Culicoides species in the Netherlands:

a comparison between tent traps and an Onderstepoort black light trap

and

the effect of an insect blanket on the biting rate

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Table of contents

Summary

Biting midges of the *Culicoides* genus (Diptera: Ceratopogonidae) are haematophagous arthropods, which can serve as vectors for orbiviruses such as bluetongue virus, equine encephalosis virus, African horse sickness virus and the recently discovered Schmallenberg virus. Biting midges also cause insect bite hypersensitivity in horses. Twenty one species of *Culicoides* have been identified in the Netherlands. Only the females suck blood and produce batches of eggs.

African horse sickness (AHS) is a non-contagious, vector-borne viral disease of equines that is transmitted by *Culicoides* spp. The main field vectors of AHS virus are *Culicoides imicola* and *Culicoides bolitinos.* African horse sickness virus (AHSV) may cause four forms of disease: horse sickness fever or mild form, cardiac form or “dikkop”, pulmonary form or “dunkop” and a mixed form. The estimated range of economic losses due to an outbreak of AHS in the Netherlands is 272-516 million euros. A form of protection against biting midges may be the use of an insect blanket, which covers the head, neck, back and belly of the horse.

The aims of the present study were to determine which species in which numbers of *Culicoides* (that potentially serve as vectors for AHSV) are attracted to horses in the Netherlands and to compare these results with the *Culicoides* species and numbers caught in the Onderstepoort black light trap during the same period. The second aim was to evaluate the use of an insect blanket on the biting rate of *Culicoides* species.

During the study a total number of 14, 032 *Culicoides* midges were caught, divided into twelve species: *C. obsoletus*, *C. punctatus*, *C. dewulfi*, *C. chiopterus*, *C. fasciipennis*, *C. festivipennis*, *C. stigma*, *C. pulicaris*, *C. nubeculosus*, *C. circumscriptus*, *C. newsteadi* and *C. salinarius*.
C. imicola and *C. bolitinos* were not found.

An insect blanket proved to be effective in preventing horses from being bitten by *Culicoides*: a horse without an insect blanket had a 2,271 times higher chance of getting bitten than a horse wearing an insect blanket.

Introduction

Biting midges of the *Culicoides* genus (Diptera: Ceratopogonidae) are haematophagous arthropods, which can serve as vectors for orbiviruses such as bluetongue virus (BTV), equine encephalosis virus (EEV) and African horse sickness virus (AHSV) (Scheffer 2012). The more recently discovered Schmallenberg virus (SBV), a novel orthobunyavirus, can also be transmitted by *Culicoides* species (Elbers et al. 2012). In horses, biting midges can also cause sweet itch or equine insect bite hypersensitivity (EIBH) (Scheffer 2012), which gives intense pruritus particularly affecting the mane and tail (dorsal midline), although in some horses a ventral distribution is seen.

There are approximately 1400 species of *Culicoides*, 21 of which have been identified as present in the Netherlands (Mellor et al. 2000, Meijswinkel et al. 2008b). Adults measure 1–5 mm in length and are generally crepuscular and/or nocturnal, with only a few species being diurnal. Only the females suck blood and produce a batch of eggs, although several species are autogenous and require a blood meal only after laying their first batch (Mullen 2002; van der Rijt et al. 2008). Adults usually stay within a few hundred metres of their larval habitats, but some species have been reported to be transported up to hundreds of kilometres via airstreams. A number of species have strong host preferences, while others feed on a wider variety of hosts (van der Rijt et al. 2008) (Fig. 1). As mentioned above, *Culicoides* species are vectors for a number of different pathogens but the focus will be on African horse sickness virus and the effect of an insect blanket in case of an outbreak of AHS in the Netherlands.

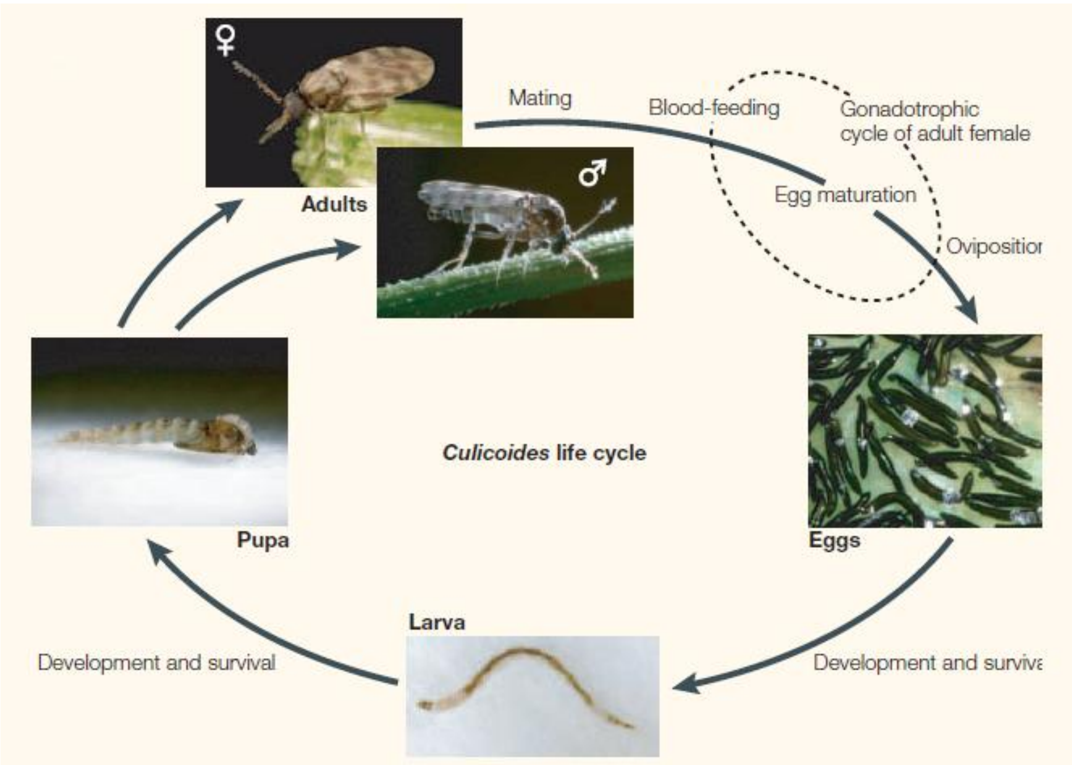

Figure 1. Lifecycle of *Culicoides* species (Purse 2005).

African horse sickness (AHS) is a non-contagious, vector-borne viral disease of equines that is transmitted by *Culicoides* spp. The main field vectors of AHS virus are *Culicoides imicola* and *Culicoides bolitinos* (Guthrie 2007; de Vos 2012). *Culicoides sonorensis* has been proven to be a competent vector of AHSV in experimental settings (Mellor and Hamblin 2004). AHSV affects all equine species including horses, donkeys, mules, hinnies and zebras. Morbidity and mortality rates vary between species, horses being most susceptible to the virus. Mortality in horses can reach up to 95%, while infections in zebras are mostly subclinical. AHSV is an *Orbivirus* belonging to the family *Reoviridae*, which also includes bluetongue virus (BTV) (de Vos 2012).
Like other orbiviruses, AHSV has a genome consisting of 10 segments of double-stranded RNA, which encode seven structural proteins (VP1–VP7) and four nonstructural proteins (NS1, NS2, NS3 and NS3a) (Wilson 2009). The core particle comprises two major proteins, VP3 and VP7, which are highly conserved among the nine serotypes, and three minor proteins VP1, VP4 and VP6. Together these proteins make up the group-specific epitopes. The outer capsid is composed of two proteins, VP2 and VP5 (Fig 2.). All nine serotypes have been reported in eastern and southern Africa, whereas serotype 9 is more widespread and appears to predominate in the northern parts of the sub-Saharan Africa (Guthrie 2007).

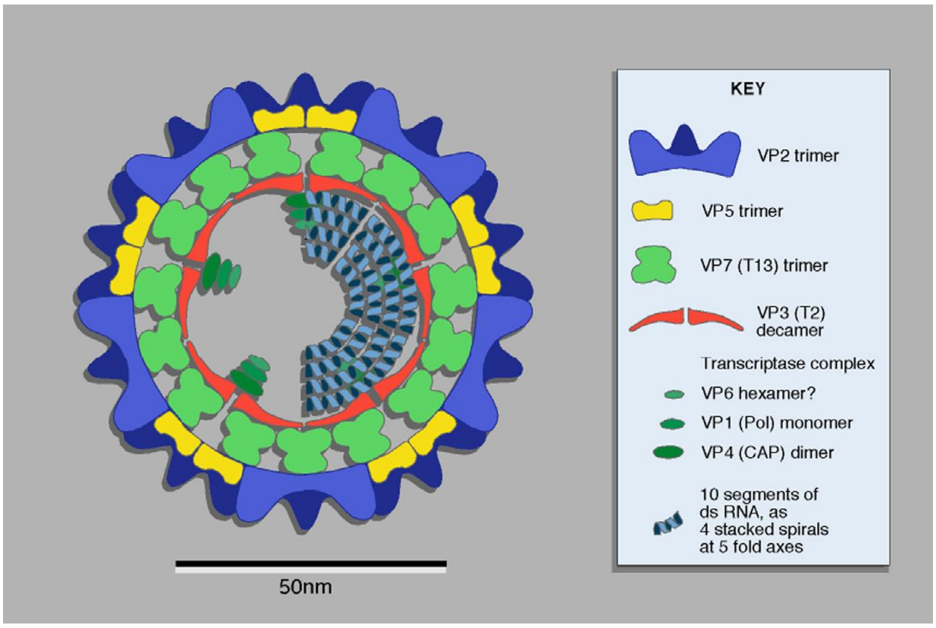

Figure 2. Diagram of orbivirus structure (Wilson 2009).

For biological transmission by *Culicoides* species, the virus must be present in peripheral blood vessels or in the skin tissues of the vertebrate host, making it accessible for the biting midge. It must then survive in the environment of the gut of the midge long enough to penetrate and infect the cells of the gut wall. It must then finally spread through the internal environment to infect the salivary glands in order to be transmitted back to the vertebrate host during subsequent blood-feeding (Fig. 3) (Wilson 2009).

