MOSQUITOES

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INTRODUCTION

Certain culicine mosquitoes are of veterinary importance because they act as vectors of arboviruses infecting livestock. These viruses are Rift Valley fever (RVF), Wesselsbron (WSL), West Nile (WN) and 3 of the equine encephalitides namely eastern equine encephalitis (EEE) virus, western equine encephalitis (WEE) virus and Venezuelan equine encephalitis (VEE) virus. All of these also cause human disease. While RVF and WSL viruses are mainly confined to Africa and the encephalitides viruses are confined to the Americas, WN virus now occurs in Africa, Asia, Europe and the Americas. This module will focus on those mosquito species transmitting these viruses. Firstly an outline will be given of mosquito morphology which will then be applied to elementary systematics to identify those genera and species that comprise the important vectors.

The main ways of collecting mosquitoes in the field and their storage for virus isolation or detection (RT-PCR) will be dealt with and the distribution of vector species in both Africa and other countries will be listed. The biology of each pertinent genus or species will be described particularly as it relates to the transmission of the virus to the vertebrate host and the survival of the virus between outbreaks. Both the dissemination of the virus in the vector mosquito and the usefulness of laboratory vector competence experiments will be considered as they relate to incriminating a particular species as a vector. The transmission cycles of each virus will be described, both the endemic cycle and the epidemic cycles. Finally, methods of mosquito control and the protection of livestock from mosquito bites will be reviewed.

MORPHOLOGY AND SYSTEMATICS

Learning to identify mosquitoes requires considerable time and experience. Thus this module can only give a framework on which the reader can build by both reading further and examining mosquito specimens. A basic account of morphology and a fundamental treatment of those genera and species that are of veterinary-medical importance are given.

Diagnosis of subfamilies Culicinae and Toxorhynchitinae

Mosquitoes belong to the order Diptera (flies), sub-order Nematocera and family Culicidae. They exhibit complete metamorphosis with a life cycle of egg to larva to pupa to adult, in which the first 3 stages are aquatic. There are 3 subfamilies (Figure.1) which can be distinguished as follows:

- Anophelinae. Adult females are blood-feeders with the maxillary palps of both sexes as long or nearly as long as the proboscis and the dorsal scutellum evenly rounded or strap-like. Adults rest with their abdomens tilted forwards at 45° to the surface, larvae have no siphon and each egg has floats.

- Culicinae. The females are blood-feeders with the palps less than half as long as the proboscis and the scutellum is tri-lobed. Adults rest with abdomens about parallel to the surface; larvae have a prominent siphon and the eggs that are deposited as rafts lack floats. Those species that are of veterinary importance all belong to this subfamily.

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- Toxorhynchitinae. The adult females do not feed on blood but only on nectar and other plant juices. They are unusually large mosquitoes with metallic coloured scales and the proboscis is strongly curved downwards. The larvae are predaceous.

Figure 1: Separation of the different life stages of subfamilies Anophelinae, Culicinae and Toxorhynchitinae

