Research Project

The normal biting rate of *Culicoides*, caught in the proximity of sheep and horses in the Netherlands

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Summary

This report is a part of the *Culicoides* project of 2011 of Utrecht University (dr. M. Sloet) and Wageningen University and Research centre (dr. G. Nodelijk). This study was conducted to answer several questions considering *Culicoides* species in the Netherlands. The part of the study discussed here focused on the following question: What is the normal biting rate of *Culicoides*, caught in the vicinity of sheep and horses? The complete project was set up to determine which tactics could be used to prevent or reduce the transmission of African Horse Sickness (AHS) by *Culicoides* species which is considered to be a potential threat for the Dutch horse population (Maclachlan & Guthrie, 2010).

In the study the experimental mosquito trapping tents designed by Van der Rijt (Rijt 2008) and Griffioen (Griffioen 2010) were used in order to collect *Culicoides* spp. and other flying insects in de proximity of horses and sheep. After an hour the tents were closed and the insects were collected. All trapped *Culicoides spp*. were microscopically counted and identified. The total number of *Culicoides* caught widely varied at the test farms.

During all trapping sessions with animals an 'Onderste Poort black light trap' was turned on to collect *Culicoides*. The line of thought behind this will be discussed later in this paper.

This study addresses the research question; "What is the normal biting rate of *Culicoides spp.*, caught in the vicinity of sheep and horses?" Moreover, it can be concluded that

- The biting rate of *Culicoides* caught in the proximity of sheep is 77% for *Culicoides spp* and 67% for *C. obsoletus.*
- The biting rate of *Culicoides* caught in the proximity of horses is 56% for *Culicoides spp*. and 58% for *C. obsoletus*.
- The biting ratio of *Culicoides spp.* is 21% in the tests with sheep in an inner tent and 77% in the tests without an inner tent. The corresponding odds ratio is 8.77. This means that the odds of blood-fed *Culicoides spp*. found was 8.77 times higher in the group of sheep without an inner tent than in the group with an inner tent.

Introduction

African Horse Sickness (AHS) is a viral disease which occurs in *Equidae* (horses, ponies, donkeys, mules and zebras). It is a vector-borne disease spread by *Culicoides* (order *Diptera*, family *Ceratopogonidae*, genus *Culicoides*). These are small insects of one to three millimetres long and are related to mosquitoes (Wittmann et al., 2000; Mellor et al., 2000). *Culicoides* species have spotted wings and this spotting pattern on the wings is type-specific and is used for identification. Worldwide more than 1400 species of *Culicoides* have been described (Mellor et al., 2000). The males feed exclusively on nectar. The females occasionally feed on nectar, but they need blood to make their eggs, which they take from mammals. The eggs need a moist place to develop (Wittmann et al., 2000; Mellor et al., 2000).

The AHS viruses (AHSV) are RNA viruses that belong to the family of the reoviruses and to the genus of the orbiviruses. AHSV are closely related to the Bluetongue virus.

When an *equid* is infected with the AHS virus by a *Culicoides*, the virus will multiply in the regional lymph nodes and from there spread throughout the body via the blood. This stage is also known as the primary viraemia (Mellor et al., 2004). Target organs and cells, such as the lungs, spleen and other lymphoid tissues, and certain endothelial cells, will be infected. The virus will multiply again in these tissues and this causes the secondary viraemia. The incubation period until the secondary viraemia is under natural circumstances less than 9 days, but in experiments it varied between 2 and 21 days. The duration of the secondary viraemia and its titre vary, depending on an number of factors, one of them being host species (Mellor et al., 2004).
AHS can occur in four different varieties; the heart, the lung, the mixed and the fever variety. Horses (and mules) are most sensitive to the virus and develop the most serious symptoms. Depending on the variety they develop, 50 to 95 percent of contaminated horses in a naïve herd will die (Mellor & Hamblin, 2004; Maclachlan & Guthrie, 2010). Donkeys are less sensitive to the illness than horses. In comparison, 5 to 10 percent of the contaminated donkeys will die. Moreover, infected zebras show only clinical symptoms in exceptional cases (Mellor & Hamblin, 2004). The incubation period - time between infection and the appearance of the first symptoms of the disease - of AHS is generally five days for horses.

AHS occurs endemically in large parts of tropical and subtropical Africa (Lord et al., 1996; Mellor & Hamblin, 2004; Maclachlan & Guthrie, 2010). Above the Sahara the illness does not occur endemically, the Sahara desert forms, as it happens, a barrier for further distribution. However, a breakout occasionally occurs beyond the endemic areas. The most recent outbreak occured in Portugal and Spain at the end of the Eighties. Until now an outbreak of AHS has never happened more northern than Spain and Portugal.
Both the number of present midges and the possibility of the AHSV to multiply in the vector are influenced by the weather conditions. At low temperatures the midges are not active and the virus can only multiply at a temperature above fifteen degrees Celsius (Mellor et al., 1998). At temperatures below this level the apparent infection rate rapidly falls to zero (Mellor et al., 1998). In theory, winter can stop an outbreak in the Netherlands for exactly this reason. However, the virus can remain latent in the vector at lower temperatures and as a result of that it is possible that the AHSV will survive winter and become active in spring (Mellor et al., 1998).