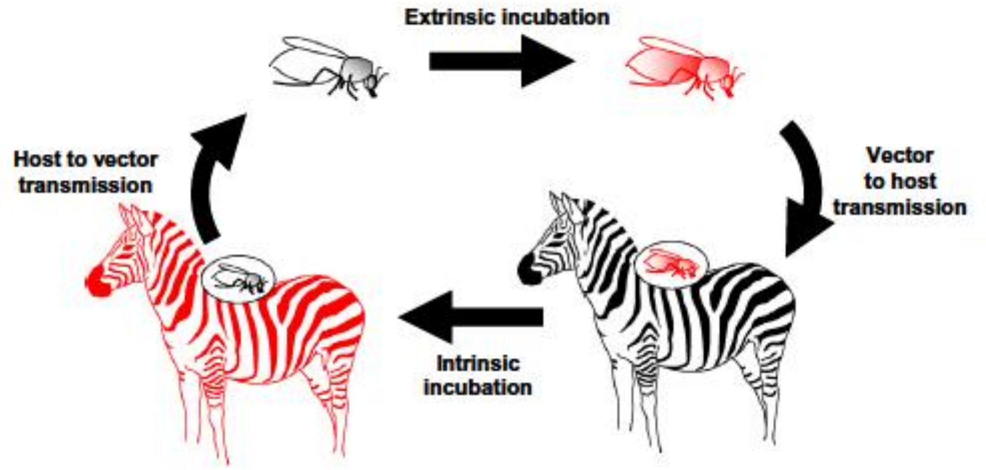

Figure 3. The AHSV transmission cycle (Wilson 2009).

After infection, initial multiplication of AHSV occurs in the regional lymph nodes and is followed by a primary viraemia, with subsequent dissemination to endothelial cells of target organs (Guthrie 2007), namely lungs, spleen and other lymphoid tissue. Virus multiplication in these tissues gives rise to a secondary viraemia. Under natural conditions the incubation period to the start of the second viraemia is less than nine days. Experimentally it has been shown to vary between 2 and 21 days (Mellor 2004). In horses a titre of up to 10^5 $TCID_{50}$ of virus/mL may be recorded but viraemia usually lasts for only four to eight days and has not been detected beyond 21 days. In donkeys and zebras the levels of viraemia are lower (< 10^3 $TCID_{50}$/mL) but may extend for up to four weeks (Guthrie 2007, Mellor 2004).

AHSV can cause four forms of disease: horse sickness fever or mild form, cardiac form or “dikkop”, pulmonary form or “dunkop” and a mixed form (Fig. 4) (Guthrie 2007, Mellor 2004). Horse sickness fever is usually observed in donkeys and zebras.
The incubation period is between 5 and 9 days, after which the temperature rises gradually over 4 to 5 days to 40°C, then drops to normal and is followed by recovery. The incubation period for the cardiac form is 5 to 7 days, followed by a fever (39°-41°C) that persists for 3 to 4 days. Typical clinical signs consist of oedema of the supraorbital fossa, conjunctiva, lips, cheeks, tongue, intermandibular space, laryngeal region and may extend down the neck. Ventral oedema and oedema of the lower limbs is not observed. The mortality rate is greater than 50% and death usually occurs within 4 to 8 days.
The pulmonary form is more per-acute than the cardiac form with the incubation period being 3 to 4 days. After 1 to 2 days fever (40°-41°C) sets in and rapidly progressive respiratory failure is seen. Death occurs within 30 minutes to a few hours after the onset of severe dyspnoea.
The mixed form is rarely clinically diagnosed but is often seen at necropsy. Initially it starts with pulmonary signs that are mild and not progressive, then it is followed by oedema and swelling of the head region. Death occurs after 3 to 6 days (Guthrie 2007).

Figure 4A and 4B. “Dikkop” (left) and “dunkop” (right) forms of African horse sickness (Guthrie 2007). Left: Severe oedema of the head. Right: froth and serous fluid at nostrils caused by severe alveolar oedema.

AHS is endemic in sub-Saharan Africa, where it is thought to persist due to the presence of zebras acting as a permanent virus reservoir (Mellor, Hamblin 2004). Incursions of AHSV in Northern Africa, the Near East and the Iberian Peninsula have occurred, but the virus was not able to persist for more than 3–4 years at most in these non-endemic areas. The AHS outbreaks in Spain in the period 1987–1990 provided evidence that Palaearctic species of *Culicoides*, especially *Culicoides pulicaris* and *Culicoides obsoletus*, may have contributed to transmission of the virus (MacLachlan and Guthrie 2010). The emergence of BTV serotype 8 in North-western Europe in 2006 demonstrated that these *Culicoides* species can indeed be competent vectors for orbiviruses, even under less favourable climatic conditions. Future incursions of AHSV in regions where *C. obsoletus* and *C. pulicaris* are abundant might thus result in an epidemic spread of the disease (MacLachlan and Guthrie 2010). Recent studies by van der Rijt et al. (2008) and Sloet van Oldruitenborgh-Oosterbaan et al. (2009) in the Netherlands demonstrated that over 90% of *Culicoides* found on horses were *C. obsoletus*. This, in combination with the potentially severe consequences of the disease, has led to increased awareness of the risk of AHS to the Netherlands (de Vos, 2012). AHS is also listed by the OIE as a notifiable disease (Wilson 2009). Horse density is relatively high in the Netherlands with an estimated population of 400.000 horses, 40% of which are kept for commercial purposes (Mourits and Saatkamp 2010). Mourits and Saatkamp (2010) have estimated the range of economic losses due to an outbreak of AHS in the Netherlands at 272–516 million Euros. Although emergency vaccination is prescribed in the draft Dutch contingency plan for African horse sickness, the currently available modified live vaccines are not considered sufficiently safe and efficacious under European conditions (de Vos, 2012)

Another form of protection against biting midges is the use of an insect blanket. These blankets cover the whole body including the head and the belly. In an experiment in Ireland in 1984 with one horse it was found that 23% of the *Culicoides* midges feed themselves on the limbs and only 1% on the belly (Townley et al., 1984). In another experiment in Israel they found that 72% of the midges landed on the belly and 27% on the dorsal side, but these specific species are not found in the Netherlands (Braverman et al., 1988).

The aims of the present study were to determine which species of *Culicoides* (that may potentially serve as vectors for AHSV) are attracted to horses in the Netherlands and to make a comparison with the *Culicoides* species caught in the Onderstepoort black light trap during the same period. The second aim was to evaluate the effect of an insect blanket on the biting rate of *Culicoides* species.

Materials and methods

In this study there were four farms selected. At each farm there were 2 horses selected for the trapping sessions. Trapping sessions were performed on dry warm days with little or no wind at evening twilight.

Farms

Four different farms in the proximity of Utrecht were selected (Table 1).

Farm	**Place**	**Environment**	**Housing situation**	**Other animals**
Farm A Fig. 5	Austerlitz	Structures of the riding school, forest, sandy soil	Individually in stables, outside in groups	Cat, dog
Farm B Fig. 6	Utrecht	Open, some trees, clay soil, open water nearby	One horse in a stable, the other one in a pasture	Sheep
Farm C Fig. 7	Stroe	Structures of the house and stables, some trees, sandy soil, open water nearby	Outside in loose house, sometimes in a pasture	Cats, sheep
Farm D Fig. 8	Bilthoven	Forest, sandy soil, open water nearby	In pasture, stabled at night	Sheep, dog, chickens

Table 1: Information of the farms selected in this study.

Figure 5. Environment of farm A.

Figure 6. Open tents and environment of farm B.

Figure 7. Open tents, Onderstepoort black light trap and environment at farm C.

Figure 8 Closed tents at farm D

Selected animals

On each farm we selected two horses or ponies (Table 2). The animals had to be calm and preferably had no sweet itch, because of the wellbeing of the animals during the study. The horses in this study were aged 7-25 years.

Farm	**Horse 1**	**Horse 2**
Riding School Austerlitz	Sunny, crossbred, age unknown (adult)	Djingiz, crossbred, age unknown (adult)
Faculty of Veterinary Medicine	Anky, Welsh pony 20 y/o	Nocturne, KWPN 17 y/o
Equus Research & Therapy	Dimmalimm frá Sörlaskjôl, Icelandic horse 7y/o	Hylling frá Hátúni, Icelandic horse 25 y/o
Sloet van Oldruitenborgh-Oosterbaan	Partner, Thoroughbred 20 y/o	Lüte, KWPN 19 y/o

Table 2: The horses used in this study

Tent traps

To collect *Culicoides*, the experimental setting of van der Rijt et al. (2008) was used. The tent traps consist of a metal frame, secured to the ground with four large metal pegs and four transport straps. The frame is covered by a large mosquito net, with mesh openings of approximately 200 µm. The nets were open at the back of the tents, the rest of the netting remained on the ground. The netting was secured to the ground using five small pegs, preventing the nets to wave.

Two tent traps were placed about two meters apart from each other, with the opening of the tent traps in the same direction. The openings were formed by rolling up the mosquito net and securing the net to the transport straps with a clamp. The openings of the tent traps were not placed facing the wind, preventing the wind from carrying large numbers of *Culicoides* into the tent traps.

Figure 9. Two open tent traps

At the front of the tent traps, one metal bar per tent was placed on top of the metal frame, to create more space in the tent for the handler and the head of the horse. For one horse we used two metal bars in the front of the tent trap, because in that way that horse stayed more calm.
The horses inside the tent trap were not tied, but held by a handler. To offer some distraction of the horses being sampled, a hay net with hay or silage was provided. (van der Rijt et al. 2008)

Figure 10. The tent trap containing a horse and handler

On each farm we used two horses, horse A and horse B. The horses were placed inside the tents according to the following schedule:

	Day 1/3		Day 2/4	
Horse↓	First hour	Second hour	First hour	Second hour
A	With an insect blanket	Without insect blanket	Without insect blanket	With an insect blanket
B	Without insect blanket	With insect blanket	With an insect blanket	Without insect blanket

Table 3. Schedule for the trapping sessions

So each horse was placed inside the tent trap for a total of two hours per day, one hour per session. On each farm we sampled for at least four days.
The insect blankets (Ivanhoe® Horse Equipment) were individually fitted to each horse. The blankets included a neck and a head piece, as shown in figure 11 and 12.