Morphological characters of culicine mosquitoes

The standard taxonomic terminology used today is that of Harbach and Knight (1980, 1981). It is followed here except that the term claw is not changed to "unguis". A generalized drawing of an adult female *Aedes* is shown in Figure.2 with inserts showing the head of a male and the tip of a foreleg.
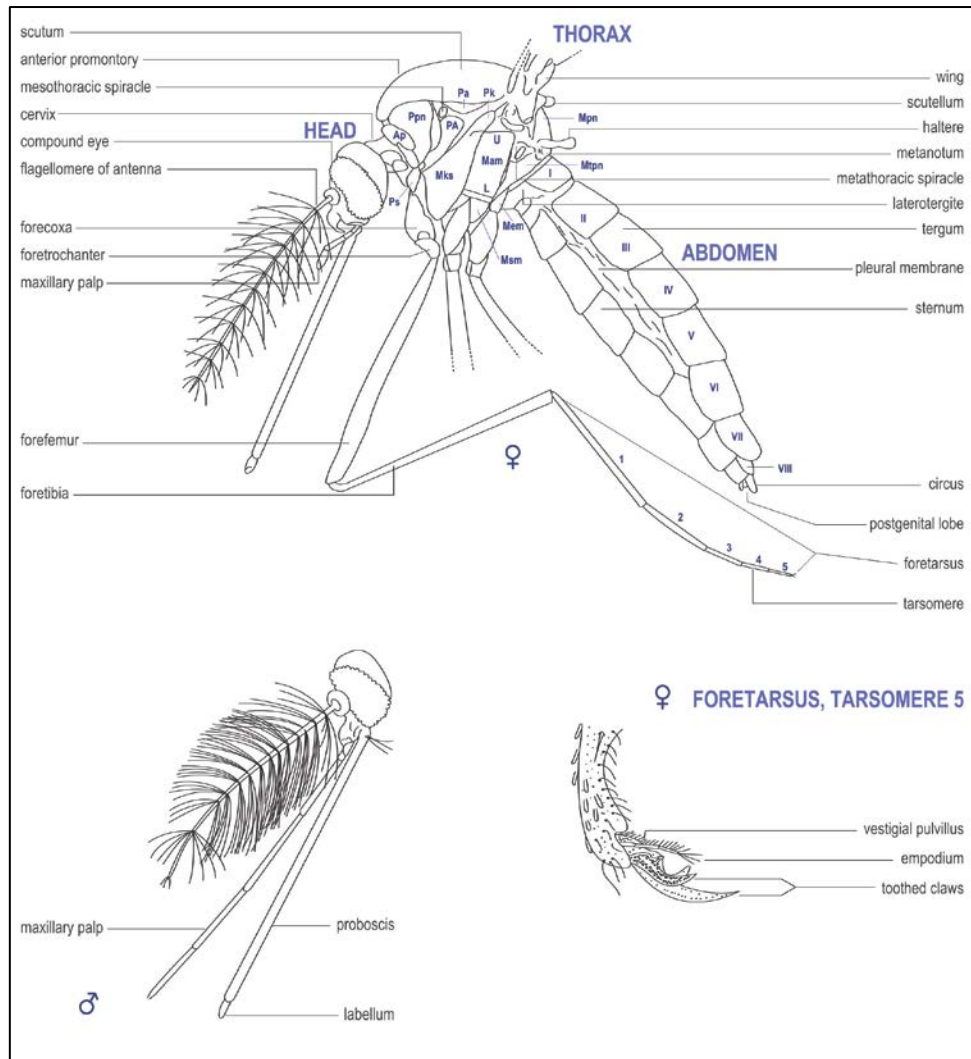
Figure 2: Adult *Aedes* mosquito (diagrammatic), left side in lateral aspect;
Ap – antepronotum, Mam – mesanepimeron (U=upper, L=lower), Mem – metameron, Mks – mesokatepisternum, Mpn – mesopostnotum, Msm – mesomeron, Mtpn – metapostnotum, Pa – paratergite, PA – postspiracular area, Pk – prealar Knob, Ppn – postpronotum, Ps – Proepisternum (modified from Harbach and Knight, 1980)

Females and males have the same appearance except that in the male the antennae are more setous (feathery), the maxillary palps longer and the genitalia are different in structure. Figure.2a is an anterodorsal view of the head of an adult female which shows more details including the different types of scale *viz* broad decumbent, narrow decumbent and erect.
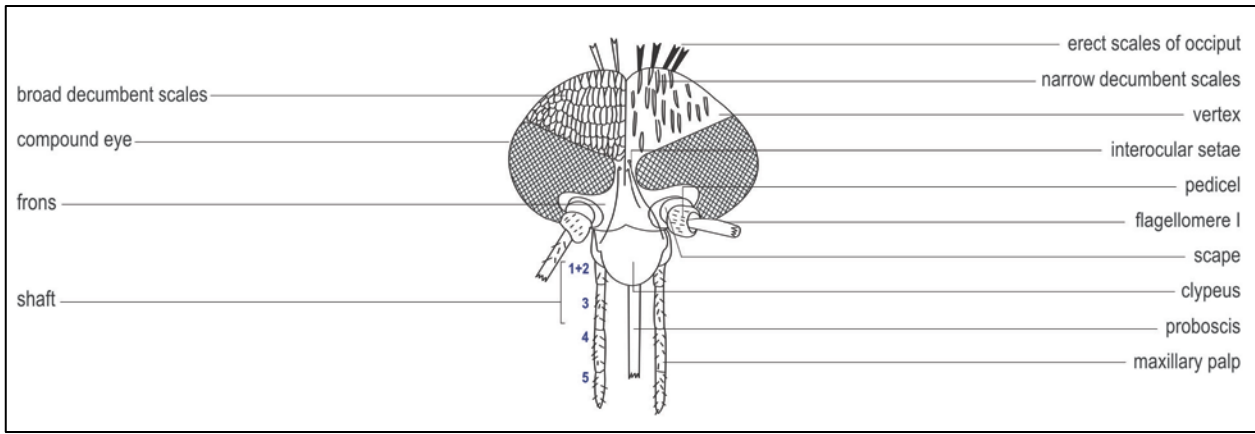
Figure 2a: Anterodorsal view of head of adult female culicine