Mainly *C. imicola*, but also *C. obsoletus* may be a possible vector for AHS (Mellor et al., 1998). *C. imicola* is a (sub)tropical species, the range of *C. obsoletus* extends much further north (Mellor et al., 1998).
Until now an outbreak of AHS has never taken place amongst the horse population of the Netherlands. Therefore the risk on such an outbreak was considered to be zero. However, Bluetongue disease in which *Culicoides spp*. acts as a vector has increasingly marched northwards from a geographical point of view (Mellor & Hamblin, 2004; Saegerman et al., 2008). The recent outbreak in the Netherlands is an example of this. Researchers suspect that climate change has a role in this development (Griffioen et al., 2010; Maclachlan & Guthrie, 2010). Climate change could also cause that the some viruses might improve in being able to survive in the vector during winter. The AHSV and the Bluetongue virusses are rather similar and for this reason it is to be feared that *Culicoides spp.* in the Netherlands may also spread AHSV. Therefore, the risk of an outbreak of AHS under horses in the Netherlands is possibly greater than formerly assumed.

AHS is an illness that is notifiable and subject to control. Notifiable means that at suspicion of contamination with AHS the owner and the veterinary surgeon of the concerning animal have to inform the government (the ‘Voedsel en Waren Autoriteit’) in the Netherlands. The Dutch government has to report an outbreak of AHS at the European Commission and at the World Organisation for Animal Health (OIE). Subject to control means that the Dutch government is obliged to take measures according to European legislation to fight AHS. According to a European suppression directive, an outbreak of AHS is spoken of when one contaminate horse is found. A number of measures have to be taken after discovering an outbreak. The basis of the suppression policies for AHS is tied up in the European directive 92/35/EEG and in the Dutch health and well-being law for animals (gezondheids- en welzijnswet voor dieren) and exists from the following head components: (1) a reporting duty for horse keepers or owners and veterinary surgeons of a suspected animal, (2) a complete transport prohibition of *Equidae* for a period of minimally 72 hours, (3) contaminated animals have to be euthanized (4) protection of the *Equidae* against the vector *Culicoides spp,* (5) the establishment of safety areas around the farm at which the contamination is found and issuing an transport prohibition in that area, (6) emergency vaccination, (7) epidemiologic research, (8) advising and informing the horse industry and (9) monitoring and surveillance. Vast information concerning aforesaid measures can be read in the draft policy scenario African horse sickness, version 1.0, December 2007 (beleidsdraaiboek Afrikaanse paardenpest, 2007). In our research attention is mainly directed at the fourth component in the enumeration, namely protecting the *Equidae* against the vector *Culicoides spp.*

Finally, the sociological consequences of an AHS outbreak in the Netherlands should not be taken lightly. A large and diverse group of people in the Netherlands have interests, relationships and emotions toward horses. Moreover, the horse industry is economically significant.

Aims of the study

The complete *Culicoides* project of 2011 of Utrecht University (dr. M. Sloet) and Wageningen University and Research centre (dr. G. Nodelijk) included three questions:

1. What types of *Culicoides spp.* are caught in the proximity of sheep and horses by means of a mosquito trapping tent in The Netherlands, and are these similar to the number and types caught at the same moment in a Onderste Poort black light trap?
2. What is the normal biting rate of *Culicoides*, caught in the vicinity of sheep and horses?
3. Does carrying an insect blanket including a neck and hood piece reduce the biting rate?

The three questions above were divided and explored by three research students of Utrecht University, participating in the project. While conducting the experiments the three students worked closely together, but each wrote their own report in an effort to answer one of the questions. In the present report the emphasis will lie on the second of the three questions.

Materials and methods

Selection of farms, animals and the moments of catching

In order to make the field study as efficient as possible several parameters have to be taken into account, for instance location, moment of the day and weather conditions. Previous studies have determined a dry, calm (windless) and warm –summer- evening the most suitable moment (van den Boom et al., 2008; van der Rijt et al., 2008; Griffioen et al., 2010). The flying times of *Culicoides* which are caught around sheep correspond to those caught in the proximity of horses (Griffioen et al., 2010)

Catching the insects happened on three in advance selected farms. At each farm four sessions of catching insects were organised on 2 or 3 different evenings. On the participating farms both horses and sheep were present.

All participating animals were in good health. Relevant data concerning the landscape and the used animals were documented. Close attention was given to surroundings factors such as: the weather circumstances, the ground type, surrounding (stationary) water and the presence of other animals in the near surroundings. Data were accurately collected about the used animals such as animal type, breed and whether or not the animal was shaved. In table 1 the information is shown about the farms and animals used in this study.

	Farm A	**Farm B**	**Farm C**
Place in The Netherlands	Zwiggelte	Westbroek	Bilthoven
Environment	Swamp and forest	Polder	Forest
Breed horse 1	American miniature horse (Fig 1a)	Dutch Warmblood	Thoroughbred
Breed horse 2	Shetland pony (Fig 1a)	Friesian horse	KWPN riding horse
Breed sheep	Zwartbles (Fig 1b)	Texel sheep	Mixed breed
Shaved / non-shaved sheep	Shaved	Non-shaved	Shaved
Pasture situation	24 hours a day Sheep: in a herd	24 hours a day Horses: with two other horses Sheep: in a herd	Housed during the night Herd of five sheep and four lambs together with the two horses
Presence of other animals	Cattle (Lakenfelders) (Fig 1c)	Horses, sheep, dogs and cats	Chicken, a dog and wildlife
Weather condition during the trapping sessions	20°C - 24°C Windless Slightly cloudy - cloudy	19°C - 24°C Light breeze - moderate breeze Slightly cloudy - cloudy	17°C - 28°C Windless - light breeze Bright - slightly cloudy

Table 1. Information about the farms and animals used in the study and the weather conditions during the trapping sessions.

Fig 1: Animals on farm A: (a) Horses, (b) Zwartbles Sheep and (c) Lakenfelders.

