

# Culicoides

Author: Dr. Gert Venter

Licensed under a [Creative Commons Attribution license](https://creativecommons.org/licenses/by/4.0/).



## TABLE OF CONTENTS

---

<b>INTRODUCTION</b> .....	<b>3</b>
<b>IMPORTANCE</b> .....	<b>3</b>
<b>DISEASE TRANSMISSION</b> .....	<b>4</b>
Biological transmission of arboviruses .....	5
Vectors and vectorship .....	7
Vector capacity and vector competence .....	7
Artificial infection methods .....	8
Vector species in southern Africa .....	9
<b>IDENTIFICATION/DIFFERENTIAL DIAGNOSTICS</b> .....	<b>12</b>
Biology/ecology/life cycle.....	14
Larval habitats.....	16
<b>DISTRIBUTION</b> .....	<b>17</b>
Geographical and seasonal abundance of livestock-associated <i>Culicoides</i> species in South Africa	17
<b>CONTROL</b> .....	<b>19</b>
Integrated control .....	20
<b>THE COLLECTION OF ADULT CULICOIDES</b> .....	<b>22</b>
Light traps .....	23
Truck traps.....	23
Aspirators and ‘sweeping’.....	24
Emergence traps .....	24
Drop traps and animal bait-traps .....	25



Storing of specimens ..... 25

**REFERENCES ..... 25**

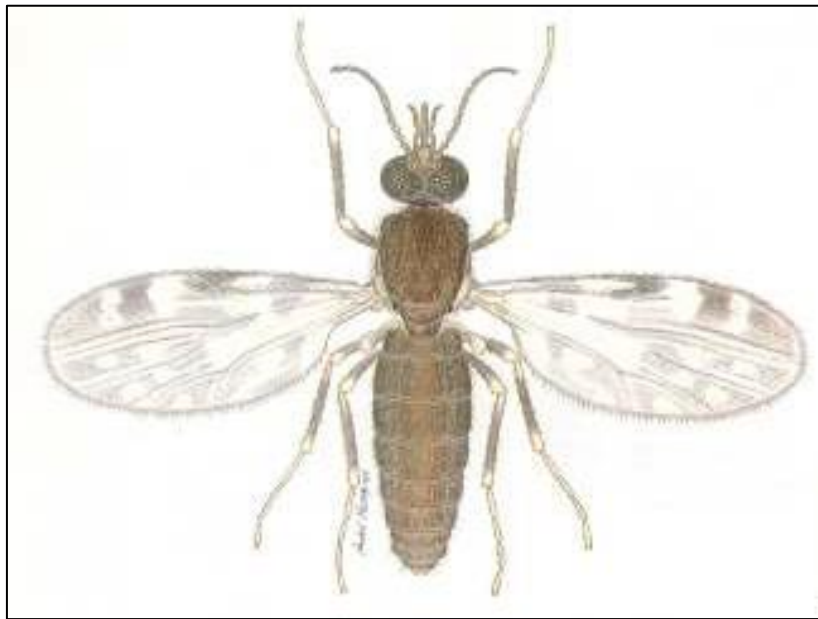
Websites: ..... 26



## INTRODUCTION

---

Biting midges in the genus *Culicoides* (Diptera: Ceratopogonidae) are like mosquitoes in that the females of nearly all species need a blood meal in order to develop eggs. These midges are smaller (1 – 3mm) than mosquitoes and can be much more abundant. More than a million blood-seeking females can be captured on a warm summer night with a single light trap near livestock. Although some European *Culicoides* species are notorious for their man biting habits most southern African species prefer to feed on animals and are night active and you will hardly notice them flying around.



***Culicoides zuluensis* female (Meiswinkel et al, 2004)**

The first reference to these insects is by reverend W. Derham who described their life history and biting habits in 1731. The first research on sub-Saharan *Culicoides* dates to 1908 when two species were described from Namibia. In 1944 Rene du Toit, from the ARC-Onderstepoort Veterinary Institute, show that these midges can play an important role in the transmission and spread of viruses that cause animal diseases such as bluetongue and African horse sickness.

Taking into account the enormous numbers in which these midges can occur, their notorious man biting habits in some countries and their ability to transmit deadly diseases it has been suggested that these midges were involved in two of the ten biblical plagues of ancient Egypt.

## IMPORTANCE

---

Female *Culicoides* midges feed on a broad spectrum of hosts including reptiles, mammals, birds, man, and even blood-engorged mosquitoes. They are a severe biting nuisance to humans in certain parts of the world, can cause an acute allergic dermatitis in horses (sweet-itch), and are biological vectors of viruses, protozoa and filarial nematodes affecting birds, humans, and other animals.

Arthropod vectors → *Culicoides*

As vectors of viruses, *Culicoides* species are of the greatest veterinary importance. More than 75 arboviruses, belonging mostly to *Bunyaviridae*, *Reoviridae* and *Rabdoviridae* families, were isolated from different *Culicoides* species worldwide. Among viruses transmitted by *Culicoides* species, those causing bluetongue (BT), African horse sickness (AHS), equine encephalosis (EE), epizootic haemorrhagic disease (EHD) and Akabane (AKA) disease are of major veterinary significance.

Bluetongue, EHD and AHS are listed by the Office Internationale des Epizooties (OIE) or the World Organisation for Animal Health as notifiable diseases. The unexpected outbreaks and apparent overwintering of BT in northern Europe, followed by outbreaks and the detection of a new orthobunyavirus, Schmallenberg virus in Germany has without doubt illustrated its devastating effect on livestock in countries with large populations of susceptible animals. African horse sickness can cause up to 90% mortality in susceptible equines. The endemic presence of AHS in southern Africa greatly impedes the movement of horses from South Africa to rest of the world. (See High Impact Diseases: African horse sickness).

## DISEASE TRANSMISSION

---

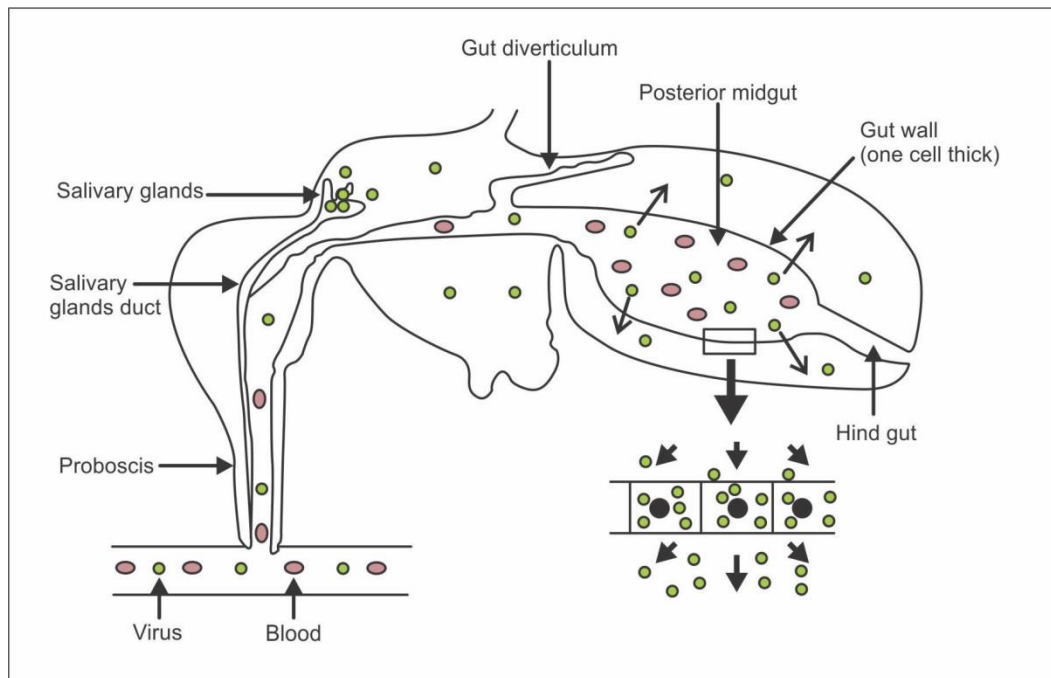
In 1943 Du Toit conducted the first successful transmission of BTV from infected *Culicoides* midges to susceptible sheep. He was able to infect healthy sheep with BT by exposing them to the bites of midges which have fed 10 days earlier on sheep suffering from BT. He also successfully infected a horse with AHSV by *Culicoides* bite. Du Toit's pioneering work involving BTV was repeated at several laboratories worldwide and it is currently accepted that both AHSV and BTV are transmitted between their hosts almost entirely by the bites of *Culicoides* midges. Distribution of these diseases is restricted to areas where competent vector species occur and transmission is limited to those times of the year when adult insects are active. In endemic areas this usually occurs during the late summer and autumn, notably when outbreaks of AHS and BT are the highest.