The thorax is the most important part of the mosquito for identification purposes. The type of setae and scaling on the dorsal <u>scutum</u> and lateral <u>pleuron</u> varies. The pleuron is composed of pleural sclerites (plates), each of which may or may not bear setae or scales. The thorax bears 3 pairs of legs *viz* 1st, 2nd and 3rd or fore, mid and hindlegs as well as the wings and <u>halteres</u>. The latter are balancing organs. The type of stripes or banding on the legs is often important. In orientating the different surfaces of the legs, each leg is regarded as fully extended outwards at right angles to the body. n this imaginary position those surfaces of the femora, tibiae and tarsi which face forwards are called <u>anterior</u>, those which face backwards are called <u>posterior</u> while the upper and lower surfaces are <u>dorsal</u> and <u>ventral</u>. Each leg consists of 9 segments: a small basal <u>coxa</u> and <u>trochanter</u>, a <u>femur</u>, a <u>tibia</u> and a <u>tarsus</u> with 5 <u>tarsomeres</u> (See figure.2). The 5th tarsomere ends in paired claws that may be "armed" or "simple" depending on whether they bear sharp <u>teeth</u> ("toothed") or not. There may be a pair of setous pads or <u>pulvilli</u> at the base of the claws ventrally (See Figure.5b).
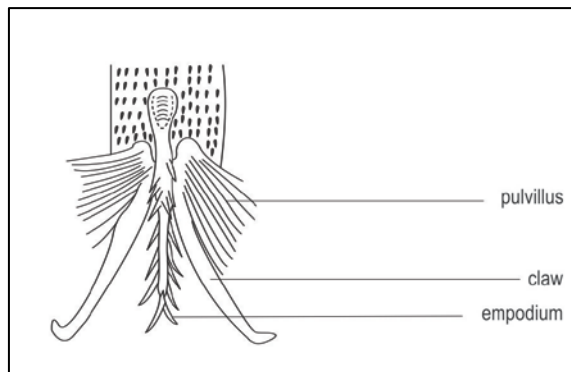
Figure 5b: Tip of 5th tarsomere of *Culex* in ventral aspect

The colour pattern provided by the abdomen's scales can be important for species identification. The abdomen (See Figure. 2) has 10 segments but only 7 or 8 are usually visible as the last 2-3 are altered for mating. In males and engorged females the dorsal sclerites, the <u>terga,</u> are usually curved around the sides and beneath the abdomen which hides the smaller ventral sclerites, the <u>sterna</u>. Because of this, care must be taken not to confuse the sides of the terga for the sterna. There is elastic tissue joining each <u>tergum</u> with the corresponding <u>sternum</u> and also joining the terga dorsally and the sterna ventrally. This permits the female mosquito to become considerably distended when engorging or when the abdomen becomes filled with eggs (<u>gravid</u>).

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The female abdomen has a pair of flaps or plates, the cerci, at its tip, which are usually visible in the dry specimen, although they may be retracted into the last segments, especially in *Culex* mosquitoes, making these difficult to see. Cerci are more elongate and pointed in *Aedes* and *Ochlerotatus* than in other genera such as *Culex* where they are short stubs. In the newly emerged female the tip of the abdomen does not rotate through 180^0 as in the male. Together with the cerci, the ventral postgenital lobe or plate constitute the external genitalia of the female.