Experimental setting

Insect trapping was carried out between the 14th of August and the 2nd of September 2011. In this period three mosquito trapping tents (2 for individual horses and 1 for 2 sheep) were used for several evenings (with circumstances as optimal as possible). In addition all three mosquito trapping tents were similarly situated, so that the wind faced the same sides of the tents. This is necessary to exclude influences like the direction of the wind as a cause of a differences in the number of caught midgets.

Sheep: mosquito trapping tent

In this study the experimental setting designed by Griffioen (Griffioen et al., 2010) was used in order to collect the *Culicoides* spp. and other flying insects.

At sunset, both sheep were placed inside the same mosquito tent trap, consisting of four sheep fences that were covered with a large mosquito net (Fig. 2a). A central pole was placed in and metal pipes were inserted in the ground as additional supports, which were approximately a meter from each corner of the sheep fences, provided support for the outer tent (Fig. 2b).

To evaluate the effect of an inner tent on biting rate of the *Culicoides spp*., an inner tent (Fig. 2c) was placed directly over the sheep in two out of four catching moments at each farm. This way insects could not get to the sheep. This inner tent also used the central pole and four additional poles which were about half a meter from each corner of the tent frame. The inner tent extended to the ground to prevent the midges from entering or leaving the inner tent. One side of the outer tent was kept partially open during the experiment, which enabled the insects to enter the outer, but not the inner tent. The inner tent can be seen as the ultimate insect blanket, because the nose and the legs of a sheep are also protected against insect biting unlike an usual insect blanket used for instance with horses which protects only the body, neck and part of the head (Fig. 2d and 2e).

Only one tent was available for the sheep sessions, while two were available for the sessions involving horses. So for the test runs with horses it was possible to conduct both the test, with an insect blanket as well as the test without an insect blanket, at the same time. In contrast, each test had to be conducted separately in the sheep sessions.

To offer some distraction to the sheep during this experiment, food was available to them during the tests.

Fig 2a: Sheep in an open mosquito trapping tent as described by Griffioen et. al. 2010 .
Fig 2b: Sheep simultaneously in the mosquito trapping tent (Griffioen et al., 2010).
Fig 2c: Sheep in a closed mosquito trapping tent as described by Griffioen et. al. 2010 (Griffioen et al., 2010).
Fig 2d and 2e: A horse wearing an insect blanket with a neck and hood piece (Ivanhoe®).

Horse: mosquito trapping tent:
In this study the experimental setting designed by Van der Rijt (van der Rijt et al., 2008) was used in order to collect the *Culicoides* spp. and other flying insects.
During sunset the horses where each placed inside individual mosquito tents (Fig 3a, b, c and d) placed 2 meters apart of each other and the sheep mosquito trapping tent. The mosquito tents consisted of a strong metal tent frame secured to the ground using metal pegs and covered with a large mosquito net (mesh opening of about 200 µm) and a seam of heavy fabric. The back of the tent was kept open, enabling the insects to enter. The rest of the netting remained on the ground.
During each of the two sessions one horse wore an individually fitted insect blanket (Ivanhoe® Horse Products, Westenhoven, the Netherlands Fig. 2d and 2e) and in a second test on the same farm the other horse was wearing the blanket (cross-over design). During sampling the horses were held by a handler inside the tent to calm the horse. This way the risk of a collapse of the tent was minimized if the horse would either panic or leave the tent. The horses displayed some signs of discomfort during trapping sessions, for example swinging their tails and stamping their hoofs. However, these are the same signs a horse would show on a pasture to chase away insects.
To offer some distraction to the horses during this experiment, hay was available to them during the tests.

Fig 3 a, b, c, and d: individual horse mosquito trapping tents as described by van der Rijt et al. (2008).

Onderste Poort black light trap:

During all sessions a Onderste Poort black light trap was used (Fig 4 a, b, c, and d). It was turned on before the trapping sessions with the animals began and turned off after the insects in the tent traps of the sheep and horses were collected. A black light trap consists of a metal frame on to which a lamp is attached at a height of two meters. This lamp is covered with a firm mosquito net of which the mesh opening is adjusted to the size of the midges. Therefore, insects larger than the midges will not be able to enter the trap, which reduces the trapping amount of insects that do not belong to the group of *Culicoides spp*.. Under the lamp there is a vent that blows the insects in the surroundings of the lamp into the trap. Then the insects land in a soft and V-shaped mosquito net of which the mesh openings are smaller than the *Culicoides spp*. Due to this V-shaped net the insects cannot leave the mosquito net because the upper part is sealed by the vent and because the sides have a mesh opening that is too small for the mosquito. The only direction the insects can go is downward but there they land in a cup of water with some soap in it, which is to break the surface tension of the water. When the insects arrive there, they drown in the water.

Black light is mainly visible during sunset or in the dark, and at that time it attracts most insects.

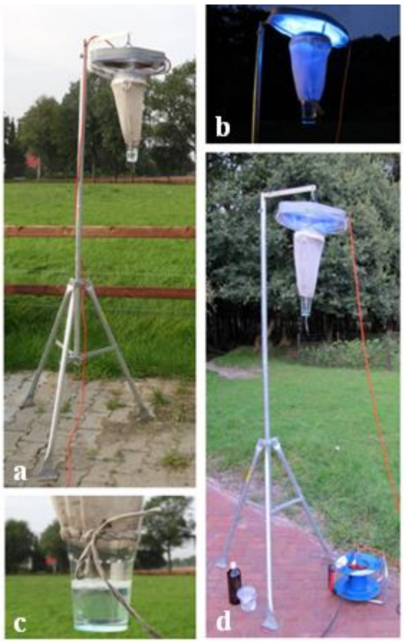


Fig 4 a, b, c and d: an Onderste Poort black light trap.