**Dr R.M. du Toit examining a *Culicoides* trap**

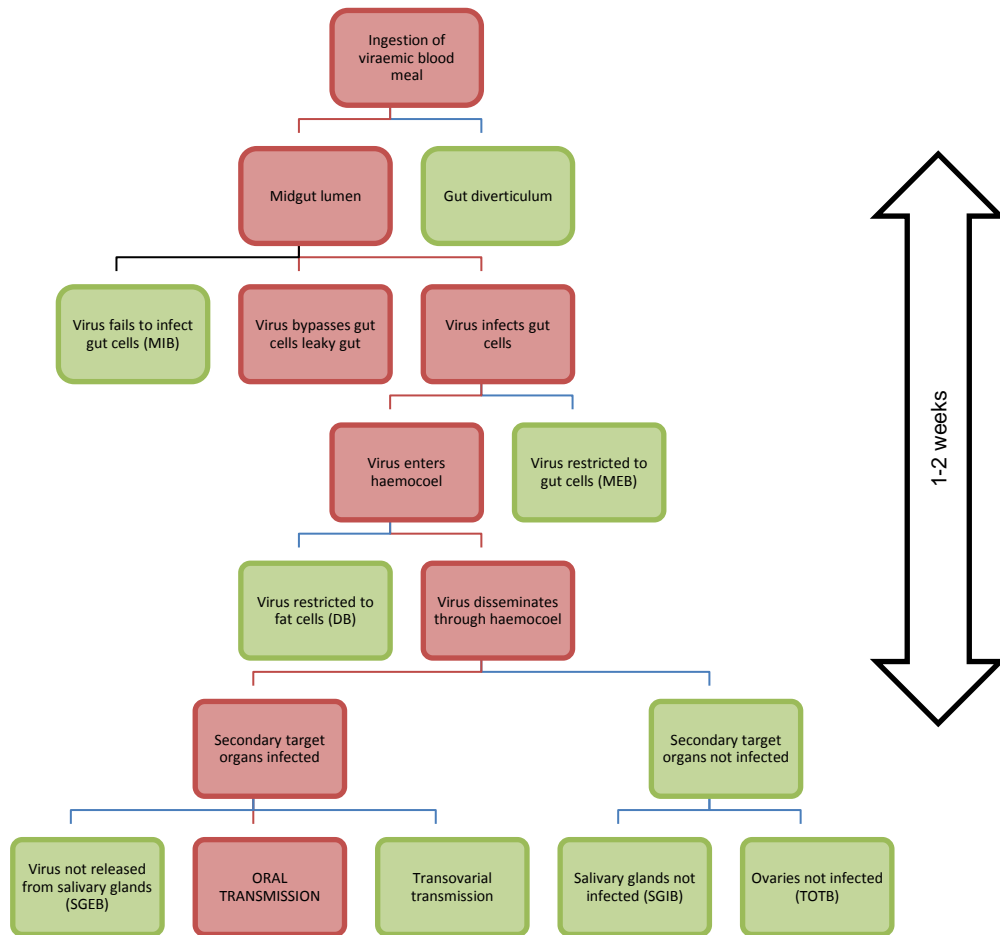
## Biological transmission of arboviruses

The period from ingestion of a virus infected blood meal to transmission capability is called the extrinsic incubation period. During this period, the virus infects and replicates in the midgut epithelial cells and then disseminates to infect secondary target organs. Virions disperse in circulating haemolymph. Once the virus is in the salivary ducts, the virus can be transmitted to vertebrates during a blood meal. The duration of extrinsic incubation in a poikilothermic vector depends on the temperature. Within limits, higher temperature shortens the extrinsic incubation period.



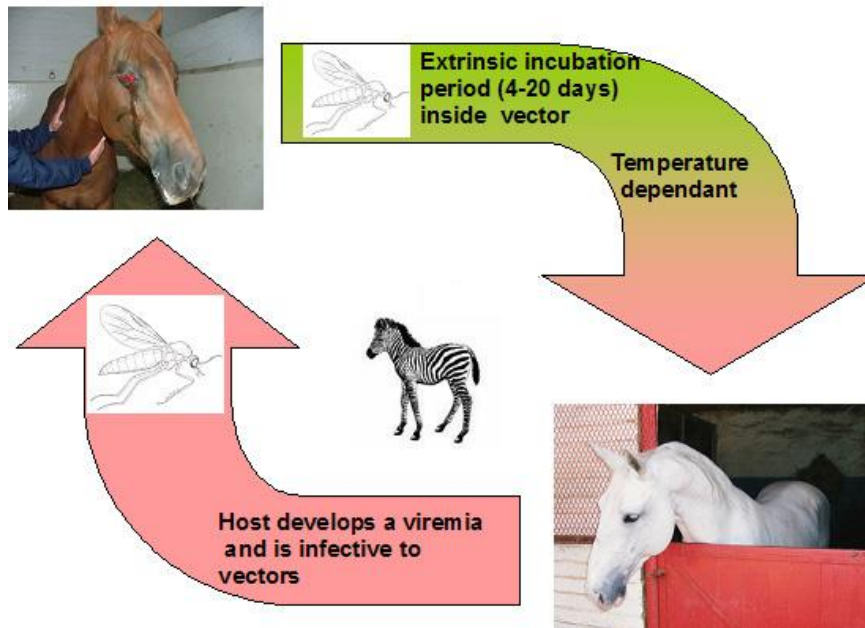
**Schematic cycle of arbovirus infection in *Culicoides* species.**

A number of barriers to arbovirus infection appear to exist, including mesenteron infection escape barriers, dissemination barriers, transovarial transmission barriers, and salivary gland infection escape barriers. In the North American *C. sonorensis*, the most important of these appeared to be the mesenteron infection barrier, which control initial establishment of persistent infection, the mesenteron escape barrier which can restrict virus to gut cells and the dissemination barrier which can prevent virus which enters the haemocoel from infecting secondary target organs. Although the expression of these barriers appeared to be genetically controlled, they can be bypassed by mechanical rupture of the midgut by e.g. filarial worms. An arbovirus must first infect and replicate in the salivary glands before it can be transmitted during subsequent feeding on a susceptible host. The time from when the vector had ingested infected blood meal to excretion of the virus in the saliva is temperature dependent and takes one to two weeks. In *C. sonorensis*, an apparent ovarian barrier prevents transovarial transmission. However, recent studies demonstrated the presence of BTV nucleic acid by nested RT-PCR in *C. sonorensis* larvae reinforcing the possibility of transovarial transmission of orbiviruses by *Culicoides* species.



**Hypothesized barriers to arbovirus infection in haematophagous insects. (Adapted from Mellor et al. 2000). MIB=midgut infection barrier, MEB=midgut escape barrier, DB=dissemination barrier, SGEB=salivary gland escape barrier, SGIB=salivary gland infection barrier and TOTB=transovarial transmission barrier.**





African horse sickness virus transmission cycle

## Vectors and vectorship

The successful transmission of an arbovirus, from an infected to a susceptible host, is dependant upon the complex relationship that exists between the virus, its insect vector, the vertebrate host, and environmental conditions. Just as finding an organism in a diseased tissue is not sufficient proof that the organism is the cause of that disease, isolation of a virus from an insect is insufficient evidence for differentiating true vectors from those species that are only incidentally infected because of the high titres of virus in the blood of the infected host. The presence of a *Culicoides* species and even the isolation of a specific virus from a species are, therefore, not evidence of vectorship or the vectorial capabilities of that species.

To prove vector status all four of the following criteria must be met:

- The isolation of the disease-producing agent from field collected specimens,
- The demonstration of its ability to become infected by feeding upon a viraemic host,
- The demonstration of its ability to transmit by bite,
- The confirmation through field evidence of the association of the infected arthropod with the vertebrate population in which the infection is occurring.

## Vector capacity and vector competence

Vectorial capacity refers to the ability of a vector population to transmit a pathogen. It can be defined as the average number of infective bites that will be delivered by a *Culicoides* midge feeding on a single host animal in one day and is a combination of a midge density in relation to the host animal, host preference, midge biting frequency, life-span of infected midge, duration of viraemia and vector competence.

Arthropod vectors → *Culicoides*

Vector competence is one of the factors which influences vectorial capacity and refers to the ability of a vector to support virus infection and replication and/or dissemination. It is a measure of the number of midges that actually become infective after feeding on a viraemic host and is dependent upon the genetic makeup of the vector midge and upon external environmental factors.

