either nulliparous or parous based on the absence or presence of a burgundy pigment in the walls of the abdomen (Dyce, 1969). This pigment is deposited after the completion of the first gonotrophic cycle and the production of the first egg batch (Dyce, 1969).

There are four main types of larval habitats, i.e. surface water and soil interface solutions; dung pats of large animals; tree-holes, plants and rock cavities; rotting fruit and plants (Meiswinkel et al., 2004). The pupae of most *Culicoides* species are aquatic and are able to float – the exception being *Culicoides imicola* Kieffer which drown when submersed (Nevill, 1970). Breeding can take place all year round in frost free areas (Becker et al., 2012; Venter et al., 2014). In South Africa, adult numbers mostly peak late summer and decline drastically after the first frost (Venter et al., 1997b). During winter, immature stages develop more slowly due to low temperatures and adult activity is decreased leading to lower overall numbers (Venter et al., 1997b; Meiswinkel et al., 2004). Decreased rainfall in winter also means less semi-aquatic larval habitats (Venter et al., 1997b).

### 2.1.4 *Culicoides* species as vectors of viruses

As a result of their blood feeding habits *Culicoides* females can transmit a large number of viruses, protozoa and nematodes (Meiswinkel et al., 2004; Purse et al., 2015). Viruses transmitted include veterinary important orbiviruses such as BTV and AHSV. To date, more than 75 arboviruses have been isolated from various *Culicoides* species globally (Meiswinkel, et al., 2004; Purse et al., 2015). *Culicoides* hypersensitivity causes "sweet-itch" in horses (Braverman et al., 1983) and in humans, they can be regarded as a biting nuisance (Carpenter et al., 2013).

Adult flight activity is dependent on factors such as environmental temperature, light intensity, relative humidity, changes in barometric pressure and lunar cycles (Mullen and Murphree, 2019). Due to their small size, wind velocity is particularly important (Mullen and Murphree, 2019).

There are two main methods by which *Culicoides* can be dispersed from emergence sites - short-distance flights of less than 5 km and semi-passive wind-aided dispersal which may cover hundreds of kilometres (Sedda et al., 2012; Burgin et al., 2013). Both methods of dispersal have been shown to aid in the spread of arboviruses (Sedda et al., 2012).

It is important to note that there is currently no evidence of vertical transmission of orbiviruses in the genus *Culicoides* (Osborne et al., 2015). Females therefore only become infected after feeding on a viraemic host. Onwards transmission of the virus will only be possible after the completion of the gonotrophic cycle, accompanied by virus replication in the salivary glands, and subsequent blood feedings on susceptible hosts.

### 2.1.5 *Culicoides* abundance and seasonality at Onderstepoort

One of the first studies conducted to determine the seasonal abundance of *Culicoides* species in South Africa was done at Onderstepoort by Nevill in 1967 (Nevill, 1967). In this study 22 species of *Culicoides* were captured, although not all species were present throughout the year (Nevill, 1967). Abundance was extremely low or absent from June to mid-August and increased steadily to September followed by a rapid increase in November and December.  A sharp increase was seen in January and February and the high numbers were maintained until the end of March or April. Numbers then gradually decreased in April and May and were absent by June.  In 1997, (Venter et al., 1997b) found a similar trend and showed that *C. imicola* remained the dominant species throughout the year.  In 2014, it was shown that although *Culicoides* numbers decreased drastically in July and August there were no midge free periods (Venter et al., 2014).

It was previously believed that *Culicoides* are most active at dawn and dusk (Kettle, 1962).  In 2009, a study done at Onderstepoort revealed that the greatest number of midges captured in fact occurred between 21h00 and 01h00 (Page et al., 2009).  The same tendency were observed in 2012 although there was a difference between midges captured in light traps and midges captured by mechanical aspiration (Scheffer et al., 2012).  It has recently been shown that more than 74% of *Culicoides* are collected two to three hours after sunset if mean night-time temperatures are less than 19 °C (Venter et al., 2019).  Numbers peaked at sunset and were sustained until after midnight when the mean night-time temperature was above 19 °C, and when temperatures dropped below 20 °C, numbers collected after midnight increased marginally (Venter et al., 2019).

## 2.2 FIELD COLLECTION OF *CULICOIDES* MIDGES

Vector surveillance is essential to the understanding, epidemiology, monitoring and control of vector-borne diseases.  The objectives of field collection are to study *Culicoides* vector distribution in areas where no information exists, to explore and understand factors influencing the distribution of *Culicoides* and to use all available data to formulate risk assessments for various diseases (Medlock et al., 2018).  If it is not possible to study laboratory colonies of *Culicoides*, then midges need to be collected alive for *inter alia* oral susceptibility studies and insecticide evaluation.

Various diverse methods exist for the surveillance of adult *Culicoides*.  These include suction light traps; truck traps; mechanical aspirators and sweep netting; drop traps and animal bait-traps; Rothamsted traps; sticky traps and carbon dioxide traps. Each method has its own advantages and disadvantages. It is important to note that different collection methods lead to substantially different estimates of relative abundance of vectors in an area (Gerry et al., 2009). A brief description of trapping methods is given below.

### 2.2.1 Mechanical aspirators and sweeping

Aspiration devices where first mentioned by Du Toit in 1944 (Du Toit, 1944) but it wasn't until 1974 that they were used with any success (Mellor and McCraig, 1974). When compared to light traps, mechanical aspiration from host animals has proven more accurate for determining midge biting rates (Gerry et al., 2009; Viennet et al., 2011; Scheffer et al., 2012). In South Africa, Scheffer et al (2012) found a marked variation in the attractiveness of one horse related to another. This could be due to smell, coat colour, body temperature and amount of exhaled carbon dioxide (Scheffer et al., 2012). These authors furthermore found that gravid females and males, although present in nearby light traps, were not captured by aspiration from animal hosts (Scheffer et al., 2012). This is likely since males and gravid females are not seeking a blood meal. Mechanical aspirators have also been used to determine and compare biting rates between different hosts (Meiswinkel and Elbers, 2016). The main advantage of mechanical aspiration is the ability to investigate a specific animal as well as pinpointing where on the animal the most midges can be found (Braverman, 1988). In addition to biting rate assessment, sweep netting can also be used to sample midges at potential larval developing sites (González et al., 2017). A disadvantage is that it is relatively labour intensive and can be, due to their small size and nocturnal habitats, affected by the experience and skill of the operator (Scheffer et al., 2012).

### 2.2.2 Truck traps

These are large net traps mounted on the roof of a moving vehicle. The vehicle is driven at a set speed through an area while the net intercepts flying insects. An advantage is that these un-baited traps can be used throughout the day and can as such capture species that may be active before sunset and after sunrise. A further advantage may be that the volume of air sampled can be calculated and as such give an indication of the density of the *Culicoides* populations in an area. Truck trapping also provides the ideal opportunity to collect in the vicinity of dangerous wildlife in the absence of 220 V electricity. A basic requirement for capture is that the insects must fly at the height of the trap (Barnard, 1980; Sanders et al., 2012). Comparable to truck trapping, midges were collected in Britain using a net suspended from a tethered helium filled balloon to establish the presence of *Culicoides* as evidence of their potential for long-distance dispersal (Sanders et al., 2011).

### 2.2.3 Drop traps and animal bait-traps

Drop traps basically consist of large net cages which are dropped over tethered animals. All *Culicoides* trapped in the cage are then sampled by mechanical aspiration. As for mechanical aspiration a disadvantage is that it is relatively labour intensive and can be, due to their small size and nocturnal habitats, affected by the experience and skill of the operator. These traps allow attacking midges to move to the host animal in a more natural manner as they are naturally found near the host (Elbers and Meiswinkel, 2016). They are also believed to be critical in interpreting the epidemiological significance of light trap collections (Gerry et al., 2009).

### 2.2.4 Rothamsted suction traps

The Rothamsted trap, originally designed for the monitoring of aphid populations, is essentially a suction trap which samples insects at a height of 12.2 m. It consists of a 9.2 m plastic pipe mounted on top of a 3 m box containing an electric fan, netting and a collection bottle (Macaulay et al., 1988). The main advantage of the Rothamsted suction traps is that they do not rely on any attractant and can be run over 24 hours. They also measure absolute abundance per unit volume of air instead of attracting insects over a wide area. The presence of *Culicoides* 12.2 m above ground level, and potential hosts, may help to explain the dispersal capacity of *Culicoides.* These traps provide an unbiased sample which allows for comparisons across a group and region (Fassotte et al., 2008). A major disadvantage of these traps is that they are relatively expensive and are a permanent structure and thus not easily transportable.

### 2.2.5 Sticky traps

A sticky trap is a glue-based trap which can be placed either directly on an animal or in the nearby vicinity. One technique involves mesh-net panels coated in petroleum jelly to form a sticky, all-body cover (Viennet et al., 2011). Thompson et al (2014) attached commercially available single-sided 200 $cm^2$ sticky traps to animals using Velcro. This study highlighted that a preference is shown by midges for certain specific colours of tape (Thompson et al., 2014). Catches utilising sticky tape are comparable to those employing direct aspiration. Sticky traps may also be used to evaluate landing rates on cattle treated with insecticides (Murchie et al., 2019) and to identify potential larval developmental sites. A major disadvantage of this method is the difficulty in removing the specimens from the trap without damaging them (Thompson et al., 2014).

### 2.2.6 Suction traps baited with carbon dioxide

As carbon dioxide ($CO_2$) is released when animals exhale, traps baited with $CO_2$ as an attractant may be attractive to host seeking blood feeding insects looking for a meal. The efficacy of $CO_2$ to attract *Culicoides* species was already shown in 1965 (Nelson, 1965). Regardless of trap type, it has been shown that traps including $CO_2$ collect a greater number of total females (particularly parous females), compared to traps without $CO_2$ (Sloyer et al., 2019). As $CO_2$ traps are not dependant on light, they may be used during the day to determine the activity of diurnal species of *Culicoides*.

The optimum release rate of $CO_2$ has yet to be determined and it is believed that the unregulated release may provide concentrations which are attractive to some species and repellent to others (Venter et al., 2016). Various climatological factors such as prevailing wind, ambient temperature and background $CO_2$ may also affect the dispersal and effectiveness of the $CO_2$ trap (Venter et al., 2016). Another disadvantage of $CO_2$ is that it is relatively expensive thereby leading to possible budgetary constraints (Venter et al., 2016). As males do not take a blood meal, it is possible that they will not be attracted to host chemical cues such as $CO_2$ (McDermott and Mullens, 2018) and may thus be under-represented in $CO_2$ traps.

Numbers of species of *Culicoides* collected may also be increased by adding specific enantiomers of octenol to $CO_2$ (Ritchie et al., 1994). It has also been shown that certain *Culicoides* species such as the Palaearctic *C. obsoletus* do not respond to $CO_2$ (Mullens et al., 2005; Gerry et al., 2009). Despite the apparent efficiency of $CO_2$ to increase trapping efficacy, studies regarding *C. imicola* and other South African livestock associated *Culicoides* species are relatively limited (Venter et al., 2016).

### 2.2.7 Suction light traps

Light traps were first described by Jerome McNeill in 1889 and have been used for almost 130 years to trap nocturnal insects (McDermott and Mullens, 2018). The first suction light traps were used for the collection of mosquitoes in 1930 (Mulhern, 1942). Currently light traps are by far the most popular traps when it comes to vector surveillance. This is due to the fact that they are reasonably cheap, are easily transportable and can be run remotely (McDermott and Mullens, 2018). Some commonly used light traps are the Onderstepoort Trap, the BG-Sentinel trap, and the CDC light trap. The efficacy of various light trap designs may differ significantly (Venter et al., 2009a). Numbers of *Culicoides* collected with the Onderstepoort and BG-Sentinel traps are far greater than those collected with the CDC light trap and this may be due to the fact that their light tubes are twice as long and more powerful (Probst et al., 2015). UV light, as opposed to white light, also increases trap efficiency (Venter and Hermanides, 2006). A major disadvantage of the Onderstepoort trap is its dependence on 220 V electricity supply.

Energy efficient light emitting diodes (LEDs) may increase the numbers collected, depending on the colour of light used (Bishop et al., 2004). Although LEDs may be more suitable for 12 V operation it has been shown that LEDs are less efficient than the traditional 220 V Onderstepoort trap under South African conditions (Venter et al., 2018).

A major disadvantage of light traps is the fact that they are only effective at night and therefore do not sample diurnal species (Mellor et al., 2000; Meiswinkel and Elbers, 2016). Only a very small proportion of the active adult population is collected with light traps and may not be representative of host attack rates (Gerry et al., 2009). Another disadvantage of light traps is the limited attractant range (Venter et al., 2012; Elbers and Meiswinkel, 2016). It has been shown that increased distance from the host negatively affects *Culicoides* abundance (Venter et al., 2012). The attractant range may be increased by increasing the wattage of the light source as well as the suction power of the fan (Elbers and Meiswinkel, 2016). Abundance data is however only comparable if types of traps, host animals and other variables are similar (Venter et al., 2012).

Studies have also shown that UV traps may collect less nulliparous females but a greater number of males (McDermott et al., 2016) which can lead to sex and parity biases. In 2018 this was attributed to the fact that *C. sonorensis* infected with BTV effectively become “blind” and are not attracted to light, thus leading to less pigmented or parous midges being caught in light traps (McDermott and Mullens, 2018) A similar effect was observed with *C. sonorensis* infected with EHDV (Mills et al., 2017). The efficacy of light traps may also decrease with ambient light and different species may be attracted to different wavelengths of light (McDermott et al., 2016). There may also be a risk of attracting infected vectors into closer proximity to the host (Bishop et al., 2006; McDermott and Mullens, 2018). Despite all the disadvantages, suction light traps are still the most widely used trap for epidemiological surveillance of adult *Culicoides* midges.

Based on their ability to collect large numbers of *Culicoides* as well as a large variety of species, we decided to use suction light traps in the present study. $CO_2$ traps enabled us to collect midges during the day as well as at night. Due to the limited information available on the efficacy of $CO_2$ to collected *C. imicola* and other South African livestock associated *Culicoides* species we also evaluated $CO_2$ traps (without a light source) in a South African setting to assess whether they could be used in future to study diurnal *Culicoides* species.

## 2.3 AFRICAN HORSE SICKNESS IN DOGS

Clinical AHSV infection of dogs is invariably fatal (O'Dell et al., 2018). The most common clinical signs are pyrexia and signs of acute respiratory distress syndrome.

Macro-pathological lesions in dogs are similar to those of the 'dunkop' form seen in horses i.e. oedema and hydrothorax (van Sittert et al., 2013).

The first reference to canine African horse sickness (cAHSV) in dogs appears in a Report of the Government Veterinary Bacteriologist of the former Transvaal in 1905-1906 (Theiler, 1907). Sir Arnold Theiler injected several dogs with blood infected with AHSV and noted that the course of ensuing disease as well as the pathological lesions were almost identical to that of AHS in horses (Theiler, 1907). He conducted similar experiments in 1910 in which 24 of 91 dogs injected with AHSV died, 53 showed reactions and recovered and 14 were asymptomatic (Theiler, 1910).

The next mention of cAHSV was in 1911. In this case dogs had been fed on a mule which had died after being immunized against AHSV (Bevan, 1911). Symptoms included tachypnoea, laboured breathing and pyrexia and death 6 to 8 hours after onset of symptoms (Bevan, 1911). Blood from two of the deceased dogs was injected subcutaneously into a horse which subsequently died 10 days later and the post mortem revealed lesions consistent with those of AHSV (Bevan, 1911). In 1951, a pack of hounds in Kenya was fed meat salvaged from horses who had died of AHSV (Piercy, 1951). Of the 35 dogs, 31 became ill and seven died with symptoms and lesions typical to those caused by AHSV (Piercy, 1951).

In 1955 blood was taken from 13 dogs in and around Onderstepoort and Kaalplaas to determine whether any dogs had neutralising antibodies against AHSV (McIntosh, 1955). One serum sample was shown to contain AHSV neutralising antibodies (McIntosh, 1955). Due to the low infection rate in dogs, and the ease with which they become infected artificially or when fed on horse meat, it was concluded that the vectors of AHSV do not readily feed on dogs (McIntosh, 1955). In 1981, it was postulated that the infective dose necessary to generate disease in the dog was extremely high leading to the assumption that dogs are naturally infected only by the consumption of infected meat (Van Rensberg et al., 1981).

This thinking started to change in 1986 when latent AHSV was isolated from *Culex pipiens* Linnaeus that had fed on dogs (El-Hussieni et al., 1986). In this experiment dogs were injected subcutaneously with AHSV as well as being fed on infected horse meat and infected mouse brains (El-Hussieni et al., 1986). Virus-free *C. pipiens* were then allowed to feed on the dogs and Indirect Fluorescent Antibody Tests (IFAT) were conducted on the salivary glands of the mosquitoes (El-Hussieni et al., 1986). The IFAT revealed the presence of AHSV which was confirmed by the isolation of virus in suckling mice (El-Hussieni et al., 1986).

The detection of AHSV infection in various African wild carnivores in 1995 highlighted a need to ascertain whether transmission of AHSV from carnivores to horses via *Culicoides* was possible (Alexander et al., 1995). Vector preference was given as a possible reason for the marked difference in the prevalence of sero-positive animals across different species (Alexander et al., 1995). However, given the fact that the carnivores tested most likely had access to infected meat means that it may not be entirely accurate to base any conclusions about vector preference on the results of this study. In Israel in 1996, it was determined that the number of insects caught in dog kennels was significantly lower than those caught in mixed animal houses containing cows, chickens, donkeys and horses (Braverman and Chizov-Ginzburg, 1996). Using a precipitin test developed by Braverman et al in 1971, a total of 401 blood meals were analysed with the results showing no canine blood meals (Braverman and Chizov-Ginzburg, 1996).

At this point there were conflicting opinions as to whether dogs are capable of acting as hosts for *Culicoides* (van Sittert et al., 2013). In 2012 a dog at the Malelane Research Centre, situated on the border of the Kruger National Park, died of cAHSV (van Sittert et al., 2013). The rest of the dogs were tested and 24 out of 56 dogs showed antibodies to AHSV on ELISA (van Sittert et al., 2013). There was no history of any of these dogs being fed infected horse meat. It was postulated that dogs are an incidental host for *Culicoides* (van Sittert et al., 2013). Between 2007-2017, 33 cases of cAHSV were identified by the Department of Pathology at the University of Pretoria, Onderstepoort (O'Dell et al., 2018). As none of the dogs had been fed on infected horse meat, it was believed that cAHSV may also be vector transmitted in dogs.

To the knowledge of the author, few definitive blood meal analyses have been done to date to confirm that the vectors of AHSV, *Culicoides* midges, do feed on domestic dogs. In 2014 and 2016, positive dog blood meals were found in Tunisia in *C. imicola* and in the eastern Cape in *Culicoides gulbenkiani* Caeiro midges respectively (Slama et al., 2015; Riddin et al., 2019). No South African studies have been done to determine to what extent South African livestock associated *Culicoides* species will feed on dogs.

## 2.4 BLOOD MEAL ANALYSIS

Blood meal analysis is essential in understanding the host-feeding patterns of vector species populations (Garros et al., 2011). Identification of blood meals allows insight into host preferences and aids in the analysis of efficacy of control measures implemented against vector populations (Mukabana et al., 2002). It may also identify potential bridge vectors that could potentially transit viruses between wildlife reservoirs and livestock (Riddin et al., 2019). Trapping a midge in the vicinity of a host does not necessarily imply that the midge has fed on that host (Scheffer et al., 2012). Direct aspiration from the host increases the level of confidence but is not definitive proof that an insect is feeding on that specific host.

There are three main challenges involved in the analysis of arthropod blood meals. Firstly, the volume of blood for analysis is extremely small. The average size of a *Culicoides* blood meal ranges from 0.023 µl to 0.062 µl in *C. imicola* (Venter et al., 2005; De Beer et al., 2020). The next challenge posed is that of digestion of the blood meal by the insect. Lastly, analysis of blood meals of mixed origin may also be difficult.

Although immunological studies began in 1899 with Bordet, it was Nuttal who perfected the use of antisera to detect antibodies for different species (Lichter, 1969). Precipitin tests were largely used in the 1950's to determine the origin of blood meals taken by various hematophagous insects (Weitz and Buxton, 1953; Weitz, 1956; Braverman et al., 1971). Immunological methods are extremely time consuming and not able to distinguish between blood meals from closely related species. The sensitivity is generally low and only one species per blood meal can be identified.

Molecular methods, DNA sequencing in particular, have now largely superseded immunological techniques (Pettersson et al., 2013; Martínez-de la Puente et al., 2015; Van Der Saag et al., 2016; MartÍnez-de la Puente et al., 2017). The main disadvantage of this method is that it is extremely costly and thereby prohibits the processing of large numbers of samples. By only making one copy per cycle the resultant quality of the product will also be poor if annealing temperatures are not optimal. Additionally, any missing sequence data may lead to mis-identification of the blood meal origin (Kent, 2009) and mixed samples may be difficult to interpret. Group specific primers have been used but are only able to detect a broad classification for the blood meal (Kent, 2009). Several of the methods used for blood meal analysis rely on the use of maternally inherited mitochondrial genes. The potentially high number of mitochondria per cell makes the amplification of mitochondrial genes by PCR ideal in analysing small blood meal volumes (Kent, 2009). The method used for this study is based on the mitochondrial cytochrome *b* gene. By designing species-specific primers, the sensitivity of the test is greatly enhanced and negates any issues that would be found using group-specific primers. The method developed by Tobe and Linacre (2008) uses two separate primer sets to amplify a species specific fragment of the cytochrome *b* gene for each species tested. Using two primer sets per species makes additional validation of the results un-necessary. The sizes of the fragments are designed to be species specific and can therefore not be confused with other mammalian species, as may be the case when using DNA sequencing, and this allows the accurate detection of multiple species from a single blood meal. The use of PCR to amplify the amplicon greatly increases the sensitivity of the test which is extremely useful where small volumes such as the blood meals need to be analysed.

It is well established that the host range of *Culicoides* includes both mammals and birds (Martínez-de la Puente et al., 2015). Despite limited studies which have concentrated on the possibility that *Culicoides* feed on dogs it is generally accepted that dogs are not a preferred host for *Culicoides* species (McIntosh, 1955; Braverman and Chizov-Ginzburg, 1996; Martínez-de la Puente et al., 2015). The increase in the detection of BTV (Oura and El Harrak, 2011) and AHSV (Van Rensberg et al., 1981; Alexander et al., 1995; van Sittert et al., 2013; O'Dell et al., 2018) in dogs, however, necessitates a re-evaluation of the situation. In this study we aimed to determine to what extent the *Culicoides* population present in South Africa will feed on domestic dogs and thereby clarify the role dogs, if any, play in the epidemiology of AHS.

# CHAPTER 3: Seasonal abundance, daytime activity and origin of blood meals of *Culicoides* at Onderstepoort

## 3.1 ABSTRACT

In this study, Onderstepoort light traps and $CO_2$ traps were used to collect *Culicoides* midges at Onderstepoort over the period of a year. Abundance, species composition, sex, and parity of collected midges was recorded. Inter-seasonal as well as daytime activity of midges was identified. Blood meal analysis of collected midges yielded definitive evidence that *Culicoides* midges will feed on dogs but to a much lesser extent than they feed on horses and other livestock.

## 3.2 INTRODUCTION

*Culicoides* Latreille (Diptera: Ceratopogonidae) biting midges are prevalent throughout most of the world. They are of great medical and veterinary importance due to their role as vectors of viruses, nematodes, and protozoa. Economically important viruses of veterinary prominence transmitted by *Culicoides* include African horse sickness- (AHSV), bluetongue- (BTV) and epizootic haemorrhagic disease virus (EHDV).

To clarify the epidemiology of diseases transmitted by these vectors numerous studies have been done in South Africa to determine the seasonal abundance and species diversity of *Culicoides* (Nevill et al., 1988; Venter et al., 1997a; Meiswinkel et al., 2004; Labuschagne et al., 2007; Labuschagne, 2016). These studies relied on light traps and were therefore biased towards the collection of night active *Culicoides* species. Based on the patterns of disease occurrence it was for many years believed that *Culicoides* midges are mainly nocturnal and only prevalent during the summer months (Meiswinkel et al., 2004; Purse et al., 2015). More recently, diurnally active species have been described (e.g. *Culicoides actoni* Smith) (Bellis et al., 2004) and it has been shown that *Culicoides* activity may move from a more nocturnal behaviour in hotter weather to a more diurnal behaviour in cooler weather (Walker, 1977). In Europe, *Culicoides obsoletus* activity was found to shift from before sunset in spring and autumn to after sunset in summer (Viennet et al., 2012).

In 2014, a study conducted in California, USA, utilising $CO_2$ baited traps confirmed daytime activity of *C. sonorensis* and that *Culicoides* can be active during the colder winter months (Mayo et al., 2014). Although $CO_2$ has been evaluated in addition to light as an attractant in South African studies (Venter et al., 2016), it has seldom been used on its own. Few studies have therefore concentrated on determining whether there is daytime activity of stock associated *Culicoides* in South Africa.

In recent years, more attention has been focused on cAHSV. Where it was previously believed to be restricted to dogs consuming infected meat, this no longer seems to be the only possible route of infection (van Sittert et al., 2013). Studies on blood meal analysis have concentrated mainly on *Culicoides* as vectors for livestock associated diseases and positive blood meals for other species such as dogs have been reported as an almost incidental finding. There is therefore little information available on the extent that *Culicoides* will feed on dogs and potentially infect them with AHSV.

This study allowed us to compare the relative species abundance of *Culicoides* midges at Onderstepoort, South Africa over a year. The numerous previous studies done at Onderstepoort serve as a comparative baseline. It was anticipated that these data would reflect the findings in the USA and demonstrate potential midge activity during the winter months. The aim was also to determine potential daytime activity of midges using $CO_2$ traps. In addition to demonstrating inter-seasonal and daytime activity the aim was to use blood meal analysis of freshly blood fed female midges to confirm to what extent stock associated *Culicoides* in South Africa feed on dogs.

## 3.3 MATERIALS AND METHODS

### 3.3.1 Study area

Collections were made in the vicinity of resident animals housed at the Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa (25,64951; 28,18541, 1222 m above sea level). It is a summer rainfall area with a moderate, dry, subtropical climate. The annual mean maximum daily temperature is 26.3 °C and the annual mean daily minimum temperature is 9.3 °C (Venter et al., 1997a). Rainfall occurs mostly from November to March and ranges from 430 mm to 1017 mm per annum. Peak rainfall occurs in January. The warmest month of the year is January with June and July being the coldest. Frost occurs between April and September with a mean of 30 days of frost per annum.

The study area contained scattered trees and a mixture of irrigated pastures and natural pastures. Indigenous wildlife such as small rodents and wild birds were most likely present at all collection sites. The camps and areas at Onderstepoort in which collections were made can be seen in Figure 1.