Collection of insects

Animal (sheep and horses) mosquito trapping tents:

Each trapping session lasted for an hour after which the horses were removed from the tents and the tents were closed. The tent of the sheep was also closed after one hour, but the sheep remained in the tents. The insects that were caught in the tent were collected by using a small household vacuum-cleaner (Fig 5a) with a small piece of the mosquito net being placed in the tubing as a filter (Fig 5b). The vacuum-cleaner was used at the lowest power setting to prevent the insects from being damaged. The catched insects were placed, along with a small piece of the mosquito net, in labelled plastic 0.5 litre cups containing a 70% ethyl–ethanol solution and thereby killing them. The insects were preserved in this solution until further processing.

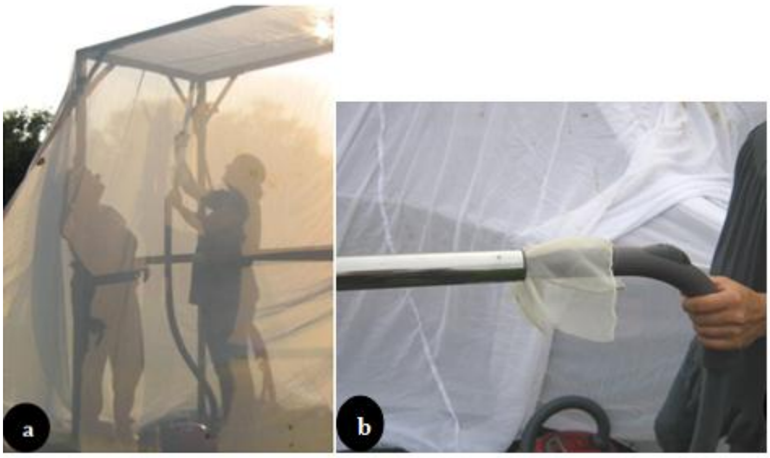


Fig 5a: collecting insects by using a small household vacuum-cleaner.
Fig 5b: mosquito net being placed in the tubing as a filter.

Onderste Poort black light trap:
At the end of the trapping session the cup of water with soap and drown insects was removed from the black light trap. Finally, the insects are filtered out of the water and preserved in labelled cups containing a 70% ethyl-ethanol solution until further processing.

Sample processing

With a pipette the insects were collected from the labelled cups, then they were placed in Petri dishes and examined under a stereo microscope. All insects other than *Culicoides spp.* were removed. All the *Culicoides* midges were identified based on size and wing pattern, after which they were manually counted and placed in separate labelled eppendorf cups. All *Culicoides* with blood in their abdomen (midgets that stung an animal) were categorised as the 'blood fed' midges. This allowed us to determine a 'normal' biting rate for the midges.

Statistical analysis

For comparing the results of the test with sheep with and without an inner tent, statistical expert dr. J. van den Broek used an odds ratio. The odd ratio is the ratio of two odds in a group that was exposed to a specific factor divided by the odds in the group not exposed to this factor.

Results

The total number of *Culicoides* caught widely varied between the three test farms. A complete overview of the results of each test run is shown in Annex I.
The results of the black light trap (tables 4, 5 and 6) are listed because the number of blood-fed midges in this trap is an indication of the number of blood fed midges present in the vicinitiy. The line of thought behind this will be discussed later in this paper.

Effect of the inner tent on the biting rate of Culicoides with sheep

We researched the effects of an inner tent on the biting rate of *Culicoides spp.* in relation to sheep. The total number as well as the blood-fed numbers *Culicoides spp. and C. obsoletus* was examined in the vicinity of sheep. The tests were conducted with an inner tent (table 2) and without an inner tent (table 3). The inner tent may be seen as the ultimate insect blanket. This makes it interesting to compare the effect of an inner tent on the biting rate for sheep to the biting rate for horses wearing an insect blanket. The effect of an insect blanket on the biting rate with horses is discussed in another part of this research project. These results and figures can be found in annex II. Next to the data of *Culicoides* in general, the results of *C. obsoletus* was also taken into consideration. Next to the data of *Culicoides* in general, the results of *C. obsoletus* was also reviewed, because the later is considered to be a possible vector of AHS (Sloet., 2011)

The results of tables 2 and 3 are graphically illustrated in graphs 1 and 2. Using the results shown in grahp 2 (more specifically the biting ratio of *Culicoides spp.* with an inner tent is 21% and without an inner tent it is 77%) an odds ratio of 8.77 for *Culicoides spp* was calculated. This means that the odds of blood-fed *Culicoides spp*. found was 8.77 times higher in the group of sheep without an inner tent than compared to the group with an inner tent.