Figure 11. Left: Horse wearing an insect blanket with neck and head piece.
Figure 12. Right: Head and neck piece close-up.

After each session, the horses were taken from the tent traps and the tent traps were closed. The trapped insects were collected using two small household vacuum-cleaners with a piece of fabric with a fine mesh placed in the tubing as a filter. The vacuum-cleaners (PrimaDonna, 1300 Watt; Best Home, 1200 Watt) were used at the lowest power setting. (van der Rijt et al. 2008) After vacuuming the tents, the collected insects were placed in 0,5 litre cups filled with a small layer of 70 % ethylethanol solution.

Figure 13. Vacuuming the tent trap

Onderstepoort black light trap

The Onderstepoort black light trap (Fig. 14-16) was turned on during the trapping sessions and during the vacuuming of the tents. On each day, we had 4 cups of insects collected from the Onderstepoort black light trap: during session 1 (V1), during session 2 (V2), during vacuuming after the first session (R1) and during vacuuming after the second session (R2).

The Onderstepoort black light trap consists of a black light lamp covered by a 2 mm mesh size net , so only small insects can pass the net. A vent situated under the lamp pulls the insects surrounding the lamp downwards. Under the vent is a V-shaped space, lined with a mosquito net, so the insects cannot escape from the trap. At the bottom of the trap is a cup of water strapped to the trap. To break down the surface tension of the water, some washing up soap (Aro®) was mixed into the water. The insects drown after they have come in contact with the water.

Ideally, the Onderstepoort black light trap is placed at a height of 2 meters. At first we used a piece of rope to secure the trap to a tree or beam available on the farm. During our time on the second farm, we started using a metal standard with a height of approximately 1,5 meters.

At the end of each evening, the cups filled with insects were filtered using a fabric with a small mesh size and also placed in 0,5 litre cups filled with a small layer of 70 % ethylethanol solution.

Figure 14. Onderstepoort black light trap in a tree.

Figure 15. Onderstepoort black light trap with the meta standard.

Figure 16. Onderstepoort black light trap in the dark.

Insect determination

The insects were processed in a laboratory, using a stereo microscope (Fig 16.). The insects were placed into a Petri dish using a pipette; a 20 ml. pipette was placed upside down onto the suction balloon, creating an opening large enough for the insects to pass. All *Culicoides spp.* were separated from the other insects. All *Culicoides spp.* were counted, identified and labelled as 'blood-fed' or 'non blood-fed'. The identification was based on size and wing pattern. The *Culicoides spp.* were placed in separate cups per session and per subspecies.

Figure 17. Stereo microscope

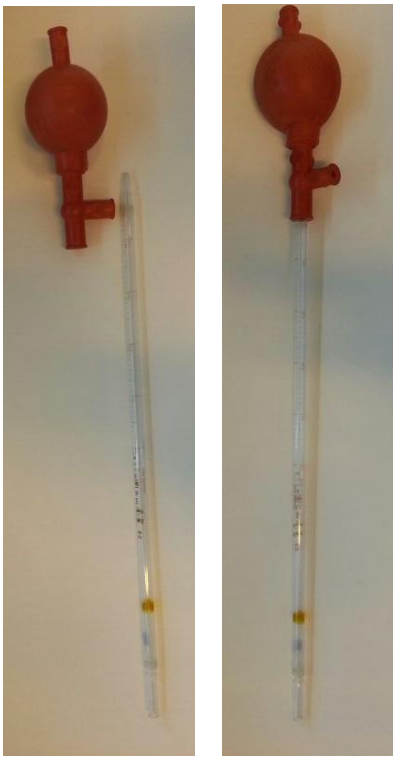

Figure 18A. (left) 20 ml pipette and suction balloon.
Figure 18B. (right) 20 ml pipette attached to the suction balloon.

Statistical analysis

A logistic regression for the number of “blood-fed” of the total number of “*Culicoides*” was performed with random horse within farm effects and with fixed “farm evening” and “effect of an insect blanket”

Results

Total number (non-blood, blood-fed, unknown) of *Culicoides* caught during the study (between July 23rd – September 4th) were 14,032.

These were divided in a total of twelve (12) different *Culicoides* species: *C. obsoletus* (12,790 = 91%), *C. punctatus* (649 = 5%), *C. dewulfi* (231 = 2%), *C. chiopterus* (141 = 1%), *C. fasciipennis* (117 = < 1%), *C. festivipennis* (51 = < 1%), *C. stigma* (22 = < 1%), *C. pulicaris* (21 = <1%), *C. nubeculosus* (5 < 1%), *C. circumscriptus* (2 = < 1%), *C. newsteadi* (2 = < 1%) and *C. salinarius* (1 = < 1%).

Figure 18 illustrates *Culicoides* species caught on all farms divided into their specific group, namely non blood-fed, blood-fed and unknown, with diagram 1 showing their absolute numbers. The *Culicoides spp*. marked as 'unknown' represent midges which we found without an abdomen, due to the strength of one of the vacuum cleaners (PrimaDonna 1300Watt), therefore it was not possible to determine whether they were blood-fed or not.

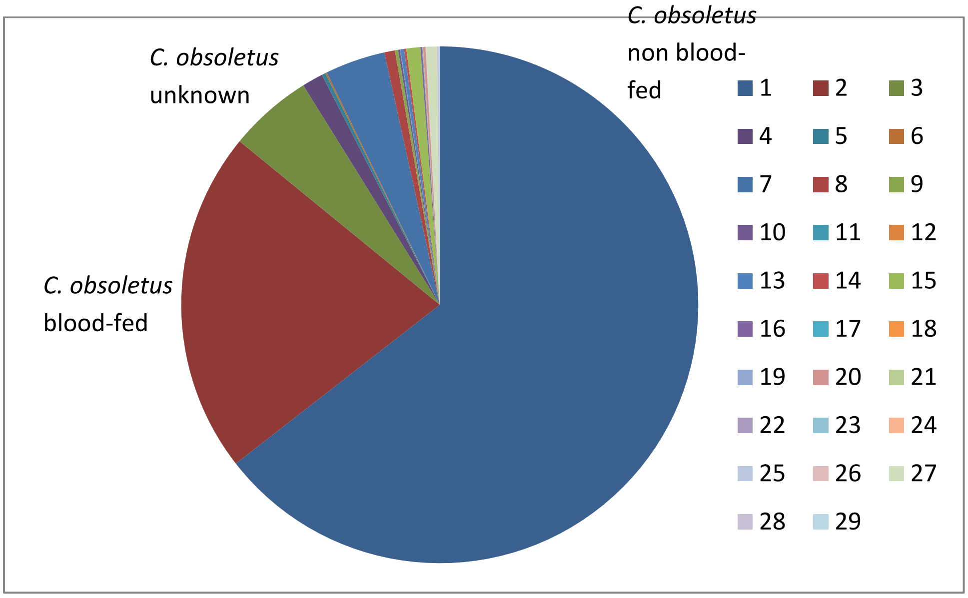

Figure 19. Culicoides species caught on all farms with a distinction between blood-fed, non blood-fed and unknown

1: *C. obsoletus* non blood
2: *C. obsoletus* blood
3: *C. obsoletus* unknown
4: *C. dewulfi* non blood
5: *C. dewulfi* blood
6: *C. dewulfi* unknown
7: *C. punctatus* non blood
8: *C. punctatus* blood
9: *C. punctatus* unknown
10: *C. pulicaris* non blood
11: *C. pulicaris* blood
12: *C. pulicaris* unknown
13: *C. festivipennis* non blood
14: *C. festivipennis* blood
15: *C. chiopterus* non blood
16: *C. chiopterus* blood
17: *C. chiopterus* unknown
18: *C. nubeculosus* non blood
19: *C. nubeculosus* blood
20: *C. stigma* non blood
21: *C. stigma* blood
22: *C. stigma* unknown
23: *C. circumscriptus* non blood
24: *C. circumscriptus* blood
25: *C. salinarius* non blood
26: *C. newsteadi* non blood
27: C. *fasciipennis* non blood
28: C. *fasciipennis* blood
29: *C. fasciipennis* unknown

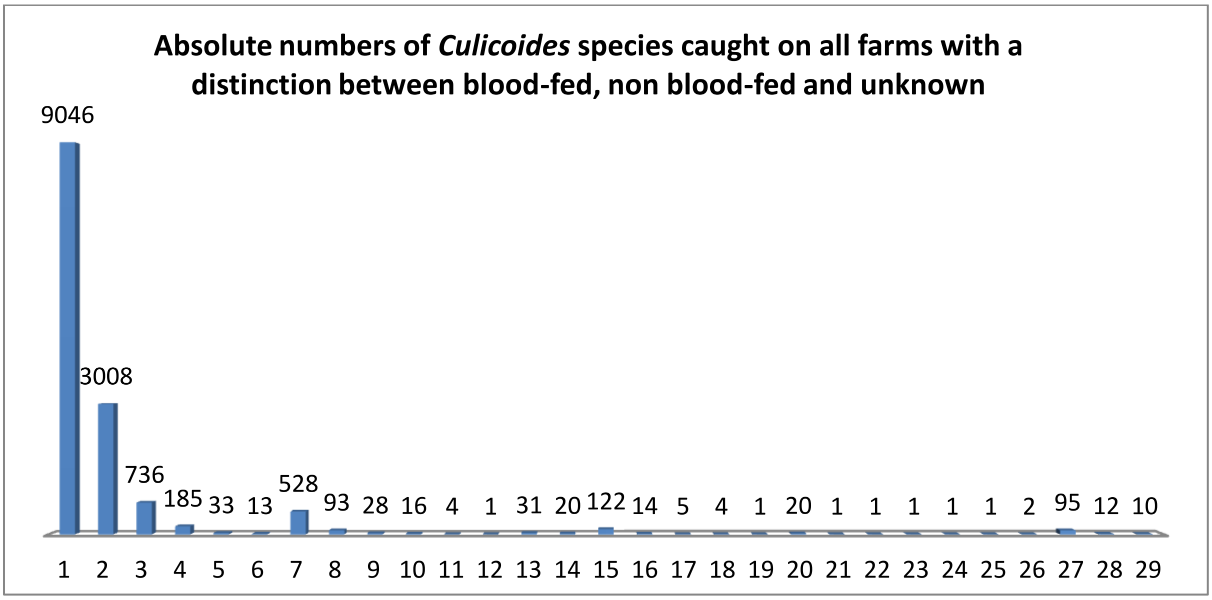

Figure 20. Numbers 1 through 29 corresponding with the legend from figure 19.