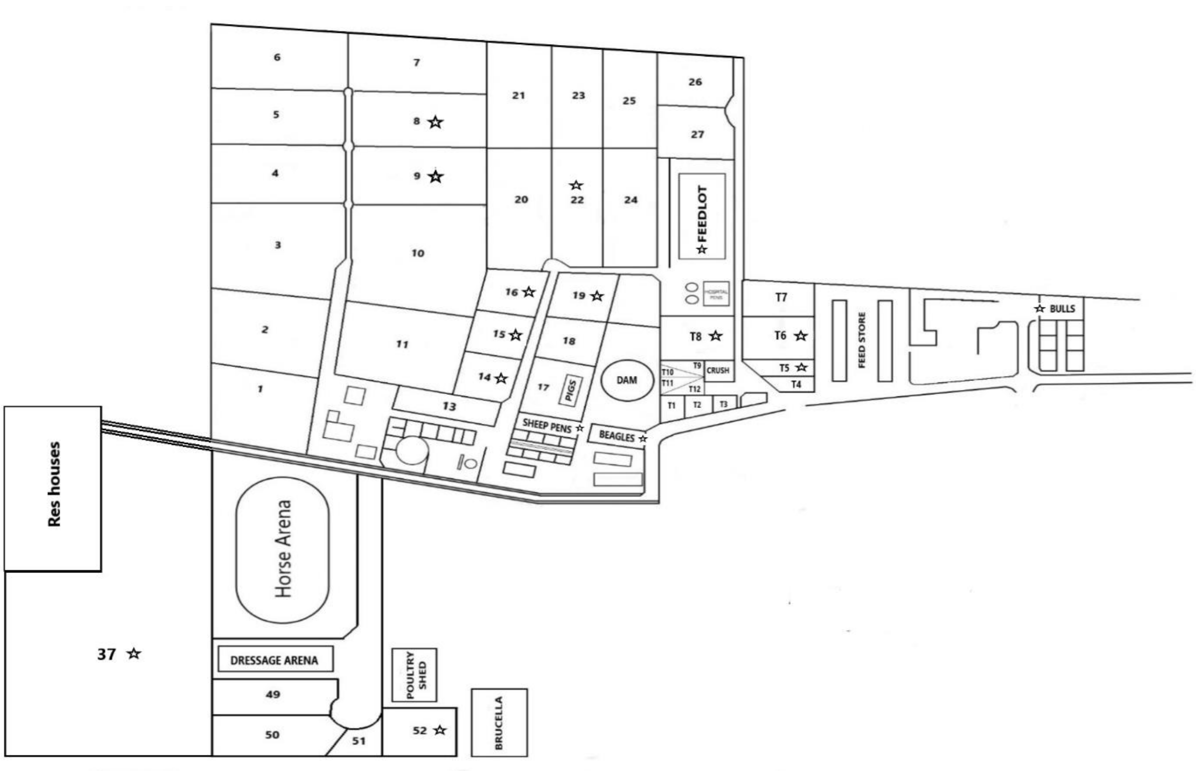



Figure 1: Sites, indicated by stars, at the Faculty of Veterinary Science, Onderstepoort where light trap and $CO_2$ collections where made to determine *Culicoides* species composition and abundance from 28 August 2019 to 28 August 2020

### 3.3.2 Experimental design

Two trapping techniques were used namely suction light traps and suction traps baited with carbon dioxide.

*3.3.2.1 Onderstepoort light traps*

Three Onderstepoort downdraught, 220 V suction light traps with 8 W, 23 cm UV light tubes were used (Venter et al., 2009a) (Figure 2).

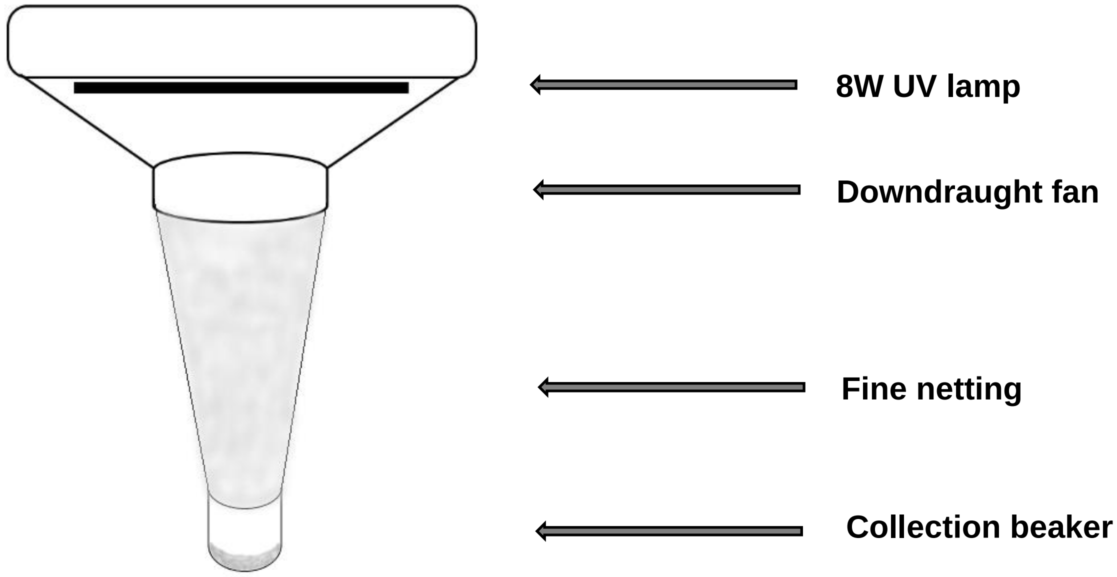



Figure 2: Diagram of an Onderstepoort light trap

To analyse the abundance and species composition of *Culicoides* midges at Onderstepoort both seasonally and inter-seasonally light traps were set up in the vicinity of different animal species at Onderstepoort. Sites were chosen near horses and sheep as well as in the dog kennels to compare the relative abundance of *Culicoides* found in the vicinity of different species of animals and thus potentially determine the host preferences of *Culicoides*. Collections were made over a year from 28 August 2019 to 28 August 2020 to compare abundance at different times of the year.

Traps were 2 m to 2.5 m above the ground depending on the structure from which they were hung. Light trap collection sites were chosen due to the need for access to electricity as well as a variety of species of host animal. By placing the traps near different host animals, i.e. horses, cattle, sheep, and dogs we hoped to collect *Culicoides* that had fed on a variety of animal species.

Light trap 1

Collections at this site were made from 28 August 2019 to 28 August 2020. Potential livestock hosts in the vicinity of the trap included horses and cattle. The trap was placed in a tree in camp T5 (Figure 1), at a height of 2.5 m. Initially, there were four horses in this camp, but they were replaced by cows on 18 September. The number of cows in T5+T6 ranged from 22 to 27. Exact numbers are shown in Appendix 1.

Due to unforeseen circumstances the electricity to this trap was interrupted on 3 December 2019. On 22 January 2020, this trap was moved to Camp 52 (Figure 1). There were between 8 and 11 horses in camps 49 to 52. Exact numbers are shown in Appendix 1. These camps are exclusively used for horses with no other animals in the immediate vicinity. There were no other light sources in the vicinity of the trap.

Light trap 2

Collections at this site were made from 28 August 2019 to 28 August 2020. Potential hosts in the vicinity of the trap include sheep and goats (see Appendix 1 for exact dates and numbers of animals). The light trap was placed in the sheep pens (Figure 1), at a height of 2.2 m. There were no other light sources in the vicinity of the trap.

Light trap 3

The light trap was placed in the dog kennels at a height of 2 m. There were ten medium-sized dogs (Beagles) which sleep in the surrounding kennels on a nightly basis. During winter, each of the five kennels had an infra-red lamp for warmth which was approximately 1.8 m from the light trap.

Light trap collections were done on a weekly basis at the above sites. Most collections were performed on a Wednesday night although this did vary depending on logistical constraints and weather conditions. The exact number of animals in each camp on a particular date is shown in Appendix 1.

*3.3.2.2 Carbon dioxide traps*

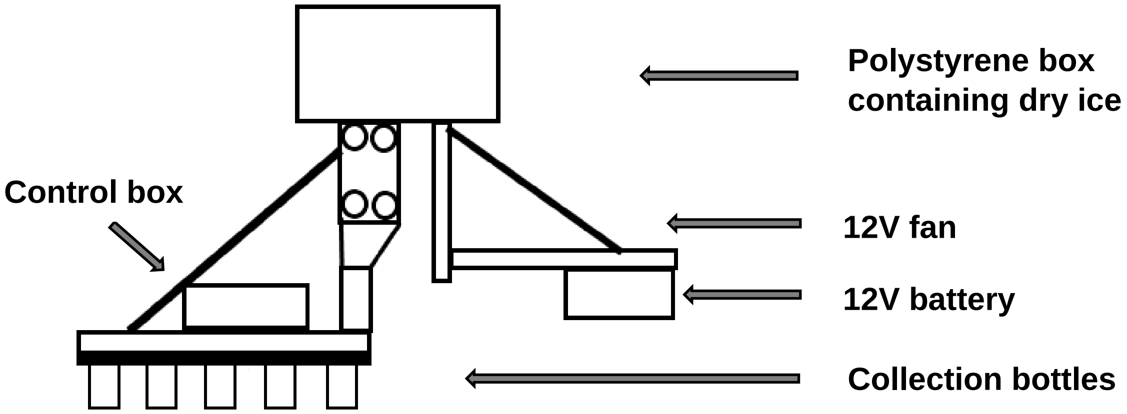
To determine the potential efficiency of $CO_2$ traps for the collection of stock associated *Culicoides* in South Africa the numbers collected with $CO_2$ traps were compared to those collected with the Onderstepoort light trap. This type of trap was also used due to the ability to be effective during the day. By using a rotational sampler, we were able to determine at what time of day *Culicoides* were most active and whether *Culicoides* were active during the day.

$CO_2$ traps and rotational samplers were custom made for this study. The trap consisted of a polystyrene box with dimensions 25.5 cm x 8.5 cm x 21 cm (Figure 3). Six holes with a diameter of 10 mm were made in the sides of the box to allow the $CO_2$ to escape. Each box contained a 2.5 kg block of dry ice (Venter et al., 2016). As the dry ice sublimates, it releases $CO_2$ through the holes. Dry ice sublimates at approximately 1-2% per hour, and 2.5 kg dry ice was sufficient to last the 24 hours for which the trap was operational.

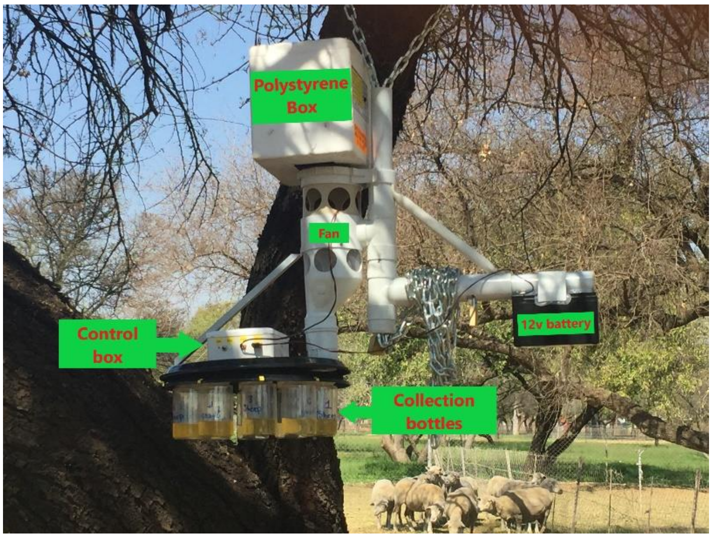
Midges attracted to the trap by the $CO_2$ were sucked in by a 12 V, 80 mm fan. Below the fan, the tubing was covered with a fine mesh to prevent insects from escaping (Figure 3). The midges then fell through the funnel into the collection bottle. The bottles contained 100 ml of water with 0.5% Savlon® solution (cetrimide 3.0 g/100 ml, chlorhexidine gluconate 0.3 g/100 ml) to reduce bacterial contamination and to break the surface tension of the water and allow the midges to sink.

An indexing system rotates the bottles in a clockwise direction every three hours allowing for time of sampling to be recorded. As there are 10 bottles, the traps may be used for up to 30 hours. It is also possible to set the indexing system to change bottles every 30 seconds. This function was added to allow easy testing of the system and ensure correct functionality and bottle positioning. We used the three-hour function and operated the traps once weekly for 24 hours. The reason we chose a three-hour cycle was to be able to differentiate between midges caught at different times of the day/night while the light trap is only operational at night. It would also allow us to compare the nightly $CO_2$ collection with that of the light traps. The fan and indexing system are run on a 12 V battery attached to the rear of the trap (Figure 3).

The unit can be mounted either on a pedestal or hung by a chain from a tree or pole. The trap situated near the dog kennels was mounted on a tripod whilst the remaining traps were suspended from a tree located in the camp in which we were trapping that week.




(a)




(b)

Figure 3: Diagram (a) and photo (b) of the carbon dioxide rotational sampler

Four $CO_2$ traps were used during this study and were run concurrently with the light traps. One trap was used exclusively at the dog kennels and was placed between the kennels used to house the dogs at night and the camp in which the dogs were running during the day. This trap was on a tripod 1.5 m above the ground. The trap was approximately 5 m from a light trap but was behind a wall and around the corner.

The remaining three traps were moved according to where each species of animal was located (namely horses, cows, and sheep). The camp number and the species within that camp were recorded for each trapping and can be found in Appendix 2. Although every effort was made to run all four traps every week this was not always possible due to traps in need of repair/maintenance etc. Traps were placed every week for a year, with the exception of 11 weeks (27 March 2020 to 30 June 2020), where South Africa was on lockdown due to COVID-19. Traps were set up between 08h00 and 10h00 once weekly and taken down 24 hours later.

### 3.3.3 Collection of *Culicoides*

Insects caught in the light traps were collected into 500 ml plastic beakers containing 100 ml water and a 0.5% Savlon® solution. In the case of the carbon dioxide traps – 250 ml plastic bottles containing 100 ml water and a 0.5% Savlon® solution were used. Once collected, the Savlon® solution was passed through a fine gauze filter and the insects were placed in 80% ethanol in sealed 250 ml plastic bottles (Goffredo and Meiswinkel, 2004). The bottles were labelled with the date, type of trap used, animals in the vicinity of the trap, and in the case of the carbon dioxide traps, the time of trapping. The collected insects were stored in the dark at room temperature until analysed.

### 3.3.4 *Culicoides* identification

Initial separation of *Culicoides* midges from other insects was achieved using a stereomicroscope. Once separated, the midges were identified to species level using appropriate identifications keys (Labuschagne, 2016) and sorted by sex and parity based on abdominal pigmentation as described by Dyce (1969). All freshly blood fed midges were identified and kept in separately labelled Eppendorf tubes containing 80% ethanol. Each separate species was then stored in a labelled Eppendorf tube containing 80% ethanol and was then further sorted and stored by sex and parity. The resulting counts were captured on a standardised Excel Spreadsheet and later transferred to an Access database.

During the months where midge numbers collected exceeded a thousand, subsampling was used to aid analysis. Samples were first passed through a 5 mm diameter mesh to exclude any large insects caught in the traps. The container was then gently shaken to suspend the remaining insects in the alcohol and equal volumes were drawn from the suspension using a pipette and placed in Bijoux bottles. One of the Bijoux bottles was then counted, and the midges stored in Eppendorf tubes as previously stated. If the total midges in the subsampled bottle was less than 1 000, then a second bottle was counted. The total number of midges in the collection was determined by multiplying the number of midges counted per Bijoux bottle by the number of Bijoux bottles into which the sample was divided.

### 3.3.5 Blood meal analysis

To analyse blood meals to assess what species of mammal, in particular dogs, *Culicoides* are feeding on, all freshly blood fed *Culicoides* collected with the light and $CO_2$ traps were sorted from the collection. Due to the large number of blood fed midges collected, not all were used for blood meal analysis. All midges collected at the dog kennels were analysed as well as a subsample of the other midges collected.

Blood fed females were stored in Eppendorf tubes in 80% alcohol in a 4 °C refrigerator prior to analysis. Individual midges were then placed in Eppendorf tubes containing 150 µl of phosphate buffered saline (PBS) and homogenised using a DWK Life Sciences Kimble™ Kontes™ Pellet Pestle™ Cordless Motor under a laminar flow hood to prevent contamination. Every effort was made to prevent contamination of the sample with human DNA by wearing gloves and sterilising all equipment used with Chlorcol®. DNA was extracted using the MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems) following the manufacturers protocol. This kit is designed for rapid purification of DNA and RNA. A magnetic bead, proteinase K (PK) mix was added to each well of a deep well plate and a lysis/binding solution was prepared. A 100 µl aliquot of the PBS solution in which the individual blood fed midges were homogenised was added to individual wells of the deep well plate. Lysis/binding solution (700 µl) was then added to each well and the sample plate was loaded on a KingFisher 96 Magnetic Bead processor and processed according to the manufacturer’s recommendations.

A total of 1 µl of the extracted DNA solution was then added to 2.8 µl of primer mix (including three universal primers and two primers for each of the following species - cat, dog, cow, horse, human, donkey, sheep, pig and goat DNA), 1.2 µl of water and 5 µl of KAPA Multiplex Master Mix and amplified via PCR. A total of 1 µl of the amplified solution was then added to 9 µl of HiDI Formamide + 0.25 µl of Genescan 500 LIZ size standard and denatured. Initial denaturation was at 95 °C for 3 minutes. This was followed by 30 cycles of 95 °C for 15 sec; 60 °C for 30 sec; 72 °C for 30 sec. Final extension occurred at 72 °C for 10 min and was then cooled to 4 °C. The final product was run on a 3500xl Sequencer – Thermo Fisher Scientific, utilizing a fragment analysis protocol with a 50cm capillary and POP-7™ polymer, and FSA (raw data) files were transferred to STRand software for analysis.

3.3.6 Climatic data

Appendix 3 shows the weather data for the days on which midge collections occurred. Minimum and maximum temperature, wind and rainfall were noted for each date on which collections occurred. Weather data was provided by the South African Weather Service and was collected at the weather station at Proefplaas (-25.7520 28.2580).

3.3.7 Statistical Analysis

Negative binomial regression models were used to assess the effect of trap type ($CO_2$ vs light trap), month and proximity to host species on *Culicoides* counts, overall and separately for *C. imicola* and *C. leucostictus*. For light traps only, negative binomial regression models were used to assess the effect of month and proximity to host species on *Culicoides* counts. For $CO_2$ traps only, negative binomial regression models were used to assess the effect of time of day, month, and proximity to host species on *Culicoides* counts. Associations between trap types and parity were assessed using Fisher's exact test, and associations between host animal species and parity were assessed using the chi squared test (this was due to the number of enumerations for Fisher's exact test being too large).

## 3.4 RESULTS

*Culicoides* midges were collected over a total of 43 trapping days spanning from 28 August 2019 to 28 August 2020. On each trapping date there were between two and seven traps running. Traps were set up weekly except during COVID-19 lockdown levels 4/5 which included April, May and the first two weeks of June 2020.

The total number of *Culicoides* collected were 461 221 with Onderstepoort light traps and 4 233 in $CO_2$ baited traps. The count ratio estimated by the negative binomial regression model was (CR = 836; 95% CI: 477.6-1464.1; $P < 0.001$). Exact numbers as well as differentiation by sex and parity can be found in Appendix 4/5.

### 3.4.1 Onderstepoort light trap collections

Species richness varied from 15 to 21 different species of *Culicoides* collected with the light traps over the 43 trapping days with a total of 23 species being collected between 28 August 2019 and 28 August 2020. *Culicoides imicola* constituted 92.4% of the total *Culicoides* collected with Onderstepoort light traps (Table 1). Although the species richness of *Culicoides* collected in the horse/cow and sheep/goat traps was similar, the richness of species collected at the dog kennels was much lower with *Culicoides leucostictus* Kieffer rather than *C. imicola* being the predominant species collected.

The numbers of *Culicoides* collected increased steadily from September 2019 to January 2020 with a sharp increase in February 2020 (Figure 4). This was followed by a marked decrease in numbers from February 2020 to March 2020. Due to COVID-19, there is no data is available for April/May 2020. When collection resumed in June 2020 the numbers were extremely low and only increased slightly in July 2020 and then increased once again in August 2020 (Figure 4).

Table 1: Mean numbers of *Culicoides* species collected with 220 V Onderstepoort light traps at various livestock species at the Faculty of Veterinary Science, Onderstepoort, from 28 August 2019 to 28 August 2020

| | **Light trap 1** | **Light trap 2** | **Light trap 3** | |
|---|---|---|---|---|
| | **Horses/Cows** | **Sheep/Goats** | **Dogs** | |
| **No of collections made** | 36 | 43 | 41 | |
| **Species Richness** | 20 | 21 | 15 | |
| ***Culicoides* species** | **Mean (%)** | **Mean (%)** | **Mean (%)** | **Total (%)** |
| *Culicoides imicola* | 7 175.4 (93.9) | 3 725.5 (94.4) | 190.7 (46.7) | 425 949 (92.4) |
| *Culicoides leucostictus* | 131.3 (1.7) | 93.2 (2.4) | 205.2 (50.3) | 17 147 (3.7) |
| *Culicoides enderleini* | 185.4 (2.4) | 33.9 (0.9) | 0.2 (0.1) | 8 143 (1.8) |
| *Culicoides subschultzei* | 60.0 (0.8) | 15.2 (0.4) | 0.3 (0.1) | 2 826 (0.6) |
| *Culicoides pycnostictus* | 35.7 (0.5) | 24.3 (0.6) | 6.2 (1.5) | 2 581 (0.6) |
| *Culicoides nivosus* | 26.9 (0.3) | 27.4 (0.7) | 2.3 (0.6) | 2 151 (0.5) |
| *Culicoides bedfordi* | 14.1 (0.2) | 10.3 (0.3) | 2.1 (0.5) | 1 035 (0.2) |
| *Culicoides zuluensis* | 5.9 (0.1) | 6.9 (0.2) | 0.1 (<0.1) | 508 (0.1) |
| Nigripennis grp | 4.5 (0.1) | 1.6 (<0.1) | 0.9 (0.2) | 265 (0.1) |
| *Culicoides brucei* | 1.5 (<0.1) | 3.3 (0.1) | 0.1 (<0.1) | 199 (<0.1) |
| *Culicoides nevilli* | 1.6 (<0.1) | 0.8 (<0.1) | 0.2 (<0.1) | 103 (<0.1) |
| *Culicoides magnus* | 0.6 (<0.1) | 1.8 (<0.1) | - | 96 (<0.1) |
| *Culicoides ravus* | 1.0 (<0.1) | 1.0 (<0.1) | <0.1 (<0.1) | 80 (<0.1) |
| *Culicoides similis* | 0.3 (<0.1) | 0.5 (<0.1) | 0.1 (<0.1) | 37 (<0.1) |
| *Culicoides olyslageri* | 0.7 (<0.1) | - | - | 24 (<0.1) |
| *Culicoides engubandei* | - | 0.4 (<0.1) | <0.1 (<0.1) | 19 (<0.1) |
| *Culicoides neavei* | 0.1 (<0.1) | 0.4 (<0.1) | <0.1 (<0.1) | 19 (<0.1) |
| *Culicoides coarctatus* | 0.3 (<0.1) | <0.1 (<0.1) | - | 11 (<0.1) |
| *Culicoides cornutus* | 0.1 (<0.1) | 0.2 (<0.1) | - | 10 (<0.1) |
| *Culicoides expectator* | <0.1 (<0.1) | 0.2 (<0.1) | - | 9 (<0.1) |
| *Culicoides bolitinos* | - | 0.1 (<0.1) | - | 4 (<0.1) |
| *Culicoides eriodendroni* | - | 0.1 (<0.1) | - | 4 (<0.1) |
| *Culicoides pretoriensis* | <0.1 (<0.1) | - | - | 1 (<0.1) |

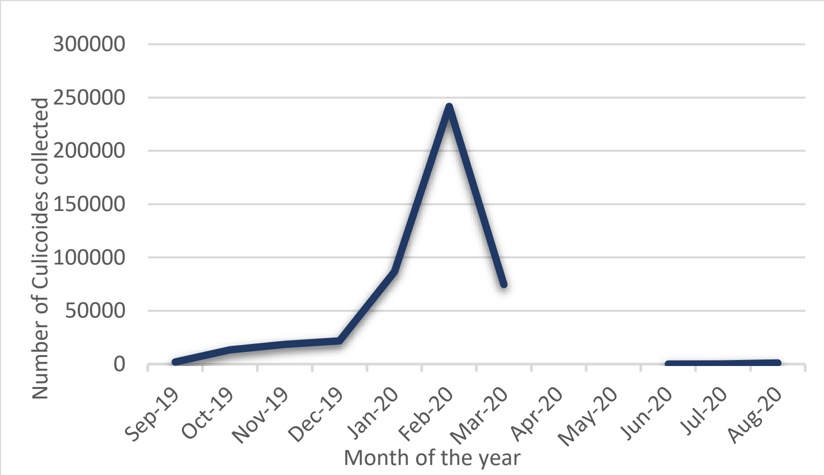



Figure 4: Monthly variation in the total number of *Culicoides* collected with 220 V Onderstepoort light traps at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

Table 2: Association between month and count ratio of *Culicoides* collected with Onderstepoort light traps at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

| | **Count Ratio (CR)** | **95% Confidence Interval** | **P-Value** |
|---|---|---|---|
| **Month** | | | |
| Aug-19 | 1 (base) | | |
| Sep-19 | 0.3 | 0.1-0.8 | 0.023 |
| Oct-19 | 2.5 | 0.9-7.4 | 0.092 |
| Nov-19 | 4.7 | 1.5-14.2 | 0.006 |
| Dec-19 | 10.4 | 3.3-33.1 | <0.001 |
| Jan-20 | 16.7 | 5.5-50.3 | <0.001 |
| Feb-20 | 38.4 | 12.4-118.7 | <0.001 |
| Mar-20 | 11.1 | 3.7-33.3 | <0.001 |
| Jun-20 | 0.0 | 0.0-0.0 | <0.001 |
| Jul-20 | 0.0 | 0.0-0.1 | <0.001 |
| Aug-20 | 0.3 | 0.1-0.8 | 0.022 |

*3.4.1.1 Light trap 1*

Light trap 1 was placed near horse/cows. Appendix 1 shows which camps were used on a specific date as well as the number of animals in the camps at the time of collection. Figure 1 shows a map of Onderstepoort including the location of each camp used. The greatest number of *Culicoides* midges collected in a single night was 76 368 on 5 February 2020. The highest monthly average was recorded in February 2020 with an average of 44 347 (Figure 5a). (Please note that the trap was not operational in December 2019 due to electrical issues).