SHEEP WITH INNER TENT				
Test run	N total *Culicoides*	N blood-fed *Culicoides*	N total *C. obsoletus*	N blood-fed *C. obsoletus*
1	4	2	1	0
2	33	23	7	3
3	43	0	3	0
4	4	0	0	0
5	15	0	6	0
6	22	0	8	0
Totaal:	121	25	25	3

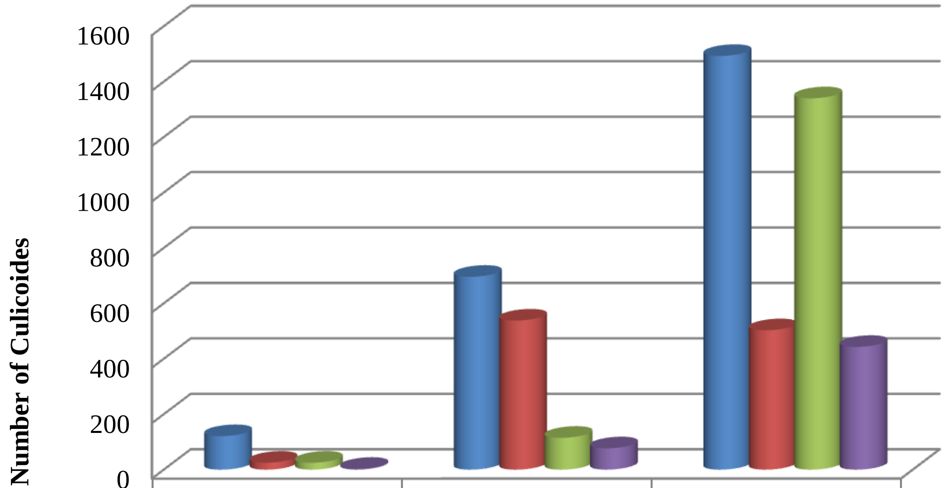
Table 2: total Culicoides caught in the sheep trap tents during all sessions with a inner tent.

SHEEP WITHOUT INNER TENT				
Test run	N total *Culicoides*	N blood-fed *Culicoides*	N total *C. obsoletus*	N blood-fed *C. obsoletus*
1	37	35	12	12
2	467	399	38	32
3	75	31	15	11
4	13	8	1	0
5	65	50	15	11
6	39	15	34	11
Totaal:	696	538	115	77

Table 3: total Culicoides caught in the sheep trap tents during all sessions without a inner tent.

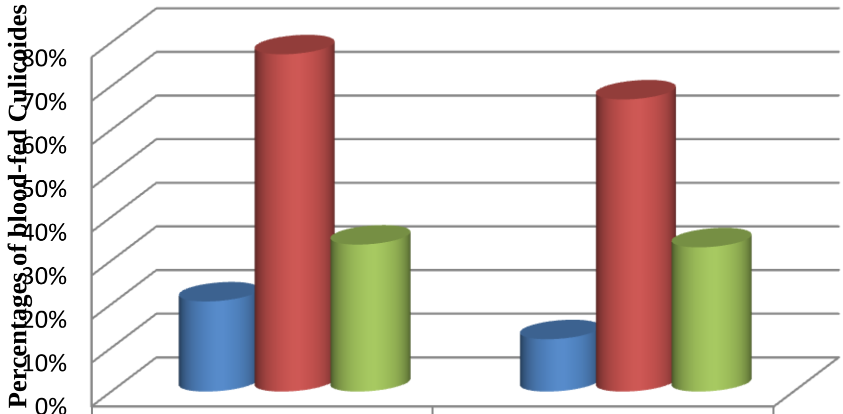
ONDERSTE POORT BLACK LIGHT TRAP				
Test run	N total *Culicoides*	N blood-fed *Culicoides*	N total *C. obsoletus*	N blood-fed *C. obsoletus*
1	155	124	127	104
2	15	11	14	10
3	0	0	0	0
4	8	1	2	0
5	2	0	0	0
6	11	0	10	0
7	1302	367	1187	329
Totaal:	1493	503	1340	443

Table 4: total Culicoides caught in the Onderste Poort black light trap during all sessions.



	Sheep with inner tent	Sheep without inner tent	Black light trap
Total Culicoides	121	696	1493
Culicoides blood-fed	25	538	503
Total C. obsoletus	25	115	1340
C. obsoletus blood-fed	3	77	443

Graph 1: Illustration of the number of Culicoides spp. and C. obsoletus caught in tent traps on 3 farms with 2 sheep per farm during 6 tests with sheep and the numbers found in the black light trap.



	Blood-fed Culicoides	Blood-fed C. obsoletus
Sheep with inner tent	21%	12%
Sheep without inner tent	77%	67%
Black light trap	34%	33%

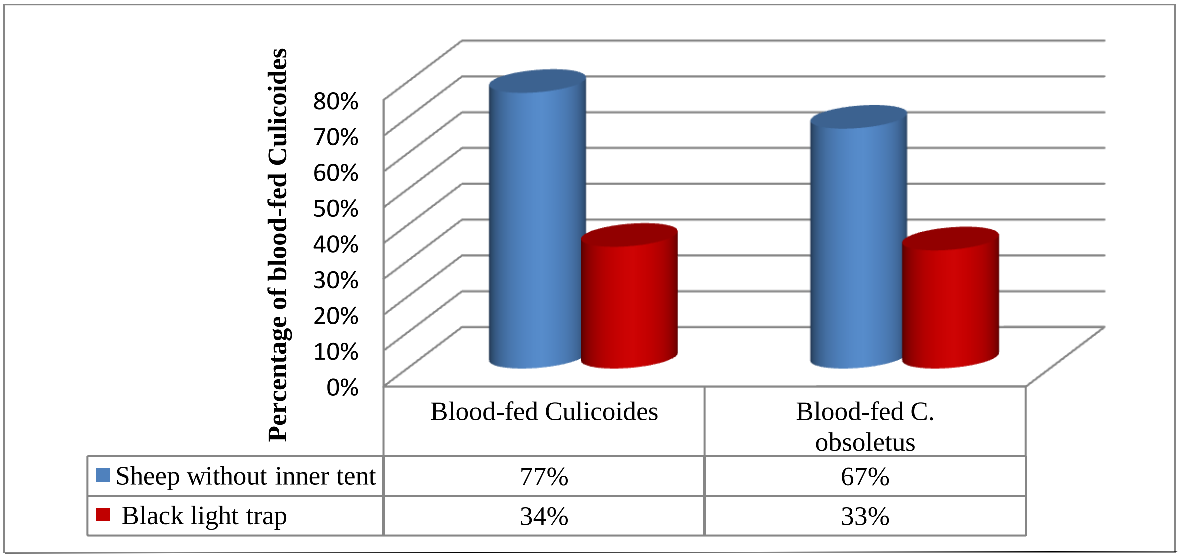
Graph 2: Illustration of the percentages of blood-fed Culicoides spp. and C. obsoletus caught in tent traps on 3 farms with 2 sheep per farm during 6 tests and the percentages found in the black light trap.