A competent vector may have a low vectorial capacity due to low biting rates or survivorship, while a vector with low competence may be more efficient in virus transmission. For example, in Australia *Culicoides brevitarsis* has a low competence for BTV, but effectively transmits the virus due to its high biting rate, while *Culicoides fulvus* which is more competent, has a lower vectorial capacity due to lower abundance and limited geographical distribution.

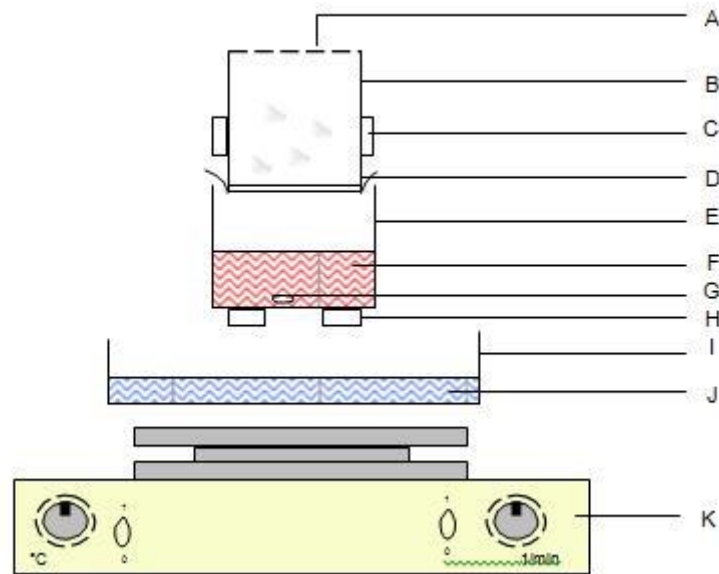
The ability of a *Culicoides* species to become infected with and transmit viruses, coupled to the seasonal abundance and host preference, is one of the factors that determine the role a *Culicoides* species will play in the occurrence and spread of the disease. Not all midges become infective and are able to transmit virus after feeding on a viraemic host. The genetic makeup of the midge and a variety of environmental factors influence this ability that can be assessed by artificial feeding of midges on blood spiked with virus followed by incubation of engorged females under defined laboratory conditions and their subsequent testing for viral infectivity. In this way it can be determined which midge species may play a role in the transmission of the viruses that cause these diseases and will help to predict outbreaks and to control them.

### Artificial infection methods

Methods for the artificial infection of *Culicoides* midges include the use of infected hosts, embryonated chicken eggs, intrathoracic inoculation of the virus directly into the haemocoel of the midge, oral infection of *Culicoides* midges with virus using fine needles and feeding of *Culicoides* midges on cotton wool pledgets drenched with virus infected blood or membrane feeding methods. Infected hosts are the most reliable method to use, but large numbers of *Culicoides* midges must be available at the time the infected host displays high viraemia levels. Therefore, this method is only feasible when a *Culicoides* laboratory colony is available. The use of susceptible animals for transmission study with orbiviruses is expensive, time consuming and requires large laboratory space and insect proof stables. An alternative method is to use embryonated chicken eggs. With intrathoracic inoculation the gut barrier is bypassed and species which are not susceptible after oral ingestion of the virus may become infected. Cotton wool pledgets soaked with a blood/virus mixture are an easy and relatively inexpensive to use in large scale laboratory trials. A drawback of this method is that many arboviruses are cell-associated and the cells settle differently in a pledget. As a result, the *Culicoides* females might be feeding only on the serum dripping from the pledget. Therefore relatively high levels of virus are required to successfully infect *Culicoides* midges.







**Blood feeding device for field-collected *Culicoides* species**

- A Gauze top
- B Feeding chamber (40mm diameter plastic “pill-bottle”)
- C Foam rubber
- D One day old chicken skin membrane
- E Blood container (45mm diameter plastic “pill-bottle”)
- F Blood/virus mixture kept at  $\pm 37^{\circ}\text{C}$
- G Magnetic stirrer bar
- H Rubber stopper support
- I Water bath
- J Water
- K Magnetic heater/stirrer

## Vector species in southern Africa

The first *Culicoides* vector competence study was conducted by Du Toit in 1944 at Onderstepoort. He fed field collected *C. imicola* on BTV-infected sheep, and after an extrinsic incubation period of 10 days, was able to transmit the disease to susceptible sheep. Similarly he also infected a horse with AHSV by *Culicoides* bite. These seminal findings by du Toit showed that *Culicoides* midges was involved in the transmission of viruses were later confirmed in the USA, Australia and England.

Subsequent oral susceptibility studies at Onderstepoort indicated that BTV can be replicated in at least 12 (seven subgenera) of more than 22 stock-associated *Culicoides* species tested in the laboratory.

Similarly it was shown that EHDV can replicate in at least 11 (seven subgenera) and that equine encephalosis virus (EEV) and AHSV can replicate in six (five subgenera) and 11 (eight subgenera) *Culicoides* species respectively.

These oral susceptibility results are supported by virus isolation from field collected midges. Field isolations of BTV were made from at least six different stock-associated field collected *Culicoides* species.

Arthropod vectors → *Culicoides*

Field isolations of AHSV and EEV were made from two different stock-associated field collected *Culicoides* species.

In addition Akabane and bovine ephemeral fever (BEF) virus have been isolated on several occasions from various field collected South African and Australian *Culicoides* species.

Oral susceptibility studies in South Africa indicated that *C. bolitinos* can be up to ten times more susceptible to infection with BTV than *C. imicola*; the latter is the most abundant *Culicoides* species and the only proven BTV vector in South Africa. BTV is able to replicate at lower temperatures in *C. bolitinos* than in *C. imicola*. While *C. imicola* can become super abundant in the warm tropical parts of South Africa, *C. bolitinos* are more adapted to cooler temperatures and can become the dominant *Culicoides* species in the cooler areas of South Africa.

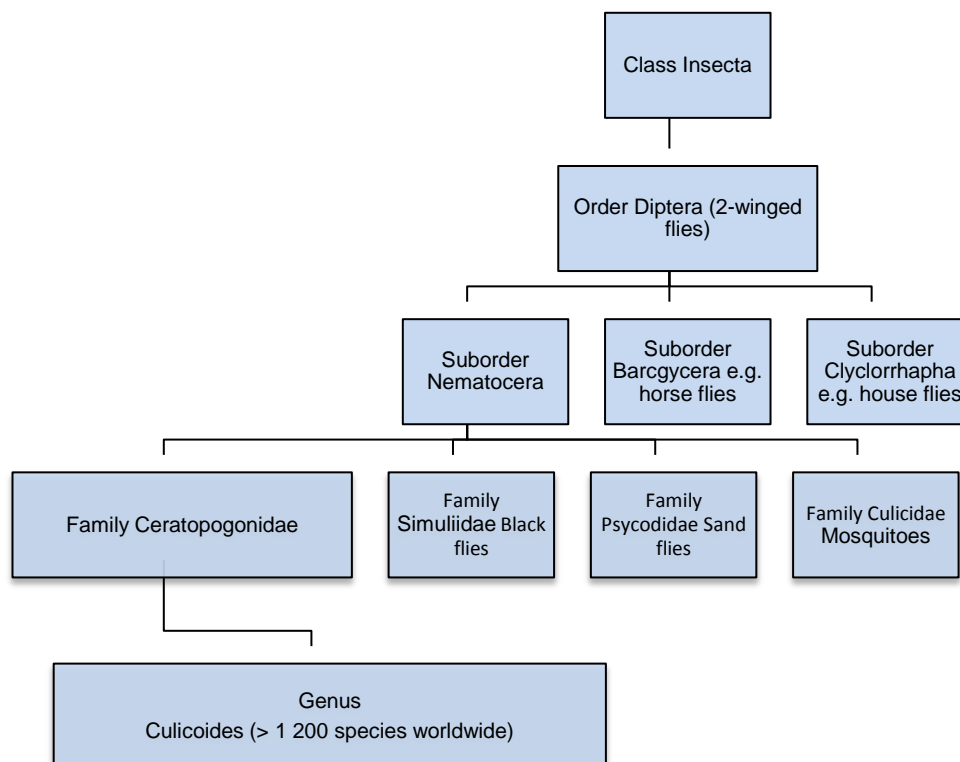
**South African *Culicoides* species from which orbiviruses could be isolated 10 days after feeding on an infected blood meal in the laboratory (lab) or from field collected specimens (field).**