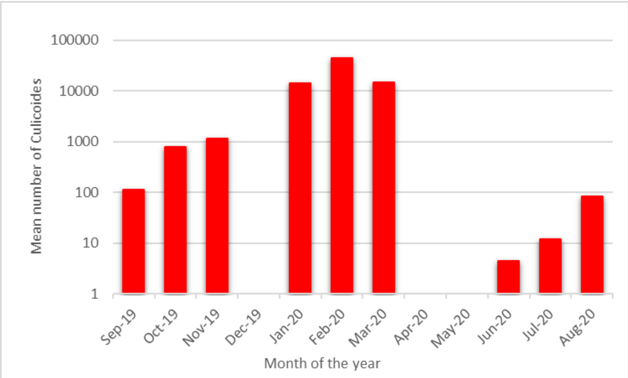
*3.4.1.2 Light trap 2*

Light trap 2 was placed near sheep/goats. Appendix 1 shows which camps were used on a specific date as well as the number of animals in the camps at the time of collection. Figure 1 shows a map of Onderstepoort including the location of each camp used. The greatest number of *Culicoides* midges collected in a single night was 31 832 on 22 January 2020. The highest monthly average was recorded in February 2020 with an average of 15 563 (Figure 5b).

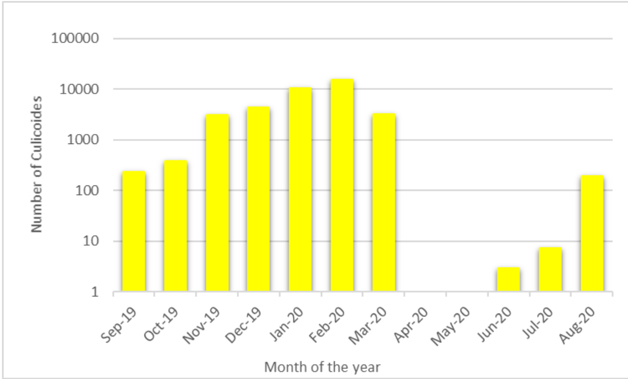
*3.4.1.3 Light trap 3*

Light trap 3 was placed in the dog kennels (Figure 1). The greatest number of *Culicoides* midges collected in a single night was 3276 on 26 December 2019. The highest monthly average was recorded in December with an average of 1 048 (Figure 5c). It is however important to note that Trap 3 was not operational for 2 weeks in February due to electrical issues.

a)




b)




c)

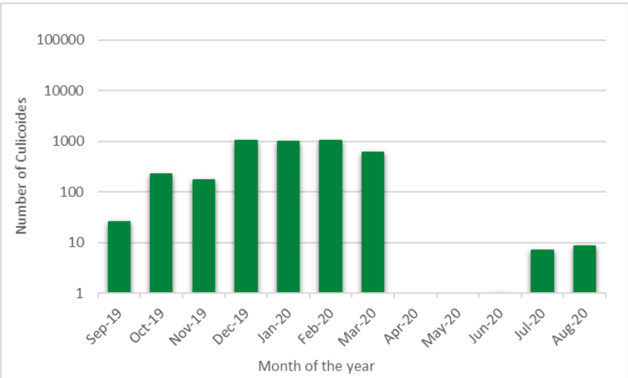



Figure 5: Monthly variation in the mean number of *Culicoides* collected with 220 V Onderstepoort light traps situated near a) horses/cows, b) sheep/goats and c) dogs at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

Table 3: Association between host animal species and count ratio of *Culicoides* collected with Onderstepoort light traps at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

| | **Count Ratio (CR)** | **95% Confidence Interval** | **P-Value** |
|---|---|---|---|
| **Host animal species** | | | |
| Dogs | 1 (base) | | |
| Cows | 3.8 | 2.0-7.2 | <0.001 |
| Horses | 8.7 | 5.2-14.5 | <0.001 |
| Sheep | 9.1 | 4.8-17.5 | <0.001 |
| Goats | 6.0 | 3.8-9.4 | <0.001 |
| None | 1.3 | 0.5-3.2 | 0.553 |

Compared to dogs, the count ratio was significantly higher at horses/cows and sheep/goats ($P < 0.05$) (Table 3).

3.4.2 Carbon dioxide trap collections

Species richness varied from 5 to 8 different species of *Culicoides* collected with $CO_2$ baited traps over the 43 trapping dates with a total of 9 species being collected between 28 August 2019 and 28 August 2020. *Culicoides leucostictus* made up 72.3% of the total *Culicoides* collected with *C. imicola* only accounting for 0.9% (Table 2).

The numbers of *Culicoides* collected increased from September 2019 to October 2019 but then decreased slightly in November 2019 followed by a sharp increase in December 2019 (Figure 6). This was followed by a sharp decline in January 2020 and numbers continued to decline in February 2020 and again in March 2020. Due to COVID-19, there is no data is available for April/May 2020 (Figure 6).

Table 4: Mean numbers of *Culicoides* collected with $CO_2$ baited traps at various livestock species at the Faculty of Veterinary Science, Onderstepoort, from 28 August 2019 to 28 August 2020

| | **$CO_2$ 1** | **$CO_2$ 2** | **$CO_2$ 3** | **$CO_2$ 4** | |
|---|---|---|---|---|---|
| | **Sheep** | **Horses** | **Cows** | **Dogs** | |
| **No of collections made** | 41 | 37 | 33 | 26 | |
| **Species Richness** | 7 | 6 | 8 | 5 | |
| ***Culicoides* species** | **Mean (%)** | **Mean (%)** | **Mean (%)** | **Mean (%)** | **Total (%)** |
| *Culicoides leucostictus* | 10.9 (67.2) | 12.4 (50.6) | 53.2 (78.3) | 13.9 (95.0) | 3 062 (72.3) |
| *Culicoides pycnostictus* | 3.1 (18.7) | 9.1 (37.2) | 6.6 (9.7) | <0.1 (0.3) | 680 (16.1) |
| *Culicoides nivosus* | 1.7 (10.5) | 2.6 (10.7) | 7.8 (11.4) | <0.1 (0.3) | 424 (10.0) |
| *Culicoides imicola* | 0.2 (0.9) | 0.3 (1.2) | 0.1 (0.2) | 0.6 (3.9) | 36 (0.9) |
| *Culicoides bedfordi* | 0.3 (1.6) | 0.1 (0.2) | 0.1 (0.1) | 0.1 (0.5) | 17 (0.4) |
| Nigripennis grp | 0.2 (0.9) | - | - | - | 6 (0.1) |
| *Culicoides enderleini* | <0.1 (<0.1) | <0.1 (<0.1) | 0.1 (0.1) | - | 5 (0.1) |
| *Culicoides magnus* | - | - | 0.1 (0.1) | - | 2 (<0.1) |
| *Culicoides subschultzei* | - | - | <0.1 (<0.1) | - | 1 (<0.1) |

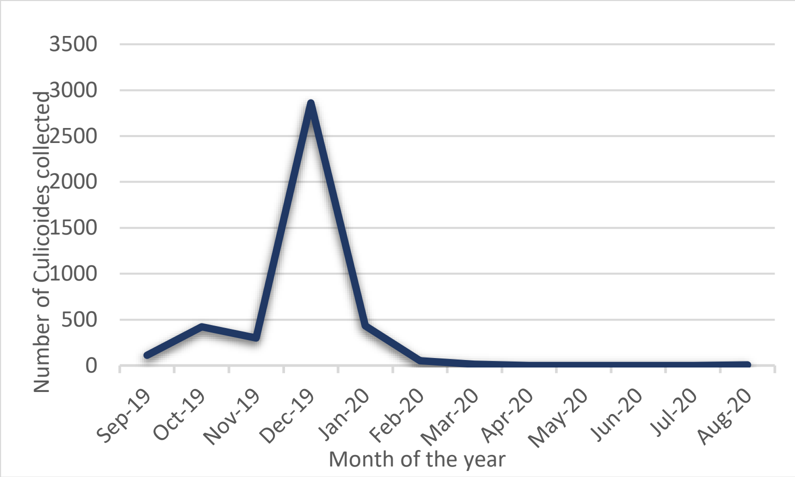



Figure 6: Monthly variation in the total number of *Culicoides* collected with $CO_2$ baited traps at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

Table 5: Association between month and count ratio of *Culicoides* collected with $CO_2$ baited traps at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

| | **Count Ratio (CR)** | **95% Confidence Interval** | **P-Value** |
|---|---|---|---|
| **Month** | | | |
| Aug-19 | 1 (base) | | |
| Sep-19 | 1.0 | 0.4-3.1 | 0.941 |
| Oct-19 | 2.6 | 0.9-7.4 | 0.081 |
| Nov-19 | 2.4 | 0.8-7.0 | 0.125 |
| Dec-19 | 16.0 | 5.4-47.5 | <0.001 |
| Jan-20 | 2.1 | 0.7-6.2 | 0.188 |
| Feb-20 | 0.4 | 0.1-1.4 | 0.148 |
| Mar-20 | 0.2 | 0.1-0.8 | 0.026 |
| Jun-20 | 0.0 | 0.0-0.0 | 0.988 |
| Jul-20 | 0.0 | 0.0-0.0 | 0.978 |
| Aug-20 | 0.1 | 0.0-0.2 | <0.001 |

Compared to August 2019, the count ratio was significantly higher in December 2019 ($P < 0.05$) but lower in March 2020 ($P < 0.05$) and August 2020 ($P < 0.05$) (Table 5).

*3.4.2.1 Carbon dioxide trap 1*

$CO_2$ trap 1 was placed near sheep. Appendix 2 shows which camps were used on a specific date as well as the number of animals in the camps at the time of collection. The greatest number of *Culicoides* midges collected in a single 24-hour period was 193 on 26 December 2019. The highest monthly average was recorded in December with a total of 255 midges being collected (Figure 7a).

*3.4.2.2 Carbon dioxide trap 2*

$CO_2$ trap 2 was placed near horses. Appendix 2 shows which camps were used on a specific date as well as the number of animals in the camps at the time of collection. The greatest number of *Culicoides* midges collected in a single 24-hour period was 431 on 26 December 2019. The highest monthly average was recorded in December with a total of 480 midges being collected (Figure 7b).

*3.4.2.3 Carbon dioxide trap 3*

$CO_2$ trap 3 was placed near cows. Appendix 2 shows which camps were used on a specific date as well as the number of animals in the camps at the time of collection. The greatest number of *Culicoides* midges collected in a single 24-hour period was 1 449 on 18 December 2019. The highest monthly average was recorded in December with a total of 1 920 midges being collected (Figure 7c).

*3.4.2.4 Carbon dioxide trap 4*

$CO_2$ trap 4 was placed in the dog kennels (Figure 1), but was only operational from December 2019 and there is therefore no data prior to that. The greatest number of *Culicoides* midges collected in a single 24-hour period was 198 on 26 December 2019. The highest monthly average was recorded in December with a total of 206 midges being collected (Figure 7d).

b)

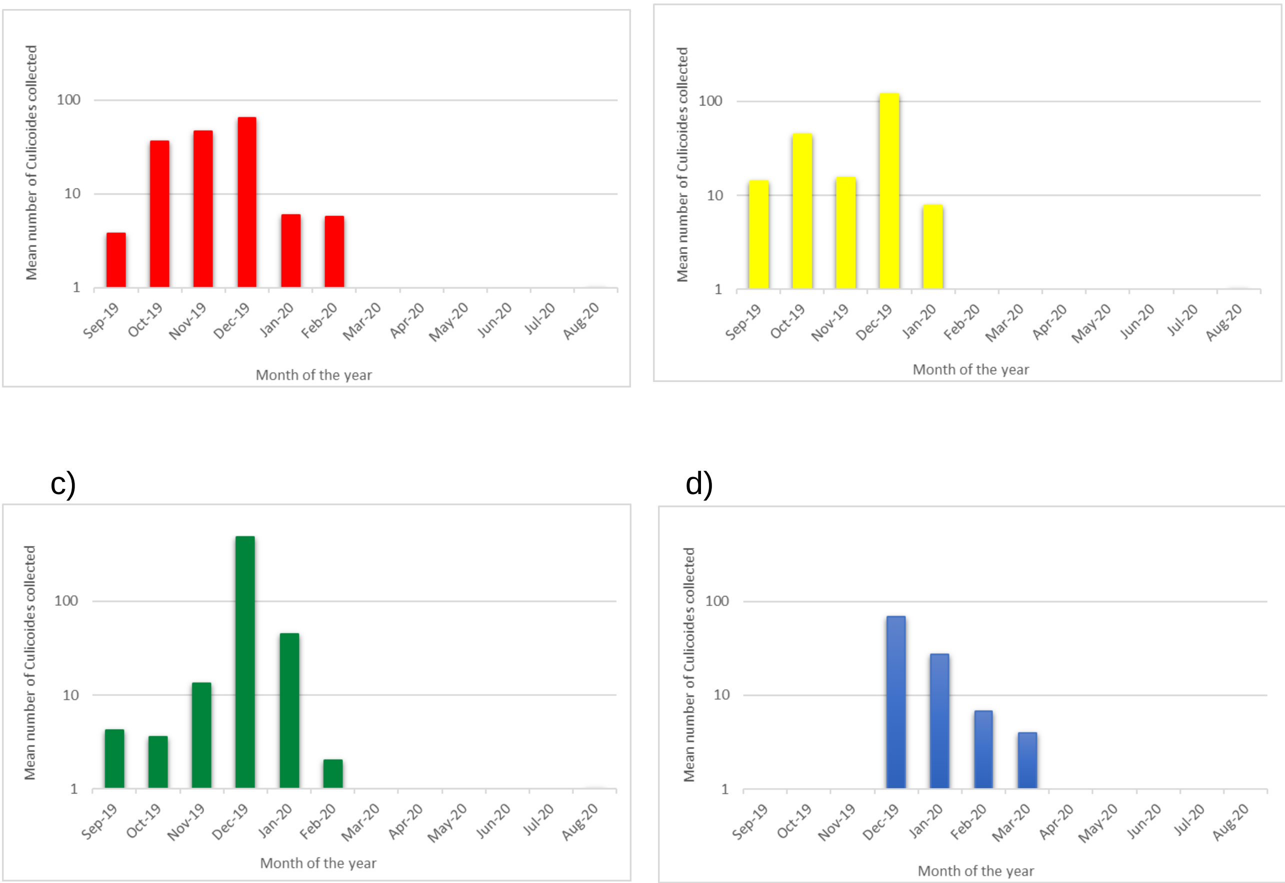



Figure 7: Monthly variation in the mean number of *Culicoides* collected with $CO_2$ baited traps situated near a) sheep, b) horses, c) cows and d) dogs at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

Table 6: Association between host animal species and count ratio of *Culicoides* collected with $CO_2$ baited traps at the Faculty of Veterinary Science, Onderstepoort from September 2019 to August 2020

| | Count Ratio (CR) | 95% Confidence Interval | P-Value |
|---|---|---|---|
| **Host animal species** | | | |
| Dogs | 1 (base) | | |
| Cows | 0.7 | 0.4-1.3 | 0.237 |
| Horses | 0.6 | 0.3-1.2 | 0.169 |
| Sheep | 0.5 | 0.3-1.0 | 0.042 |

The count ratio at the dogs was significantly different from that at the sheep (P = 0.042) (Table 6). The count ratio at the cows (P = 0.237) and horses (P = 0.169) were not significantly different from those at the dogs (Table 6).

3.4.3 Time of day of collections using carbon dioxide

The carbon dioxide traps were designed to change bottle every three hours thereby allowing us to determine at which time of day the midges were collected. Each trapping started at between 08h00 and 10h00 in the morning with the average start time being 09h30. Appendix 6 shows the numbers and species of *Culicoides* collected at different times of the day for each trap.

Table 7: Total number of *Culicoides* midges collected with $CO_2$ baited traps at various livestock species at the Faculty of Veterinary Science, Onderstepoort, using a rotational sampler operating at three-hour intervals starting from 09h30 and ending at 09h30 on the following day

| | **09h30-12h30** | **12h30-15h30** | **15h30-18h30** | **18h30-21h30** | **21h30-00h30** | **00h30-03h30** | **03h30-06h30** | **06h30-09h30** |
|---|---|---|---|---|---|---|---|---|
| **Sheep** | 9 | 13 | 12 | 94 | 164 | 282 | 120 | 13 |
| **Horses** | 6 | 8 | 43 | 223 | 310 | 216 | 80 | 18 |
| **Cows** | 13 | 27 | 38 | 638 | 698 | 557 | 223 | 47 |
| **Dogs** | 6 | 27 | 25 | 27 | 166 | 72 | 51 | 7 |
| **Total** | 34 | 75 | 118 | 982 | 1 338 | 1 127 | 474 | 85 |
| **%** | 0.8% | 1.8% | 2.8% | 23.2% | 31.6% | 26.6% | 11.2% | 2.0% |

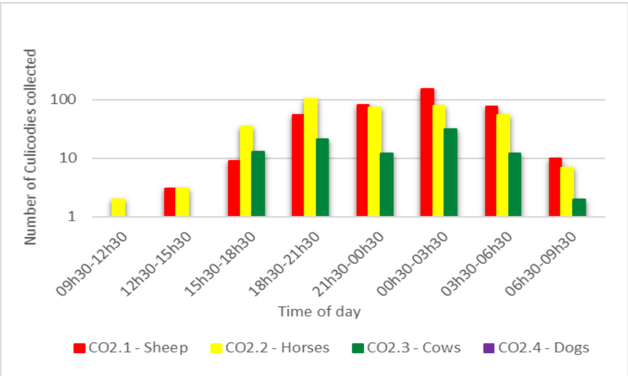
Table 7 shows that the greatest number of midges were collected between 21h30 and 00h30 with 00h30 to 03h30 and 18h30 to 21h30 showing slightly lower numbers. Daytime collections (06h30 to 18h30) constitute 7.4% of total midges collected.

Table 8: Variation of the number of *Culicoides* collected at various livestock species at the Faculty of Veterinary Science, Onderstepoort when compared to the time of day

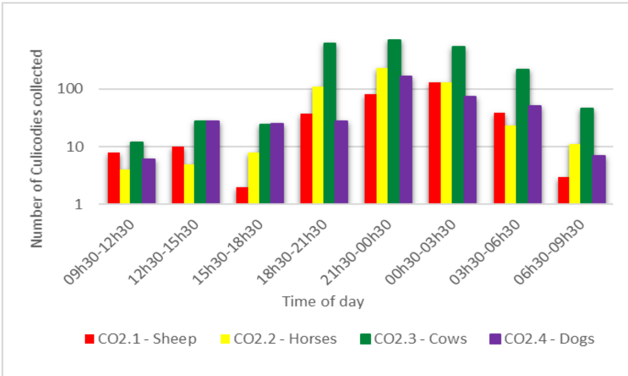
| | Count Ratio (CR) | 95% Confidence Interval | P-Value |
|---|---|---|---|
| **Time of collection** | | | |
| 09h30-12h30 | 1 (base) | | |
| 12h30-15h30 | 2.8 | 1.2-6.4 | 0.018 |
| 15h30-18h30 | 10.0 | 4.2-23.8 | <0.001 |
| 18h30-21h30 | 34.9 | 15.9-73.4 | <0.001 |
| 21h30-00h30 | 33.9 | 15.6-73.4 | <0.001 |
| 00h30-03h30 | 43.0 | 19.7-93.7 | <0.001 |
| 03h30-06h30 | 22.5 | 10.3-49.5 | <0.001 |
| 06h30-09h30 | 3.8 | 1.6-8.7 | 0.002 |

Using the time period 09h30 to 12h30 as a base, the count increased and peaked during the night (18h30 to 06h30). All values were significantly higher than the base ($P < 0.05$).

a)




b)




c)

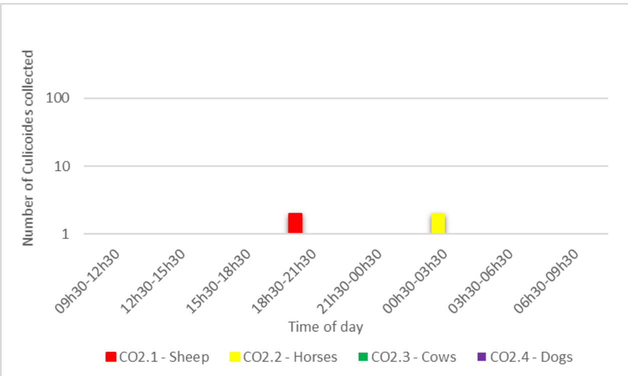



Figure 8: Total number of *Culicoides* collected at three-hour intervals with $CO_2$ baited traps situated near sheep, horses, cows and dogs at the Faculty of Veterinary Science, Onderstepoort from a) September 2019 to November 2019, December 2019 to March 2020 and c) June 2020 to August 2020

Figure 8 shows the difference in time of collection at different times of the year. In the period of September 2019 to November 2019 the greatest number of midges were collected between 00h30 and 03h30 (Figure 8a). From December 2019 to March 2020 this changed, and more midges were collected from 21h30 to 00h30 (Figure 8b). Too few midges were collected from June 2020 to August 2020 to draw any real conclusions (Figure 8c).

### 3.4.4 Parity of *Culicoides imicola* and *Culicoides leucostictus* with respect to trap type and host animal

*Culicoides imicola* and *Culicoides leucostictus* were the dominant species collected with Onderstepoort light traps and $CO_2$ traps, respectively. Nulliparous females were predominant in both trap types, accounting for 49.6% in Onderstepoort light traps and 36.1% in $CO_2$ trap collections (Table 4). The proportion of males collected with $CO_2$ traps was far greater than those caught with light traps with 30.6% found in $CO_2$ traps and only 4.4% found in light traps (Table 9). The proportion of blood feds collected with $CO_2$ traps appears greater than those collected with light traps but due to the overall low numbers of *C. imicola* collected with $CO_2$ traps this value is not statistically significant. Significant variation in parity is seen between trap types ($P < 0.01$) (Table 9).

Table 9: *Culicoides imicola* collected with Onderstepoort light traps and $CO_2$ traps at various livestock species at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020 differentiated by sex and parity

| | | **Total number collected (%)** | | | |
|---|---|---|---|---|---|
| **Trap type** | **Nulliparous** | **Parous/Gravid** | **Blood fed** | **Males** | **Total** |
| Light | 211 408 (49.6) | 191 406 (44.9) | 4 387 (1.0) | 18 748 (4.4) | 425 949 |
| $CO_2$ | 13 (36.1) | 11 (30.6) | 1 (2.8) | 11 (30.6) | 36 |
| **Total** | 211 421 (49.6) | 191 417 (44.9) | 4 388 (1.0) | 18 759 (4.4) | 425 985 |

The parity distribution in collected *C. leucostictus* was very different to that seen in *C. imicola*. Nulliparous females accounted for 60.1% in $CO_2$ traps but only 37.2% in light trap collections (Table 10). The predominant parity in the light traps collections was made up of parous/gravid *Culicoides* (52.8%). Males were also less represented in

$CO_2$ traps (2.2%) versus light traps (9.8%) (Table 10). Significant variation in parity is seen between trap types ($P < 0.01$).

Table 10: *Culicoides leucostictus* collected with Onderstepoort light traps and $CO_2$ traps at various livestock species at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020 differentiated by sex and parity

| | Total number collected (%) | | | | |
|---|---|---|---|---|---|
| **Trap type** | **Nulliparous** | **Parous/Gravid** | **Blood fed** | **Males** | **Total** |
| Light | 6 380 (37.2) | 9 056 (52.8) | 26 (0.2) | 1 685 (9.8) | 17 147 |
| $CO_2$ | 1 839 (60.1) | 1155 (37.7) | 1 (<0.1) | 67 (2.2) | 3 062 |
| **Total** | 819 (40.7) | 10 211 (50.5) | 27 (0.1) | 1 752 (8.7) | 20 209 |

The proportion of nulliparous *C. imicola* at the various hosts ranged from 47.6% as determined at the horses to 65.7% where no animals were in the immediate vicinity. Similarly, the parous rate ranged from 25.7% where no animals were around to 47.3% at the horses (Table 11). The proportion of parous females collected was of statistical significance with $P < 0.01$. While the proportional representation of nulliparous females collected at the cows (51.6%), goats (52.7%) and sheep (51.5%) did not vary significantly, it was significantly lower at the horses (47.6%) and significantly higher where no animals were present (65.7%) (Table 11). The proportion of male *C. imicola* at the various hosts ranged from 4.3% at the horses to 9.0% at the sheep (Table 11).

Table 11: *Culicoides imicola* collected in the vicinity of various livestock species at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020 differentiated by sex and parity

| | Total number collected (%) | | | | |
|---|---|---|---|---|---|
| **Host species** | **Nulliparous** | **Parous/Gravid** | **Blood fed** | **Males** | **Total** |
| Cows | 3 178 (51.6) | 2 420 (39.3) | 161 (2.6) | 402 (6.5) | 6 161 |
| Dogs | 3 859 (51.8) | 3 181 (42.7) | 60 (0.8) | 354 (4.8) | 7 454 |
| Goats | 82 103 (52.7) | 64 825 (41.6) | 2 091 (1.3) | 6 901 (4.4) | 155 920 |
| Horses | 120 012 (47.4) | 119 336 (47.3) | 2 023 (0.8) | 10 710 (4.3) | 252 081 |
| Sheep | 2 177 (51.5) | 1 619 (38.3) | 53 (1.3) | 380 (9.0) | 4 229 |
| None | 92 (65.7) | 36 (25.7) | 0 (0.0) | 12 (8.6) | 140 |
| **Total** | 211 421 (49.6) | 191 417 (44.9) | 4 388 (1.0) | 18 759 (4.4) | 425 985 |

*Culicoides leucosticus* midges collected showed an altogether different pattern. Parous/gravid midges were predominant at all the host animal species except for cows (Table 12). Proportions of males collected also differed vastly between host animal species. Significant variation in parity is therefore seen between different host animal species ($P < 0.01$).