Biting rate of Culicoides in sheep

A part of my research question was to determine the normal biting rate of *Culicoides spp* and *C. obsoletus* in relation to sheep. This was done by calculating the percentage of blood-fed midges in the surroundings of sheep. Therefore, the test was conducted without an inner tent (table 5) to allow the *Culicoides spp* and *C. obsoletus* to behave normally. For *Culicoides spp*. a biting rate of 77% was found. In comparison this was 10% lower for the *C. obsoletus*, because this was 67%.
The average feeding rate of all *Culicoides* midges and *C. obsoletus* in the tests without an inner tent was compared to the biting rate of the midges caught in the black-light traps, namely 34% and 33% respectively. All results are shown in table 5 and are graphically represented in graph 3.

THE BITING RATE OF *CULICOIDES* WITH SHEEP		
	Blood-fed *Culicoides spp.*	Blood-fed *C. obsoletus*
Sheep without inner tent	77%	67%
Black light trap	34%	33%

Table 5: the biting rate of Culicoides en C. obsoletus with sheep, shown by the percentage of blood-fed midges trapped in the sheep mosquito trapping tents without an inner tent and in the black light trap.



Graph 4: Illustration of the percentages of blood-fed Culicoides spp. and C. obsoletus caught in tent traps with sheep without an inner tent. The results of the black light trap are shown in red.

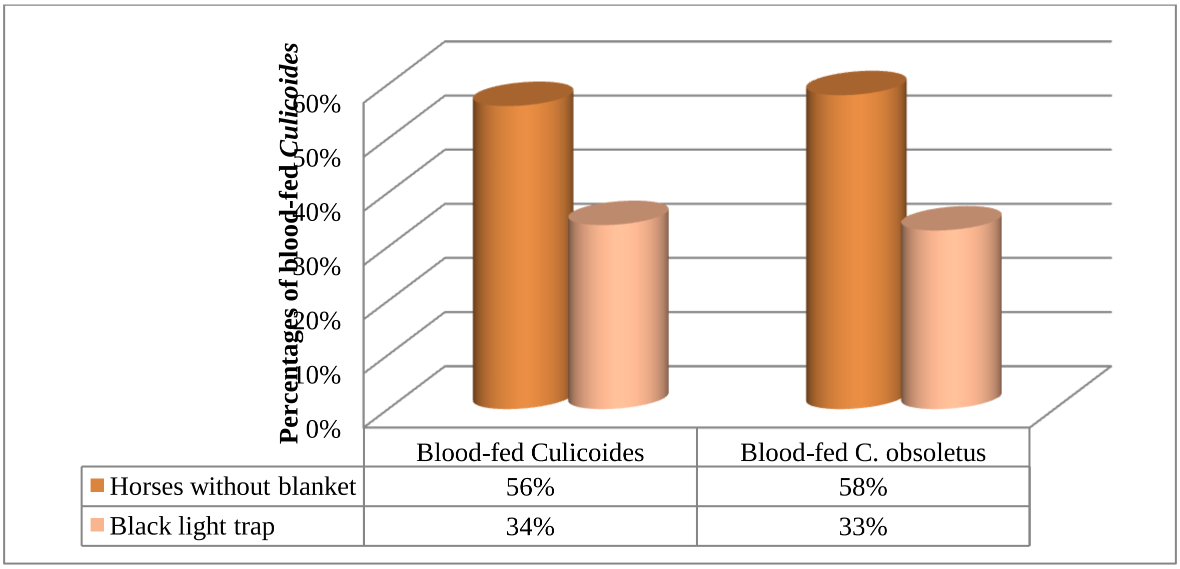
Biting rate of Culicoides in horses

The second part of the research question was to determine the normal biting rate of *Culicoides spp* and *C. obsoletus* in horses. This was done by calculating the percentage of blood-fed midges in the vicinity of horses without a blanket (table 6). For *Culicoides spp*. a biting rate of 56% was found. The percentage for *C. obsoletus* was 58%.

In the direct surroundings of horses without insect blankets the average feeding rate of all *Culicoides* midges and *C. obsoletus* was compared with the percentage of blood-fed midges caught in the black-light trap, namely 34% and 33% respectively. The results are shown in table 6 and Graph 5.

BITING RATE OF *CULICOIDES* WITH HORSES		
	Blood-fed *Culicoides spp.*	Blood-fed *C. obsoletus*
Horses without blanket	56%	58%
Black light trap	34%	33%

Table 6: the biting rate of Culicoides and C. obsoletus with horses, shown by the percentage of blood-fed midges trapped in de horse trap tents and without using an insect blanket and in the black light trap.



Graph 5: Illustration of the percentages of blood-fed Culicoides spp. and C. obsoletus caught in tent traps with horses without using an insect blanket and in the black light trap.