	BT		EHD		AHS		EEV	
	lab	field	lab	field	lab	field	lab	field
<i>C. imicola</i>	+	+	+		+	+	+	+
<i>C. bolitinos</i>	++	+	+		+	+	+	
<i>C. gulbenkiani</i> <i>C. tutti-frutti</i> <i>Hoffmania</i>	+		+					
<i>C. zuluensis</i>								+
<i>C. milnei</i> <i>C. magnus</i> <i>C. brucei</i> <i>Remmia</i>	+		+		+		+	
<i>C. enderleni</i>					+			
<i>C. nevillei</i>	+							
<i>C. schultzei</i> <i>Meijerehelea</i> <i>C.</i> <i>leucostictus</i> <i>Beltranmyia</i> <i>C.</i> <i>nivosus</i>			+		+			
<i>C. pycnostictus</i>			+					
<i>Pontoculicoides</i>	+	+					+	

Arthropod vectors → *Culicoides*

<i>C. engubandei</i>				+
<i>Synhelea C. dutoiti</i>				
<i>Monoculicoides C. cornutus</i>				
<i>C. bedfordi</i>				+
<i>C. huambensis</i>	+			
<i>C. expectator</i>	+	+		+

---



**Systematic classification of the genus *Culicoides***

The genus *Culicoides* resort under the suborder Nematocera. The Nematocera tend to be small, fragile insects with long antennae, from which they derive their name (Gr. *nema*, thread; Gr. *heras*, horn.) The family Ceratopogonidae consists of the midges. The important blood-sucking varieties are confined to the genera *Culicoides* and *Leptoconops*. Flight is limited but they may travel long distances with the prevailing wind. Feeding is largely restricted to the night and, being pool feeders, the bites are painful. In this group the immature stages are always aquatic or semi-aquatic and the adult females are bloodsuckers. Both the males and females feed on plant juices. The family Ceratopogonidae is distinguished by their 15-segmented antennae, which are characterized by sexual dimorphism, and their distinctive wing venation.

*Culicoides* biting midges must not be confused with black flies (*Simulium* species), which can also occur in immense numbers, causing severe irritation and disruption in the normal activities of both man and beast

Arthropod vectors → *Culicoides*

with their bites. Black flies are short stout midges; also about 3 mm long but in contrast to *Culicoides* species, are day active, and have short horn-like antennae with a strongly humped thorax, broad clear wings and short legs.

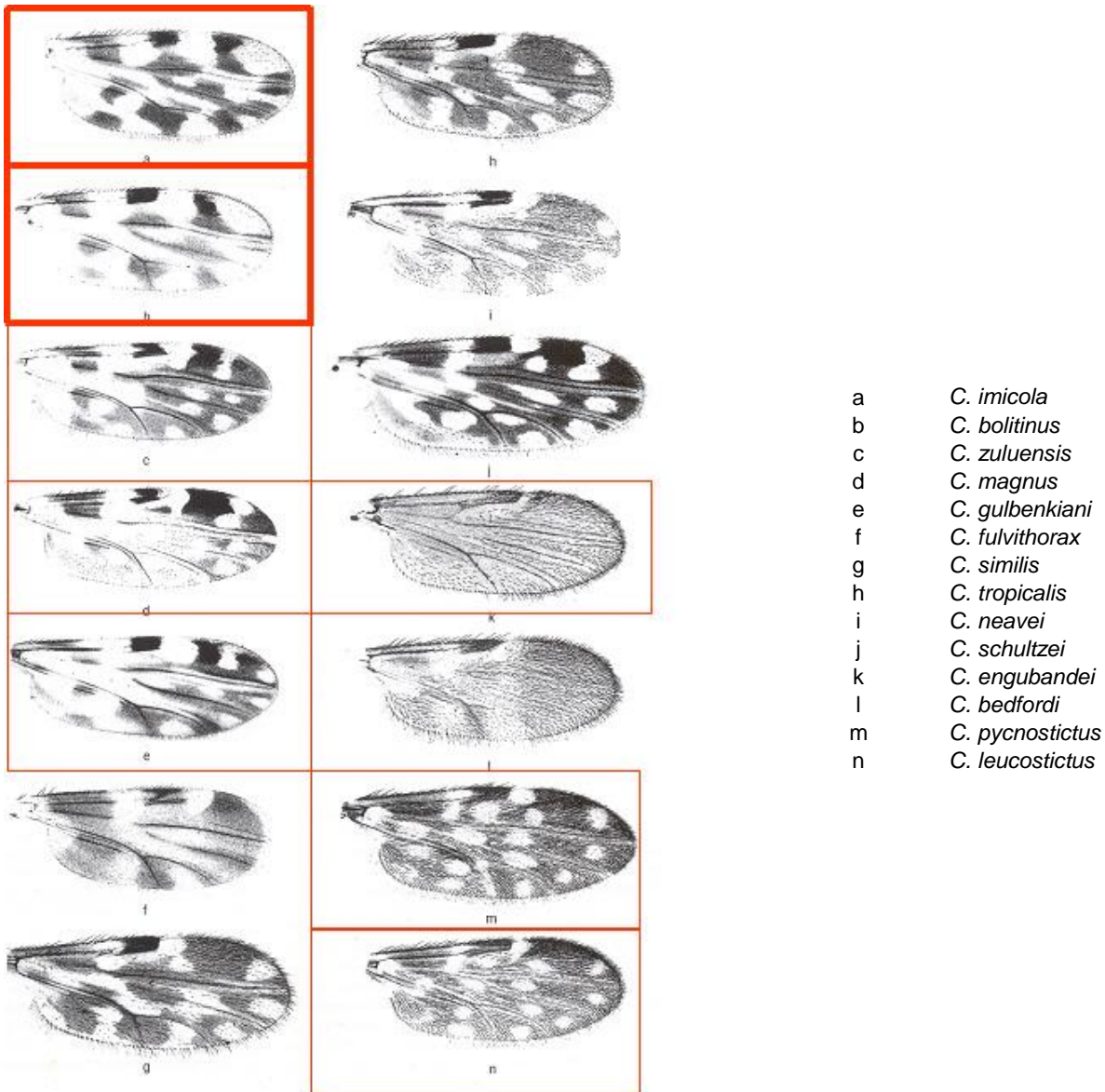
## IDENTIFICATION/DIFFERENTIAL DIAGNOSTICS

---

At least 120, of the more than 1 320 species of *Culicoides* species described worldwide, are found in South Africa. Each of these is unique concerning the animals they will bite, the places where they will breed and disease agents they are able to transmit.

Most *Culicoides* midges have a wing pattern that is composed of grey and white spots; these patterns are unique to each species. These patterns can be fairly easily observed under a dissecting microscope and many species are quite easily separable on wing pattern. Within subgenera the patterns become more broadly similar. Subgenera can often comprise five or more species complexes. Within species complexes identifications based on wing patterns alone become unreliable. Ten per cent of African *Culicoides* species lack a wing pattern, and so for reliable identification such species (indeed for all species in all complexes) specimens must be dissected and mounted on microscope slides and examined at 100-400x magnification.