Table 12: *Culicoides leucostictus* collected in the vicinity of various livestock species at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020 differentiated by sex and parity

| | **Total number collected (%)** | | | | |
|---|---|---|---|---|---|
| **Host species** | **Nulliparous** | **Parous/Gravid** | **Blood fed** | **Males** | **Total** |
| Cows | 1 307 (62.7) | 672 (32.2) | 1 (0.1) | 105 (5.0) | 2 085 |
| Dogs | 3 870 (44.1) | 4 608 (52.5) | 13 (0.2) | 284 (3.2) | 8 775 |
| Goats | 1 177 (30.6) | 2 094 (54.5) | 9 (0.2) | 565 (14.7) | 3 845 |
| Horses | 1 590 (33.3) | 2 509 (52.5) | 0 (0.0) | 679 (14.2) | 4 778 |
| Sheep | 266 (40.9) | 295 (45.3) | 4 (0.6) | 86 (13.2) | 651 |
| None | 9 (12.0) | 33 (44.0) | 0 (0.0) | 33 (44.0) | 75 |
| **Total** | 8 219 (40.6) | 10 211 (50.5) | 27 (0.1) | 1 752 (8.7) | 20 215 |

### 3.4.4 Freshly blood fed female *Culicoides* collected

In total, 4 451 blood fed females were collected which accounted for 1% of the total midges collected. Only 0.1% of the blood fed midges were collected with the $CO_2$ traps. 98.6% of the blood fed midges collected with Onderstepoort light traps belonged to the species *C. imicola* (Figure 9). Of the four blood fed midges collected with $CO_2$ traps, only one belonged to the species *C. imicola* (Figure 10). The probability of collecting blood fed females was therefore 10 times more likely with Onderstepoort light trap collections when compared to collections made using $CO_2$ traps.

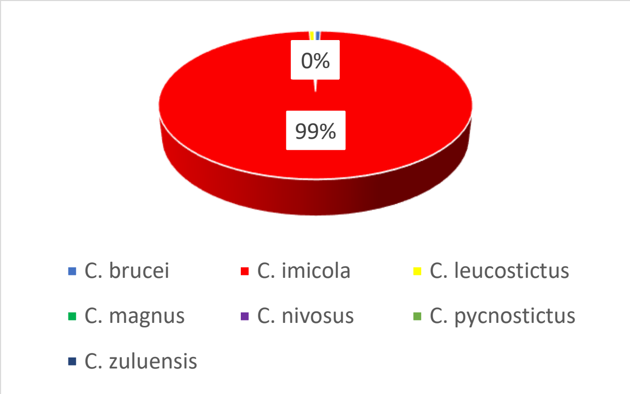



Figure 9: Relative proportion of different species of blood fed female *Culicoides* collected with Onderstepoort light traps at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020

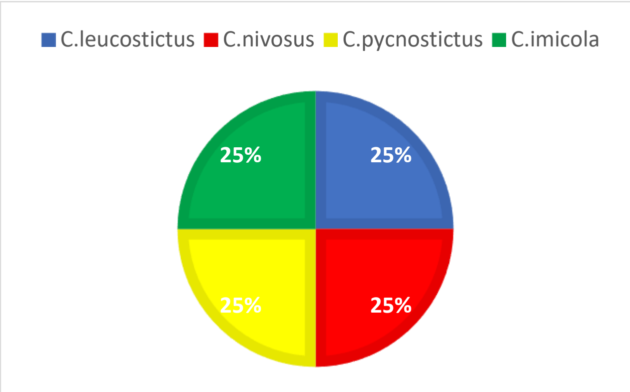



Figure 10: Relative proportion of different species of blood fed female *Culicoides* collected with $CO_2$ baited traps at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020

### 3.4.5 Blood meal analysis

Freshly blood fed female *Culicoides* were separated according to which host animal species was closest to the trap. The blood meals of 310 blood fed *Culicoides* were analysed using Mitochondrial cytochrome *b* analysis.

Table 13: Results of blood meal analysis of 310 blood fed *Culicoides* midges collected in the vicinity of various livestock species at the Faculty of Veterinary Science, Onderstepoort from 28 August 2019 to 28 August 2020

| **Animals species closest to trap site** | **Animal species detected in blood meal** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Cows** | **Dogs** | **Goats** | **Horses** | **Sheep** | **No result** | **Total** |
| Cows | 67.2% | 0.0% | 0.0% | 16.4% | 3.3% | 13.1% | 100.0% |
| Dogs | 25.8% | 12.9% | 0.0% | 33.9% | 12.9% | 14.5% | 100.0% |
| Goats | 11.1% | 0.0% | 27.0% | 33.3% | 17.5% | 11.1% | 100.0% |
| Horses | 9.7% | 0.0% | 0.0% | 74.2% | 1.6% | 14.5% | 100.0% |
| Sheep | 12.9% | 0.0% | 11.3% | 29.0% | 32.3% | 14.5% | 100.0% |
| **Total** | 25.2% | 2.6% | 7.7% | 37.4% | 13.6% | 13.6% | 100.0% |

Results show that *C. imicola* will feed on domestic dogs although they are not a preferred host (Table 13). For midges collected near cows, sheep and horses, the predominant species fed on was the one closest to the trap (Table 13). For midges collected near both dogs and goats, the predominant species fed on was horses. Overall, horses were the preferred host in this study. No result could be obtained for 13.6% of the blood meals tested. This could be due to a number of reasons i.e. the blood meal could be too small or degraded or it originated from a species of animal that was not included in the test panel e.g. birds or rodents.

Two of the blood meal analyses showed two different host species were fed on. One showed dog and horse and the second showed dog and cat. For ease of statistical analysis they were both reported as dog blood meals in Table 13.

All the midges used for blood meal analysis were *C. imicola* except for three which belonged to the species *C. leucostictus*. Interestingly, all three *C. leucostictus* analysed were collected at the dog kennels and all three showed positive dog blood meals.

## 3.5 DISCUSSION

*Culicoides* biting midges were collected weekly from 28 August 2019 to 28 August 2020 at various hosts in the Onderstepoort area in South Africa using Onderstepoort 220 V UV light and $CO_2$ traps concurrently. Seasonal abundance and species diversity as determined with the light traps were highly comparable to previous light trap surveys conducted in the area (Venter et al., 1997a; Labuschagne, 2016), and as such provided a baseline for evaluation of the results obtained in the present study. In agreement with previous studies the light trap collections at horse/cows and sheep/goats show a higher abundance of *Culicoides*, and especially *C. imicola*, towards the end of summer i.e. January to March with numbers peaking in February, indicating a higher risk of transmission of *Culicoides* transmitted viruses for this period.

Previous light trap surveys conducted in the Onderstepoort area did not focus on dogs and/or smaller mammals, and the present study showed a marked difference in *Culicoides* abundance and species composition at dog kennels compared to that collected at bigger livestock species ($P < 0.001$). In line with previous studies *C. imicola* was dominant and abundant at the larger mammals (Meiswinkel et al., 2004) but not so at the dogs. The dominant species collected at the dogs was *C. leucostictus* and when compared to the other traps, the total number of midges collected was 9.6% and 5.9% of the midges collected at sheep/goats and horses/cows, respectively. These results indicate that dogs may not attract *C. imicola* and may not be a preferred host.

The $CO_2$ traps collected at least two logs less *Culicoides* compared to the Onderstepoort light traps (CR = 836; 95% CI = 477-1464; $P < 0.001$). Whereas the light trap collections showed an increase in *Culicoides* collected from January to March with a peak in February, the $CO_2$ traps showed an increase from November to January with a peak in December. The dominant species, accounting for 72.3% of the total number of midges collected with $CO_2$ traps was *C. leucostictus*. A possible reason for this is that while $CO_2$ may act as an attractant for some species of *Culicoides*, it may be repellent to others (Venter et al., 2016). High background levels of $CO_2$ at collection sites due to the presence of animals could mean that *C. imicola* were already abundant in the area and were not specifically attracted to the trap but rather by the animals. Studies in South Africa adding $CO_2$ to light traps, has previously shown an increase in the efficiency of the traps when compared to un-baited traps (Venter et al., 2016).

$CO_2$ on its own has been shown to be a powerful attractant in some species, e.g. *C. sonorensis* in the USA (Mayo et al., 2014). The present study demonstrates that that is not the case in the Onderstepoort area and that traps baited only with $CO_2$ are likely not useful for livestock associated *Culicoides* surveillance and study in this area. This is accentuated by the fact that *C. imicola* accounted for less than 1% of the total number of midges collected with the $CO_2$ traps. This was remarkable as *C. imicola* accounted for 92.4% of the midges collected with light traps. In agreement with our results, the addition of $CO_2$ to light traps also proved largely ineffective in improving the collections of *Culicoides*, including *C. imicola*, at dog and cat shelters in Spain (González et al., 2020). Poor responses to $CO_2$ were also reported for the Palaearctic *Culicoides obsoletus* (Meigen) in Europe (Mullens et al., 2005; Gerry et al., 2009). As *C. imicola* is one of the main vectors for transmission of orbiviruses in South Africa this result suggests that $CO_2$ traps for vector surveillance in South Africa may not be appropriate. It should also be considered that $CO_2$ is relatively expensive, and it may not always be feasible to use it in large-scale surveillance programmes, especially in rural areas.

The predominant species collected with the $CO_2$ traps was *C. leucostictus*. Although *C. leucostictus* is considered an ornithophilic species (Meiswinkel et al., 2004), it has been shown to feed on zebras and donkeys (Riddin et al., 2019). All three available *C. leucostictus* females tested in the present study were positively identified as having taken a blood meal from a dog. Oral susceptibility studies have previously shown that *C. leucostictus* is more susceptible to infection with AHSV than *C. imicola* (Venter et al., 2009b). However, to date, field infections of *C. leucostictus* with AHSV are still lacking.

In addition to the significantly lower numbers collected with the $CO_2$ traps, species diversity was also lower. While 23 species were collected with the light traps only nine were collected with the $CO_2$ traps. Considering that a variety of biologically diverse *Culicoides* species could be involved in the transmission of viruses (Carpenter et al., 2008; Venter et al., 2011; Del Rio López et al., 2012; Ruder et al., 2012), it becomes obvious that the accurate detection of all potential vectors in livestock situations is crucial to clarify the epidemiology of the related diseases. The relative inefficiency of $CO_2$ to attract *Culicoides*, as found in the present study, does not exclude the possibility that it may act synergically with other chemicals in the attraction of *Culicoides.* It was shown in Australia that octenol combined with $CO_2$ increases the number of midges collected and thereby increases trap sensitivity (Ritchie et al., 1994).

*Culicoides imicola*, considered a proven vector of AHSV, was found throughout the year and no midge-free periods were recorded. The presence of males and nulliparous females throughout the year indicate that breeding continued throughout winter. Due to the apparent lack of transovarial transmission of orbiviruses in the genus *Culicoides* (Osborne et al., 2015), it is vital to know the number of parous females in a population in order to ascertain their vector potential. An association between trap type and parity was shown ($P < 0.01$). Parous females represented 44.9% and 30.6% of the midges collected with the light and $CO_2$ traps, respectively. Parous females are therefore less likely to be collected with $CO_2$ traps and this may lead to an under-estimation of the risk of disease transmission.

Similarly, an association between host animal and parity rates exists ($P < 0.01$). The proportion of parous females collected with traps where no animals were in the immediate vicinity of the trap was considerably lower (25.7%) than with traps near potential hosts. The different parous rates between sites indicate that *Culicoides* are not homogenously distributed in an area (González et al., 2017) and emphasizes the relatively short attraction range, < 5 m, of the light trap (Venter et al., 2012; Elbers and Meiswinkel, 2016). Factors that may play a role here include the presence of potential breeding sites in the vicinity and the random movement of host animals in relation to the trap.

The rotational sampler, as developed in the present study, allowed us to determine that the greatest proportion of midges were collected between 21h30 and 00h30. These findings are in agreement with a study conducted at Onderstepoort in 2009 (Page et al., 2009). As was found by (Venter et al., 2019) this peak can depend on the season and is influenced by seasonal temperatures. Peak collecting time from September to November was between 00h30 and 03h30 whereas collections peaked from 21h30 to 00h30 from December to March.

The numbers of *Culicoides* collected during the day accounted for 7.4% of the total numbers collected. Of the 36 *C. imicola* collected using $CO_2$ traps, only one (2.8%) was collected during the day. Once again, highlighting the inefficiency of $CO_2$ traps for the collection of *C. imicola*. These results are in agreement with that of (Venter et al., 2019) who also found low levels of daytime activity using relatively inefficient light traps. Daytime activity of *Culicoides* midges is therefore present albeit in a much lower proportion to that of night-time activity. Daytime collections from September to November were slightly higher than the average with 10.2% being collected during the day, and then slightly lower when it became hotter from December to March where daytime collections accounted for 6.7% of the total collections. Only three *Culicoides* were collected with $CO_2$ traps from June to August which makes evaluation statistically difficult. The results would however suggest that *Culicoides* are more active during the day in more temperate weather. The potential influence of cloud cover on *Culicoides* flight activity still needs to be determined.

Blood fed midges collected with the light traps accounted for less than 1.0% of the total number of midges collected. The numbers collected with the $CO_2$ traps was however only 0.1% of the total collection. Low numbers of blood feds are expected as midges which have recently fed will not be host seeking. In agreement with the abundance as determined in the light traps, 98.6% of the blood feds collected with the light traps belong to *C. imicola*. Of four blood fed midges collected with $CO_2$ traps only one was *C. imicola.* This highlights a further drawback of using $CO_2$ traps for studies related to blood meal analysis.

Only 12.9% of the blood fed females collected at dog kennels actually fed on the dogs, showing that they will feed on dogs but to a much lesser extent than on bigger livestock species. Although vector transmitted AHSV in dogs may therefore be possible it is unlikely that dogs will act as reservoir or cycling hosts for equine AHSV.

Although it has been shown that light trap results may not be a true representation of the attack rates on hosts (Gerry et al., 2009; Viennet et al., 2011; Scheffer et al., 2012), blood meal analysis of midges collected near cows, horses, and sheep revealed that midges will indeed feed on the animal species closest to them. *Culicoides* collected near goats fed on goats 27% of the time and were slightly more inclined to feed on horses (33.3%). A possible reason for this is the proximity of the stables which are a short distance from the sheep pens in which the trap was located. This would suggest that the midges would rather go into the stables and feed on the horses than feed on the goats. This was not the case when sheep were kept in the sheep pens and those collections yielded 32.2% and 29.0% positive blood meals for sheep and horses, respectively.

Of the 62 midges collected and analysed from the dog kennels, three were *C. leucostictus* and the rest were *C. imicola*. Very interestingly, all three *C. leucostictus* revealed positive dog blood meals which accounted for 37.5% of the total dog blood meals found. As *C. leucostictus* was the predominant species collected at the dog kennels it would suggest that dogs are a preferred host for a species which was previously believed to be ornithophilic (Meiswinkel et al., 2004). Further investigation of *C. leucostictus* as a vector for AHSV may also be warranted.

This study demonstrated both daytime as well as inter-seasonal activity of *C. imicola* as well as other *Culicoides* species, in the Onderstepoort area, South Africa. Although only low numbers were collected during the colder winter months it enforced the possibility that viruses can overwinter in adult *Culicoides* species in the area. Blood meal analyses showed that although *Culicoides* do feed on dogs under natural conditions, low feeding rates as obtained in the present study showed that dogs are most likely not a preferred host. Although canines may die as a result of *Culicoides* transmitted virus, dogs seem not to play a significant role in the epidemiology of AHSV. Comparisons of light and $CO_2$ trap data emphasizes that a great number of factors can influence the number of *Culicoides* midges collected with various trapping methods, and that care should be taken in interpreting and extrapolating trap data.

# CHAPTER 4: GENERAL CONCLUSIONS

This study has shown:

- Seasonal and inter-seasonal abundance of *Culicoides* midges at Onderstepoort remains consistent with previous data by GJ Venter (Venter et al., 1997a). It confirms that daytime transmission by *Culicoides* is unlikely to play a major role in the transmission of AHSV and that potential overwintering of viruses transmitted by *Culicoides* in the Onderstepoort area may be possible (Venter et al., 2014; Steyn et al., 2015).
- The use of $CO_2$ traps confirmed daytime activity of *Culicoides* biting midges in South Africa. However, due to the low species composition and numbers collected using these traps, it is unlikely that they will be useful in future vector and disease surveillance.
- Blood meal analysis confirmed that *Culicoides* biting midges will feed on dogs and are thus able to infect them with AHSV. However, there was clear evidence that dogs are not a preferred host for *C. imicola* and given the choice, the midges would rather feed on larger livestock.
- Blood meal analysis on *Culicoides leucostictus* confirmed that they also feed on dogs.
- Data presented over the last couple of years emphasizes the fact that a great number of factors can influence the number of insects, including *Culicoides* midges, collected with light traps and that we need to be careful when interpreting and extrapolating such data.

Questions still to be investigated:

- Numbers of *Culicoides* associated with dogs where there is no other livestock in a 5 to 10 km radius (for example, collecting midges at a dog kennel located in a city).
- Identification of factors related to the attraction of *Culicoides* to livestock. The relative inefficiency of $CO_2$ as determined in the present study, highlights the need to identify factors that may attract *Culicoides imicola* and other livestock associated *Culicoides* in South Africa.

- In an outbreak of AHS, surveillance of *Culicoides* should include species not previously believed to transmit AHSV such as *C. leucostictus*.
- Further blood meal analysis of *C. leucostictus* to include dogs and other livestock species to determine host preferences.
- Methods to collect specifically freshly blood fed females need to be developed and improved.

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## APPENDIX 1: Location of light traps and animals present in the camps

| | Light 1 | | | Light 2 | | | Light 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Date | Camp no | Species | No of animals | Camp no | Species | No of animals | Camp no | Species | No of animals |
| 28/08/2019 | T5 | Horses | 2 + 2 in T6 | Sheep pens | Sheep | 15 + lambs | Beagles | Dogs | 10 |
| 04/09/2019 | T5 | Horses | 2 + 2 in T6 | Sheep pens | Sheep | 15 + lambs | Beagles | Dogs | 10 |
| 12/09/2019 | T5 | Horses | 0 | Sheep pens | Sheep | 15 + lambs | Beagles | Dogs | 10 |
| 18/09/2019 | T5 | Cattle | 27 in T6 | Sheep pens | Sheep | 15 + lambs | Beagles | Dogs | 10 |
| 25/09/2019 | T5 | Cattle | T5-8, T6-19 | Sheep pens | Sheep | 15 + lambs | Beagles | Dogs | 10 |
| 30/09/2019 | T5 | Cattle | T5-10, T6-14 | Sheep pens | Sheep | Sheep in 17 | Beagles | Dogs | 10 |
| 02/10/2019 | T5 | Cattle | T5-10, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 07/10/2019 | T5 | Cattle | T5-8, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 15/10/2019 | T5 | Cattle | T5-8, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 21/10/2019 | T5 | Cattle | T5-8, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 30/10/2019 | T5 | Cattle | T5-8, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 05/11/2019 | T5 | Cattle | T5-8, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 14/11/2019 | T5 | Cattle | T5-8, T6-14 | Sheep pens | Goats | 7 goats and sheep in 17 | Beagles | Dogs | 10 |
| 20/11/2019 | T5 | Cattle | 0 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 26/11/2019 | T5 | Cattle | 24 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 03/12/2019 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 11/12/2019 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 18/12/2019 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 26/12/2019 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 01/01/2020 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 08/01/2020 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 15/01/2020 | T5 | | No electricity | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 22/01/2020 | 55 | Horses | 11 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 29/01/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 05/02/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 12/02/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | | | No electricity |
| 19/02/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | | | No electricity |
| 26/02/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 04/03/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 11/03/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 18/03/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 25/03/2020 | 55 | Horses | 8 horses | Sheep pens | Goats | 7 goats + kids | Beagles | Dogs | 10 |
| 17/06/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 24/06/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 01/07/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 08/07/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 15/07/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 7 goats | Beagles | Dogs | 10 |
| 22/07/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 0 (goats in 13) | Beagles | Dogs | 10 |
| 29/07/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 0 (goats in 13) | Beagles | Dogs | 10 |
| 05/08/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Goats | 0 (goats in 13) | Beagles | Dogs | 10 |
| 12/08/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Sheep | 11 | Beagles | Dogs | 10 |
| 18/02/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Sheep | 11 | Beagles | Dogs | 10 |
| 28/08/2020 | 52 | Horses | 7 horses in 49-51 | Sheep pens | Sheep | 11 | Beagles | Dogs | 10 |

## APPENDIX 2: Location of $CO_2$ traps and animals present in the camps

| | $CO_2$ 1 (Sheep) | | $CO_2$ 2 (Horses) | | $CO_2$ 3 (Cattle) | | $CO_2$ 4 (Dogs) | |
|---|---|---|---|---|---|---|---|---|
| Date | Camp no | No of animals | Camp no | No of animals | Camp no | No of animals | Camp no | No of animals |
| 28/08/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | Bulls | 4 | | |
| 04/09/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 8 | | |
| 12/09/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 8 | | |
| 18/09/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 8 | | |
| 25/09/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 8 | | |
| 30/09/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 8 | | |
| 02/10/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 8 | | |
| 07/10/2019 | 14 | 28 (btwn 14/15) | 22 | 7 (+ surrounding camps) | T8 | 6 | | |
| 15/10/2019 | 14 | 29 in 14 | 22 | 7 (+ surrounding camps) | T8 | 3 + 1 calf | | |
| 21/10/2019 | 14 | 29 in 14 | 22 | 7 (+ surrounding camps) | T8 | 3 + 1 calf | | |
| 30/10/2019 | 14 | 29 in 14 | 22 | 7 (+ surrounding camps) | T8 | 2 + 2 calves | | |
| 05/11/2019 | 14 | 29 in 14 | 22 | 7 (+ surrounding camps) | T6 | 14 | | |
| 14/11/2019 | 15 | 18 | 22 | 7 (+ surrounding camps) | T4 | 3 in T4, 6 in T5 | | |
| 20/11/2019 | 15 | 5 sheep and 7 lambs | 22 | 7 (+ surrounding camps) | 8 | 11 cows + calves | | |
| 26/11/2019 | 15 | 5 sheep and 7 lambs | 22 | 7 (+ surrounding camps) | 8 | 0 | | |
| 03/12/2019 | 15 | 5 sheep and 7 lambs | 22 | 7 (+ surrounding camps) | 8 | 0 | | |
| 11/12/2019 | 19 | 6 rams | 37 | 20+ | 16 | 12 cows + calves | Beagles | 10 |
| 18/12/2019 | 19 | 6 rams | 37 | 20+ | 8 | 12 cows + calves | Beagles | 10 |
| 26/12/2019 | 19 | 6 rams | 37 | 20+ | 8 | cows in 5 + 9 | Beagles | 10 |
| 01/01/2020 | 19 | 6 rams | 37 | 20+ | 8 | cows in 5 + 9 | Beagles | 10 |
| 08/01/2020 | 19 | 6 rams | 22 | 20+ | 8 | 10 + calves in 9 | Beagles | 10 |
| 15/01/2020 | 19 | 6 rams | 22 | 7 (+ surrounding camps) | 8 | Cows in 4 + 6 | Beagles | 10 |
| 22/01/2020 | 19 | 6 rams | 22 | 7 (+ surrounding camps) | 9 | 34 cows & calves in 5,8 | Beagles | 10 |
| 29/01/2020 | 19 | 6 rams | 22 | 7 (+ surrounding camps) | 9 | 25 + calves in 9,10 | Beagles | 10 |
| 05/02/2020 | 19 | 6 rams | 22 | 7 (+ surrounding camps) | 8 | cows in 4 + 6 | Beagles | 10 |
| 12/02/2020 | 19 | 6 rams | 22 | 7 (+ surrounding camps) | | Not working | Beagles | 10 |
| 19/02/2020 | 15 | 6 rams | | Not working | | Not working | Beagles | 10 |
| 26/02/2020 | 15 | 15 | | Not working | | Not working | Beagles | 10 |
| 04/03/2020 | 15 | 6 rams | | Not working | | Not working | Beagles | 10 |
| 11/03/2020 | | Not working | | Not working | | Not working | Beagles | 10 |
| 18/03/2020 | Sheep pens | 15 | | Not working | | Not working | Beagles | 10 |
| 25/03/2020 | | Not working | | Not working | | Not working | | Not working |
| 17/06/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | | Not working | Beagles | 10 |
| 24/06/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | | Not working | Beagles | 10 |
| 01/07/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | | Not working | Beagles | 10 |
| 08/07/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | T6 | 14 cows | Beagles | 10 |
| 15/07/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | T6 | 14 cows | Beagles | 10 |
| 22/07/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | Feedlot | 11 calves | Beagles | 10 |
| 29/07/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | Feedlot | 11 calves | Beagles | 10 |
| 05/08/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | Feedlot | 9 calves | Beagles | 10 |
| 12/08/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | Feedlot | 9 calves | Beagles | 10 |
| 18/02/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | Feedlot | 9 calves | Beagles | 10 |
| 28/08/2020 | 14 | 30+ | 22 | 7 (+ surrounding camps) | Feedlot | 9 calves | Beagles | 10 |

## APPENDIX 3: Weather data for dates on which collections occurred

| Date | Minimum Temperature (°C) | Maximum Temperature (°C) | Rain(mm) | Average Wind Speed (m/s) |
|---|---|---|---|---|
| 28/08/2019 | 9.6 | 28.4 | - | 0 |
| 04/09/2019 | 9.3 | 28 | - | 0 |
| 12/09/2019 | 10.2 | 30 | - | 0 |
| 18/09/2019 | 16.4 | 32.9 | - | 0 |
| 25/09/2019 | 8.3 | 20.2 | - | 3.6 |
| 30/09/2019 | 13.9 | 28.4 | - | 3.3 |
| 02/10/2019 | 9.5 | 23.6 | 0.2 | 1.3 |
| 07/10/2019 | 16.3 | 31.9 | - | 0 |
| 15/10/2019 | 16.5 | 32.6 | - | 3.6 |
| 21/10/2019 | 19.8 | 36.9 | - | 2.4 |
| 30/10/2019 | 12.9 | 29.4 | - | 0 |
| 05/11/2019 | 17.7 | 30.7 | *** | 1.1 |
| 14/11/2019 | 14.3 | 28.6 | 0.2 | 1.3 |
| 20/11/2019 | 16.4 | 31 | 29.8 | 1.7 |
| 26/11/2019 | 17.4 | 31.6 | - | 0 |
| 03/12/2019 | 18 | 34.9 | 19.6 | 3 |
| 11/12/2019 | 13.9 | 26.9 | - | 1.5 |
| 18/12/2019 | 15.1 | 29.9 | - | 0 |
| 26/12/2019 | 20.5 | 34.2 | - | 0 |
| 01/01/2020 | 17.7 | 30.3 | 4.8 | 0 |
| 08/01/2020 | 20.2 | 28.2 | *** | 0 |
| 15/01/2020 | 16.3 | 29.2 | - | 0 |
| 22/01/2020 | 18.5 | 29.9 | - | 1.4 |
| 29/01/2020 | 14.9 | 30 | - | 0 |
| 05/02/2020 | 17.7 | 32.1 | 2.8 | 0 |
| 12/02/2020 | 15.2 | 25.5 | - | 1.4 |
| 19/02/2020 | 19.4 | 30.7 | - | 1.7 |
| 26/02/2020 | 13 | 30 | - | 0 |
| 04/03/2020 | 14.8 | 28.2 | - | 0 |
| 11/03/2020 | 17 | 31 | - | 1.3 |
| 18/03/2020 | 14.8 | 26.2 | 0.2 | 0 |
| 25/03/2020 | 14.2 | 30.5 | - | 1.5 |
| 17/06/2020 | 6.3 | 15.3 | - | 2.8 |
| 24/06/2020 | 2.1 | 20.6 | - | 0 |
| 01/07/2020 | 6.5 | 19.6 | - | 1.6 |
| 08/07/2020 | 8.5 | 19.1 | - | 1.5 |
| 15/07/2020 | 1 | 16.5 | - | 1.2 |
| 22/07/2020 | 4.7 | 21.8 | - | 1.3 |
| 29/07/2020 | 3.3 | 21.3 | - | 1.3 |
| 05/08/2020 | 4.3 | 23.5 | - | 0 |
| 12/08/2020 | 10.6 | 25.2 | *** | 1.2 |
| 18/08/2020 | 4.9 | 19.7 | - | 4 |
| 28/08/2020 | 10.4 | 25 | *** | 1.3 |