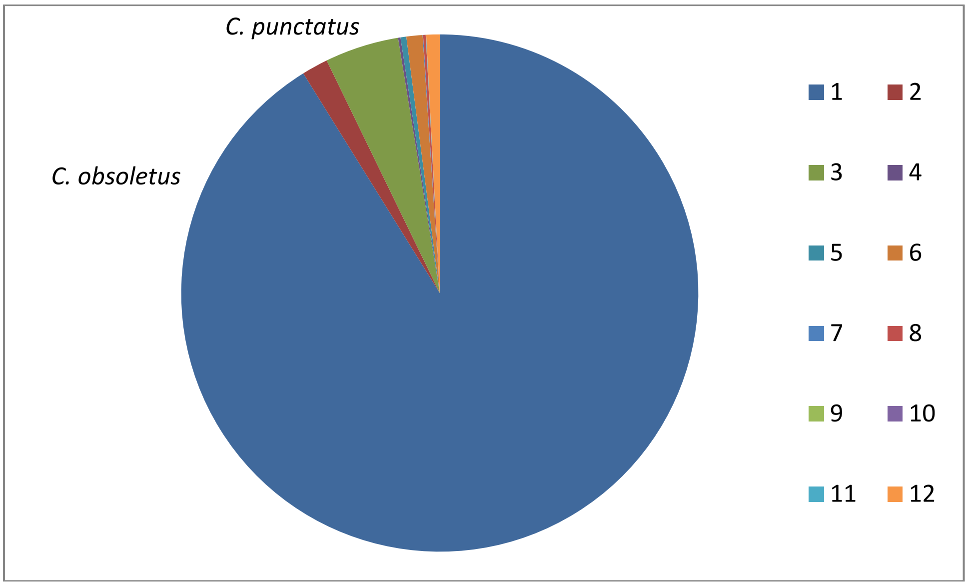

Figure 21. The various *Culicoides* species caught on all farms

1: *C. obsoletus*	6: *C. chiopterus*	11: *C. newsteadi*
2: *C. dewulfi*	7: *C. nubeculosus*	12: C. *fasciipennis*
3: *C. punctatus*	8: *C. stigma*	
4: *C. pulicaris*	9: *C. circumscriptus*	
5: *C. festivipennis*	10: *C. salinarius*	

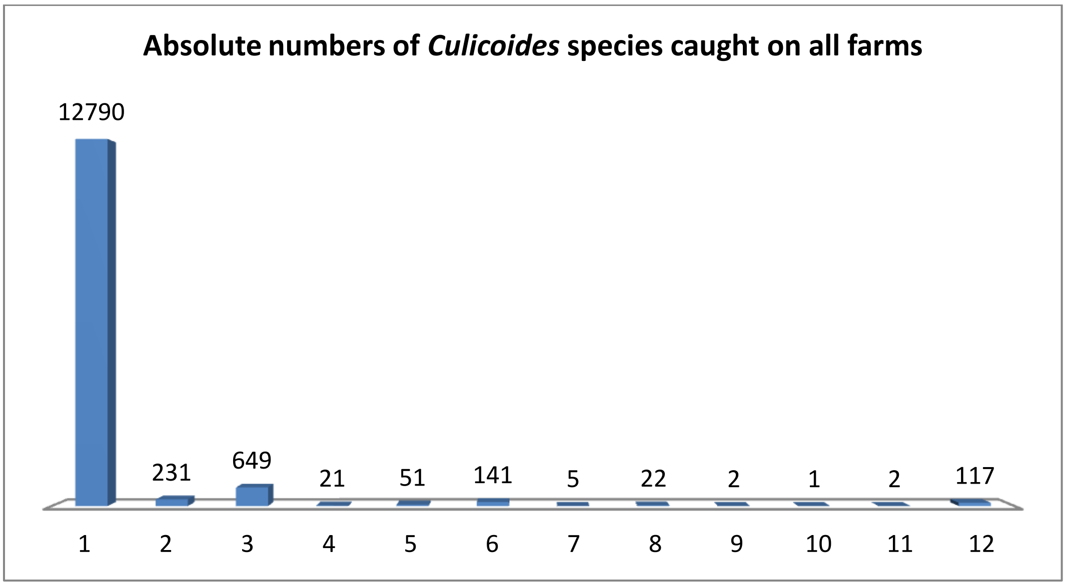

Figure 22. The various *Culicoides* species caught on all farms in absolute numbers, corresponding with figure 21.

The total number of *Culicoides* midges caught in this study varied widely per farm, ranging from 124 up to 12536. The following figures C through F show the variation of *Culicoides* species caught on each individual farm.

In the Onderstepoort black light trap there were 12 *Culicoides* spp. caught during all the trapping sessions. The total number of midges caught is 766. In table 4 all species found and their absolute numbers are shown. The coloured rows represent the *Culicoides* spp. that were only found in the Onderstepoort black light trap and not in the tent traps.

C. obsoletus non blood	548
C. obsoletus blood	88
C. obsoletus unknown	4
C. dewulfi non blood	14
C. dewulfi blood	2
C. punctatus non blood	24
C. punctatus blood	21
C. pulicaris non blood	2
C. pulicaris blood	1
C. pulicaris unknown	1
C. festivipennis non blood	30
C. festivipennis blood	20
C. chiopterus non blood	2
C. nubeculosus non blood	1
C. nubeculosus blood	1
C. stigma blood	1
C. circumscriptus non blood	1
C. circumscriptus blood	1
C. newsteadi non blood	2
C. *fasciipennis* non blood	1
C. salinarius non blood	1

Table 4. *Culicoides* spp. caught in the Onderstepoort black light trap.

Farm A

Three (3) species caught at farm A: *C. obsoletus (98%)*, *C. dewulfi* (1%) and *C. punctatus (1%)*. Total number caught: 124

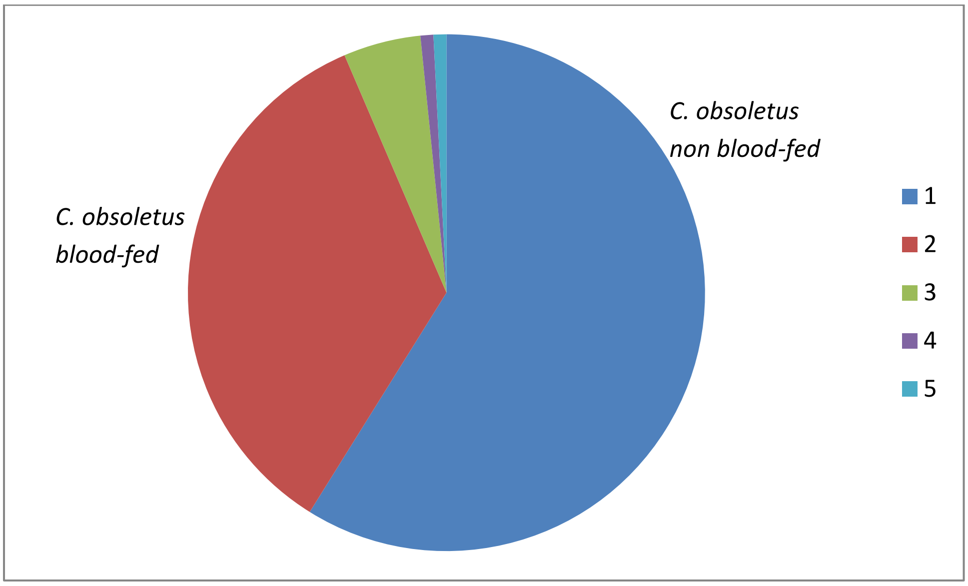

Figure 23. Total numbers of species caught at farm A.

1: *C. obsoletus* non blood (73)
2: *C. obsoletus blood* (43)
3: *C. obsoletus* unknown (6)
4: *C. dewulfi* non blood (1)
5: *C. punctatus* non blood (1)

Farm B

Eleven (11) species caught at farm B: *C. obsoletus (85%), C. punctatus (7%), C. festivipennis (4%), C. dewulfi (1%), C. chiopterus (< 1%), C. fasciipennis (< 1%), C. stigma (< 1%), C. pulicaris (< 1%), C. nubeculosus (< 1%), C. circumscriptus (< 1%), C. newsteadi (< 1%).* Total number caught: 1070

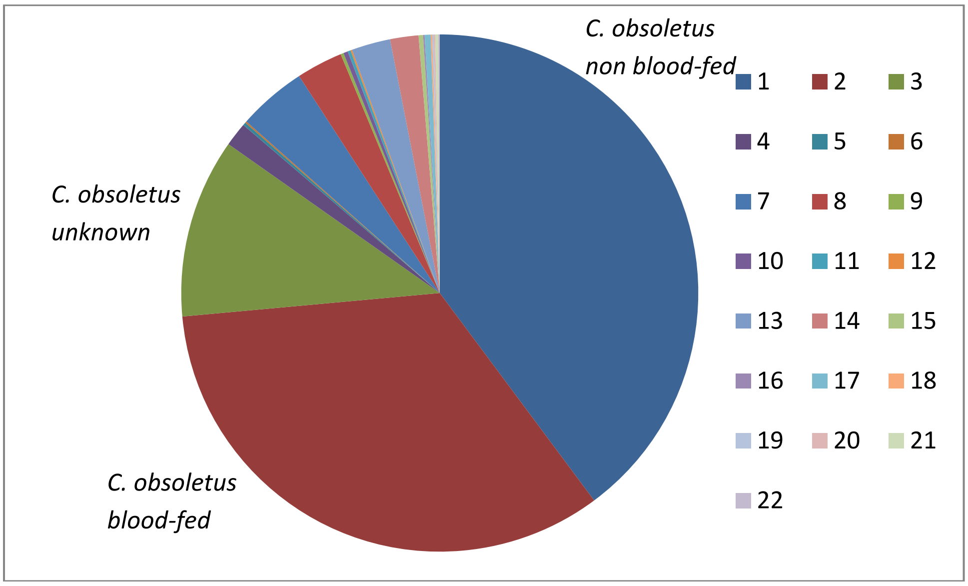

Figure 24. Total numbers of species caught at farm B.