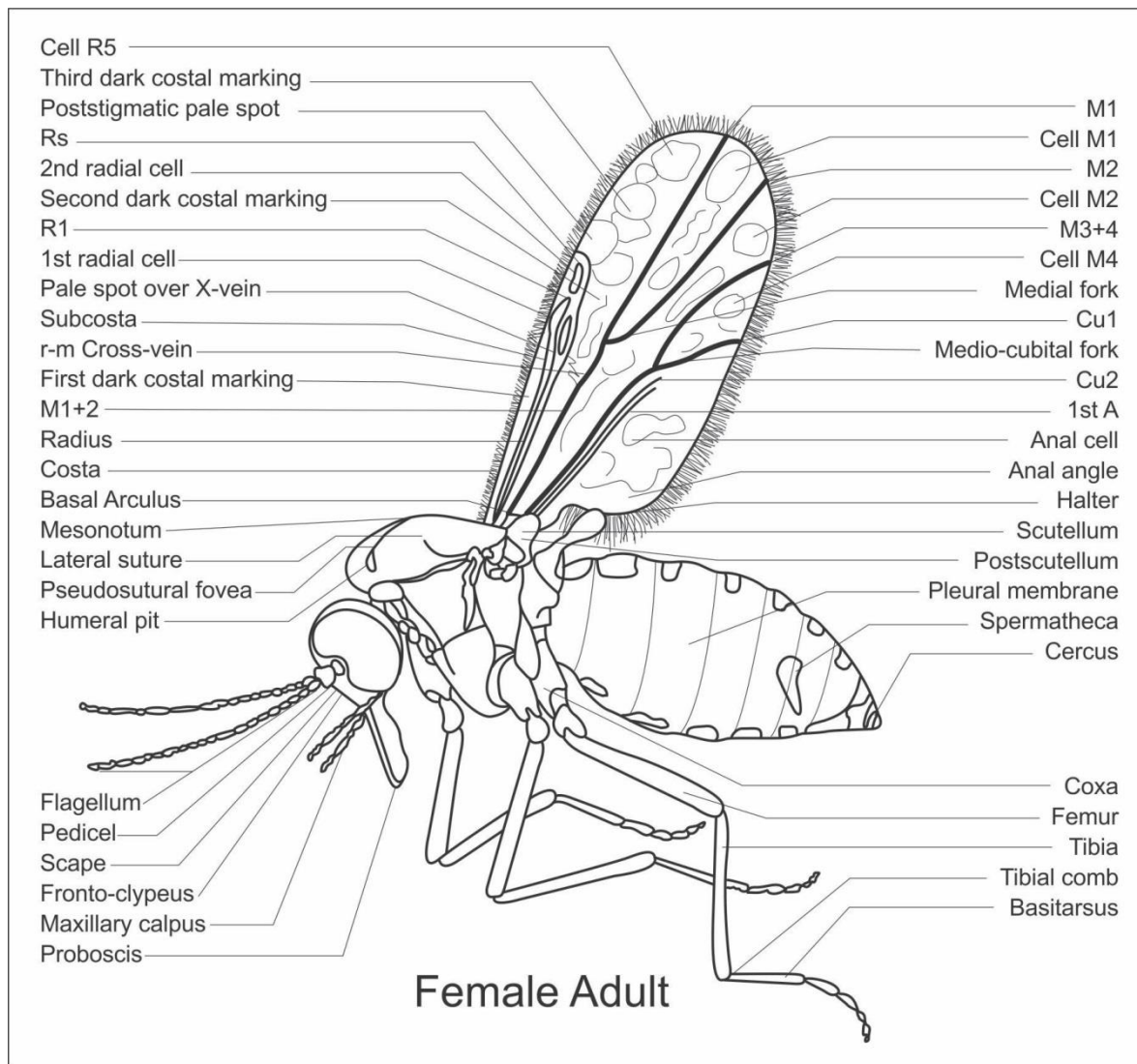




**A wing of each of the 14 species of *Culicoides* commonly found associated with livestock in southern Africa**

In slide-mounted specimens identification of the female is based on the shape of the spermathecae and their number, the shape of the third palpal segment and the manner in which the sensillae are distributed upon it, on the conformation of the space between the eyes, and on whether the chitinous areas between the ocelli are adorned with hairs or not. Perhaps the most useful taxonomic aid for the identification of females is the precise number and arrangement of each of the seven types of sensillae to be found on the antenna. The shapes of the various parts of the genitalia are highly species-specific and are always used in identification.





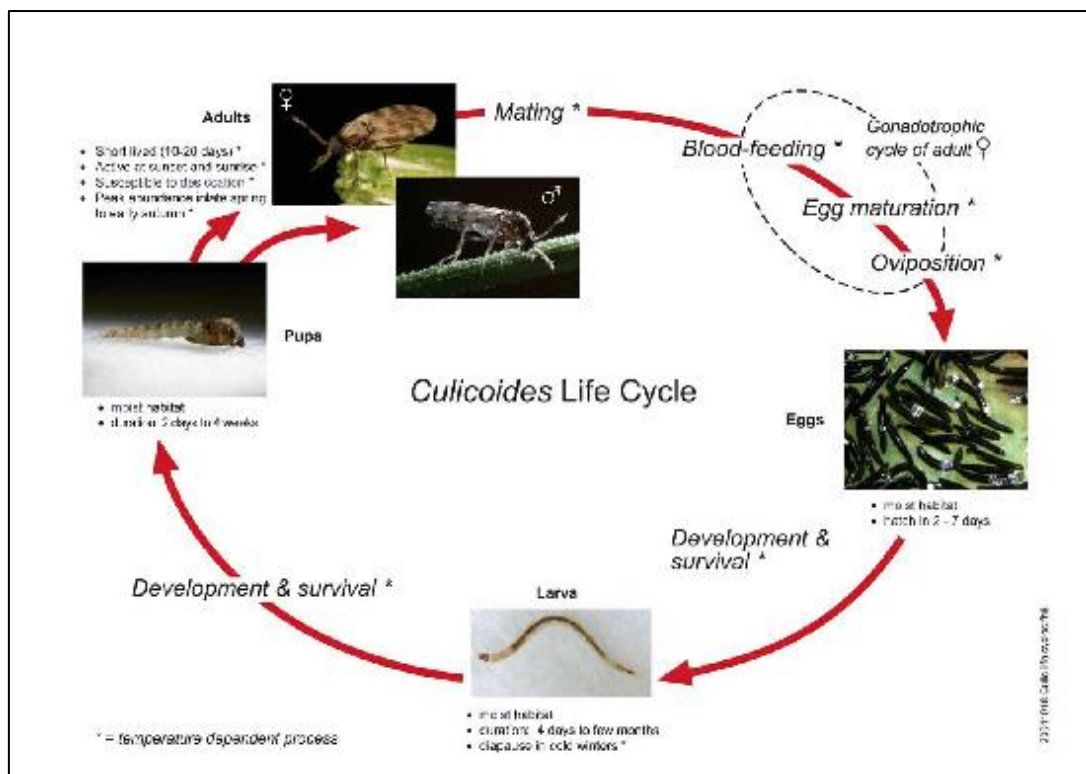
**Anatomy/morphology of a female adult *Culicoides***

## Biology/ecology/life cycle

### Life cycle

All *Culicoides* species display a typical holometabolous life cycle and only the females, who need blood for the completion of the gonotrophic cycle, are haematophagous. No individuals are seen with partly developed eggs together with a fresh blood meal, nor with partly developed eggs without a partly digested blood meal, indicating normal gonotrophic harmony and a lack of autogeny in most South African species.





The life cycle of *Culicoides* vector: This diagram shows the biological processes (in italics) involved in passing between the egg, larval, pupal, and adult stages of the *Culicoides* life cycle, and list important features of each stage. \* indicates a temperature dependent process or stage.

## Eggs

The eggs are usually about 0,25 mm in length, often pale when laid (turning to glossy black), elongate, curved, and pointed at each end. Some species possess characteristic sculpture or markings. In *C. imicola* the maturation of eggs takes two to four days, depending on the environmental temperature, after a blood meal had been taken.

## Larva

The larvae are vermiform, usually pale, and with or without prolegs. They have a characteristically distinctive sclerotized, prognathous head capsule with toothed mandibles and eyespots. There are three thoracic and nine abdominal segments. The larvae undergo four stages, are eel-like in their movements, and burrow in and out of their breeding medium. The larvae of some species are carnivorous and feed on protozoa, rotifers and nematodes. The fourth stage larvae of some species may even be cannibalistic on second stage larvae.

## Pupa

The pupae are comma-shaped and light brown to black, with a pair of dorsal respiratory horned protruding from the prothorax. There are numerous spines, setae, protuberances, and processes that can be used as diagnostic characters. The pupae of most *Culicoides* species are aquatic and have the ability to float. However, the pupae of all *Avaritia* species, including the *Imicola* Complex, drown when submersed. On immersion, the pupae of all species, except *C. imicola*, wriggle free of the

breeding medium and float to the surface. *Culicoides imicola* pupae, however, lay on the substrate below the water surface and drown within two days at room temperature. It has been shown that soaking rains have no adverse effect on the eggs, larvae and pupae of most species, but the pupae of *C. imicola* do drown. The larvae of *C. imicola* will, however, not pupate until conditions are dry enough. Depending on the temperature adult *Culicoides* females may survive for up to 63 days.

## Larval habitats

It is believed that all *Culicoides* species only breed in moist low-lying areas. Although this is true for some species, many have more specialized larval habitats. The basic requirements are moisture and a medium containing organic matter. Therefore, *Culicoides* species may breed in situations varying from those which are almost aquatic, e.g. pond margins; to those where no free water is present but the humidity is close to 100%, e.g. interior of dung pads and decomposing fruit. The various larval habitats can be roughly grouped into four main types.

### Surface water and oil interface situations

About half the known *Culicoides* species in southern Africa make use of various combinations of soil and water as a medium in which to lay their eggs. Soil may vary from coarse sand to the finest clay, and the basic medium may be enriched to a greater or lesser extent with decomposing plant matter, varying from intact material to humus, or with fresh to well decomposed dung, such as is often found on irrigated pastures. The water may range from fresh flowing streams to polluted stagnant pools with varying degrees of acidity, alkalinity or salinity. The degree of light and the presence or absence of plant cover, which may be either tall or kept short by grazing animals, are additional important factors that govern the larval habitat of certain *Culicoides* species. In southern Africa most of the major stock-associated species (*C. imicola*, *C. zuluensis*, *C. magnus*, *C. schultzei* group, *C. pycnostictus*, *C. leucostictus*, and *C. nivosus*) use one or another of the above combinations as their larval habitat.