*** No data recorded at weather station

Weather data provided by the South African Weather Service

## APPENDIX 4a: *Culicoides* counts from light trap 1 (near horses/cows) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. cornutus* | 1 | 0 | 0 | 0 | 1 |
| | *C. enderleini* | 0 | 3 | 0 | 2 | 5 |
| | *C. imicola* | 270 | 161 | 8 | 88 | 527 |
| | *C. leucostictus* | 10 | 8 | 0 | 2 | 20 |
| | *C. nivosus* | 1 | 2 | 0 | 1 | 4 |
| | *C. pycnostictus* | 1 | 17 | 0 | 0 | 18 |
| | *C. zuluensis* | 0 | 0 | 0 | 2 | 2 |
| | **Total** | 283 | 191 | 8 | 95 | **577** |
| 04-Sep-19 | *C. imicola* | 53 | 74 | 2 | 12 | 141 |
| | *C. leucostictus* | 1 | 1 | 0 | 1 | 3 |
| | *C. nivosus* | 2 | 2 | 0 | 0 | 4 |
| | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. similis* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 56 | 79 | 2 | 13 | **150** |
| 12-Sep-19 | *C. brucei* | 0 | 0 | 1 | 0 | 1 |
| | *C. imicola* | 22 | 26 | 1 | 6 | 55 |
| | *C. leucostictus* | 0 | 2 | 0 | 0 | 2 |
| | *C. nivosus* | 0 | 1 | 0 | 1 | 2 |
| | *C. pycnostictus* | 0 | 2 | 0 | 0 | 2 |
| | **Total** | 22 | 31 | 2 | 7 | **62** |
| 18-Sep-19 | *C. bedfordi* | 0 | 3 | 0 | 0 | 3 |
| | *C. brucei* | 11 | 0 | 0 | 0 | 11 |
| | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 112 | 111 | 4 | 35 | 262 |
| | *C. leucostictus* | 2 | 24 | 0 | 0 | 26 |
| | *C. magnus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 2 | 6 | 0 | 0 | 8 |
| | *C. pycnostictus* | 0 | 36 | 0 | 0 | 36 |
| | *C. similis* | 0 | 2 | 0 | 0 | 2 |
| | *C. zuluensis* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 127 | 185 | 4 | 35 | **351** |
| 25-Sep-19 | *C. imicola* | 2 | 0 | 0 | 1 | 3 |
| | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 4 | 0 | 0 | 1 | **5** |
| 30-Sep-19 | *C. imicola* | 2 | 0 | 1 | 0 | 3 |
| | *C. leucostictus* | 0 | 0 | 0 | 1 | 1 |
| | *C. nivosus* | 0 | 1 | 0 | 1 | 2 |
| | **Total** | 2 | 1 | 1 | 2 | **6** |
| 02-Oct-19 | *C. bedfordi* | 0 | 12 | 0 | 1 | 13 |
| | *C. brucei* | 1 | 3 | 0 | 0 | 4 |
| | *C. imicola* | 139 | 145 | 4 | 14 | 302 |
| | *C. leucostictus* | 8 | 10 | 0 | 5 | 23 |
| | *C. magnus* | 0 | 1 | 0 | 1 | 2 |
| | *C. nivosus* | 6 | 32 | 0 | 8 | 46 |
| | *C. pycnostictus* | 1 | 16 | 0 | 0 | 17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. zuluensis* | 0 | 0 | 0 | 3 | 3 |
| | **Total** | 155 | 219 | 4 | 32 | **410** |
| 07-Oct-19 | *C. bedfordi* | 4 | 3 | 0 | 0 | 7 |
| | *C. brucei* | 3 | 0 | 0 | 1 | 4 |
| | *C. enderleini* | 3 | 2 | 0 | 2 | 7 |
| | *C. imicola* | 293 | 226 | 20 | 42 | 581 |
| | *C. leucostictus* | 4 | 10 | 0 | 7 | 21 |
| | *C. magnus* | 0 | 0 | 0 | 1 | 1 |
| | *C. neavei* | 0 | 1 | 0 | 0 | 1 |
| | *C. nevilli* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 5 | 14 | 0 | 2 | 21 |
| | *C. pycnostictus* | 3 | 20 | 0 | 1 | 24 |
| | *C. zuluensis* | 0 | 0 | 0 | 3 | 3 |
| | **Total** | 316 | 276 | 20 | 59 | **671** |
| 15-Oct-19 | *C. bedfordi* | 5 | 11 | 0 | 7 | 23 |
| | *C. brucei* | 1 | 9 | 0 | 8 | 18 |
| | *C. enderleini* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 437 | 504 | 12 | 71 | 1 024 |
| | *C. leucostictus* | 8 | 7 | 0 | 17 | 32 |
| | *C. magnus* | 0 | 2 | 0 | 0 | 2 |
| | *C. nivosus* | 11 | 24 | 2 | 14 | 51 |
| | *C. pycnostictus* | 5 | 17 | 0 | 2 | 24 |
| | *C. subschultzei* | 0 | 0 | 0 | 1 | 1 |
| | *C. zuluensis* | 0 | 2 | 0 | 0 | 2 |
| | **Total** | 468 | 576 | 14 | 120 | **1 178** |
| 21-Oct-19 | *C. bedfordi* | 4 | 8 | 0 | 4 | 16 |
| | *C. enderleini* | 0 | 1 | 0 | 1 | 2 |
| | *C. imicola* | 45 | 99 | 1 | 54 | 199 |
| | *C. leucostictus* | 8 | 25 | 0 | 21 | 54 |
| | *C. nivosus* | 10 | 49 | 0 | 26 | 85 |
| | *C. pycnostictus* | 14 | 41 | 1 | 14 | 70 |
| | *C. similis* | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 81 | 223 | 2 | 121 | **427** |
| 30-Oct-19 | *C. bedfordi* | 5 | 6 | 0 | 0 | 11 |
| | *C. brucei* | 1 | 0 | 0 | 0 | 1 |
| | *C. enderleini* | 1 | 1 | 0 | 0 | 2 |
| | *C. imicola* | 662 | 226 | 48 | 121 | 1 057 |
| | *C. leucostictus* | 11 | 21 | 0 | 15 | 47 |
| | *C. nivosus* | 7 | 64 | 0 | 16 | 87 |
| | *C. pycnostictus* | 6 | 25 | 0 | 4 | 35 |
| | **Total** | 693 | 343 | 48 | 156 | **1 240** |
| 05-Nov-19 | *C. bedfordi* | 5 | 5 | 0 | 1 | 11 |
| | *C. brucei* | 0 | 1 | 0 | 0 | 1 |
| | *C. enderleini* | 4 | 4 | 0 | 2 | 10 |
| | *C. expectator* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 957 | 680 | 19 | 36 | 1 692 |
| | *C. leucostictus* | 8 | 18 | 0 | 3 | 29 |
| | *C. neavei* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 4 | 48 | 0 | 5 | 57 |
| | *C. pycnostictus* | 2 | 13 | 0 | 0 | 15 |
| | *C. similis* | 1 | 1 | 0 | 0 | 2 |
| | *C. subschultzei* | 0 | 0 | 0 | 6 | 6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. zuluensis* | 1 | 0 | 0 | 1 | 2 |
| | **Total** | 983 | 771 | 19 | 54 | **1 827** |
| 14-Nov-19 | *C. bedfordi* | 1 | 9 | 0 | 0 | 10 |
| | *C. enderleini* | 3 | 0 | 0 | 0 | 3 |
| | *C. imicola* | 96 | 107 | 12 | 54 | 269 |
| | *C. leucostictus* | 5 | 16 | 1 | 17 | 39 |
| | *C. nevilli* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 8 | 69 | 0 | 11 | 88 |
| | *C. pretoriensis* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 5 | 33 | 0 | 6 | 44 |
| | *C. similis* | 1 | 2 | 0 | 1 | 4 |
| | *C. subschultzei* | 0 | 1 | 0 | 1 | 2 |
| | *C. zuluensis* | 2 | 0 | 0 | 2 | 4 |
| | Nigripennis grp | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 122 | 238 | 13 | 93 | **466** |
| 20-Nov-19 | *C. bedfordi* | 6 | 3 | 0 | 0 | 9 |
| | *C. imicola* | 43 | 31 | 0 | 12 | 86 |
| | *C. leucostictus* | 9 | 33 | 0 | 33 | 75 |
| | *C. magnus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 13 | 59 | 0 | 21 | 93 |
| | *C. pycnostictus* | 4 | 11 | 0 | 5 | 20 |
| | *C. zuluensis* | 0 | 1 | 0 | 0 | 1 |
| | Nigripennis grp | 2 | 2 | 0 | 0 | 4 |
| | **Total** | 77 | 141 | 0 | 71 | **289** |
| 26-Nov-19 | *C. bedfordi* | 3 | 2 | 0 | 3 | 8 |
| | *C. brucei* | 1 | 2 | 0 | 1 | 4 |
| | *C. cornutus* | 0 | 2 | 0 | 0 | 2 |
| | *C. enderleini* | 4 | 15 | 0 | 1 | 20 |
| | *C. imicola* | 869 | 826 | 51 | 43 | 1 789 |
| | *C. leucostictus* | 18 | 58 | 0 | 13 | 89 |
| | *C. magnus* | 0 | 2 | 0 | 0 | 2 |
| | *C. neavei* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 1 | 53 | 0 | 16 | 70 |
| | *C. pycnostictus* | 2 | 21 | 0 | 1 | 24 |
| | *C. similis* | 0 | 1 | 0 | 0 | 1 |
| | *C. zuluensis* | 0 | 1 | 0 | 1 | 2 |
| | Nigripennis grp | 3 | 6 | 0 | 1 | 10 |
| | **Total** | 902 | 989 | 51 | 80 | **2 022** |
| 22-Jan-20 | *C. bedfordi* | 72 | 12 | 0 | 24 | 108 |
| | *C. brucei* | 12 | 0 | 0 | 0 | 12 |
| | *C. enderleini* | 252 | 1 056 | 0 | 48 | 1 356 |
| | *C. imicola* | 10 488 | 9 120 | 240 | 552 | 20 400 |
| | *C. leucostictus* | 336 | 468 | 0 | 192 | 996 |
| | *C. nivosus* | 0 | 0 | 0 | 12 | 12 |
| | *C. pycnostictus* | 48 | 36 | 0 | 24 | 108 |
| | *C. subschultzei* | 12 | 36 | 0 | 264 | 312 |
| | *C. zuluensis* | 24 | 12 | 0 | 0 | 36 |
| | Nigripennis grp | 12 | 12 | 0 | 12 | 36 |
| | **Total** | 11 256 | 10 752 | 240 | 1 128 | **23 376** |
| 29-Jan-20 | *C. bedfordi* | 4 | 0 | 0 | 4 | 8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. enderleini* | 20 | 104 | 0 | 4 | 128 |
| | *C. imicola* | 2 064 | 2 418 | 32 | 182 | 4 696 |
| | *C. leucostictus* | 36 | 34 | 0 | 22 | 92 |
| | *C. nivosus* | 0 | 2 | 0 | 0 | 2 |
| | *C. pycnostictus* | 8 | 2 | 0 | 0 | 10 |
| | *C. subschultzei* | 0 | 6 | 0 | 24 | 30 |
| | Nigripennis grp | 2 | 0 | 0 | 0 | 2 |
| | **Total** | 2 134 | 2 566 | 32 | 236 | **4 968** |
| 05-Feb-20 | *C. enderleini* | 96 | 480 | 0 | 24 | 600 |
| | *C. imicola* | 39 936 | 32 688 | 456 | 1 176 | 74 256 |
| | *C. leucostictus* | 384 | 432 | 0 | 24 | 840 |
| | *C. nevilli* | 24 | 0 | 0 | 0 | 24 |
| | *C. nivosus* | 96 | 24 | 0 | 24 | 144 |
| | *C. olyslageri* | 24 | 0 | 0 | 0 | 24 |
| | *C. pycnostictus* | 96 | 72 | 0 | 24 | 192 |
| | *C. subschultzei* | 24 | 96 | 0 | 144 | 264 |
| | Nigripennis grp | 0 | 24 | 0 | 0 | 24 |
| | **Total** | 40 680 | 33 816 | 456 | 1 416 | **76 368** |
| 12-Feb-20 | *C. bedfordi* | 36 | 24 | 0 | 0 | 60 |
| | *C. enderleini* | 96 | 204 | 0 | 0 | 300 |
| | *C. imicola* | 9 252 | 14 820 | 324 | 4 980 | 29 376 |
| | *C. leucostictus* | 180 | 288 | 0 | 168 | 636 |
| | *C. nevilli* | 12 | 0 | 0 | 0 | 12 |
| | *C. nivosus* | 12 | 0 | 0 | 0 | 12 |
| | *C. pycnostictus* | 156 | 96 | 0 | 0 | 252 |
| | *C. subschultzei* | 0 | 0 | 0 | 168 | 168 |
| | *C. zuluensis* | 0 | 0 | 0 | 12 | 12 |
| | **Total** | 9 744 | 15 432 | 324 | 5 328 | **30 828** |
| 19-Feb-20 | *C. bedfordi* | 24 | 12 | 0 | 12 | 48 |
| | *C. enderleini* | 276 | 192 | 0 | 0 | 468 |
| | *C. imicola* | 12 024 | 10 020 | 288 | 1 500 | 23 832 |
| | *C. leucostictus* | 144 | 156 | 0 | 72 | 372 |
| | *C. nivosus* | 24 | 0 | 0 | 0 | 24 |
| | *C. pycnostictus* | 72 | 36 | 0 | 24 | 132 |
| | *C. subschultzei* | 12 | 12 | 0 | 288 | 312 |
| | Nigripennis grp | 0 | 24 | 0 | 0 | 24 |
| | **Total** | 12 576 | 10 452 | 288 | 1 896 | **25 212** |
| 26-Feb-20 | *C. bedfordi* | 0 | 0 | 0 | 20 | 20 |
| | *C. enderleini* | 420 | 340 | 0 | 20 | 780 |
| | *C. imicola* | 19 140 | 23 780 | 140 | 560 | 43 620 |
| | *C. leucostictus* | 40 | 160 | 0 | 40 | 240 |
| | *C. nivosus* | 0 | 40 | 0 | 0 | 40 |
| | *C. pycnostictus* | 0 | 40 | 0 | 0 | 40 |
| | *C. ravus* | 0 | 20 | 0 | 0 | 20 |
| | *C. subschultzei* | 0 | 0 | 0 | 180 | 180 |
| | *C. zuluensis* | 20 | 0 | 0 | 0 | 20 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Nigripennis grp | 20 | 0 | 0 | 0 | 20 |
| | **Total** | 19 640 | 24 380 | 140 | 820 | **44 980** |
| 04-Mar-20 | *C. coarctatus* | 10 | 0 | 0 | 0 | 10 |
| | *C. enderleini* | 230 | 340 | 0 | 0 | 570 |
| | *C. imicola* | 4 650 | 4 740 | 70 | 100 | 9 560 |
| | *C. leucostictus* | 30 | 50 | 0 | 30 | 110 |
| | *C. magnus* | 10 | 0 | 0 | 0 | 10 |
| | *C. nivosus* | 10 | 0 | 0 | 0 | 10 |
| | *C. pycnostictus* | 10 | 20 | 0 | 0 | 30 |
| | *C. subschultzei* | 0 | 20 | 0 | 30 | 50 |
| | *C. zuluensis* | 20 | 10 | 0 | 10 | 40 |
| | Nigripennis grp | 20 | 0 | 0 | 0 | 20 |
| | **Total** | 4 990 | 5 180 | 70 | 170 | **10 410** |
| 11-Mar-20 | *C. bedfordi* | 0 | 40 | 0 | 0 | 40 |
| | *C. enderleini* | 900 | 760 | 0 | 120 | 1 780 |
| | *C. imicola* | 11 340 | 13 120 | 400 | 1 000 | 25 860 |
| | *C. leucostictus* | 100 | 180 | 0 | 60 | 340 |
| | *C. nevilli* | 0 | 20 | 0 | 0 | 20 |
| | *C. pycnostictus* | 0 | 60 | 0 | 0 | 60 |
| | *C. subschultzei* | 20 | 20 | 0 | 540 | 580 |
| | Nigripennis grp | 20 | 0 | 0 | 0 | 20 |
| | **Total** | 12 380 | 14 200 | 400 | 1 720 | **28 700** |
| 18-Mar-20 | *C. bedfordi* | 40 | 24 | 0 | 0 | 64 |
| | *C. enderleini* | 200 | 160 | 0 | 8 | 368 |
| | *C. imicola* | 3 696 | 5 504 | 16 | 320 | 9 536 |
| | *C. leucostictus* | 64 | 352 | 0 | 0 | 416 |
| | *C. nivosus* | 16 | 8 | 0 | 8 | 32 |
| | *C. pycnostictus* | 24 | 80 | 0 | 0 | 104 |
| | *C. ravus* | 0 | 16 | 0 | 0 | 16 |
| | *C. subschultzei* | 0 | 8 | 0 | 128 | 136 |
| | *C. zuluensis* | 16 | 8 | 0 | 8 | 32 |
| | **Total** | 4 056 | 6 160 | 16 | 472 | **10 704** |
| 25-Mar-20 | *C. bedfordi* | 32 | 16 | 0 | 0 | 48 |
| | *C. enderleini* | 88 | 184 | 0 | 0 | 272 |
| | *C. imicola* | 6 400 | 2 272 | 32 | 136 | 8 840 |
| | *C. leucostictus* | 16 | 136 | 0 | 24 | 176 |
| | *C. nivosus* | 0 | 16 | 0 | 0 | 16 |
| | *C. pycnostictus* | 0 | 24 | 0 | 0 | 24 |
| | *C. subschultzei* | 8 | 24 | 0 | 88 | 120 |
| | *C. zuluensis* | 32 | 8 | 0 | 0 | 40 |
| | **Total** | 6 576 | 2 680 | 32 | 248 | **9 536** |
| 17-Jun-20 | *C. imicola* | 2 | 4 | 0 | 0 | 6 |
| | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 2 | 6 | 0 | 0 | **8** |
| 24-Jun-20 | *C. imicola* | 1 | 0 | 0 | 0 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Total** | 1 | 0 | 0 | 0 | **1** |
| 08-Jul-20 | *C. imicola* | 1 | 1 | 0 | 0 | 2 |
| | *C. leucostictus* | 1 | 1 | 0 | 0 | 2 |
| | *C. magnus* | 0 | 1 | 0 | 0 | 1 |
| | *C. zuluensis* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 3 | 3 | 0 | 0 | **6** |
| 22-Jul-20 | *C. imicola* | 10 | 1 | 0 | 0 | 11 |
| | **Total** | 10 | 1 | 0 | 0 | **11** |
| 29-Jul-20 | *C. imicola* | 27 | 4 | 0 | 4 | 35 |
| | *C. leucostictus* | 2 | 0 | 0 | 3 | 5 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. ravus* | 1 | 0 | 0 | 0 | 1 |
| | *C. zuluensis* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 32 | 4 | 0 | 7 | **43** |
| 05-Aug-20 | *C. imicola* | 16 | 1 | 0 | 1 | 18 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 17 | 1 | 0 | 1 | **19** |
| 12-Aug-20 | *C. brucei* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 58 | 3 | 2 | 6 | 69 |
| | *C. leucostictus* | 4 | 2 | 0 | 5 | 11 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. zuluensis* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 63 | 7 | 2 | 11 | **83** |
| 28-Aug-20 | *C. enderleini* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 124 | 74 | 0 | 7 | 205 |
| | *C. leucostictus* | 13 | 8 | 0 | 6 | 27 |
| | *C. nevilli* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 2 | 0 | 0 | 0 | 2 |
| | *C. similis* | 0 | 1 | 0 | 0 | 1 |
| | *C. zuluensis* | 3 | 0 | 0 | 0 | 3 |
| | **Total** | 144 | 83 | 0 | 13 | **240** |
| | **Grand Total** | **128 595** | **129 986** | **2 188** | **14 405** | **275 174** |