1: *C. obsoletus* non blood (426)
2: *C. obsoletus* blood (361)
3: *C. obsoletus* unknown (120)
4: *C. dewulfi* non blood (16)
5: *C. dewulfi* blood (2)
6: *C. dewulfi* unknown (1)
7: *C. punctatus* non blood (46)
8: *C. punctatus* blood (31)
9: *C. punctatus* unknown (2)
10: *C. pulicaris* non blood (3)
11: *C. pulicaris* blood (2)
12: *C. pulicaris* unknown (1)
13: *C. festivipennis* non blood (26)
14: *C. festivipennis* blood (19)
15: *C. chiopterus* non blood (3)
16: *C. chiopterus* blood (1)
17: *C. nubeculosus* non blood (4)
18: *C. nubeculosus* blood (1)
19: *C. stigma* blood (1)
20: *C. circumscriptus* non blood (1)
21: *C. newsteadi* non blood (2)
22: C. *fasciipennis* non blood (1)

Farm C

Seven (7) species caught at farm C: *C. obsoletus (94%), C. dewulfi (3%), C. festivipennis (2%), C. punctatus (1%), C. chiopterus (*< 1%*), C. circumscriptus (*< 1%*)* and *C. salinarius (*< 1%*).* Total number caught: 302

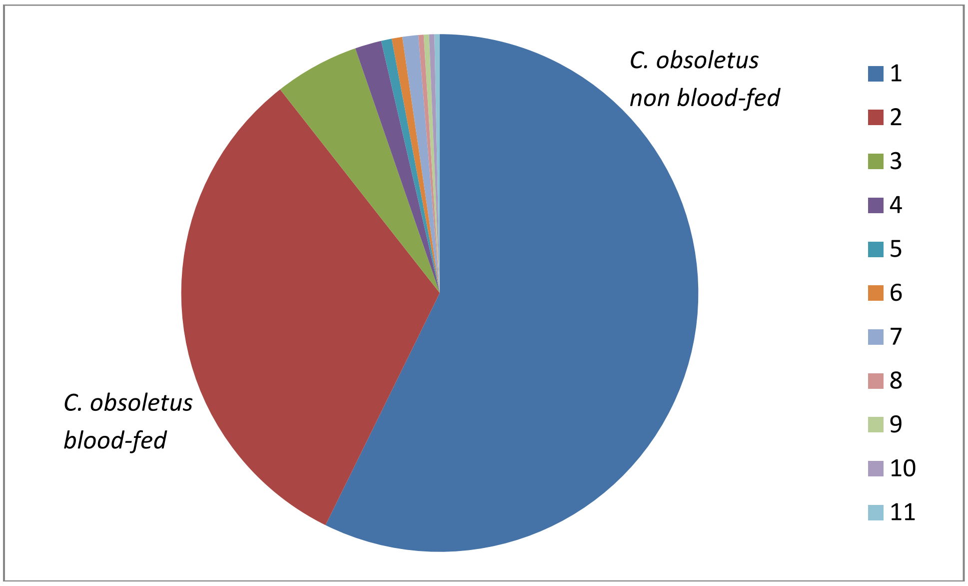

Figure 25. Total numbers of species caught at farm C.

1: *C. obsoletus* non blood (173)
2: *C. obsoletus* blood (97)
3: C. obsoletus unknown (16)
4: *C. dewulfi* non blood (5)
5: *C. dewulfi* blood (2)
6: *C. punctatus* non blood (2)
7: *C. festivipennis* non blood (3)
8: *C. festivipennis* blood (1)
9: *C. chiopterus* non blood (1)
10: *C. circumscriptus* blood (1)
11: *C. salinarius* non blood (1)

Farm D

Eight (8) species caught at farm D: *C. obsoletus (92%), C. punctatus (4%), C. dewulfi (1%), C. chiopterus (1%), C. fasciipennis (1%), C. festivipennis* (< 1%), *C. stigma* (< 1%), *C. pulicaris* (< 1%). Total number caught: 12536

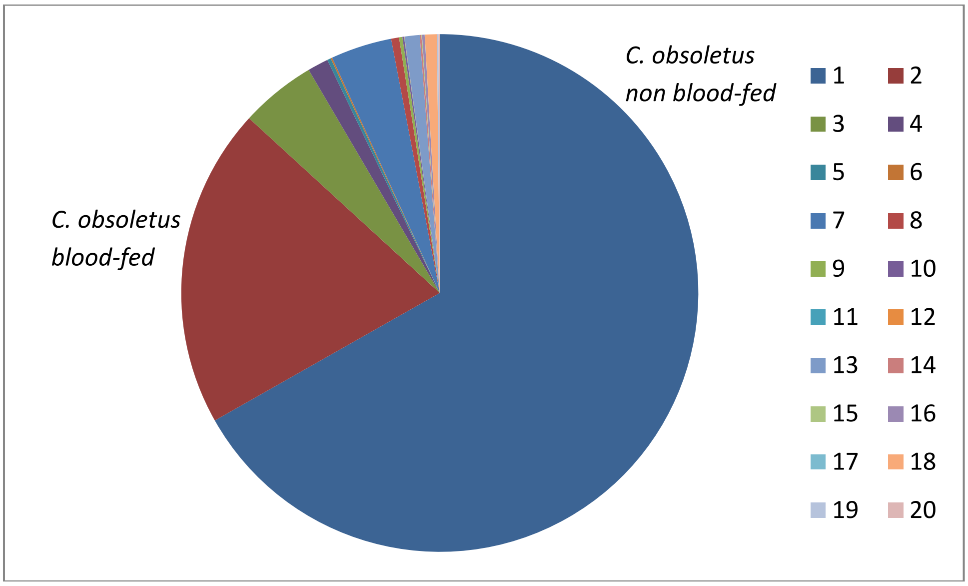

Figure 26. Total numbers of species caught at farm D.

1: *C. obsoletus* non blood (8374)
2: *C. obsoletus* blood (2507)
3: *C. obsoletus* unknown (594)
4: *C. dewulfi* non blood (163)
5: *C. dewulfi* blood (29)
6: *C. dewulfi* unknown (12)
7: *C. punctatus* non blood (479)
8: *C. punctatus* blood (62)
9: *C. punctatus* unknown (26)
10: *C. pulicaris* non blood (13)
11: *C. pulicaris* blood (2)
12: *C. festivipennis* non blood (2)
13: *C. chiopterus* non blood (118)
14: *C. chiopterus* blood (13)
15: *C. chiopterus* unknown (5)
16: *C. stigma* non blood (20)
17: *C. stigma* unknown (1)
18: C. *fasciipennis* non blood (94)
19: C. *fasciipennis* blood (12)
20: *C. fasciipennis* unknown (10)

The figure below illustrates the number of *Culicoides* midges and *C. obsoletus* midges caught on all farms in the various experimental setups. Also the number of blood-fed midges are shown.

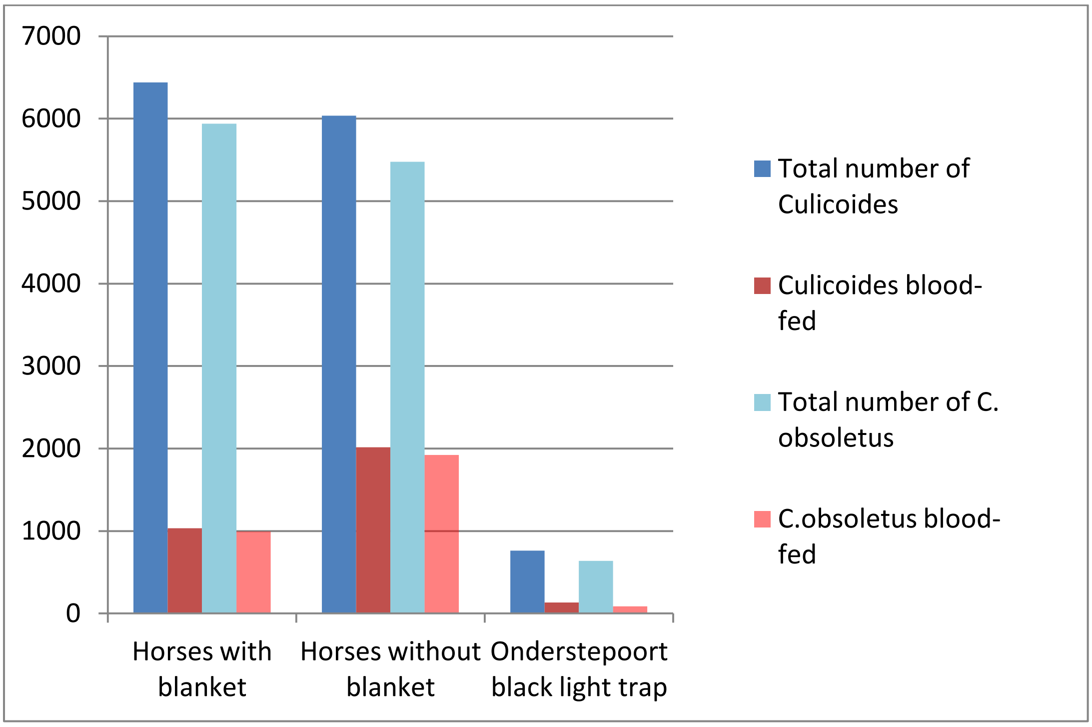

Figure 27. Total numbers of *Culicoides* (total and blood-fed) and *C. obsoletus* (total and blood-fed)

The two figures below illustrates the difference in percentages between a horse with and without a blanket on the number of blood-fed *C. obsoletus*.