### Dung pats of large animals

At least ten *Culicoides* species, all in the subgenus *Avaritia*, require the fresh dung of certain animals to complete their life cycles. For example, the dung of the Cape buffalo, cattle and sometimes blue wildebeest is used by *C. bolitinos*, which apparently also feeds on these hosts. Other species breed in the dung of the elephant, the black and white rhinoceros, and the plains zebra.

### Tree-holes, plant and rock activities

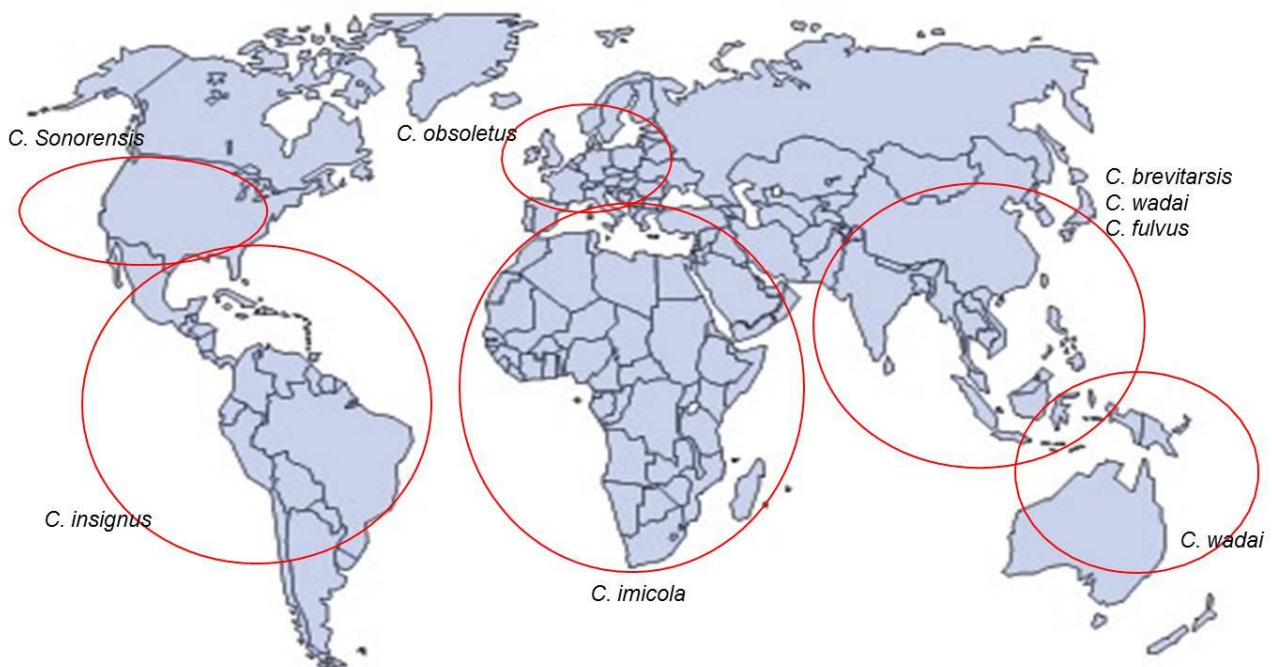
These larval habitats vary from deep, dark, water-filled holes to shallow, exposed but moist hollows which may contain various amounts of water, decomposing leaf litter and sediment. Tree-holes are not restricted to dense forests, as even the more sparsely treed savannahs are rich in tree-hole-associated *Culicoides* species. About 15% of African *Culicoides* species (including *C. accraensis*, *C. clarkei*, *C. olysageri*, *C. eriodendroni*, *C. punctithorax* and *C. nigripennis*) are known or suspected to breed in these habitats. Owing to the restricted size and availability of such rain-dependent habitats these species never become abundant and so are rarely collected. Birds are thought to be their primary source of blood.

## Rotting fruits and plants

These larval habitats have still to be investigated thoroughly. In South Africa a new *Avaritia* species, closely related to *C. pseudopallidipennis* from West Africa, has been reared from the rotting fallen fruits of the sausage tree (*Kigelia africana*) and the maroela tree (*Sclerocarya caffra*). In West Africa the larvae of *C. grahamii* (also of the subgenus *Avaritia*) have been found in the rotting stems of the banana plant.

## DISTRIBUTION

With the exception of Antarctica and New Zealand, *Culicoides* midges are found on virtually all large landmasses ranging from the tropics to the tundra. The most important *Culicoides* vectors of orbiviruses include *C. imicola* in Africa, *C. sonorensis* in North America, *C. insignis* in South and Central America, *C. wadai*, *C. brevitarsis*, *C. actoni* in Australia, *C. fulvus*, *C. schultzei* in Asia, *C. imicola*, *C. pulicaris* and *C. obsoletus* in Europe.



Worldwide distribution of the major *Culicoides* vectors.

## Geographical and seasonal abundance of livestock-associated *Culicoides* species in South Africa

Over the last 35 years more than 112 *Culicoides* species were identified in South Africa. Following the initial work of du Toit 1941 the first identification key for South African *Culicoides* species was compiled by O.G.H. Fiedler in 1951. He recorded 22 species, of which one (*C. onderstepoortensis*) was described for the first time, from South Africa. In 1971 *C. imicola* was shown to be the most abundant livestock *Culicoides* species in the Onderstepoort area of South Africa. The results of subsequent studies showed *C. imicola* to be the most abundant livestock-associated *Culicoides* species in the summer rainfall area of

## Arthropod vectors → *Culicoides*

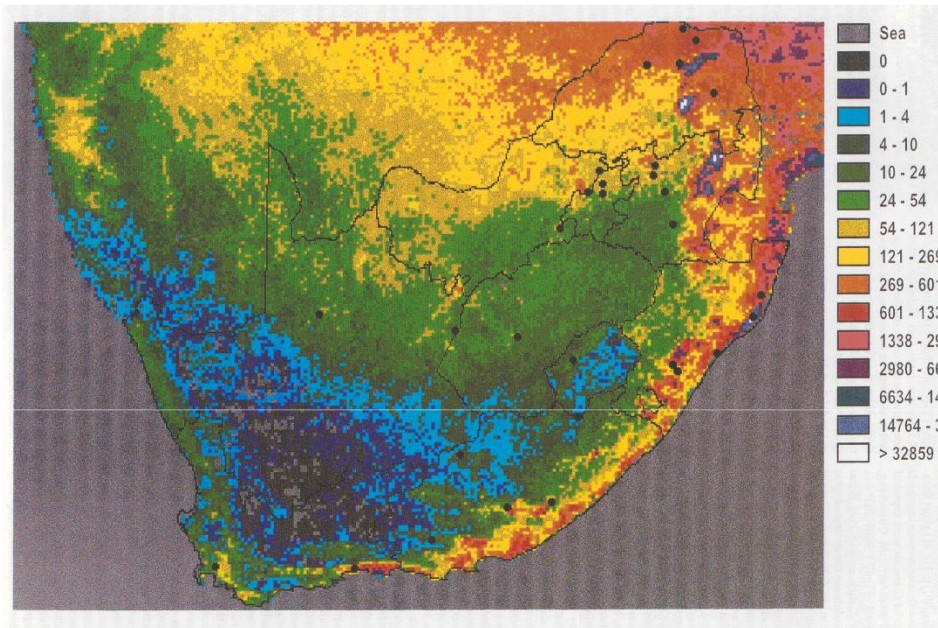
South Africa, especially in the warm, frost-free summer rainfall areas of the country. *Culicoides imicola* is relatively uncommon in warm/dry and cool/wet areas and therefore cannot be regarded as the only vector of orbiviruses in South Africa. The most abundant species in the latter areas were members of the *C. schultzei* group and *C. zuluensis*.

A seven year study on the seasonal abundance of *C. imicola* at the ARC-Onderstepoort Veterinary Institute showed a drop in adult numbers during sustained rainy periods followed by a sharp increase in populations during the drier periods that followed. A three year light trap survey indicated adults of *Culicoides* species, and especially *C. imicola*, to be present throughout the year in frost-free areas of the country and that breeding takes place throughout the winter in these areas. In the most parts of South Africa *Culicoides* numbers reach a peak in late summer and drop sharply after the first frost. Low numbers of adult *Culicoides* midges during the winter months may not only be due to low temperatures but also to lower winter rainfall. Relatively large *Culicoides* collections can be made during winter in the winter rainfall areas. No seasonal fluctuation of the dominant species in most summer rainfall areas was found.