Discussion

This study reaffirmed the usefulness of the tent traps designed by Van der Rijt et al. and Griffioen et al. It allowed us to gather insect in the vicinity of horses and sheep with as little damage to the insects as possible. Because of that the distinction between *Culicoides* and other insects as well as the determination of the different *Culicoides* species could be made more accurately and swiftly. Another advantage of the mosquito trapping tents is that *Culicoides* are attracted by real animals instead of artificial attractants like UV-light or CO_2 (Griffioen, 2010). A disadvantage of these tents is the amount of manual labor involved in preparing and dismembering them. Furthermore, constant supervision is needed to assure the safety and welfare of the animals used in the experiment. During this study it became clear that it would not be possible to do this experiment without tame animals.

Unfortunately, our study was done at the end of the summer season in August and September at which time only few evenings had the right weather conditions for our tests. Moreover, it should be noted that the summer of 2011 was not the best of summers for insects in general due to pour weather conditions. This had a negative influence on the total amount of trapped *Culicoides* midges.

In our study, the black light trap proved to attract most insects both during and after sunset. The study of Meiswinkel et al. affirmed that active *Culicoides* will not be attracted by a black light trap, when the trap has to compete against natural light (Meiswinkel et al., 2008b). During the day the vent will only draw down insects that accidentally fly by. So, the sessions with the animals ended approximately at sunset, but the black light trap was not turned off until almost an hour after sunset. At that moment all *Culicoides* were already collected from the mosquito trapping tents. However, most insects in the black light trap were not trapped during, but after the trapping sessions with animals.

<u>Biting rate:</u>

In this paper the normal biting rate of *Culicoides*, caught in the vicinity of sheep and horses has been described. The biting rate is a very important parameter of ailments, which are transferred by biting insects (Dye, 1992; Gubbins et al., 2008) such as AHS (Lord et al., 1996). Thus several studies were conducted concerning the biting rate of midges in a variety of –farm- animals (Skierska, 1973; Carpenter et al., 2008). Likewise, the biting rate of the midges proves to be an important parameter for the degree of distribution of AHS in horses (Gerry et al., 2009). Furthermore, it is to be expected that protection measures aimed at reducing the biting rate are most efficient to fight AHS.

A possible solution might be accommodating horses along with sheep. This lessens the density of the herd of horses, because less horses can be in the same place when the herd is mixed with sheep (dilution tactic). *Culicoides spp.* also bite sheep, but sheep are immune for AHS. This dilution tactic is probably efficient as long as the number of *Culicoides* attracted by the mixed herd remains constant. However, the latter is still debatable. For example, the degree at which sheep attract *Culicoides* and the biting rate at horses with and without the presence of sheep is still undetermined and it might be possible that the different midges have a host-preference.

These uncertainties cause that the value of a dilution tactic as a protection measure against the distribution of AHS could not be determined in the present study.

Black light trap as a control:

To evaluate the biting rate of *Culicoides* midges in the vicinity of sheep and horses, it must be taken into account that a certain percentage of blood-fed midges are present in the area of the traps. So there is no guarantee that the blood found in the insects in de animal tent traps is from the tested animals in the tents. It might be that the insects fed on other animals before entering the mosquito trapping tent and were accidentally trapped. This is confirmed by the average percentages of blood-fed *Culicoides* (34%) and *C. obsoletus* (33%) in the Onderste Poort black light trap. So therefore, it is assumed that these are the standard numbers of blood-fed midges in an midges population.

Recommendations:

The number of *Culicoides* caught are relatively low during the present study, which makes it difficult to draw hard conclusions. If the number of *Culicoides* caught had been higher, the conclusions would have been more clearly supported by data. Previous experiments had shown that a dry, windless and warm (summer) evening would be the best time to catch *Culicoides* (van den Boom et al., 2008; van der Rijt et al., 2008; Griffioen et al., 2010). A recommendation for another field study would be to start catching the *Culicoides* earlier in the mosquito season, which is from May to September. Then the odds will be higher that the weather conditions are suitable to perform the tests and so ensure more successful trapping sessions. Another advantage of starting earlier in the season is that there is more time to organize a larger number of trapping sessions during the whole season which will provide a higher amount of *Culicoides* which means that more data can be found. Moreover, fluctuations in numbers of caught *Culicoides* over the season can be integrated into the study, which corresponds with test results of a Belgian study, in which midges were captured from May to November with a peak in June and September (Fassotte et al., 2008).

Another recommendation is to organize only one session per evening which should be conducted during sunset. The best time to trap the midges is around sunset, because the midges are most active at that time of the day (van den Boom et al., 2008; van der Rijt et al., 2008; Griffioen et al., 2010). Moreover, it is easier to compare sessions and numbers of trapped *Culicoides* when all test are preformed at the same moment of the day.

In addition, the percentages blood-fed *Culicoides* in the area around the animals can only be accurately determined when the black lights are turned off at the same moment the trapping session in the tent ends. Our study failed to do that, but if another study would take up my advice the percentage of the *Culicoides* in the black light trap could be compared to the percentages found in the tent traps because of the identical time span.

Conclusions

In the present study the biting rate of *Culicoides* caught in the proximity of sheep is 77% for *Culicoides spp*. and 67% for *C. obsoletus*. The biting rate of *Culicoides* caught in the proximity of horses is 56% for *Culicoides spp*. and 58% for *C. obsoletus* in this research. The biting rate of *Culicoides spp*. is 21% in the tests with an inner tent and 77 % in the tests without an inner tent. The corresponding odd ratio is 8.77 for *Culicoides spp*.