## APPENDIX 4b: *Culicoides* counts from light trap 2 (near sheep) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. bolitinos* | 2 | 1 | 0 | 1 | 4 |
| | *C. brucei* | 1 | 0 | 0 | 0 | 1 |
| | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 527 | 278 | 13 | 39 | 857 |
| | *C. leucostictus* | 9 | 15 | 0 | 0 | 24 |
| | *C. magnus* | 2 | 1 | 0 | 0 | 3 |
| | *C. nivosus* | 1 | 3 | 1 | 0 | 5 |
| | *C. pycnostictus* | 2 | 15 | 0 | 7 | 24 |
| | *C. zuluensis* | 4 | 0 | 0 | 0 | 4 |
| | **Total** | **548** | **314** | **14** | **47** | **923** |
| 04-Sep-19 | *C. brucei* | 2 | 0 | 0 | 0 | 2 |
| | *C. imicola* | 159 | 134 | 6 | 14 | 313 |
| | *C. leucostictus* | 1 | 2 | 0 | 1 | 4 |
| | *C. pycnostictus* | 0 | 2 | 0 | 1 | 3 |
| | *C. similis* | 1 | 0 | 0 | 0 | 1 |
| | *C. zuluensis* | 0 | 1 | 0 | 2 | 3 |
| | **Total** | **163** | **139** | **6** | **18** | **326** |
| 12-Sep-19 | *C. imicola* | 62 | 58 | 2 | 8 | 130 |
| | *C. leucostictus* | 4 | 2 | 0 | 2 | 8 |
| | *C. nivosus* | 1 | 4 | 0 | 0 | 5 |
| | *C. pycnostictus* | 1 | 8 | 0 | 1 | 10 |
| | *C. zuluensis* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **68** | **73** | **2** | **11** | **154** |
| 18-Sep-19 | *C. bedfordi* | 4 | 2 | 0 | 0 | 6 |
| | *C. brucei* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 216 | 219 | 5 | 19 | 459 |
| | *C. leucostictus* | 7 | 16 | 0 | 3 | 26 |
| | *C. magnus* | 1 | 3 | 0 | 0 | 4 |
| | *C. nivosus* | 0 | 16 | 0 | 1 | 17 |
| | *C. pycnostictus* | 2 | 23 | 0 | 0 | 25 |
| | *C. similis* | 0 | 1 | 0 | 0 | 1 |
| | *C. zuluensis* | 1 | 0 | 0 | 1 | 2 |
| | **Total** | **231** | **281** | **5** | **24** | **541** |
| 25-Sep-19 | *C. bedfordi* | 0 | 0 | 0 | 3 | 3 |
| | *C. brucei* | 1 | 0 | 0 | 1 | 2 |
| | *C. imicola* | 17 | 6 | 0 | 8 | 31 |
| | *C. leucostictus* | 4 | 1 | 0 | 2 | 7 |
| | *C. nivosus* | 0 | 2 | 0 | 0 | 2 |
| | *C. pycnostictus* | 2 | 2 | 0 | 1 | 5 |
| | **Total** | **24** | **11** | **0** | **15** | **50** |
| 30-Sep-19 | *C. bedfordi* | 2 | 0 | 0 | 1 | 3 |
| | *C. imicola* | 16 | 5 | 0 | 56 | 77 |
| | *C. leucostictus* | 2 | 2 | 0 | 2 | 6 |
| | *C. nivosus* | 1 | 1 | 0 | 0 | 2 |
| | *C. pycnostictus* | 0 | 3 | 0 | 0 | 3 |
| | **Total** | **21** | **11** | **0** | **59** | **91** |
| 02-Oct-19 | *C. bedfordi* | 5 | 3 | 0 | 0 | 8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. brucei* | 1 | 2 | 1 | 0 | 4 |
| | *C. eriodendroni* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 492 | 244 | 12 | 21 | 769 |
| | *C. leucostictus* | 6 | 10 | 0 | 5 | 21 |
| | *C. magnus* | 4 | 2 | 0 | 1 | 7 |
| | *C. nivosus* | 1 | 3 | 0 | 0 | 4 |
| | *C. pycnostictus* | 1 | 12 | 0 | 0 | 13 |
| | **Total** | **511** | **276** | **13** | **27** | **827** |
| 07-Oct-19 | *C. bedfordi* | 5 | 12 | 0 | 8 | 25 |
| | *C. brucei* | 2 | 1 | 0 | 0 | 3 |
| | *C. enderleini* | 0 | 1 | 0 | 1 | 2 |
| | *C. imicola* | 725 | 539 | 13 | 61 | 1 338 |
| | *C. leucostictus* | 23 | 18 | 0 | 21 | 62 |
| | *C. magnus* | 1 | 1 | 1 | 0 | 3 |
| | *C. nevilli* | 1 | 0 | 0 | 1 | 2 |
| | *C. nivosus* | 10 | 19 | 0 | 2 | 31 |
| | *C. pycnostictus* | 29 | 54 | 0 | 3 | 86 |
| | *C. similis* | 0 | 1 | 0 | 0 | 1 |
| | *C. subschultzei* | 1 | 1 | 0 | 0 | 2 |
| | *C. zuluensis* | 2 | 1 | 0 | 1 | 4 |
| | Nigripennis grp | 1 | 3 | 0 | 1 | 5 |
| | **Total** | **800** | **651** | **14** | **99** | **1 564** |
| 15-Oct-19 | *C. bedfordi* | 13 | 45 | 0 | 17 | 75 |
| | *C. brucei* | 20 | 11 | 0 | 8 | 39 |
| | *C. coarctatus* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 759 | 686 | 21 | 222 | 1 688 |
| | *C. leucostictus* | 15 | 11 | 3 | 47 | 76 |
| | *C. magnus* | 4 | 1 | 0 | 0 | 5 |
| | *C. neavei* | 0 | 3 | 0 | 0 | 3 |
| | *C. nivosus* | 11 | 31 | 0 | 46 | 88 |
| | *C. pycnostictus* | 15 | 35 | 2 | 14 | 66 |
| | *C. zuluensis* | 1 | 1 | 0 | 8 | 10 |
| | Nigripennis grp | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **840** | **824** | **26** | **362** | **2 052** |
| 21-Oct-19 | *C. bedfordi* | 14 | 21 | 0 | 29 | 64 |
| | *C. brucei* | 1 | 1 | 0 | 3 | 5 |
| | *C. enderleini* | 1 | 2 | 0 | 0 | 3 |
| | *C. expectator* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 398 | 269 | 3 | 363 | 1 033 |
| | *C. leucostictus* | 37 | 55 | 0 | 76 | 168 |
| | *C. nevilli* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 29 | 94 | 0 | 29 | 152 |
| | *C. pycnostictus* | 37 | 69 | 0 | 13 | 119 |
| | *C. similis* | 2 | 5 | 0 | 1 | 8 |
| | *C. subschultzei* | 1 | 0 | 0 | 0 | 1 |
| | *C. zuluensis* | 1 | 2 | 0 | 2 | 5 |
| | **Total** | **521** | **520** | **3** | **516** | **1 560** |
| 30-Oct-19 | *C. bedfordi* | 7 | 3 | 0 | 9 | 19 |
| | *C. brucei* | 1 | 1 | 0 | 0 | 2 |
| | *C. enderleini* | 3 | 0 | 0 | 1 | 4 |
| | *C. imicola* | 1 405 | 464 | 23 | 129 | 2 021 |
| | *C. leucostictus* | 22 | 18 | 0 | 19 | 59 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. nivosus* | 7 | 26 | 0 | 11 | 44 |
| | *C. pycnostictus* | 12 | 24 | 0 | 8 | 44 |
| | *C. similis* | 1 | 0 | 0 | 0 | 1 |
| | *C. zuluensis* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **1 459** | **536** | **23** | **177** | **2 195** |
| 05-Nov-19 | *C. bedfordi* | 12 | 20 | 0 | 8 | 40 |
| | *C. brucei* | 1 | 1 | 0 | 0 | 2 |
| | *C. enderleini* | 5 | 4 | 0 | 3 | 12 |
| | *C. imicola* | 1 692 | 1 111 | 59 | 80 | 2 942 |
| | *C. leucostictus* | 20 | 27 | 0 | 21 | 68 |
| | *C. magnus* | 2 | 7 | 0 | 1 | 10 |
| | *C. nivosus* | 7 | 55 | 0 | 8 | 70 |
| | *C. pycnostictus* | 8 | 23 | 0 | 2 | 33 |
| | *C. similis* | 2 | 0 | 0 | 0 | 2 |
| | *C. zuluensis* | 4 | 2 | 0 | 2 | 8 |
| | **Total** | **1 753** | **1 250** | **59** | **125** | **3 187** |
| 14-Nov-19 | *C. bedfordi* | 4 | 2 | 0 | 3 | 9 |
| | *C. brucei* | 4 | 0 | 0 | 1 | 5 |
| | *C. enderleini* | 4 | 1 | 0 | 1 | 6 |
| | *C. imicola* | 703 | 485 | 36 | 118 | 1 342 |
| | *C. leucostictus* | 16 | 52 | 0 | 15 | 83 |
| | *C. magnus* | 2 | 1 | 0 | 0 | 3 |
| | *C. nivosus* | 3 | 49 | 0 | 12 | 64 |
| | *C. pycnostictus* | 18 | 46 | 0 | 8 | 72 |
| | *C. similis* | 0 | 0 | 0 | 1 | 1 |
| | *C. subschultzei* | 0 | 0 | 0 | 1 | 1 |
| | *C. zuluensis* | 3 | 1 | 0 | 3 | 7 |
| | Nigripennis grp | 0 | 2 | 0 | 0 | 2 |
| | **Total** | **757** | **639** | **36** | **163** | **1 595** |
| 20-Nov-19 | *C. bedfordi* | 16 | 7 | 0 | 0 | 23 |
| | *C. brucei* | 4 | 3 | 0 | 0 | 7 |
| | *C. cornutus* | 0 | 1 | 0 | 0 | 1 |
| | *C. enderleini* | 3 | 0 | 0 | 0 | 3 |
| | *C. imicola* | 2 068 | 857 | 49 | 610 | 3 584 |
| | *C. leucostictus* | 164 | 145 | 2 | 19 | 330 |
| | *C. magnus* | 6 | 4 | 0 | 1 | 11 |
| | *C. nivosus* | 11 | 25 | 0 | 1 | 37 |
| | *C. pycnostictus* | 18 | 40 | 0 | 1 | 59 |
| | *C. subschultzei* | 2 | 1 | 0 | 1 | 4 |
| | *C. zuluensis* | 3 | 4 | 0 | 5 | 12 |
| | Nigripennis grp | 13 | 6 | 0 | 1 | 20 |
| | **Total** | **2 308** | **1 093** | **51** | **639** | **4 091** |
| 26-Nov-19 | *C. bedfordi* | 2 | 0 | 0 | 0 | 2 |
| | *C. brucei* | 2 | 0 | 0 | 0 | 2 |
| | *C. enderleini* | 4 | 8 | 0 | 0 | 12 |
| | *C. imicola* | 1 774 | 1 436 | 40 | 56 | 3 306 |
| | *C. leucostictus* | 58 | 66 | 2 | 10 | 136 |
| | *C. nivosus* | 18 | 28 | 0 | 22 | 68 |
| | *C. pycnostictus* | 0 | 6 | 0 | 0 | 6 |
| | *C. subschultzei* | 0 | 0 | 0 | 4 | 4 |
| | *C. zuluensis* | 4 | 2 | 0 | 0 | 6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Nigripennis grp | 6 | 8 | 0 | 0 | 14 |
| | **Total** | **1 868** | **1 554** | **42** | **92** | **3 556** |
| 03-Dec-19 | *C. bedfordi* | 3 | 2 | 0 | 10 | 15 |
| | *C. brucei* | 0 | 1 | 0 | 0 | 1 |
| | *C. enderleini* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 1 269 | 597 | 33 | 99 | 1 998 |
| | *C. leucostictus* | 43 | 31 | 2 | 39 | 115 |
| | *C. magnus* | 1 | 3 | 0 | 1 | 5 |
| | *C. nivosus* | 4 | 6 | 0 | 24 | 34 |
| | *C. pycnostictus* | 5 | 4 | 0 | 2 | 11 |
| | *C. similis* | 0 | 0 | 0 | 1 | 1 |
| | *C. subschultzei* | 0 | 0 | 0 | 4 | 4 |
| | *C. subschultzei* | 0 | 0 | 0 | 0 | 0 |
| | *C. zuluensis* | 5 | 3 | 0 | 8 | 16 |
| | Nigripennis grp | 1 | 2 | 0 | 1 | 4 |
| | **Total** | **1 332** | **649** | **35** | **189** | **2 205** |
| 11-Dec-19 | *C. bedfordi* | 2 | 0 | 0 | 0 | 2 |
| | *C. brucei* | 3 | 3 | 0 | 0 | 6 |
| | *C. enderleini* | 0 | 2 | 0 | 0 | 2 |
| | *C. imicola* | 836 | 3 751 | 64 | 405 | 5 056 |
| | *C. leucostictus* | 12 | 270 | 0 | 7 | 289 |
| | *C. magnus* | 1 | 1 | 0 | 0 | 2 |
| | *C. nevilli* | 0 | 0 | 0 | 1 | 1 |
| | *C. nivosus* | 18 | 68 | 0 | 3 | 89 |
| | *C. pycnostictus* | 2 | 66 | 0 | 0 | 68 |
| | *C. ravus* | 1 | 0 | 0 | 0 | 1 |
| | *C. subschultzei* | 0 | 0 | 0 | 1 | 1 |
| | *C. zuluensis* | 3 | 1 | 0 | 5 | 9 |
| | **Total** | **878** | **4 162** | **64** | **422** | **5 526** |
| 18-Dec-19 | *C. brucei* | 3 | 0 | 0 | 0 | 3 |
| | *C. cornutus* | 0 | 3 | 0 | 0 | 3 |
| | *C. imicola* | 3 306 | 1 401 | 42 | 174 | 4 923 |
| | *C. leucostictus* | 81 | 204 | 0 | 42 | 327 |
| | *C. magnus* | 6 | 0 | 0 | 0 | 6 |
| | *C. nivosus* | 3 | 21 | 0 | 6 | 30 |
| | *C. pycnostictus* | 9 | 27 | 0 | 0 | 36 |
| | *C. ravus* | 3 | 0 | 0 | 0 | 3 |
| | *C. zuluensis* | 6 | 12 | 0 | 3 | 21 |
| | Nigripennis grp | 0 | 3 | 0 | 0 | 3 |
| | **Total** | **3 417** | **1 671** | **42** | **225** | **5 355** |
| 26-Dec-19 | *C. bedfordi* | 6 | 0 | 0 | 3 | 9 |
| | *C. cornutus* | 3 | 0 | 0 | 0 | 3 |
| | *C. enderleini* | 3 | 3 | 0 | 0 | 6 |
| | *C. expectator* | 0 | 3 | 0 | 0 | 3 |
| | *C. imicola* | 2 676 | 1 281 | 45 | 72 | 4 074 |
| | *C. leucostictus* | 63 | 240 | 0 | 45 | 348 |
| | *C. magnus* | 3 | 0 | 0 | 0 | 3 |
| | *C. nivosus* | 21 | 24 | 0 | 18 | 63 |
| | *C. pycnostictus* | 12 | 21 | 0 | 0 | 33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. ravus* | 3 | 0 | 0 | 0 | 3 |
| | *C. similis* | 6 | 0 | 0 | 0 | 6 |
| | *C. subschultzei* | 3 | 0 | 0 | 21 | 24 |
| | *C. zuluensis* | 0 | 0 | 0 | 6 | 6 |
| | **Total** | **2 799** | **1 572** | **45** | **165** | **4 581** |
| 01-Jan-20 | *C. bedfordi* | 6 | 0 | 0 | 0 | 6 |
| | *C. enderleini* | 3 | 9 | 0 | 0 | 12 |
| | *C. eriodendroni* | 0 | 0 | 0 | 3 | 3 |
| | *C. imicola* | 3 945 | 1 260 | 237 | 147 | 5 589 |
| | *C. leucostictus* | 45 | 111 | 3 | 18 | 177 |
| | *C. magnus* | 3 | 0 | 0 | 0 | 3 |
| | *C. nivosus* | 6 | 0 | 0 | 6 | 12 |
| | *C. pycnostictus* | 3 | 12 | 0 | 0 | 15 |
| | *C. subschultzei* | 0 | 0 | 0 | 3 | 3 |
| | *C. zuluensis* | 0 | 3 | 0 | 12 | 15 |
| | **Total** | 4 011 | 1 395 | 240 | 189 | **5 835** |
| 08-Jan-20 | *C. enderleini* | 1 | 11 | 0 | 0 | 12 |
| | *C. imicola* | 1 085 | 856 | 8 | 31 | 1 980 |
| | *C. leucostictus* | 51 | 50 | 0 | 7 | 108 |
| | *C. nivosus* | 5 | 4 | 0 | 4 | 13 |
| | *C. pycnostictus* | 2 | 8 | 0 | 0 | 10 |
| | *C. subschultzei* | 1 | 1 | 0 | 2 | 4 |
| | *C. zuluensis* | 6 | 2 | 0 | 1 | 9 |
| | Nigripennis grp | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **1 152** | **932** | **8** | **45** | **2 137** |
| 15-Jan-20 | *C. bedfordi* | 2 | 4 | 0 | 4 | 10 |
| | *C. brucei* | 0 | 0 | 0 | 4 | 4 |
| | *C. enderleini* | 10 | 60 | 0 | 6 | 76 |
| | *C. engubandei* | 0 | 2 | 0 | 0 | 2 |
| | *C. imicola* | 1 338 | 3 716 | 70 | 218 | 5 342 |
| | *C. leucostictus* | 84 | 96 | 0 | 28 | 208 |
| | *C. magnus* | 0 | 2 | 0 | 0 | 2 |
| | *C. nevilli* | 2 | 2 | 0 | 4 | 8 |
| | *C. nivosus* | 12 | 8 | 0 | 2 | 22 |
| | *C. pycnostictus* | 12 | 22 | 0 | 2 | 36 |
| | *C. subschultzei* | 2 | 24 | 0 | 20 | 46 |
| | *C. zuluensis* | 2 | 6 | 0 | 4 | 12 |
| | Nigripennis grp | 6 | 4 | 0 | 0 | 10 |
| | **Total** | 1 470 | 3 946 | 70 | 292 | **5 778** |
| 22-Jan-20 | *C. bedfordi* | 24 | 32 | 0 | 0 | 56 |
| | *C. enderleini* | 144 | 392 | 0 | 32 | 568 |
| | *C. engubandei* | 8 | 0 | 0 | 0 | 8 |
| | *C. imicola* | 17 264 | 11 640 | 120 | 1 120 | 30 144 |
| | *C. leucostictus* | 232 | 344 | 0 | 120 | 696 |
| | *C. neavei* | 0 | 0 | 0 | 8 | 8 |
| | *C. nivosus* | 8 | 64 | 0 | 0 | 72 |
| | *C. pycnostictus* | 0 | 40 | 0 | 0 | 40 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. subschultzei* | 40 | 72 | 0 | 80 | 192 |
| | *C. zuluensis* | 8 | 32 | 0 | 0 | 40 |
| | Nigripennis grp | 0 | 8 | 0 | 0 | 8 |
| | **Total** | **17 728** | **12 624** | **120** | **1 360** | **31 832** |
| 29-Jan-20 | *C. enderleini* | 20 | 192 | 0 | 0 | 212 |
| | *C. engubandei* | 0 | 8 | 0 | 0 | 8 |
| | *C. expectator* | 0 | 4 | 0 | 0 | 4 |
| | *C. imicola* | 3 300 | 3 436 | 132 | 320 | 7 188 |
| | *C. leucostictus* | 0 | 96 | 0 | 24 | 120 |
| | *C. nevilli* | 4 | 0 | 0 | 0 | 4 |
| | *C. nivosus* | 24 | 16 | 0 | 4 | 44 |
| | *C. pycnostictus* | 4 | 44 | 0 | 0 | 48 |
| | *C. subschultzei* | 0 | 12 | 0 | 36 | 48 |
| | *C. zuluensis* | 4 | 8 | 0 | 4 | 16 |
| | **Total** | **3 356** | **3 816** | **132** | **388** | **7 692** |
| 05-Feb-20 | *C. enderleini* | 40 | 60 | 0 | 20 | 120 |
| | *C. imicola* | 11 240 | 11 640 | 200 | 760 | 23 840 |
| | *C. leucostictus* | 60 | 100 | 0 | 0 | 160 |
| | *C. nivosus* | 80 | 80 | 0 | 0 | 160 |
| | *C. pycnostictus* | 0 | 80 | 0 | 0 | 80 |
| | *C. ravus* | 20 | 0 | 0 | 0 | 20 |
| | *C. subschultzei* | 0 | 20 | 0 | 20 | 40 |
| | *C. zuluensis* | 20 | 0 | 0 | 0 | 20 |
| | **Total** | 11 460 | 11 980 | 200 | 800 | **24 440** |
| 12-Feb-20 | *C. bedfordi* | 4 | 0 | 0 | 0 | 4 |
| | *C. enderleini* | 8 | 14 | 0 | 0 | 22 |
| | *C. imicola* | 824 | 1 308 | 34 | 134 | 2 300 |
| | *C. leucostictus* | 14 | 24 | 0 | 6 | 44 |
| | *C. magnus* | 0 | 2 | 0 | 0 | 2 |
| | *C. nivosus* | 2 | 6 | 0 | 2 | 10 |
| | *C. pycnostictus* | 6 | 2 | 0 | 2 | 10 |
| | *C. subschultzei* | 0 | 4 | 0 | 2 | 6 |
| | *C. zuluensis* | 0 | 0 | 0 | 2 | 2 |
| | **Total** | **858** | **1 360** | **34** | **148** | **2 400** |
| 19-Feb-20 | *C. bedfordi* | 24 | 24 | 0 | 0 | 48 |
| | *C. brucei* | 0 | 24 | 24 | 0 | 48 |
| | *C. enderleini* | 0 | 120 | 0 | 0 | 120 |
| | *C. imicola* | 16 416 | 10 128 | 672 | 1 296 | 28 512 |
| | *C. leucostictus* | 96 | 0 | 0 | 0 | 96 |
| | *C. pycnostictus* | 24 | 0 | 0 | 0 | 24 |
| | *C. subschultzei* | 0 | 0 | 0 | 96 | 96 |
| | **Total** | **16 560** | **10 296** | **696** | **1 392** | **28 944** |
| 26-Feb-20 | *C. bedfordi* | 6 | 0 | 0 | 0 | 6 |
| | *C. enderleini* | 18 | 84 | 0 | 0 | 102 |
| | *C. imicola* | 2 508 | 3 348 | 42 | 240 | 6 138 |
| | *C. leucostictus* | 12 | 60 | 0 | 6 | 78 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. nivosus* | 0 | 6 | 0 | 0 | 6 |
| | *C. pycnostictus* | 6 | 30 | 0 | 0 | 36 |
| | *C. subschultzei* | 24 | 18 | 0 | 60 | 102 |
| | **Total** | **2 574** | **3 546** | **42** | **306** | **6 468** |
| 04-Mar-20 | *C. brucei* | 0 | 0 | 0 | 1 | 1 |
| | *C. enderleini* | 9 | 12 | 0 | 0 | 21 |
| | *C. imicola* | 1 238 | 1 004 | 16 | 107 | 2 365 |
| | *C. leucostictus* | 2 | 10 | 0 | 4 | 16 |
| | *C. magnus* | 2 | 0 | 0 | 0 | 2 |
| | *C. nevilli* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 1 | 11 | 0 | 0 | 12 |
| | *C. subschultzei* | 0 | 0 | 0 | 6 | 6 |
| | *C. zuluensis* | 1 | 2 | 0 | 0 | 3 |
| | Nigripennis grp | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **1 256** | **1 039** | **16** | **118** | **2 429** |
| 11-Mar-20 | *C. bedfordi* | 0 | 4 | 0 | 4 | 8 |
| | *C. enderleini* | 32 | 28 | 0 | 0 | 60 |
| | *C. imicola* | 2 692 | 2 496 | 128 | 164 | 5 480 |
| | *C. leucostictus* | 24 | 32 | 0 | 24 | 80 |
| | *C. neavei* | 0 | 4 | 0 | 0 | 4 |
| | *C. nevilli* | 4 | 8 | 0 | 0 | 12 |
| | *C. ravus* | 0 | 4 | 0 | 4 | 8 |
| | *C. subschultzei* | 4 | 0 | 0 | 32 | 36 |
| | *C. zuluensis* | 4 | 0 | 0 | 0 | 4 |
| | **Total** | **2 760** | **2 576** | **128** | **228** | **5 692** |
| 18-Mar-20 | *C. bedfordi* | 0 | 2 | 0 | 0 | 2 |
| | *C. enderleini* | 24 | 18 | 0 | 2 | 44 |
| | *C. imicola* | 1 040 | 1 044 | 4 | 92 | 2 180 |
| | *C. leucostictus* | 6 | 20 | 0 | 0 | 26 |
| | *C. pycnostictus* | 0 | 8 | 0 | 0 | 8 |
| | *C. ravus* | 0 | 2 | 0 | 0 | 2 |
| | *C. subschultzei* | 0 | 2 | 0 | 6 | 8 |
| | *C. zuluensis* | 0 | 6 | 0 | 2 | 8 |
| | **Total** | 1 070 | 1 102 | 4 | 102 | **2 278** |
| 25-Mar-20 | *C. bedfordi* | 0 | 1 | 0 | 0 | 1 |
| | *C. brucei* | 0 | 1 | 0 | 0 | 1 |
| | *C. enderleini* | 18 | 17 | 0 | 0 | 35 |
| | *C. imicola* | 1 843 | 509 | 9 | 83 | 2 444 |
| | *C. leucostictus* | 6 | 14 | 0 | 9 | 29 |
| | *C. nivosus* | 1 | 4 | 0 | 0 | 5 |
| | *C. pycnostictus* | 1 | 4 | 0 | 0 | 5 |
| | *C. ravus* | 0 | 5 | 0 | 0 | 5 |
| | *C. subschultzei* | 0 | 0 | 0 | 25 | 25 |
| | *C. zuluensis* | 22 | 6 | 0 | 0 | 28 |
| | **Total** | **1 891** | **561** | **9** | **117** | **2 578** |
| 24-Jun-20 | *C. imicola* | 5 | 0 | 0 | 1 | 6 |
| | **Total** | **5** | **0** | **0** | **1** | **6** |
| 01-Jul-20 | *C. imicola* | 11 | 1 | 0 | 0 | 12 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Total** | **11** | **1** | **0** | **0** | **12** |
| 08-Jul-20 | *C. imicola* | 10 | 4 | 0 | 0 | 14 |
| | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **10** | **5** | **0** | **0** | **15** |
| 22-Jul-20 | *C. imicola* | 4 | 0 | 0 | 0 | 4 |
| | **Total** | **4** | **0** | **0** | **0** | **4** |
| 29-Jul-20 | *C. imicola* | 10 | 0 | 0 | 0 | 10 |
| | **Total** | **10** | **0** | **0** | **0** | **10** |
| 05-Aug-20 | *C. imicola* | 35 | 5 | 0 | 0 | 40 |
| | *C. zuluensis* | 1 | 1 | 0 | 0 | 2 |
| | **Total** | **36** | **6** | **0** | **0** | **42** |
| 12-Aug-20 | *C. imicola* | 72 | 10 | 1 | 3 | 86 |
| | *C. leucostictus* | 1 | 1 | 0 | 2 | 4 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. zuluensis* | 4 | 0 | 0 | 1 | 5 |
| | **Total** | **77** | **12** | **1** | **6** | **96** |
| 28-Aug-20 | *C. brucei* | 1 | 0 | 0 | 0 | 1 |
| | *C. enderleini* | 3 | 0 | 0 | 0 | 3 |
| | *C. imicola* | 347 | 219 | 5 | 11 | 582 |
| | *C. leucostictus* | 4 | 1 | 0 | 3 | 8 |
| | *C. magnus* | 2 | 3 | 0 | 0 | 5 |
| | *C. nevilli* | 3 | 1 | 0 | 1 | 5 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 3 | 0 | 0 | 3 |
| | *C. zuluensis* | 13 | 3 | 0 | 2 | 18 |
| | **Total** | **373** | **231** | **5** | **17** | **626** |
| | **Grand Total** | **86 970** | **71 654** | **2 185** | **8 884** | **169 693** |

## APPENDIX 4c: *Culicoides* counts from light trap 3 (near dogs) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. imicola* | 39 | 18 | 2 | 4 | 63 |
| | *C. leucostictus* | 8 | 8 | 0 | 1 | 17 |
| | *C. nivosus* | 3 | 1 | 0 | 1 | 5 |
| | *C. pycnostictus* | 0 | 4 | 0 | 0 | 4 |
| | **Total** | 50 | 31 | 2 | 6 | **89** |
| 04-Sep-19 | *C. imicola* | 9 | 9 | 2 | 0 | 20 |
| | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | *C. pycnostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 12 | 9 | 2 | 0 | **23** |
| 12-Sep-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 6 | 3 | 0 | 0 | 9 |
| | *C. leucostictus* | 3 | 4 | 0 | 5 | 12 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 6 | 0 | 0 | 6 |
| | **Total** | 10 | 14 | 0 | 5 | **29** |
| 18-Sep-19 | *C. bedfordi* | 0 | 0 | 0 | 1 | 1 |
| | *C. imicola* | 16 | 12 | 0 | 2 | 30 |
| | *C. leucostictus* | 2 | 7 | 0 | 0 | 9 |
| | **Total** | 18 | 19 | 0 | 3 | **40** |
| 25-Sep-19 | *C. bedfordi* | 2 | 1 | 0 | 0 | 3 |
| | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 4 | 2 | 0 | 0 | **6** |
| 30-Sep-19 | *C. bedfordi* | 3 | 0 | 0 | 2 | 5 |
| | *C. brucei* | 0 | 0 | 0 | 1 | 1 |
| | *C. imicola* | 6 | 7 | 0 | 3 | 16 |
| | *C. leucostictus* | 1 | 1 | 0 | 2 | 4 |
| | *C. nivosus* | 1 | 1 | 0 | 0 | 2 |
| | *C. pycnostictus* | 0 | 4 | 0 | 0 | 4 |
| | **Total** | 11 | 13 | 0 | 8 | **32** |
| 02-Oct-19 | *C. bedfordi* | 3 | 0 | 0 | 0 | 3 |
| | *C. brucei* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 38 | 17 | 0 | 0 | 55 |
| | *C. leucostictus* | 5 | 15 | 0 | 1 | 21 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 4 | 0 | 0 | 4 |
| | **Total** | 47 | 37 | 0 | 1 | **85** |
| 07-Oct-19 | *C. bedfordi* | 4 | 0 | 0 | 4 | 8 |
| | *C. imicola* | 62 | 40 | 1 | 9 | 112 |
| | *C. leucostictus* | 15 | 8 | 0 | 5 | 28 |
| | *C. nivosus* | 4 | 4 | 0 | 0 | 8 |
| | *C. pycnostictus* | 10 | 16 | 0 | 3 | 29 |
| | *C. similis* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 95 | 69 | 1 | 21 | **186** |
| 15-Oct-19 | *C. bedfordi* | 5 | 4 | 0 | 1 | 10 |
| | *C. imicola* | 51 | 27 | 1 | 61 | 140 |
| | *C. leucostictus* | 16 | 18 | 0 | 10 | 44 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. nivosus* | 0 | 2 | 0 | 2 | 4 |
| | *C. pycnostictus* | 19 | 9 | 0 | 2 | 30 |
| | **Total** | 91 | 60 | 1 | 76 | **228** |
| 21-Oct-19 | *C. bedfordi* | 4 | 3 | 0 | 14 | 21 |
| | *C. imicola* | 18 | 18 | 0 | 25 | 61 |
| | *C. leucostictus* | 34 | 64 | 0 | 28 | 126 |
| | *C. nivosus* | 9 | 4 | 0 | 11 | 24 |
| | *C. pycnostictus* | 13 | 20 | 0 | 10 | 43 |
| | **Total** | 78 | 109 | 0 | 88 | **275** |
| 30-Oct-19 | *C. bedfordi* | 3 | 2 | 0 | 9 | 14 |
| | *C. imicola* | 152 | 60 | 8 | 7 | 227 |
| | *C. leucostictus* | 27 | 15 | 0 | 20 | 62 |
| | *C. nivosus* | 0 | 4 | 0 | 5 | 9 |
| | *C. pycnostictus* | 12 | 11 | 0 | 6 | 29 |
| | **Total** | 194 | 92 | 8 | 47 | **341** |
| 05-Nov-19 | *C. bedfordi* | 2 | 4 | 0 | 1 | 7 |
| | *C. imicola* | 112 | 81 | 1 | 14 | 208 |
| | *C. leucostictus* | 132 | 118 | 0 | 7 | 257 |
| | *C. nivosus* | 1 | 0 | 0 | 2 | 3 |
| | *C. pycnostictus* | 31 | 12 | 0 | 3 | 46 |
| | **Total** | 278 | 215 | 1 | 27 | **521** |
| 14-Nov-19 | *C. bedfordi* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 25 | 17 | 1 | 5 | 48 |
| | *C. leucostictus* | 34 | 39 | 0 | 6 | 79 |
| | *C. nivosus* | 1 | 3 | 0 | 1 | 5 |
| | *C. pycnostictus* | 5 | 14 | 0 | 4 | 23 |
| | *C. similis* | 0 | 0 | 0 | 1 | 1 |
| | Nigripennis grp | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 65 | 74 | 1 | 18 | **158** |
| 20-Nov-19 | *C. bedfordi* | 2 | 0 | 0 | 0 | 2 |
| | *C. imicola* | 76 | 42 | 2 | 16 | 136 |
| | *C. leucostictus* | 10 | 14 | 0 | 0 | 24 |
| | *C. nivosus* | 0 | 3 | 0 | 0 | 3 |
| | *C. pycnostictus* | 0 | 2 | 0 | 0 | 2 |
| | Nigripennis grp | 7 | 0 | 0 | 0 | 7 |
| | **Total** | 95 | 61 | 2 | 16 | **174** |
| 26-Nov-19 | *C. imicola* | 67 | 44 | 1 | 8 | 120 |
| | *C. leucostictus* | 169 | 185 | 0 | 4 | 358 |
| | *C. nivosus* | 2 | 6 | 0 | 0 | 8 |
| | *C. pycnostictus* | 5 | 7 | 0 | 0 | 12 |
| | Nigripennis grp | 0 | 12 | 0 | 0 | 12 |
| | **Total** | 243 | 254 | 1 | 12 | **510** |
| 03-Dec-19 | *C. imicola* | 69 | 31 | 0 | 4 | 104 |
| | *C. leucostictus* | 11 | 2 | 0 | 1 | 14 |
| | *C. similis* | 0 | 0 | 0 | 1 | 1 |
| | Nigripennis grp | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 80 | 34 | 0 | 6 | **120** |
| 11-Dec-19 | *C. imicola* | 11 | 74 | 1 | 3 | 89 |
| | *C. leucostictus* | 14 | 32 | 0 | 1 | 47 |
| | *C. pycnostictus* | 2 | 5 | 0 | 0 | 7 |
| | Nigripennis grp | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 27 | 112 | 1 | 4 | **144** |