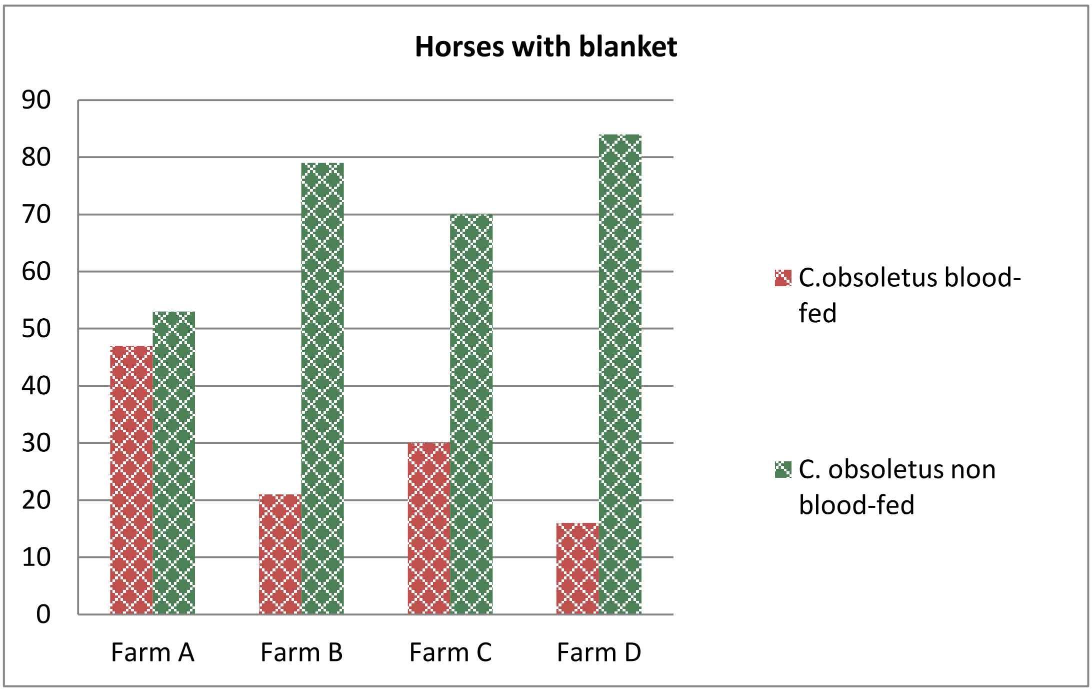

Figure 28. Percentage *of C. obsoletus*, divided into blood-fed and non blood-fed found at horses with an insect blanket.

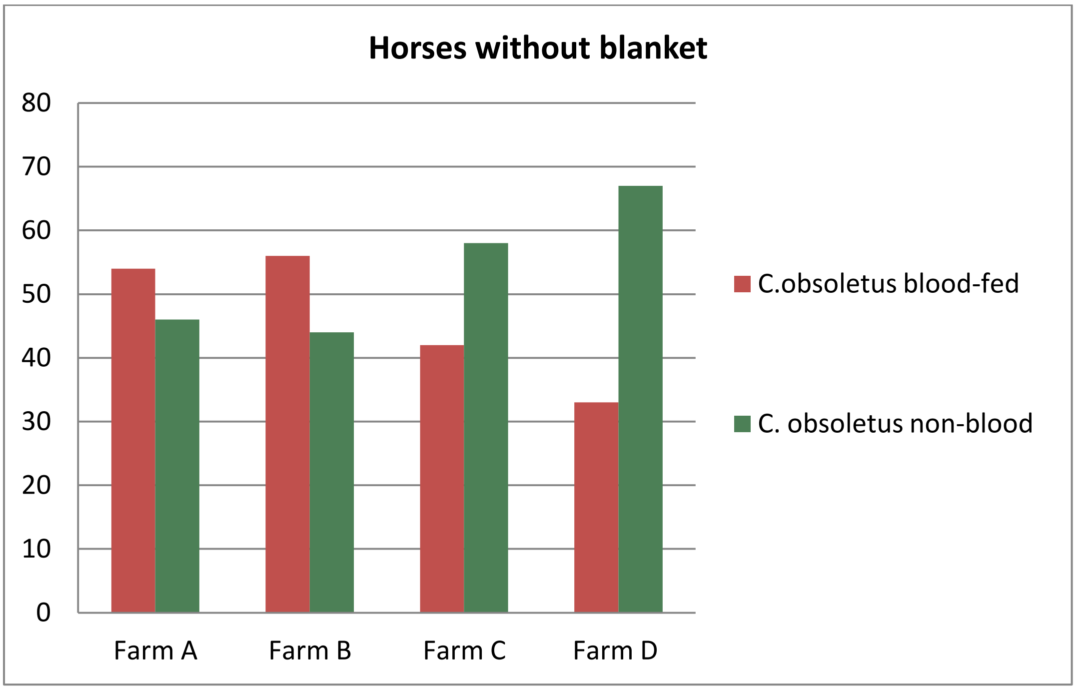

Figure 29. Percentage of *C. obsoletus*, divided into blood-fed and non blood-fed found at horses without an insect blanket.

The table below illustrates the number of *C. obsoletus* in percentages with a blood meal flying in the surroundings caught by the Onderstepoort black light trap.

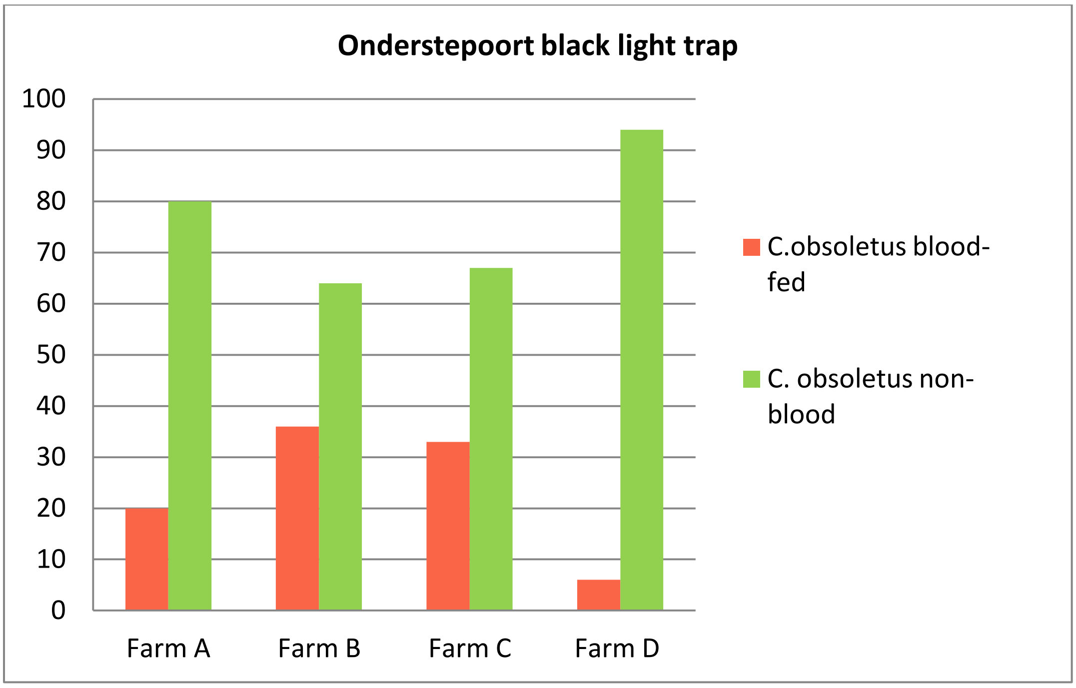

Figure 30. Blood-fed and non blood-fed *C. obsoletus* found in the Onderstepoort black light trap.

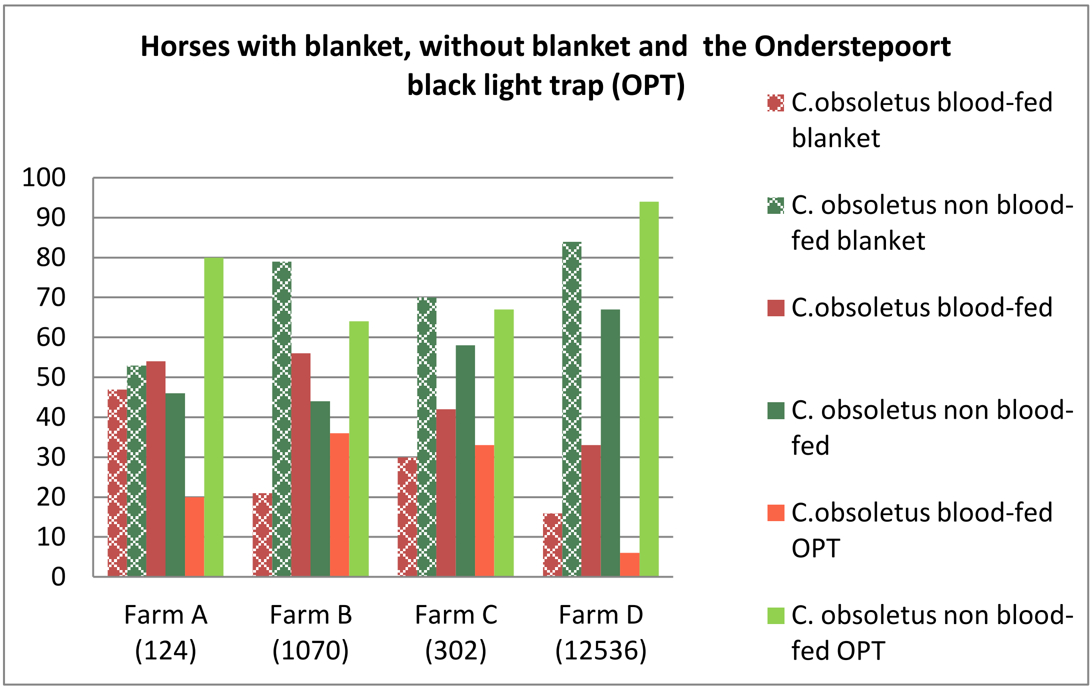

Figure 31. Figures 28-30 combined. (between brackets on the X-axis the total number of *Culicoides* caught during all experiments on this farm)

In the surroundings *C. obsoletus* midges are mostly flying around without a blood meal in their gut. The average percentage of blood-fed *C. obsoletus* flying around is 24% and thus 76% are flying around without having had a recent blood meal.