*Culicoides imicola* was absent in light trap collections made in the sheep farming area in the Karoo region of South Africa which is endemic for BT. This suggested that other livestock-associated *Culicoides* species may play a role in the epidemiology of the disease. *Culicoides imicola* is uncommon in the colder high-lying BT endemic areas of South Africa where *C. bolitinos* was found to be the most abundant *Culicoides* species. *Culicoides bolitinos* was also shown to be abundant at some locations in the winter rainfall region of the Western Cape Province, and the dominant *Culicoides* species, in the absence of *C. imicola*, in the sandy dunefields adjoining Port Elizabeth in the Eastern Cape Province. The absence of *C. imicola* at Port Elizabeth and in light trap collections made at Struisbaai and Alexanderbay on the southern and western coastline were attributed to the sandiness of the soil. Limited records suggest that *C. bolitinos* is most probably also widespread in most parts of Africa but, unlike *C. imicola*, is not known to occur outside the Afrotropical Region.

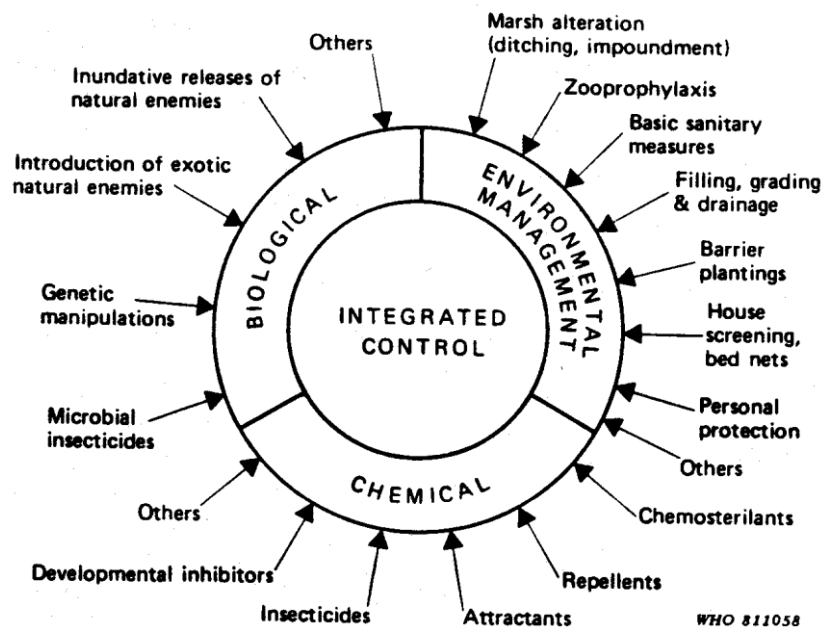
Some of the abundant and more widely distributed *Culicoides* species have a limited host preference and will thus be less important as potential vectors of orbiviruses. According to these surveys, the more abundant and widespread species, which have the greatest potential as arbovirus vectors, are *C. imicola*, the *C. schultzei* group, *C. zuluensis*, *C. pycnostictus*, *C. leucostictus*, *C. bedfordi*, *C. magnus*, *C. ravus*, *C. gulbenkiani*, *C. similis* and *C. bolitinos*.





Predicted abundance of *C. imicola* in southern Africa based on the 2-variable model combining minimum LST and minimum NDVI. Values are the predicted annual mean light-trap catch of the vector.

## CONTROL



Integrated control methodologies comprises chemical, biological and environmental procedures used Jointly or sequentially against a background of an exhaustive ecological understanding of the selected target pest or vector, so as to maximise efficacy, and be fully acceptable from the health and environmental standpoints

## Integrated control

The most important control measure is the protection of animals from contact with *Culicoides* midges. Recommended measures to prevent diseases associated with *Culicoides* midges include vaccination, stabling at night, meshing of stables, and application of insect repellents both to the animal and its stable environment. Around livestock *Culicoides* midges can occur in remarkable high numbers especially on warmer nights and during periods of excessive rainfall. At such times more than 1 000 000 blood seeking *C. imicola* females can be captured in a single light-trap and if the estimate is correct that this may represent less than 1% of the number of midges active on a particular night, clearly illustrates the intensity of attacks that must on occasion be endured nightly by exposed animals. It is impossible to eradicate such numbers of *Culicoides* and so, in Africa specifically, the first line of defense against *Culicoides*-borne orbiviruses must remain vaccination.

### Vaccination

A potential problem with commercially available AHSV and BTV vaccines is that as live attenuated preparations they induce a low viraemia in some vaccinated animals, and so may infect vector *Culicoides*. Because of this fairly scarce possibility many countries, especially those outside of Africa, prohibit the use of live attenuated vaccines. In addition there is the fear that *Culicoides* may ingest vaccine viruses from vaccinated animals and after reversion to virulence on passage through the vector, these viruses may be transmitted in the field. Another concern is that these vaccine viruses may re-assort with wild type viruses, and so lead to the possible creation of new strains of virus with different virulence characteristics.

### Housing livestock in screened buildings

Although it is known that stabled horses are relative safe from infection with AHSV very little is known about the factors that either attract or repel *Culicoides* species and there are no clear directives regarding the definition of a safe stable. Results, generated in Europe, indicate that *C. imicola* and other *Culicoides* species will enter stables and that under certain environmental conditions the number of *C. imicola* collected inside a stable can exceed the numbers collected on the outside. This tendency seems to be linked to environmental temperature and will increase towards the onset of winter.

### Treating of either resting sites, such as animal housing, or host animals with insecticides

The chemical control of adult biting midges by direct treatment of livestock with pesticides is not practical under extensive farming conditions, but it may be justifiable for valuable animals such as racehorses whose immunity to *Culicoides*-transmitted diseases (AHS and EE) is in doubt.

Many pyrethroid insecticides are effective against Diptera, and, depending on the formulation used, have a reasonably long residual effect. Sprays can be used weekly while in cattle insecticide-impregnated eartags may be effective for four to six weeks, or even as long as 10 weeks against *Culicoides* midges. In Australia it has been found that, after Hereford cattle had been given a single subcutaneous injection of Ivermectin at a dose of 200 mcg/kg, the mean mortality of engorged *Culicoides* females 48 hours after feeding was 99% for 10 days after treatment. An adverse effect is



the impact of Ivermectin on the dung beetle fauna so important for sanitation. A study in North America reported very limited success against *C. sonorensis* following application of 5% permethrin or 27% pirimiphos-methyl to the dorsum of calves. Engorged *Culicoides* females that exhibited sublethal intoxication, recovered and subsequently produced matured batches of eggs of normal size. However, a belly spray of 0,2% permethrin substantially reduced numbers of engorged females, and lowered engorgement levels 3 and 7 days post-treatment, but by day 10 little effect was noted. The chemical control of *Culicoides* adults merit additional research, especially in Africa where attacks rates are very high. In bioassay determinations of the efficacy of permethrins against *C. sonorensis* it was shown that midges were able to feed, and thus potentially transmit pathogens, before being incapacitated.

### **Environmental interventions to remove larval breeding sites or the application of insecticides and pathogens to habitats where larvae develop**

The elimination of the larval habitat of *C. imicola*, has received no attention in Africa perhaps because little success has been achieved with other species of *Culicoides* elsewhere in the world. Under restricted situations it may be feasible to reduce *Culicoides* adult numbers by treatment of their breeding sites with compounds such as Temephos. However, as regards *C. imicola* its explosive increase in numbers, and its rapid radiation over large areas as soils become suitably moist under continuous rains, would make the widespread application of Temephos not only expensive but impracticable.

Increasing concerns of the impact of chemicals on the environment resulted in a decline in the number of agents available for livestock pest management. Reliance on only a few active ingredients may create problems with insecticide resistance.

### **The use of repellents or host kairomones to lure and kill adult midges**

Different modes of action have been proposed for repellents, namely

- inhibition of response to an otherwise attractive signal;
- switching of the sensory message from attraction to repulsion;
- activation of a receptor system that controls a competing behaviour;
- activation of a noxious odour receptor; and
- activation of different receptor types simultaneously causing loss of the specific signal for host location.

The ideal insect repellent would repel multiple species of biting arthropods, remain effective for at least eight hours, cause no irritation to the skin or mucous membranes, cause no systemic toxicity, be resistant to abrasion and rub-off, and be greaseless and odourless. Assessment of efficacy of repellents applied to host animals against *Culicoides* species and especially *C. imicola* is hampered by their relatively small size and their nocturnal activity which make direct observation difficult.