| | | | | | | |
|---|---|---|---|---|---|---|
| 18-Dec-19 | *C. imicola* | 276 | 155 | 2 | 10 | 443 |
| | *C. leucostictus* | 167 | 27 | 0 | 9 | 203 |
| | *C. nevilli* | 1 | 0 | 0 | 0 | 1 |
| | *C. subschultzei* | 1 | 0 | 0 | 0 | 1 |
| | *C. zuluensis* | 0 | 1 | 0 | 1 | 2 |
| | Nigripennis grp | 0 | 1 | 0 | 0 | 1 |
| | ***Total*** | 445 | 184 | 2 | 20 | **651** |
| 26-Dec-19 | *C. bedfordi* | 2 | 0 | 0 | 0 | 2 |
| | *C. imicola* | 258 | 120 | 4 | 16 | 398 |
| | *C. leucostictus* | 888 | 1 942 | 6 | 34 | 2 870 |
| | *C. nivosus* | 0 | 4 | 0 | 0 | 4 |
| | *C. zuluensis* | 0 | 0 | 1 | 0 | 1 |
| | Nigripennis grp | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 1 148 | 2 067 | 11 | 50 | **3 276** |
| 01-Jan-20 | *C. imicola* | 362 | 112 | 6 | 11 | 491 |
| | *C. leucostictus* | 364 | 348 | 1 | 7 | 720 |
| | *C. nivosus* | 1 | 0 | 0 | 1 | 2 |
| | Nigripennis grp | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 728 | 460 | 7 | 19 | **1 214** |
| 08-Jan-20 | *C. imicola* | 274 | 252 | 4 | 5 | 535 |
| | *C. leucostictus* | 166 | 118 | 1 | 3 | 288 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | Nigripennis grp | 0 | 1 | 0 | 0 | 1 |
| | **Total** | 440 | 372 | 5 | 8 | **825** |
| 15-Jan-20 | *C. bedfordi* | 1 | 2 | 0 | 0 | 3 |
| | *C. engubandei* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 109 | 241 | 0 | 15 | 365 |
| | *C. leucostictus* | 385 | 433 | 1 | 28 | 847 |
| | *C. nivosus* | 2 | 1 | 0 | 0 | 3 |
| | *C. pycnostictus* | 2 | 1 | 0 | 0 | 3 |
| | Nigripennis grp | 0 | 4 | 0 | 1 | 5 |
| | **Total** | 500 | 682 | 1 | 44 | **1 227** |
| 22-Jan-20 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 137 | 115 | 1 | 1 | 254 |
| | *C. leucostictus* | 415 | 494 | 1 | 22 | 932 |
| | *C. zuluensis* | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 553 | 610 | 2 | 24 | **1 189** |
| 29-Jan-20 | *C. imicola* | 124 | 118 | 1 | 3 | 246 |
| | *C. leucostictus* | 94 | 103 | 0 | 45 | 242 |
| | *C. pycnostictus* | 0 | 3 | 0 | 0 | 3 |
| | Nigripennis grp | 0 | 2 | 0 | 0 | 2 |
| | **Total** | 218 | 226 | 1 | 48 | **493** |
| 05-Feb-20 | *C. imicola* | 127 | 155 | 6 | 11 | 299 |
| | *C. leucostictus* | 305 | 182 | 2 | 10 | 499 |
| | **Total** | 432 | 337 | 8 | 21 | **798** |
| 26-Feb-20 | *C. enderleini* | 2 | 0 | 0 | 0 | 2 |
| | *C. imicola* | 480 | 503 | 5 | 57 | 1 045 |
| | *C. leucostictus* | 110 | 96 | 1 | 9 | 216 |
| | *C. nevilli* | 1 | 4 | 0 | 0 | 5 |
| | *C. subschultzei* | 1 | 2 | 0 | 0 | 3 |
| | **Total** | 594 | 605 | 6 | 66 | **1 271** |

| | | | | | | |
|---|---|---|---|---|---|---|
| 04-Mar-20 | *C. enderleini* | 4 | 2 | 0 | 0 | 6 |
| | *C. imicola* | 299 | 264 | 2 | 23 | 588 |
| | *C. leucostictus* | 119 | 37 | 0 | 10 | 166 |
| | *C. neavei* | 0 | 0 | 0 | 1 | 1 |
| | *C. nevilli* | 1 | 0 | 0 | 0 | 1 |
| | *C. subschultzei* | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 423 | 303 | 2 | 35 | **763** |
| 11-Mar-20 | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 297 | 342 | 4 | 23 | 666 |
| | *C. leucostictus* | 136 | 64 | 0 | 5 | 205 |
| | *C. nivosus* | 2 | 1 | 0 | 0 | 3 |
| | *C. pycnostictus* | 1 | 0 | 0 | 1 | 2 |
| | *C. subschultzei* | 0 | 0 | 0 | 4 | 4 |
| | *C. zuluensis* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 437 | 408 | 4 | 33 | **882** |
| 18-Mar-20 | *C. bedfordi* | 1 | 1 | 0 | 0 | 2 |
| | *C. imicola* | 228 | 268 | 2 | 11 | 509 |
| | *C. leucostictus* | 30 | 29 | 0 | 2 | 61 |
| | *C. nevilli* | 1 | 2 | 0 | 0 | 3 |
| | *C. pycnostictus* | 1 | 2 | 0 | 0 | 3 |
| | *C. subschultzei* | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 261 | 302 | 2 | 14 | **579** |
| 25-Mar-20 | *C. imicola* | 77 | 25 | 2 | 5 | 109 |
| | *C. leucostictus* | 36 | 5 | 0 | 0 | 41 |
| | *C. ravus* | 0 | 1 | 0 | 0 | 1 |
| | *C. subschultzei* | 0 | 0 | 0 | 2 | 2 |
| | Nigripennis grp | 0 | 2 | 0 | 0 | 2 |
| | **Total** | 113 | 33 | 2 | 7 | **155** |
| 24-Jun-20 | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 1 | 0 | 0 | 0 | **1** |
| 01-Jul-20 | *C. imicola* | 2 | 1 | 0 | 0 | 3 |
| | **Total** | 2 | 1 | 0 | 0 | **3** |
| 15-Jul-20 | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | 1 | 0 | 0 | 0 | **1** |
| 22-Jul-20 | *C. imicola* | 5 | 0 | 0 | 0 | 5 |
| | *C. leucostictus* | 0 | 0 | 0 | 1 | 1 |
| | **Total** | 5 | 0 | 0 | 1 | **6** |
| 29-Jul-20 | *C. imicola* | 21 | 1 | 0 | 0 | 22 |
| | *C. leucostictus* | 2 | 1 | 0 | 0 | 3 |
| | **Total** | 23 | 2 | 0 | 0 | **25** |
| 05-Aug-20 | *C. imicola* | 9 | 2 | 0 | 0 | 11 |
| | *C. leucostictus* | 3 | 1 | 0 | 1 | 5 |
| | **Total** | 12 | 3 | 0 | 1 | **16** |
| 12-Aug-20 | *C. imicola* | 6 | 1 | 1 | 2 | 10 |
| | *C. leucostictus* | 8 | 0 | 0 | 0 | 8 |
| | **Total** | 14 | 1 | 1 | 2 | **18** |
| | **Grand Total** | **7 748** | **7 801** | **74** | **731** | **16 354** |

## APPENDIX 5a: *Culicoides* counts from $CO_2$ trap 1 (near sheep) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. leucostictus* | 3 | 2 | 0 | 0 | 5 |
| | *C. pycnostictus* | 2 | 2 | 0 | 0 | 4 |
| | **Total** | **5** | **4** | **0** | **0** | **9** |
| 4-Sep-19 | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **2** | **1** | **0** | **0** | **3** |
| 12-Sep-19 | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 1 | 1 | 0 | 0 | 2 |
| | **Total** | **2** | **2** | **0** | **0** | **4** |
| 18-Sep-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 2 | 2 | 0 | 0 | 4 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **5** | **2** | **0** | **0** | **7** |
| 30-Sep-19 | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 0 | 2 | 0 | 0 | 2 |
| | *C. pycnostictus* | 0 | 2 | 0 | 0 | 2 |
| | **Total** | **1** | **4** | **0** | **0** | **5** |
| 2-Oct-19 | *C. leucostictus* | 1 | 1 | 0 | 0 | 2 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 3 | 0 | 0 | 3 |
| | **Total** | **1** | **5** | **0** | **0** | **6** |
| 7-Oct-19 | *C. bedfordi* | 2 | 0 | 0 | 0 | 2 |
| | *C. imicola* | 0 | 1 | 0 | 0 | 1 |
| | *C. leucostictus* | 16 | 5 | 0 | 2 | 23 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 6 | 7 | 0 | 0 | 13 |
| | **Total** | **24** | **14** | **0** | **2** | **40** |
| 15-Oct-19 | *C. leucostictus* | 4 | 6 | 0 | 0 | 10 |
| | *C. nivosus* | 3 | 1 | 0 | 0 | 4 |
| | *C. pycnostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **9** | **7** | **0** | **0** | **16** |
| 21-Oct-19 | *C. bedfordi* | 3 | 0 | 0 | 1 | 4 |
| | *C. leucostictus* | 17 | 9 | 0 | 2 | 28 |
| | *C. nivosus* | 11 | 9 | 0 | 0 | 20 |
| | *C. pycnostictus* | 19 | 5 | 0 | 0 | 24 |
| | **Total** | **50** | **23** | **0** | **3** | **76** |
| 30-Oct-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 8 | 11 | 0 | 1 | 20 |
| | *C. nivosus* | 3 | 7 | 0 | 0 | 10 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. pycnostictus* | 5 | 7 | 0 | 0 | 12 |
| | **Total** | **18** | **25** | **0** | **1** | **44** |
| 5-Nov-19 | *C. bedfordi* | 2 | 0 | 0 | 0 | 2 |
| | *C. leucostictus* | 24 | 19 | 0 | 7 | 50 |
| | *C. nivosus* | 2 | 4 | 0 | 0 | 6 |
| | *C. pycnostictus* | 8 | 7 | 0 | 0 | 15 |
| | **Total** | **36** | **30** | **0** | **7** | **73** |
| 14-Nov-19 | *C. leucostictus* | 3 | 2 | 0 | 1 | 6 |
| | *C. nivosus* | 0 | 3 | 0 | 0 | 3 |
| | *C. pycnostictus* | 6 | 2 | 0 | 0 | 8 |
| | **Total** | **9** | **7** | **0** | **1** | **17** |
| 20-Nov-19 | *C. leucostictus* | 8 | 12 | 1 | 0 | 21 |
| | *C. nivosus* | 3 | 1 | 0 | 0 | 4 |
| | *C. pycnostictus* | 4 | 1 | 0 | 0 | 5 |
| | Nigrpennis grp | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **16** | **14** | **1** | **0** | **31** |
| 26-Nov-19 | *C. leucostictus* | 17 | 32 | 0 | 4 | 53 |
| | *C. nivosus* | 0 | 4 | 0 | 0 | 4 |
| | *C. pycnostictus* | 1 | 2 | 0 | 0 | 3 |
| | Nigrpennis grp | 0 | 4 | 0 | 0 | 4 |
| | **Total** | **18** | **42** | **0** | **4** | **64** |
| 3-Dec-19 | *C. leucostictus* | 11 | 6 | 0 | 0 | 17 |
| | Nigrpennis grp | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **11** | **7** | **0** | **0** | **18** |
| 18-Dec-19 | *C. leucostictus* | 20 | 14 | 0 | 2 | 36 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 4 | 3 | 0 | 0 | 7 |
| | **Total** | **25** | **17** | **0** | **2** | **44** |
| 26-Dec-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 58 | 108 | 0 | 4 | 170 |
| | *C. nivosus* | 1 | 5 | 0 | 0 | 6 |
| | *C. pycnostictus* | 8 | 8 | 0 | 0 | 16 |
| | **Total** | **68** | **121** | **0** | **4** | **193** |
| 1-Jan-20 | *C. leucostictus* | 0 | 2 | 0 | 0 | 2 |
| | *C. pycnostictus* | 0 | 2 | 0 | 0 | 2 |
| | **Total** | **0** | **4** | **0** | **0** | **4** |
| 8-Jan-20 | *C. leucostictus* | 5 | 3 | 0 | 0 | 8 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **6** | **3** | **0** | **0** | **9** |
| 15-Jan-20 | *C. leucostictus* | 2 | 3 | 0 | 0 | 5 |
| | *C. pycnostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **3** | **3** | **0** | **0** | **6** |
| 22-Jan-20 | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 2 | 2 | 0 | 0 | 4 |
| | *C. imicola* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **2** | **4** | **0** | **0** | **6** |
| 29-Jan-20 | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. imicola* | 1 | 1 | 0 | 0 | 2 |
| | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **3** | **2** | **0** | **0** | **5** |
| 5-Feb-20 | *C. imicola* | 0 | 1 | 0 | 0 | 1 |
| | *C. leucostictus* | 2 | 1 | 0 | 0 | 3 |
| | *C. pycnostictus* | 1 | 2 | 0 | 0 | 3 |
| | **Total** | **3** | **4** | **0** | **0** | **7** |
| 19-Feb-20 | *C. leucostictus* | 11 | 3 | 0 | 0 | 14 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **12** | **4** | **0** | **0** | **16** |
| 4-Mar-20 | *C. leucostictus* | 0 | 1 | 0 | 1 | 2 |
| | **Total** | **0** | **1** | **0** | **1** | **2** |
| 12-Aug-20 | *C. leucostictus* | 1 | 1 | 0 | 0 | 2 |
| | **Total** | **1** | **1** | **0** | **0** | **2** |
| | **Grand Total** | **330** | **351** | **1** | **25** | **707** |

## APPENDIX 5b: *Culicoides* counts from $CO_2$ trap 2 (near horses) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 12 | 13 | 0 | 0 | 25 |
| | **Total** | **13** | **14** | **0** | **0** | **27** |
| 4-Sep-19 | *C. leucostictus* | 4 | 3 | 0 | 0 | 7 |
| | *C. nivosus* | 1 | 1 | 0 | 1 | 3 |
| | *C. pycnostictus* | 12 | 20 | 0 | 0 | 32 |
| | **Total** | **17** | **24** | **0** | **1** | **42** |
| 12-Sep-19 | *C. imicola* | 0 | 0 | 0 | 1 | 1 |
| | *C. leucostictus* | 1 | 1 | 0 | 0 | 2 |
| | *C. nivosus* | 1 | 2 | 0 | 0 | 3 |
| | *C. pycnostictus* | 2 | 5 | 0 | 0 | 7 |
| | **Total** | **4** | **8** | **0** | **1** | **13** |
| 18-Sep-19 | *C. leucostictus* | 0 | 3 | 0 | 0 | 3 |
| | *C. nivosus* | 1 | 3 | 0 | 0 | 4 |
| | *C. pycnostictus* | 4 | 5 | 0 | 0 | 9 |
| | **Total** | **5** | **11** | **0** | **0** | **16** |
| 2-Oct-19 | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 5 | 2 | 0 | 0 | 7 |
| | *C. pycnostictus* | 4 | 9 | 0 | 0 | 13 |
| | **Total** | **10** | **11** | **0** | **0** | **21** |
| 7-Oct-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 6 | 3 | 0 | 5 | 14 |
| | *C. nivosus* | 1 | 2 | 0 | 1 | 4 |
| | *C. pycnostictus* | 31 | 29 | 0 | 0 | 60 |
| | **Total** | **40** | **34** | **0** | **6** | **80** |
| 15-Oct-19 | *C. leucostictus* | 2 | 4 | 0 | 0 | 6 |
| | *C. nivosus* | 3 | 2 | 0 | 2 | 7 |
| | *C. pycnostictus* | 3 | 2 | 0 | 0 | 5 |
| | **Total** | **8** | **8** | **0** | **2** | **18** |
| 21-Oct-19 | *C. imicola* | 0 | 0 | 0 | 1 | 1 |
| | *C. leucostictus* | 0 | 0 | 0 | 1 | 1 |
| | *C. nivosus* | 2 | 3 | 0 | 1 | 6 |
| | *C. pycnostictus* | 11 | 9 | 0 | 0 | 20 |
| | **Total** | **13** | **12** | **0** | **3** | **28** |
| 30-Oct-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 7 | 3 | 0 | 1 | 11 |
| | *C. nivosus* | 12 | 9 | 0 | 1 | 22 |
| | *C. pycnostictus* | 19 | 22 | 0 | 0 | 41 |
| | **Total** | **39** | **34** | **0** | **2** | **75** |
| 5-Nov-19 | *C. leucostictus* | 2 | 2 | 0 | 0 | 4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. nivosus* | 2 | 2 | 0 | 0 | 4 |
| | *C. pycnostictus* | 8 | 9 | 0 | 0 | 17 |
| | **Total** | **12** | **13** | **0** | **0** | **25** |
| 20-Nov-19 | *C. imicola* | 0 | 1 | 0 | 7 | 8 |
| | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | *C. nivosus* | 0 | 3 | 0 | 0 | 3 |
| | *C. pycnostictus* | 9 | 5 | 0 | 0 | 14 |
| | **Total** | **11** | **9** | **0** | **7** | **27** |
| 26-Nov-19 | *C. leucostictus* | 1 | 1 | 0 | 0 | 2 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. pycnostictus* | 2 | 5 | 0 | 0 | 7 |
| | **Total** | **3** | **7** | **0** | **0** | **10** |
| 18-Dec-19 | *C. leucostictus* | 16 | 0 | 0 | 1 | 17 |
| | *C. nivosus* | 2 | 0 | 0 | 0 | 2 |
| | *C. pycnostictus* | 17 | 13 | 0 | 0 | 30 |
| | **Total** | **35** | **13** | **0** | **1** | **49** |
| 26-Dec-19 | *C. leucostictus* | 163 | 193 | 0 | 5 | 361 |
| | *C. nivosus* | 8 | 16 | 0 | 0 | 24 |
| | *C. pycnostictus* | 23 | 21 | 1 | 1 | 46 |
| | **Total** | **194** | **230** | **1** | **6** | **431** |
| 1-Jan-20 | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **0** | **1** | **0** | **0** | **1** |
| 8-Jan-20 | *C. leucostictus* | 14 | 6 | 0 | 0 | 20 |
| | *C. nivosus* | 2 | 2 | 0 | 0 | 4 |
| | *C. pycnostictus* | 6 | 1 | 0 | 0 | 7 |
| | **Total** | **22** | **9** | **0** | **0** | **31** |
| 15-Jan-20 | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **0** | **2** | **0** | **0** | **2** |
| 22-Jan-20 | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 1 | 1 | 0 | 0 | 2 |
| | **Total** | **2** | **2** | **0** | **0** | **4** |
| 29-Jan-20 | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **0** | **1** | **0** | **0** | **1** |
| 12-Aug-20 | *C. leucostictus* | 2 | 1 | 0 | 0 | 3 |
| | **Total** | **2** | **1** | **0** | **0** | **3** |
| | | | | | | |
| | **Grand Total** | **430** | **444** | **1** | **29** | **904** |

## APPENDIX 5c: *Culicoides* counts from $CO_2$ trap 3 (near cows) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 4-Sep-19 | *C. leucostictus* | 3 | 2 | 0 | 0 | 5 |
| | *C. pycnostictus* | 4 | 8 | 0 | 0 | 12 |
| | **Total** | **7** | **10** | **0** | **0** | **17** |
| 12-Sep-19 | *C. leucostictus* | 0 | 0 | 0 | 1 | 1 |
| | **Total** | **0** | **0** | **0** | **1** | **1** |
| 18-Sep-19 | *C. pycnostictus* | 2 | 1 | 0 | 0 | 3 |
| | **Total** | **2** | **1** | **0** | **0** | **3** |
| 2-Oct-19 | *C. pycnostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **2** | **0** | **0** | **0** | **2** |
| 7-Oct-19 | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **2** | **1** | **0** | **0** | **3** |
| 15-Oct-19 | *C. pycnostictus* | 2 | 2 | 0 | 0 | 4 |
| | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **3** | **2** | **0** | **0** | **5** |
| 30-Oct-19 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 2 | 1 | 0 | 0 | 3 |
| | *C. nivosus* | 1 | 1 | 0 | 0 | 2 |
| | *C. pycnostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **6** | **2** | **0** | **0** | **8** |
| 14-Nov-19 | *C. nivosus* | 1 | 0 | 0 | 0 | 1 |
| | *C. pycnostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **3** | **0** | **0** | **0** | **3** |
| 20-Nov-19 | *C. leucostictus* | 8 | 3 | 0 | 1 | 12 |
| | *C. nivosus* | 0 | 4 | 0 | 0 | 4 |
| | *C. pycnostictus* | 4 | 3 | 0 | 0 | 7 |
| | **Total** | **12** | **10** | **0** | **1** | **23** |
| 26-Nov-19 | *C. leucostictus* | 2 | 5 | 0 | 0 | 7 |
| | *C. nivosus* | 0 | 3 | 0 | 0 | 3 |
| | *C. pycnostictus* | 4 | 13 | 0 | 0 | 17 |
| | **Total** | **6** | **21** | **0** | **0** | **27** |
| 3-Dec-19 | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | *C. nivosus* | 0 | 2 | 0 | 0 | 2 |
| | *C. pycnostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **3** | **2** | **0** | **0** | **5** |
| 18-Dec-19 | *C. leucostictus* | 1 021 | 206 | 0 | 16 | 1 243 |
| | *C. nivosus* | 79 | 28 | 1 | 1 | 109 |
| | *C. pycnostictus* | 83 | 14 | 0 | 0 | 97 |
| | **Total** | **1 183** | **248** | **1** | **17** | **1 449** |
| 26-Dec-19 | *C. leucostictus* | 150 | 205 | 0 | 4 | 359 |
| | *C. nivosus* | 31 | 58 | 0 | 0 | 89 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *C. pycnostictus* | 7 | 11 | 0 | 0 | 18 |
| | **Total** | **188** | **274** | **0** | **4** | **466** |
| 1-Jan-20 | *C. imicola* | 1 | 0 | 1 | 1 | 3 |
| | *C. leucostictus* | 31 | 12 | 0 | 0 | 43 |
| | *C. magnus* | 0 | 1 | 0 | 0 | 1 |
| | *C. nivosus* | 4 | 7 | 0 | 1 | 12 |
| | *C. pycnostictus* | 10 | 6 | 0 | 0 | 16 |
| | **Total** | **46** | **26** | **1** | **2** | **75** |
| 8-Jan-20 | *C. leucostictus* | 3 | 5 | 0 | 1 | 9 |
| | *C. magnus* | 1 | 0 | 0 | 0 | 1 |
| | *C. nivosus* | 2 | 2 | 0 | 0 | 4 |
| | **Total** | **6** | **7** | **0** | **1** | **14** |
| 15-Jan-20 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | *C. leucostictus* | 7 | 10 | 0 | 0 | 17 |
| | *C. nivosus* | 3 | 11 | 0 | 0 | 14 |
| | *C. pycnostictus* | 6 | 3 | 0 | 0 | 9 |
| | **Total** | **17** | **25** | **0** | **0** | **42** |
| 22-Jan-20 | *C. enderleini* | 0 | 1 | 0 | 0 | 1 |
| | *C. leucostictus* | 9 | 36 | 0 | 0 | 45 |
| | *C. nivosus* | 6 | 8 | 0 | 0 | 14 |
| | *C. pycnostictus* | 5 | 16 | 0 | 0 | 21 |
| | **Total** | **20** | **61** | **0** | **0** | **81** |
| 29-Jan-20 | *C. imicola* | 0 | 0 | 0 | 1 | 1 |
| | *C. leucostictus* | 2 | 4 | 0 | 0 | 6 |
| | *C. nivosus* | 0 | 0 | 0 | 1 | 1 |
| | *C. pycnostictus* | 5 | 1 | 0 | 0 | 6 |
| | **Total** | **7** | **5** | **0** | **2** | **14** |
| 5-Feb-20 | *C. subschultzei* | 0 | 1 | 0 | 0 | 1 |
| | *C. enderleini* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **1** | **1** | **0** | **0** | **2** |
| 28-Aug-20 | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **0** | **1** | **0** | **0** | **1** |
| | | | | | | |
| | **Grand Total** | **1 514** | **697** | **2** | **28** | **2 241** |