Using the logistic regression for the number of "blood-fed" of the total number of *Culicoides*, we compared the horses with a blanket with the horses without a blanket. This showed an odds ratio of 2,271. This means that the chance of being bitten by *Culicoides spp*. is 2,271 times higher without the horse wearing a blanket, compared with horses who did wear a blanket.

Discussion

In the present study the use of the experimental design of the tent traps from van der Rijt *et al.* (2008) proved to be successful to collect *Culicoides* species from horses. The *Culicoides* species that were collected from the tent traps using the vacuum cleaner were sometimes damaged too much to determine whether they were blood-fed or not because one of the vacuums could not be set to a lower suction grade.

The *Culicoides* species collected during the present study differ from previous studies performed in the Netherlands. Van der Rijt et al. (2008) caught 94,1 % *C. obsoletus* and 5,8% *C. pulicaris* and only three midges of two other species, namely *C. stigma* and *C.vexans*. The total number of *Culicoides* caught was 3614. Griffioen *et al*. (2011) collected Culicoides species around sheep and found 54,1% *C. chiopterus* and 42,7% *C. obsoletus*. There is a different distribution of species when choosing a horse or a sheep as your animal of interest. In the present study the total number of *Culicoides* caught was 14,032 with a total of twelve different species. 91% was *C. obsoletus*, 5% *C. punctatus*, 2% *C. dewulfi*, 1% *C. chiopterus* and the other species (*C. fasciipennis, C. festivipennis, C. stigma, C. pulicaris, C. nubeculosus, C. circumscriptus, C. newsteadi* and *C. salinarius*) were below 1%. The differences between these studies may be explained by geographical differences, habitat on farms, slight weather differences and in which year the study was performed. The fact that we went back to the entomologist with *Culicoides* species that we could not determine may have contributed to the fact that we have determined more species than there were found in earlier studies. *C. imicola* and *C. bolitinos* were not found in the present study.

Meiswinkel et al. (2008b) used the Onderstepoort black light trap to collect *Culicoides* during the night in the Netherlands. The most collected *Culicoides* were *Culicoides* of the Pulicaris complex (40,1%) (mainly *C. punctatus*) and the Obsoletus complex (37,5%). *C. dewulfi* (13,0%) and *C. chiopterus* (7,1%) were the following *Culicoides* spp. most collected. In the present study the Onderstepoort black light trap caught a total of 766 *Culicoides* and all twelve species found in this study. The majority was *C. obsoletus* (640 = 84%), followed by *C. festivipennis* (50 = 7%) and *C. punctatus* (45 = 7%). Because *C. obsoletus* midges are our highest percentage of midges caught we calculated the percentage of *C. obsoletus* flying around with a blood meal. The average percentage of blood-fed C. obsoletus is 24% and thus 76% are flying around without having had a recent blood meal.
In table 4 it is shown that not all *Culicoides* spp. that are found in the Onderstepoort black light trap are also found in the tent traps. This illustrates the fact that not all *Culicoides* spp. are attracted to horses. For instance, *C. festivipennis* has birds as preferred hosts instead of horses (Hendry, 2011).

In a previous study performed by de Jong, Wessels and Stoop (unpublished) found that the use of an insect blanket does help against biting midges (Sloet 2012).

We collected more *Culicoides* species in the tent traps in which the horse had an insect blanket on. Our suggestion is that a horse with an insect blanket on will show less reaction to insects because the horse does not feel them on the skin and thus less tail flicking, skin twitching and stamping with hoofs. The number of blood-fed *Culicoides* is lower in the tent trap with a horse with an insect blanket on compared to a tent trap with an uncovered horse. After statistical analysis the chance that a horse gets bitten by a midge without an insect blanket on is 2,271 times higher (P: <0,01) than if a horse has an insect blanket on.

The table of the horses with blanket at farm A stands out because the percentage of *C. obsoletus* blood-fed and non-blood are similar. On the other three farms the percentage of non-blood *C. obsoletus* is much higher than the percentage of blood-fed *C. obsoletus*. This can be explained by the very low numbers of midges caught at farm A, compared to the numbers caught at the other farms, making these results less reliable.

Recommendations for a follow-up study would be to start early in de season, preferably May, so there is enough time to catch on warm days with little or no wind. Starting earlier means also more trapping sessions and thus catching more *Culicoides*. Use a vacuum cleaner which can be adjusted to a lower suction rate so the *Culicoides* will not get damaged.
The two microscopes used during this research were not ideal to determine *Culicoides*; due to its low magnification it was not possible to see all the details on the wings of the *Culicoides* spp. With the microscope used to make pictures there was higher magnification possible, so the details on the wings were more clear. For follow-up studies we recommend to use a microscope with high magnification possibilities.

At farm A we thought to have a great location for a trapping session, but the numbers of *Culicoides* caught were very low. Because it is difficult to predict whether a location is suitable to catch large numbers of midges, we recommend to use the Onderstepoort black light trap in advance to measure the number of midges in the area. For instance, you could use the Onderstepoort black light trap a few days at evening twilight at the preferred farm before starting the trapping sessions at that farm.

For future research the aims of the study can be expanded by measuring the effect of permethrin (Tektonik®) pour-on on the biting rate of *Culicoides* spp. These results can be compared with the effect of the insect blanket, to find a more optimal protection for horses against biting midges and the diseases they transmit.

Conclusion

In total, twelve *Culicoides* species were caught during the trapping sessions that were performed between July 23rd – September 4th in the Netherlands, namely *C. obsoletus*, *C. punctatus*, *C. dewulfi*, *C. chiopterus*, *C. fasciipennis*, *C. festivipennis*, *C. stigma*, *C. pulicaris*, *C. nubeculosus*, *C. circumscriptus*, *C. newsteadi* and *C. salinarius*.
C. circumscriptus, *C. newsteadi* and *C. salinarius* were exclusively found in the Onderstepoort black light trap.
C. imicola and *C. bolitinos* were not found during the present study.

The most caught species in the Onderstepoort black light trap was *C. obsoletus*. The Onderstepoort black light trap gives us information about the percentage of *Culicoides* present in the area with a recent blood meal in their gut. For *C. obsoletus* this was 24%.

The use of an insect blanket is effective in preventing the horses from getting bitten by *Culicoides*. After statistical analysis it was proven that a horse without an insect blanket has a 2,271 times higher chance of getting bitten than a horse wearing an insect blanket.

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Appendices

1. Pictures *Culicoides* species

C. nubeculosus non blood-fed (female)

C. nubeculosus without body (male)

C. salinarius non blood-fed (female)

C. dewulfi non blood-fed (male)

C. newsteadi non blood-fed (male)

C. circumscriptus blood-fed (female)

C. fasciipennis non blood-fed (female)

2. Table containing all collected data

Adres	datum	tijd	vangstcode	C.obsoletus zonder	C.obsoletus bloed	C. obsoletus onbekend	C. dewulfi zonder	C. dewulfi bloed	C. dewulfi onbekend	C. punctatus zonder	C. punctatus bloed	C. punctatus onbekend	C.pulicaris zonder	C. pulicaris bloed	C. pulicaris onbekend	C. festivipennis zonder	C. festivipennis bloed	C. chiopterus zonder	C. chiopterus bloed	C.chiopterus onbekend	C. nubeculosus zonder	C. nubeculosus bloed	C.stigma zonder	C. stigma bloed	C.stigma onbekend	C.circumscriptus zond	C.circumscriptus bloed	C.salinarius zonder	C.newsteadi zonder	C.fasciipennis zonder	C.fasciipennis bloed	C.fasciipennis onbeker
Austerlitz	23-7-2012	21.00 - 22.00	Sunny deken	3	2	2																										
	23-7-2012	21.00 - 22.00	Djingiz zonder deken	3	3																											
	23-7-2012	22.45 - 23.45	Sunny zonder deken	4	2	1																										
	23-7-2012	22.45 - 23.45	Djingiz deken	1																												
	23-7-2012	21.00 - 22.00	Onderstepoortval V1																													
	23-7-2012	45 min	Onderstepoortval R1																													
	23-7-2012	22.45 - 23.45	Onderstepoortval V2	16																												
	23-7-2012	30 min	Onderstepoortval R2	2																												
	24-7-2012	20.00 - 21.00	Sunny zonder deken																													
	24-7-2012	20.00 - 21.00	Djingiz deken	3	1																											
	24-7-2012	21.45 - 22.45	Sunny deken	3	3	2																										
	24-7-2012	21.45 - 22.45	Djingiz zonder deken	5	9																											
	24-7-2012	20.00 - 21.00	Onderstepoortval V1																													
	24-7-2012	45 min	Onderstepoortval R1																													
	24-7-2012	21.45 - 22.45	Onderstepoortval V2	10	2																											
	24-7-2012	50 min	Onderstepoortval R2	7	1																											
	25-7-2012	19.55 - 20.55	Sunny deken																													
	25-7-2012	19.55 - 20.55	Djingiz zonder deken																													
	25-7-2012	21.30 - 22.30	Sunny zonder deken		2																											
	25-7-2012	21.30 - 22.30	Djingiz deken	1	1																											
	25-7-2012	19.55 - 20.55	Onderstepoortval V1																													
	25-7-2012	30 min	Onderstepoortval R1																													
	25-7-2012	21.30 - 22.30	Onderstepoortval V2	2	2																											
	25-7-2012	20 min	Onderstepoortval R2	4	1	1																										
	26-7-2012	20.00 - 21.00	Sunny zonder deken	3																												
	26-7-2012	20.00 - 21.00	Djingiz deken	2	3																											
	26-7-2012	21.30 - 22.30	Sunny deken	3	4					1																						
	26-7-2012	21.30 - 22.30	Djingiz zonder deken	1	3																											
	26-7-2012	20.00 - 21.00	Onderstepoortval V1																													
	26-7-2012	20 min	Onderstepoortval R1																													
	26-7-2012	21.30 - 22.30	Onderstepoortval V2																													
	26-7-2012	50 min	Onderstepoortval R2		4		1																									
Faculteit	10-8-2012	20.25 - 21.25	Anky deken	3														1														
	10-8-2012	20.25 - 21.25	Nocturne zonder deke	147	66	13	2			2											1											
	10-8-2012	22.15 - 22.45	Anky zonder deken	10	7													1														
	10-8-2012	22.15 - 22.45	Nocturne deken	31	3	2	1			1																						
	10-8-2012	20.25 - 21.25	Onderstepoortval V1																													
	10-8-2012	40 min	Onderstepoortval R1	1									1																			
	10-8-2012	22.15 - 22.45	Onderstepoortval V2	2	2					2						2	2															
	10-8-2012	20 min	Onderstepoortval R2		1						1																1					
	11-8-2012	19.00 - 20.00	Anky zonder deken																													
	11-8-2012	19.00 - 20.00	Nocturne deken	13												1																