In recent study in South Africa utilizing light traps and repellent impregnated polyester netting, repellency has been assessed by comparison of the numbers of *Culicoides* midges caught in the light traps over a period of time. These studies concluded that N, N-diethyl-3-methylbenzamide DEET and a mixture of organic fatty acids C8910 [15% (w/w) mixture of octanoic, nonanoic and

decanoic acids in light mineral oil] had a significant repellent effect against *Culicoides* species, including *C. imicola*, for all catches made from after sunset to before sunrise, when applied to polyester mesh as tested with a down-draught suction light trap. No significant repellent effect against *Culicoides* was found for the citronella oil or the  $\alpha$ -cyano-cypermethrin.

### Decoy hosts

Under certain conditions the presence of cattle near sheep may serve to reduce the level of BT infection in the sheep, apparently because the vector *Culicoides* prefer to feed on the cattle. The presence of decoy hosts can, however, increased the animal biomass on a farm which translates into increased feeding opportunities for *Culicoides* midges. It would seem thus inevitable that their population levels will rise in accord, which, devolves into increased virus transmission risk.

Smoking of stables is a farmers remedy applied in an effort to repel midges, however, light traps that have been operated in the palls of smoke have still yielded enormous catches of *Culicoides* midges, with these catches as large as any made at smokeless stables.

Research to assess and improve the efficacy of control methods is required and, in the longer term, efforts should be made to develop better bait systems for monitoring, and possibly controlling, midges. For all these studies we need better methods to analyse the ecology and behaviour of midges in the field. The paucity of control options and basic knowledge provide a warning that we must be better prepared for the emergence of midge-borne diseases.

### Strategies to protect animals from *Culicoides* midges when transporting animals

- Treating animals with chemical repellents prior to and during transportation;
- Loading, transporting and unloading animals at times of low vector activity (i.e. bright sunshine, low temperature);
- Ensuring that vehicles do not stop en route during dawn or dusk, or overnight, unless the animals are held behind insect proof netting;
- Darkening the interior of the vehicle, for example by covering the roof and/or sides of vehicles with shade cloth;
- Surveillance for vectors at common stopping and offloading points to gain information on seasonal variations; and
- Using historical or modeling information to identify low risk ports and transport routes.

## THE COLLECTION OF ADULT CULICOIDES

---

The majority of investigations conducted on *Culicoides* species world-wide deal primarily with the monitoring of disease vectors as their acknowledged role in epidemics of disease. Monitoring is mostly aimed at adult activity in the vicinity of vertebrate hosts. Since adult midges spend more than 90% of their time resting, for example developing the oocytes, digesting a bloodmeal and developing eggs (Mullens et al 2004), this group represents less than 10% of the adult population.

## Light traps

Despite the emphasis placed on the collection of *Culicoides* midges only a limited number of suction light traps, the primary monitoring tools used for this purpose, are commercially available and the majority of these were originally designed for the collection of mosquitoes. Although all these traps make use of a light source to attract *Culicoides* midges and a fan to draw them into a holding cage or container the variation in the trap types used by different laboratories/research groups makes direct comparison between investigations difficult.

Factors may influence the number of *Culicoides* specimens as well as the number of each age grade collected with light traps include the presence of breeding sites and other light sources near the light trap, the height of the trap above ground level, wind-speed, the phase of the moon, and even the tides. Climatic conditions such as temperature and wind velocity, rainfall, relative humidity, and the age of the population during the trapping night may also influence the numbers of *Culicoides* midges collected.

The numbers of *Culicoides* midges and the species diversity collected with light traps are seldom comparable to the *Culicoides* biting rate on the livestock host.

Traps baited with CO<sub>2</sub> have also been used to collect *Culicoides*. The advantage of these traps is their collection of diurnal species. A limitation of CO<sub>2</sub> traps is the need for dry ice. The unregulated release of CO<sub>2</sub> may provide concentrations that are attractive to some species and repellent to others.

The relatively strong attraction of the light source renders light traps less useful for the study of some important behavioural aspects of *Culicoides* species e.g. the entering behaviour of *Culicoides* species into stables and the evaluation of repellents against *Culicoides* midges. Several studies have shown that the numbers of *Culicoides* midges collected with UV light traps is not always comparable to host attack rate. To define the vector capacity of a specific *Culicoides* species or population accurately it will be necessary to determine a relationship between biting rate and light trap abundance. Results obtained with light traps need to be compared with other non-attractant collections methods e.g. animal-baited collection, truck traps, suction traps, drop traps or even electrocuting grids.

## Truck traps



Vehicle-mounted trap

Arthropod vectors → *Culicoides*

Vehicle-mounted traps (commonly referred to as 'truck-traps') can be used to capture flying *Culicoides* midges throughout the day, and in this way hourly activity rates may be determined. The results can also be related to prevailing meteorological conditions including temperature, relative humidity and windiness. Vehicle-mounted traps are particularly rewarding for the collection of large numbers of biting midges around dusk and dawn. Also, male swarms, which may not be attracted to light traps, can be captured in large numbers in this way.

### Aspirators and 'sweeping'

Aspirators (or 'pooters') and hand operated sweep nets are used in specialised host preference studies when live *Culicoides* need to be captured off tethered animals or humans. A hand-held pooter can be used but requires that each individual midge be located, using a red torchlight, prior to capture. More commonly the 'sweeping' of marked areas of the host with a small hand-held domestic vacuum cleaner is the preferred method used. In this way more *Culicoides* midges may be captured and more rapidly, which is necessary to determine which areas of the host are being attacked within a specified time slot.

### Emergence traps

These are made of fine netting, are conical in shape, and have a collection bottle at the apex. The latter is lined with a sticky substance or containing a liquid. An emergence trap (built to cover a specific unit area) is placed over a suspected *Culicoides* larval habitat, to remain *in situ* where it can be monitored hourly, daily or weekly. In this way emergence rates, and species association profiles, can be obtained. Furthermore, during emergence, if larvae or pupae need to be retrieved, samples of the substrate can be extracted and removed for further studies in the laboratory. Here a saturated sugar solution is added to the sample to alter the specific gravity of the medium, and so the larvae and pupae are induced to float to the surface. The pupae are then retrieved with a spatula and placed in individual vials for eclosion. The resultant adult and its associated pupal pelt can then be mounted on a single glass slide for identification.



Emergence traps



## Drop traps and animal bait-traps

An efficient type of animal-bait trap for small and medium-sized animals is the "closure" type which allows the attacking midges to move to the host animal in a normal way and in a relatively normal environmental situation. All midges attacking the animal at the time of trap closure are collected, including those that are actually feeding at the time.



Drop trap for collecting *Culicoides* attracted to tethered calves

## Storing of specimens

The choice of method for storing specimens depends on the purpose for which they are collected. If they are to be used for virus isolation or for DNA studies then the use of preservatives such as formalin must be avoided; and freezing in liquid nitrogen may be required.

## REFERENCES

1. Meiswinkel, R., Venter, G.J. & Nevill, E.M. (2004) Vectors: *Culicoides* spp. In: *Infectious Diseases of Livestock* (ed. by J.A.W. Coetzer and R.C. Tustin), pp. 93-136. Oxford University Press, Cape Town
2. Mellor, P.S., Boorman, J. & Baylis, M. (2000) *Culicoides* biting midges: their role as arbovirus vectors. *Annual Review of Entomology*, 45, 307-340.
3. Wittmann, E.J. (2000) Temperature and the transmission of arboviruses by *Culicoides*. PhD Thesis, University of Bristol, Bristol

4. Wirth, W.W. & Hubert, A.A. (1989) The *Culicoides* of southeast Asia (Diptera: Ceratopogonidae). Memoirs of the America Entomological Institute, Gainesville, U.S.A.
5. (Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C. & Baylis, M. (2005) Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews Microbiology*, 3, 171-181.)
6. Nevill, E.M. (1967) Biological studies on some South African *Culicoides* species (Diptera: Ceratopogonidae) and the morphology of their immature stages. M.Sc. (Agric) Thesis. University of Pretoria.
7. (Tabachnick, W.J. (2004) *Culicoides* and the global epidemiology of bluetongue virus infection. *Veterinaria Italiana*, 40, 145-150.)
8. (Baylis, M., Meiswinkel, R. & Venter, G.J. (1998) A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in southern Africa. *Journal of the South African Veterinary Association*, 70, 80-89.
9. Venter, G.J. (1991) 'n Studie van die verspreiding en seisoenale voorkoms van *Culicoides* species (Diptera: Ceratopogonidae) en gekoppelde virussiektes in die R.S.A. MSc thesis University of the Orange Free State.
10. Wirth, W.W. & Hubert, A.A. (1989) The *Culicoides* of Southeast Asia (Diptera: Ceratopogonidae). Memoirs of the America Entomological Institute, Gainesville, U.S.A.

## Websites:

<http://www.culicoides.net/>