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Annex I: the results of each test run

Red is blood-fed

RS proef vangst

14/aug/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
6	4	31	8	3	0	0	0	52	1	52

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
4	8	13	8	0	0	2	0	35	1	35

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
2	6	5	8	0	0	0	2	23	1	23

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. nubeculosu	TOTAAL	Aantal uur	Culicoides per uur
48	2	42	1	0	0	1	0	1	95	5,25	18,10

RS vangst 1

20/aug/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
0	12	2	23	0	0	0	0	37	1	37

Paard 1 Deken

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
1	6	3	21	1	0	0	0	32	1	32

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
1	8	12	20	0	0	0	0	41	1	41

Onderste poort val loopt door in vangst 2

RS vangst 2

20/aug/11

Schaap Binnentent

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
1	0	1	2	0	0	0	0	4	1	4

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
1	4	4	19	1	0	0	0	1	30	1	30

Paard 2 Deken

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
4	1	0	7	2	0	0	0	14	1	14

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
23	104	1	7	6	9	0	4	1	155	4,08	37,96

RS vangst 3

22/aug/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
6	32	60	367	0	0	0	0	2	467	1,08	431,08

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
0	1	10	35	0	0	0	0	46	1	46

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
0	6	2	9	1	0	0	0	2	20	1	20

Onderste poort val loopt door in vangst 4

RS vangst 4

22/aug/11

Schaap Binnentent

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
4	3	6	18	0	0	0	2		33	1,08	30,46

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
8	31	13	37	2	0	1	0		92	1	92

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. pulicaris	TOTAAL	Aantal uur	Culicoides per uur
3	17	1	22	0	0	0	0	1	44	1	44

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
4	10	0	1	0	0	0	0		15	3,92	3,83

MP vangst 1

15/aug/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
4	11	37	20	3	0	0	0	75	1,37	54,88

Paard 1 MISLUKT

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
x	x	x	x	x	x	x	x	x	x	x

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
8	2	13	5	0	0	0	0	28	1,02	27,54

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
0	0	0	0	0	0	0	0	0	4,08	-

MP vangst 2

16/aug/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
1	0	4	8	0	0	0	0	13	0,88	14,72

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
0	0	3	0	0	0	0	0	3	1	3

Paard 2 Deken

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
0	0	8	2	0	0	0	0	10	1	10

Onderste poort val loopt door in vangst 3

MP vangst 3

16/aug/11

Schaap Binnentent

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
3	0	40	0	0	0	0	0	43	1,22	35,34

Paard 1 Deken

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
5	0	5	3	0	0	0	0	13	1	13

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
8	3	58	21	0	0	0	0	90	1,03	87,10

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	TOTAAL	Aantal uur	Culicoides per uur
2	0	3	0	0	0	2	1	8	4,92	1,63

MP vangst 4

17/aug/11

Schaap Binnentent

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
0	0	3	0	0	0	0	0	1	4	1	4

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
0	0	11	3	0	0	0	0		14	1	14

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
0	0	15	4	0	0	0	0		19	1,03	18,39

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
0	0	2	0	0	0	0	0		2	2,92	0,69

MS vangst 1

1/sep/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
4	11	10	39	0	0	0	0	1	65	1,3	50

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
3	7	6	4	4	1	0	0		25	1	25

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
18	15	27	23	1	0	0	1		85	1	85

Onderste poort val loopt door in vangst 2

MS vangst 2

1/sep/11

Schaap

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
23	11	0	4	1	0	0	0		39	1,12	34,93

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
232	94	4	47	18	6	0	0	2	403	1	403

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
438	206	6	20	33	15	11	1		730	1	730

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
10	0	0	0	1	0	0	0		11	4,5	2,44

MS vangst 3

2/sep/11

Schaap Binnentent

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
6	0	9	0	0	0	0	0		15	1,18	12,68

Paard 1 Deken

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
1	1	1	1	0	0	0	0	1	5	1	5

Paard 2

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
7	3	7	0	0	0	0	0	1	18	1	18

Onderste poort val loopt door in vangst 4

MS vangst 4

2/sep/11

Schaap Binnentent

C. obsoletus	C.obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
8	0	13	0	1	0	0	0		22	1,12	19,70

Paard 1

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus		TOTAAL	Aantal uur	Culicoides per uur
83	167	15	15	2	0	0	0		282	1	282

Paard 2 Deken

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. vexans	TOTAAL	Aantal uur	Culicoides per uur
196	80	42	8	0	0	0	0	2	328	1	328

Onderste poort val

C. obsoletus	C. obsoletus	C. chiopterus	C. chiopterus	C. dewulfi	C. dewulfi	C. punctatus	C. punctatus	C. nubeculosu	TOTAAL	Aantal uur	Culicoides per uur
858	329	67	35	7	1	2	2	1	1302	4,93	263,92

Annex II: results Petra de Jong

(de Jong., 2012)

In the direct surrounding of horses wearing insect blankets the average feeding rate of all *Culicoides* midges was comparable with that of the midges caught in the black-light trap, namely 32% and 34%. Also the average feeding rate of *C. obsoletus* was comparable with that of the midges caught in the black-light trap, namely 30% and 33%. In the direct surrounding of horses without a blanket the average feeding rate of all *Culicoides* midges was increased to 56 % and for the *C. obsoletus* it was increased to 58%.
This means that the odds of blood-fed *Culicoides spp*. found was 3.57 times higher in the group without a blanket than with a blanket. This means that using an insect blanket significantly decreased the percentage of blood-fed *Culicoides* spp..