## APPENDIX 5d: *Culicoides* counts from $CO_2$ trap 4 (near dogs) sorted by sex and parity

| Date | *Culicoides* Species | Nulliparous | Parous/Gravid | Blood fed | Males | Total |
|---|---|---|---|---|---|---|
| 18-Dec-19 | *C. leucostictus* | 6 | 1 | 0 | 0 | 7 |
| | *C. imicola* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **6** | **2** | **0** | **0** | **8** |
| 26-Dec-19 | *C. nivosus* | 0 | 1 | 0 | 0 | 1 |
| | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 67 | 128 | 0 | 1 | 196 |
| | **Total** | **68** | **129** | **0** | **1** | **198** |
| 1-Jan-20 | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 9 | 6 | 0 | 0 | 15 |
| | **Total** | **10** | **6** | **0** | **0** | **16** |
| 8-Jan-20 | *C. leucostictus* | 2 | 0 | 0 | 0 | 2 |
| | **Total** | **2** | **0** | **0** | **0** | **2** |
| 15-Jan-20 | *C. bedfordi* | 1 | 0 | 0 | 0 | 1 |
| | *C. imicola* | 2 | 3 | 0 | 0 | 5 |
| | *C. leucostictus* | 7 | 10 | 0 | 2 | 19 |
| | **Total** | **10** | **13** | **0** | **2** | **25** |
| 22-Jan-20 | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 40 | 39 | 0 | 1 | 80 |
| | **Total** | **41** | **39** | **0** | **1** | **81** |
| 29-Jan-20 | *C. leucostictus* | 7 | 3 | 0 | 2 | 12 |
| | **Total** | **7** | **3** | **0** | **2** | **12** |
| 5-Feb-20 | *C. leucostictus* | 3 | 0 | 0 | 0 | 3 |
| | **Total** | **3** | **0** | **0** | **0** | **3** |
| 12-Feb-20 | *C. leucostictus* | 1 | 0 | 0 | 0 | 1 |
| | **Total** | **1** | **0** | **0** | **0** | **1** |
| 19-Feb-20 | *C. imicola* | 1 | 0 | 0 | 0 | 1 |
| | *C. leucostictus* | 12 | 8 | 0 | 1 | 21 |
| | *C. pycnostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **13** | **9** | **0** | **1** | **23** |
| 4-Mar-20 | *C. leucostictus* | 1 | 1 | 0 | 0 | 2 |
| | *C. imicola* | 3 | 2 | 0 | 0 | 5 |
| | **Total** | **4** | **3** | **0** | **0** | **7** |
| 11-Mar-20 | *C. leucostictus* | 2 | 1 | 0 | 0 | 3 |
| | **Total** | **2** | **1** | **0** | **0** | **3** |
| 18-Mar-20 | *C. bedfordi* | 0 | 1 | 0 | 0 | 1 |
| | *C. leucostictus* | 0 | 1 | 0 | 0 | 1 |
| | **Total** | **0** | **2** | **0** | **0** | **2** |
| | **Grand Total** | **167** | **207** | **0** | **7** | **381** |

## APPENDIX 6a: *Culicoides* counts from $CO_2$ trap 1 (near sheep) at different times of the day

| Date | *Culicoides* Species | 09.30-12.30 | 12.30-15.30 | 15.30-18.30 | 18.30-21.30 | 21.30-00.30 | 00.30-03.30 | 03.30-06.30 | 06.30-09.30 |
|---|---|---|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. leucostictus* | | | 1 | | 2 | | 2 | |
| | *C. pycnostictus* | | | | | 2 | | 2 | |
| | **Total** | | | **1** | | **4** | | **4** | |
| 04-Sep-19 | *C. leucostictus* | | | 2 | | | | | |
| | *C. pycnostictus* | | | 1 | | | | | |
| | **Total** | | | **3** | | | | | |
| 12-Sep-19 | *C. leucostictus* | | | | 1 | | | | |
| | *C. nivosus* | | | | | | | | 1 |
| | *C. pycnostictus* | | | | 1 | 1 | | | |
| | **Total** | | | | **2** | **1** | | | **1** |
| 18-Sep-19 | *C. bedfordi* | | | | | | 1 | | |
| | *C. leucostictus* | | 1 | | 1 | 1 | | 1 | |
| | *C. nivosus* | | | | | | | 1 | |
| | *C. pycnostictus* | | | | | | 1 | | |
| | **Total** | | **1** | | **1** | **1** | **1** | **2** | |
| 30-Sep-19 | *C. leucostictus* | | | | | | | 1 | |
| | *C. nivosus* | | | | 1 | | 1 | | |
| | *C. pycnostictus* | | | | 1 | | 1 | | |
| | **Total** | | | | **2** | | **2** | **1** | |
| 02-Oct-19 | *C. leucostictus* | | | 1 | | | | | 1 |
| | *C. nivosus* | | | | | | | 1 | |
| | *C. pycnostictus* | | | 1 | | | | 1 | 1 |
| | **Total** | | | **2** | | | | **2** | **2** |
| 07-Oct-19 | *C. bedfordi* | | | | | 1 | | | 1 |
| | *C. imicola* | | | | | 1 | | | |
| | *C. leucostictus* | | 1 | 2 | 5 | 1 | 1 | 11 | 2 |
| | *C. nivosus* | | 1 | | | | | | |
| | *C. pycnostictus* | | | | 1 | 2 | 3 | 6 | 1 |
| | **Total** | | **2** | **2** | **6** | **5** | **4** | **17** | **4** |
| 15-Oct-19 | *C. leucostictus* | | | | | 4 | 2 | 4 | |
| | *C. nivosus* | | | | | 2 | 2 | | |
| | *C. pycnostictus* | | | | | 2 | | | |
| | **Total** | | | | | **8** | **4** | **4** | |
| 21-Oct-19 | *C. bedfordi* | | | 1 | 1 | | 2 | | |
| | *C. leucostictus* | | | | 2 | 8 | 18 | | |
| | *C. nivosus* | | | | 1 | 3 | 16 | | |
| | *C. pycnostictus* | | | | | 9 | 15 | | |
| | **Total** | | | **1** | **4** | **20** | **51** | | |
| 30-Oct-19 | *C. bedfordi* | | | | 1 | | | | |
| | *C. imicola* | | | | | | | 1 | |
| | *C. leucostictus* | | | | 3 | 8 | 4 | 5 | |
| | *C. nivosus* | | | | 1 | 2 | 6 | 1 | |
| | *C. pycnostictus* | | | | 4 | 1 | 4 | 3 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Total** | | | | **9** | **11** | **14** | **10** | |
| 05-Nov-19 | *C. bedfordi* | | | | 1 | | | 1 | |
| | *C. leucostictus* | | | | 23 | 3 | 11 | 13 | |
| | *C. nivosus* | | | | | 1 | 3 | 2 | |
| | *C. pycnostictus* | 1 | | | 4 | | 6 | 3 | 1 |
| | **Total** | **1** | | | **28** | **4** | **20** | **19** | **1** |
| 14-Nov-19 | *C. leucostictus* | | | | | 2 | 2 | 1 | 1 |
| | *C. nivosus* | | | | | | 2 | 1 | |
| | *C. pycnostictus* | | | | | | 6 | 1 | 1 |
| | **Total** | | | | | **2** | **10** | **3** | **2** |
| 20-Nov-19 | *C. leucostictus* | | | 1 | | 15 | 3 | 2 | |
| | *C. nivosus* | | | | | 3 | 1 | | |
| | *C. pycnostictus* | | | | | 2 | 3 | | |
| | Nigripennis grp | | | | | 1 | | | |
| | **Total** | | | **1** | | **21** | **7** | **2** | |
| 26-Nov-19 | *C. leucostictus* | | | | 3 | 5 | 30 | 15 | |
| | *C. nivosus* | | | | | | 4 | | |
| | *C. pycnostictus* | | | | | | 1 | 2 | |
| | Nigripennis grp | | | | | 2 | 2 | | |
| | **Total** | | | | **3** | **7** | **37** | **17** | |
| 03-Dec-19 | *C. leucostictus* | 7 | 9 | | | | | | 1 |
| | Nigripennis grp | | 1 | | | | | | |
| | **Total** | **7** | **10** | | | | | | **0** |
| 18-Dec-19 | *C. leucostictus* | | | 1 | 10 | 17 | 5 | 3 | |
| | *C. nivosus* | | | | | 1 | | | |
| | *C. pycnostictus* | | | | 1 | 5 | 1 | | |
| | **Total** | | | **1** | **11** | **23** | **6** | **3** | |
| 26-Dec-19 | *C. bedfordi* | | | | | | 1 | | |
| | *C. leucostictus* | 1 | | 1 | 14 | 26 | 103 | 24 | 1 |
| | *C. nivosus* | | | | 1 | 1 | 3 | 1 | |
| | *C. pycnostictus* | | | | 2 | 6 | 5 | 3 | |
| | **Total** | **1** | | **1** | **17** | **33** | **112** | **28** | **1** |
| 01-Jan-20 | *C. leucostictus* | | | | | | 2 | | |
| | *C. pycnostictus* | | | | 1 | | 1 | | |
| | **Total** | | | | **1** | | **3** | | |
| 08-Jan-20 | *C. leucostictus* | | | | 4 | 4 | | | |
| | *C. nivosus* | | | | | 1 | | | |
| | **Total** | | | | **4** | **5** | | | |
| 15-Jan-20 | *C. leucostictus* | | | | | 1 | 2 | 1 | 1 |
| | *C. pycnostictus* | | | | | | | 1 | |
| | **Total** | | | | | **1** | **2** | **2** | **1** |
| 22-Jan-20 | *C. imicola* | | | | | | 1 | | |
| | *C. leucostictus* | | | | | 1 | | | |
| | *C. nivosus* | | | | | 2 | 2 | | |
| | **Total** | | | | | **3** | **3** | | |
| 29-Jan-20 | *C. enderleini* | | | | | 1 | | | |
| | *C. imicola* | | | | 1 | 1 | | | |
| | *C. leucostictus* | | | | 2 | | | | |
| | **Total** | | | | **3** | **2** | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 05-Feb-20 | *C. imicola* | | | | | | 1 | | |
| | *C. leucostictus* | | | | | | 2 | 1 | |
| | *C. pycnostictus* | | | | | | | 3 | |
| | **Total** | | | | | | **3** | **4** | |
| 19-Feb-20 | *C. leucostictus* | | | | | 10 | 2 | 2 | |
| | *C. nivosus* | | | | | 1 | | | |
| | *C. pycnostictus* | | | | | 1 | | | |
| | **Total** | | | | | **12** | **2** | **2** | |
| 04-Mar-20 | *C. leucostictus* | | | | 1 | 1 | | | |
| | **Total** | | | | **1** | **1** | | | |
| 12-Aug-20 | *C. leucostictus* | | | | 2 | | | | |
| | **Total** | | | | **2** | | | | |
| | **Grand Total** | **9** | **13** | **12** | **94** | **164** | **282** | **120** | **13** |

## APPENDIX 6b: *Culicoides* counts from $CO_2$ trap (near horses) at different times of the day

| Date | ***Culicoides* Species** | **09.30-12.30** | **12.30-15.30** | **15.30-18.30** | **18.30-21.30** | **21.30-00.30** | **00.30-03.30** | **03.30-06.30** | **06.30-09.30** |
|---|---|---|---|---|---|---|---|---|---|
| 28-Aug-19 | *C. leucostictus* | | | | 1 | | | | |
| | *C. nivosus* | | | | 1 | | | | |
| | *C. pycnostictus* | | | | 11 | 6 | 6 | 2 | |
| | **Total** | | | | **13** | **6** | **6** | **2** | |
| 04-Sep-19 | *C. leucostictus* | | | 4 | 3 | | | | |
| | *C. nivosus* | | | | 2 | | | | 1 |
| | *C. pycnostictus* | | | 25 | 7 | | | | |
| | **Total** | | | **29** | **12** | | | | **1** |
| 12-Sep-19 | *C. imicola* | | | | 1 | | | | |
| | *C. leucostictus* | | | | 2 | | | | |
| | *C. nivosus* | | | | 2 | 1 | | | |
| | *C. pycnostictus* | | | | 6 | 1 | | | |
| | **Total** | | | | **11** | **2** | | | |
| 18-Sep-19 | *C. leucostictus* | | | | | | 2 | | 1 |
| | *C. nivosus* | | | | | 1 | 1 | 1 | 1 |
| | *C. pycnostictus* | | | | 3 | 1 | 4 | 1 | |
| | **Total** | | | | **3** | **2** | **7** | **2** | **2** |
| 02-Oct-19 | *C. leucostictus* | | | | 1 | | | | |
| | *C. nivosus* | | | | 4 | 2 | 1 | | |
| | *C. pycnostictus* | | | 1 | 10 | 1 | 1 | | |
| | **Total** | | | **1** | **15** | **3** | **2** | | |
| 07-Oct-19 | *C. bedfordi* | | | | | 1 | | | |
| | *C. imicola* | | | | | | | 1 | |
| | *C. leucostictus* | | | | 4 | 5 | 5 | | |
| | *C. nivosus* | | | | 1 | 1 | | 2 | |
| | *C. pycnostictus* | | | 2 | 16 | 12 | 10 | 19 | 1 |
| | **Total** | | | **2** | **21** | **19** | **15** | **22** | **1** |
| 15-Oct-19 | *C. leucostictus* | | | | | 3 | 1 | 2 | |
| | *C. nivosus* | | | | | 1 | 2 | 4 | |
| | *C. pycnostictus* | | | | | 4 | 1 | | |
| | **Total** | | | | | **8** | **4** | **6** | |
| 21-Oct-19 | *C. imicola* | 1 | | | | | | | |
| | *C. leucostictus* | | | | | | 1 | | |
| | *C. nivosus* | | | | | 2 | 2 | 1 | 1 |
| | *C. pycnostictus* | | | 1 | 5 | 1 | 5 | 8 | |
| | **Total** | **1** | | **1** | **5** | **3** | **8** | **9** | **1** |
| 30-Oct-19 | *C. bedfordi* | | | | 1 | | | | |
| | *C. leucostictus* | | | | 3 | 6 | 2 | | |
| | *C. nivosus* | 1 | | 1 | 6 | 6 | 3 | 5 | |
| | *C. pycnostictus* | | | | 13 | 16 | 10 | 2 | |
| | **Total** | **1** | | **1** | **23** | **28** | **15** | **7** | |
| 05-Nov-19 | *C. leucostictus* | | | | 2 | 2 | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *C. nivosus* | | | | 2 | | 1 | | 1 |
| | *C. pycnostictus* | | 2 | | 8 | 3 | | 3 | 1 |
| | **Total** | | **2** | | **12** | **5** | **1** | **3** | **2** |
| 20-Nov-19 | *C. imicola* | | | | | | 7 | 1 | |
| | *C. leucostictus* | | | | | | 2 | | |
| | *C. nivosus* | | | | | 2 | 1 | | |
| | *C. pycnostictus* | | 1 | | | 2 | 7 | 4 | |
| | **Total** | | **1** | | | **4** | **17** | **5** | |
| 26-Nov-19 | *C. leucostictus* | | | | | | 2 | | |
| | *C. nivosus* | | | | | | 1 | | |
| | *C. pycnostictus* | | | | | | 6 | 1 | |
| | **Total** | | | | | | **9** | **1** | |
| 18-Dec-19 | *C. nivosus* | | | | 1 | 1 | | | |
| | *C. pycnostictus* | | | 3 | 5 | 12 | 4 | 1 | 5 |
| | *C. leucostictus* | 1 | | | 6 | 5 | 3 | 2 | |
| | **Total** | **1** | | **3** | **12** | **18** | **7** | **3** | **5** |
| 26-Dec-19 | *C. nivosus* | | 1 | | 3 | 10 | 7 | 2 | 1 |
| | *C. pycnostictus* | 2 | 1 | 1 | 20 | 12 | 4 | 5 | 1 |
| | *C. leucostictus* | 1 | 3 | 2 | 45 | 189 | 107 | 13 | 1 |
| | **Total** | **3** | **5** | **3** | **68** | **211** | **118** | **20** | **3** |
| 01-Jan-20 | *C. leucostictus* | | | | | | 1 | | |
| | **Total** | | | | | | **1** | | |
| 08-Jan-20 | *C. leucostictus* | | | | 18 | | | | 2 |
| | *C. nivosus* | | | | 4 | | | | |
| | *C. pycnostictus* | | | | 5 | 1 | 1 | | |
| | **Total** | | | | **27** | **1** | **1** | | **2** |
| 15-Jan-20 | *C. nivosus* | | | | | | 1 | | |
| | *C. pycnostictus* | | | | 1 | | | | |
| | **Total** | | | | **1** | | **1** | | |
| 22-Jan-20 | *C. leucostictus* | | | | | | 1 | | |
| | *C. nivosus* | | | 1 | | | | | |
| | *C. pycnostictus* | | | 1 | | | 1 | | |
| | **Total** | | | **2** | | | **2** | | |
| 29-Jan-20 | *C. enderleini* | | | | | | | | 1 |
| | **Total** | | | | | | | | **1** |
| 12-Aug-20 | *C. leucostictus* | | | 1 | | | 2 | | |
| | **Total** | | | **1** | | | **2** | | |
| | **Grand Total** | **6** | **8** | **43** | **223** | **310** | **216** | **80** | **18** |

## APPENDIX 6c: *Culicoides* counts from $CO_2$ trap 3 (near cows) at different times of the day

| Date | *Culicoides* Species | 09.30-12.30 | 12.30-15.30 | 15.30-18.30 | 18.30-21.30 | 21.30-00.30 | 00.30-03.30 | 03.30-06.30 | 06.30-09.30 |
|---|---|---|---|---|---|---|---|---|---|
| 04-Sep-19 | *C. leucostictus* | | | 5 | | | | | |
| | *C. pycnostictus* | | | 8 | 4 | | | | |
| | **Total** | | | **13** | **4** | | | | |
| 12-Sep-19 | *C. leucostictus* | | | | 1 | | | | |
| | **Total** | | | | **1** | | | | |
| 18-Sep-19 | *C. pycnostictus* | | | | 3 | | | | |
| | **Total** | | | | **3** | | | | |
| 02-Oct-19 | *C. pycnostictus* | | | | 1 | | 1 | | |
| | **Total** | | | | **1** | | **1** | | |
| 07-Oct-19 | *C. leucostictus* | | | | 1 | | | | |
| | *C. nivosus* | | | | 1 | | | | |
| | *C. pycnostictus* | | | | | | | 1 | |
| | **Total** | | | | **2** | | | **1** | |
| 15-Oct-19 | *C. leucostictus* | | | | | | 1 | | |
| | *C. pycnostictus* | | | | | 2 | 2 | | |
| | **Total** | | | | | **2** | **3** | | |
| 30-Oct-19 | *C. bedfordi* | | | | | | 1 | | |
| | *C. leucostictus* | | | | 1 | 1 | 1 | | |
| | *C. nivosus* | | | | | | | 2 | |
| | *C. pycnostictus* | | | | | | 2 | | |
| | **Total** | | | | **1** | **1** | **4** | **2** | |
| 14-Nov-19 | *C. nivosus* | | | | | 1 | | | |
| | *C. pycnostictus* | | | | | | 2 | | |
| | **Total** | | | | | **1** | **2** | | |
| 20-Nov-19 | *C. leucostictus* | | | | 4 | 4 | 3 | | 1 |
| | *C. nivosus* | | | | 1 | 1 | 2 | | |
| | *C. pycnostictus* | 1 | | | 1 | 1 | 2 | 2 | |
| | **Total** | **1** | | | **6** | **6** | **7** | **2** | **1** |
| 26-Nov-19 | *C. leucostictus* | | | | | 1 | 5 | 1 | |
| | *C. nivosus* | | | | 1 | | 1 | 1 | |
| | *C. pycnostictus* | | | | 2 | 1 | 8 | 5 | 1 |
| | **Total** | | | | **3** | **2** | **14** | **7** | **1** |
| 03-Dec-19 | *C. leucostictus* | | | 2 | | | | | |
| | *C. nivosus* | | | 1 | 1 | | | | |
| | *C. pycnostictus* | | | | 1 | | | | |
| | **Total** | | | **3** | **2** | | | | |
| 18-Dec-19 | *C. leucostictus* | 2 | 4 | 6 | 462 | 543 | 131 | 87 | 8 |
| | *C. nivosus* | | 2 | 1 | 16 | 48 | 22 | 10 | 10 |
| | *C. pycnostictus* | 3 | 7 | 5 | 17 | 12 | 46 | 2 | 5 |
| | **Total** | **5** | **13** | **12** | **495** | **603** | **199** | **99** | **23** |
| 26-Dec-19 | *C. leucostictus* | 4 | 7 | 2 | 25 | 35 | 213 | 67 | 6 |
| | *C. nivosus* | 2 | 3 | 1 | 14 | 17 | 39 | 7 | 6 |
| | *C. pycnostictus* | | | 3 | 5 | 1 | 5 | 1 | 3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Total** | **6** | **10** | **6** | **44** | **53** | **257** | **75** | **15** |
| 01-Jan-20 | *C. imicola* | | | | | 2 | 1 | | |
| | *C. leucostictus* | | | | 1 | 5 | 27 | 10 | |
| | *C. magnus* | | | | 1 | | | | |
| | *C. nivosus* | | 1 | | | 1 | 8 | 2 | |
| | *C. pycnostictus* | | | | | 1 | 9 | 6 | |
| | **Total** | | **1** | | **2** | **9** | **45** | **18** | |
| 08-Jan-20 | *C. leucostictus* | | | | 9 | | | | |
| | *C. magnus* | | | | 1 | | | | |
| | *C. nivosus* | | 1 | | 3 | | | | |
| | **Total** | | **1** | | **13** | | | | |
| 15-Jan-20 | *C. bedfordi* | | | | | | 1 | | |
| | *C. enderleini* | | | | 1 | | | | |
| | *C. leucostictus* | | | | 1 | 2 | 6 | 7 | 1 |
| | *C. nivosus* | | 1 | | | 1 | 3 | 6 | 3 |
| | *C. pycnostictus* | | 1 | | 1 | | 6 | | 1 |
| | **Total** | | **2** | | **3** | **3** | **16** | **13** | **5** |
| 22-Jan-20 | *C. enderleini* | | | | | | | 1 | |
| | *C. leucostictus* | 1 | | 1 | 30 | 7 | 6 | | |
| | *C. nivosus* | | | | | 7 | 3 | 3 | 1 |
| | *C. pycnostictus* | | | | 17 | 2 | | 2 | |
| | **Total** | **1** | | **1** | **47** | **16** | **9** | **6** | **1** |
| 29-Jan-20 | *C. imicola* | | | | 1 | | | | |
| | *C. leucostictus* | | | | 6 | | | | |
| | *C. nivosus* | | | 1 | | | | | |
| | *C. pycnostictus* | | | 1 | 4 | 1 | | | |
| | **Total** | | | **2** | **11** | **1** | | | |
| 05-Feb-20 | *C. enderleini* | | | | | | | | 1 |
| | *C. subschultzei* | | | | | 1 | | | |
| | **Total** | | | | | **1** | | | **1** |
| 28-Aug-20 | *C. leucostictus* | | | 1 | | | | | |
| | **Total** | | | **1** | | | | | |
| | **Grand Total** | **13** | **27** | **38** | **638** | **698** | **557** | **223** | **47** |

## APPENDIX 6d: *Culicoides* counts from $CO_2$ trap 4 (near dogs) at different times of the day

| Date | *Culicoides* Species | 09.30-12.30 | 12.30-15.30 | 15.30-18.30 | 18.30-21.30 | 21.30-00.30 | 00.30-03.30 | 03.30-06.30 | 06.30-09.30 |
|---|---|---|---|---|---|---|---|---|---|
| 18-Dec-19 | *C. imicola* | | | | | | 1 | | |
| | *C. leucostictus* | | | | 2 | 3 | 2 | | |
| | **Total** | | | | **2** | **3** | **3** | | |
| 26-Dec-19 | *C. imicola* | | | | 1 | | | | |
| | *C. leucostictus* | 4 | 6 | 10 | 20 | 140 | 11 | 4 | 1 |
| | *C. nivosus* | | | | | | 1 | | |
| | **Total** | **4** | **6** | **10** | **21** | **140** | **12** | **4** | **1** |
| 01-Jan-20 | *C. imicola* | | | | | | 1 | | |
| | *C. leucostictus* | | | | | | 12 | 3 | |
| | **Total** | | | | | | **13** | **3** | |
| 08-Jan-20 | *C. leucostictus* | | | | 1 | | 1 | | |
| | **Total** | | | | **1** | | **1** | | |
| 15-Jan-20 | *C. bedfordi* | | | | | | 1 | | |
| | *C. imicola* | | | | | 1 | 3 | 1 | |
| | *C. leucostictus* | | | | | 2 | 11 | 6 | |
| | **Total** | | | | | **3** | **15** | **7** | |
| 22-Jan-20 | *C. imicola* | | | | | 1 | | | |
| | *C. leucostictus* | 2 | 20 | | | 11 | 14 | 29 | 4 |
| | **Total** | **2** | **20** | | | **12** | **14** | **29** | **4** |
| 29-Jan-20 | *C. leucostictus* | | | 12 | | | | | |
| | **Total** | | | **12** | | | | | |
| 05-Feb-20 | *C. leucostictus* | | | 3 | | | | | |
| | **Total** | | | **3** | | | | | |
| 12-Feb-20 | *C. leucostictus* | | | | 1 | | | | |
| | **Total** | | | | **1** | | | | |
| 19-Feb-20 | *C. imicola* | | | | | 1 | | | |
| | *C. leucostictus* | | | | | 7 | 7 | 6 | 1 |
| | *C. pycnostictus* | | | | | | | | 1 |
| | **Total** | | | | | **8** | **7** | **6** | **2** |
| 04-Mar-20 | *C. imicola* | | | | | | 5 | | |
| | *C. leucostictus* | | | | 2 | | | | |
| | **Total** | | | | **2** | | **5** | | |
| 11-Mar-20 | *C. leucostictus* | | 1 | | | | 1 | 1 | |
| | **Total** | | **1** | | | | **1** | **1** | |
| 18-Mar-20 | *C. bedfordi* | | | | | | 1 | | |
| | *C. leucostictus* | | | | | | | 1 | |
| | **Total** | | | | | | **1** | **1** | |
| | **Grand Total** | **6** | **27** | **25** | **27** | **166** | **72** | **51** | **7** |