	11-8-2012	20.35 - 21.35	Anky deken				1														1											
	11-8-2012	20.35 - 21.35	Nocturne zonder deke	17	63	5				1	1	1		1																		
	11-8-2012	19.00 - 20.00	Onderstepoortval V1																													
	11-8-2012	20 min	Onderstepoortval R1																													
	11-8-2012	20.35 - 21.35	Onderstepoortval V2																													
	11-8-2012	30 min	Onderstepoortval R2	1							4					4	6															
	14-8-2012	18.45 - 19.45	Anky deken		1																											
	14-8-2012	18.45 - 19.45	Nocturne zonder deken		1																											
	14-8-2012	20.15 - 21.15	Anky zonder deken	4	7	1																										
	14-8-2012	20.15 - 21.15	Nocturne deken	31	4	5	7	1		5		1	1																			
	14-8-2012	18.45 - 19.45	Onderstepoortval V1																													
	14-8-2012	15 min	Onderstepoortval R1																													
	14-8-2012	20.15 - 21.15	Onderstepoortval V2	1	1																											
	14-8-2012	35 min	Onderstepoortval R2																													
	16-8-2012	18.50 - 19.50	Anky zonder deken	3	3			1													1											
	16-8-2012	18.50 - 19.50	Nocturne deken	29	8	2																										
	16-8-2012	20.20 - 21.20	Anky deken	22	2																											
	16-8-2012	20.20 - 21.20	Nocturne zonder deke	40	142	84				8	6																					
	16-8-2012	18.50 - 19.50	Onderstepoortval V1																													
	16-8-2012	20 min	Onderstepoortval R1																													
	16-8-2012	20.20 - 21.20	Onderstepoortval V2																													
	16-8-2012	40 min	Onderstepoortval R2	2	2	1				1	1					8	7				1									1		
	18-8-2012	19.00 - 20.00	Anky deken	1																												
	18-8-2012	19.00 - 20.00	Nocturne zonder deke	6		2			1																							
	18-8-2012	20.30 - 21.30	Anky zonder deken	7	7					7	2							1	1													
	18-8-2012	20.30 - 21.30	Nocturne deken	18	22	5	1			2	2																					
	18-8-2012	19.00 - 20.00	Onderstepoortval V1																													
	18-8-2012	30 min	Onderstepoortval R1																													
	18-8-2012	20.30 - 21.30	Onderstepoortval V2	1	1					1																						
	18-8-2012	45 min	Onderstepoortval R2	36	18		4			16	14		1	1	1	11	4					1		1					2			
Stroe	20-8-2012	18.40 - 19.40	Catootje deken																													
	20-8-2012	18.40 - 19.40	Oma zonder	1																												
	20-8-2012	20.15 - 21.15	Catootje zonder deker	21	27	1																										
	20-8-2012	20.15 - 21.15	Oma met deken	38	7	6																										
	20-8-2012	18.40 - 19.40	Onderstepoortval V1													1																
	20-8-2012	15 min	Onderstepoortval R1																													
	20-8-2012	20.15 - 21.15	Onderstepoortval V2																													
	20-8-2012	20 min	Onderstepoortval R2	6																												
	21-8-2012	18.40 - 19.40	Catootje zonder deker	2	2																											
	21-8-2012	18.40 - 19.40	Oma deken			1												1														
	21-8-2012	20.05 - 21.05	Catootje deken	3	12	1																										
	21-8-2012	20.05 - 21.05	Oma zonder deken	10	3	2																										
	21-8-2012	18.40 - 19.40	Onderstepoortval V1																													
	21-8-2012	10 min	Onderstepoortval R1																													
	21-8-2012	20.05 - 21.05	Onderstepoortval V2					1																								
	21-8-2012	20 min	Onderstepoortval R2	3	1		1																									
	23-8-2012	18.30 - 19.30	Catootje deken	5	3		1																									
	23-8-2012	18.30 - 19.30	Oma zonder	6	1	1																										
	23-8-2012	20.00 - 21.00	Catootje zonder deker	2	5	1																										
	23-8-2012	20.00 - 21.00	Oma met deken	1																												
	23-8-2012	18.30 - 19.30	Onderstepoortval V1																													
	23-8-2012	15 min	Onderstepoortval R1																													
	23-8-2012	20.00 - 21.00	Onderstepoortval V2	1																												
	23-8-2012	15 min	Onderstepoortval R2																													
	24-8-2012	18.30 - 19.30	Catootje zonder deker	4	2																											

	24-8-2012	20.00 - 21.00	Oma zonder deken	14	4	1	1																									
	24-8-2012	18.30 - 19.30	Onderstepoortval V1																													
	24-8-2012	10 min	Onderstepoortval R1																													
	24-8-2012	20.00 - 21.00	Onderstepoortval V2	5	2																											
	24-8-2012	45 min	Onderstepoortval R2	43	26		2	1		2						2	1										1	1				
Bilthoven	27-8-2012	18.30 - 19.30	Lûte deken	1322	179	179	6		7	50	2	3	6					44	4	2			15							57	4	5
	27-8-2012	18.30 - 19.30	Partner zonder deken	310	218	9	14	14										2														
	27-8-2012	20.10 - 21.10	Lûte zonder deken	1142	338	230				231	31	20	6	2				8	2	2			1		1					7	5	3
	27-8-2012	20.10 - 21.10	Partner met deken	652	243	14	5	2	1	13	3	1																		1	1	
	27-8-2012	18.30 - 19.30	Onderstepoortval V1															1														
	27-8-2012	30 min	Onderstepoortval R1																													
	27-8-2012	20.10 - 21.10	Onderstepoortval V2	4	2																											
	27-8-2012	30 min	Onderstepoortval R2	321	13	2	1																									
	28-8-2012	18.00 - 19.00	Lûte zonder deken	27	15	7	3		2	2																						1
	28-8-2012	18.00 - 19.00	Partner met deken	9	6		1																									
	28-8-2012	19.45 - 20.45	Lûte met deken	960	147	80	17			92	4	2											1							6	1	
	28-8-2012	19.45 - 20.45	Partner zonder deken	229	145	4	2			2																						
	28-8-2012	18.20 - 19.00	Onderstepoortval V1																													
	28-8-2012	30 min	Onderstepoortval R1	2																												
	28-8-2012	19.45 - 20.45	Onderstepoortval V2																													
	28-8-2012	30 min	Onderstepoortval R2	18	2											1		1														
	29-8-2012	18:00 - 19.00	Lûte deken	37	14													3														
	29-8-2012	18:00 - 19.00	Partner zonder deken	3	6	1	1																									
	29-8-2012	19.40 - 20.40	Lûte zonder deken	565	252	15	8	1	1	48	8		1					19	4	1			2							13		1
	29-8-2012	19.40 - 20.40	Partner met deken	406	113	6	4			2	2																			1		
	29-8-2012	18:00 - 19.00	Onderstepoortval V1																													
	29-8-2012	20 min	Onderstepoortval R1																													
	29-8-2012	19.40 - 20.40	Onderstepoortval V2	1																												
	29-8-2012	35 min	Onderstepoortval R2	25	4		2			2	1																					
	2-9-2012	17.55 - 18.55	Lûte zonder deken	6	7		1																									
	2-9-2012	17.55 - 18.55	Partner met deken	22	17	4	2	1																								
	2-9-2012	19.30 - 20.30	Lûte met deken	485	88	11			1	1								3	1													
	2-9-2012	19.30 - 20.30	Partner zonder deken	372	340	5	19	2		4	5																					
	2-9-2012	17.55 - 18.55	Onderstepoortval V1																													
	2-9-2012	25 min	Onderstepoortval R1																													
	2-9-2012	19.30 - 20.30	Onderstepoortval V2	5	1																											
	2-9-2012	35 min	Onderstepoortval R2	11	2		1																									
	3-9-2012	17.50 - 18.50	Lûte deken	637	73	6	51	7		2								37	2				1							9	1	
	3-9-2012	17.50 - 18.50	Partner zonder deken	7	14		3	1																								
	3-9-2012	19.30 - 20.30	Lûte zonder deken	456	128	16	2			29	5																					
	3-9-2012	19.30 - 20.30	Partner met deken	88	12	1	1				1																					
	3-9-2012	18.00 - 18.50	Onderstepoortval V1																													
	3-9-2012	25 min	Onderstepoortval R1				1																									
	3-9-2012	19.30 - 20.30	Onderstepoortval V2																													
	3-9-2012	20 min	Onderstepoortval R2	7																												
	4-9-2012	17.50 - 18.50	Lûte zonder deken	1																												
	4-9-2012	17.50 - 18.50	Partner met deken	3	2		3																									
	4-9-2012	19.25 - 20.25	Lûte met deken	103	24	2	2	1		1																						
	4-9-2012	19.25 - 20.25	Partner zonder deken	126	102	2	12																									
	4-9-2012	17.50 - 18.50	Onderstepoortval V1																													
	4-9-2012	30 min	Onderstepoortval R1																													

	4-9-2012	19.25 - 20.25	Onderstepoortval V2	1																												
	4-9-2012	60 min	Onderstepoortval R2	11			1									1																
####																																

Totaal: 14032