The male genitalia are often most important for species identification and in some cases are the only means of separating closely related species morphologically. Abdominal segments IX and X are highly modified to form the genitalia and are often retracted into segment VIII, making them difficult to see. Thus a proper study of the genitalia requires their removal from the rest of the abdomen and mounting on a slide for examination under a compound microscope (see Jupp, 1996, pp 17-20). The different genera display considerable variation in the structure of the male genitalia. Males differ from females in that just after emergence segments VIII-X commence rotating so that after 24hrs they have turned through 180^0. Because of this, the sterna of segments VIII-X become dorsal and the terga ventral. In Figure.3b, d & h male genitalia of *Culex (Cux.) pipiens/quinquefasciatus* are shown in sternal view which was originally ventral i.e. the pre-rotation position but which is now dorsal.
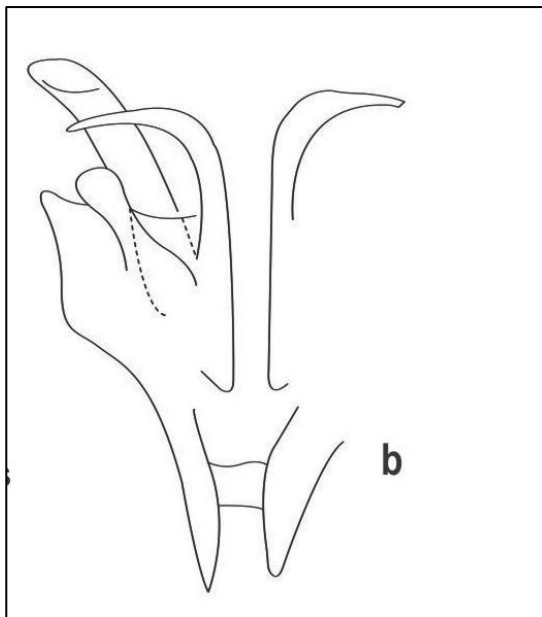
Figure 3b: Male *pipiens* phallosome details
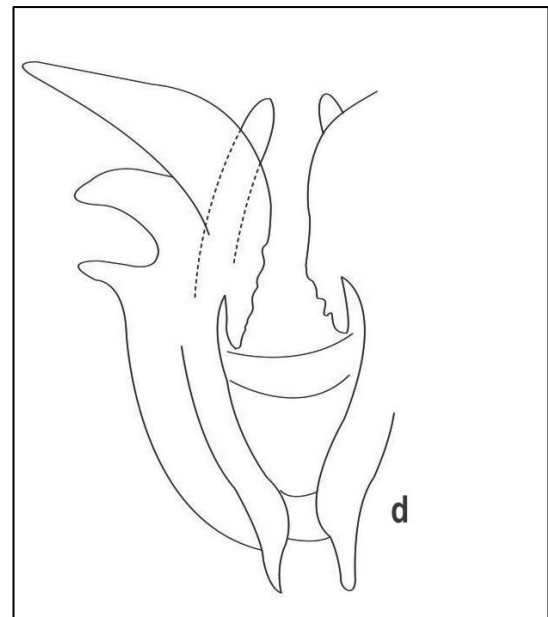
Figure 3d: Male *quinquefasciatus* phallosome details
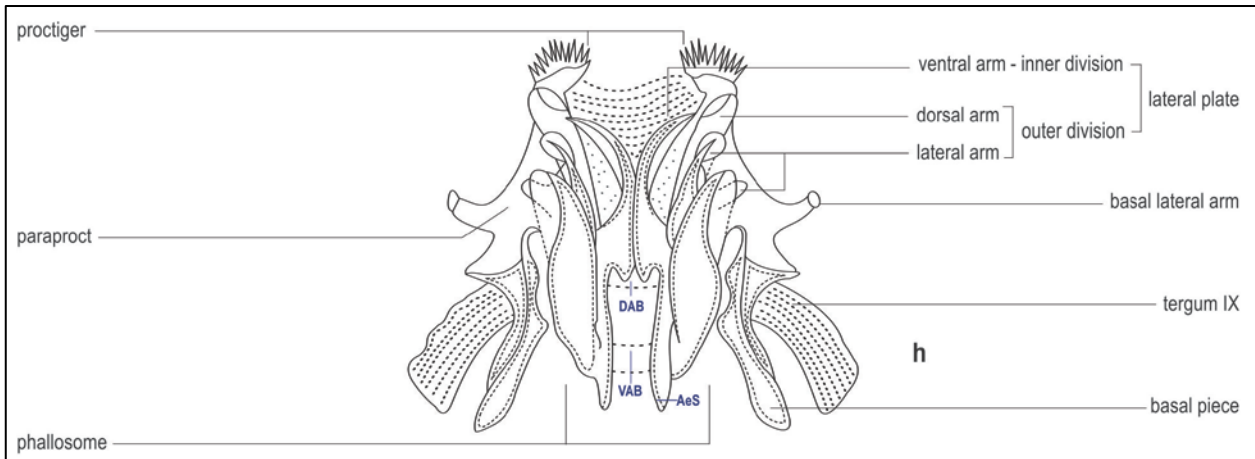
Figure 3h: Aedeagus of *Cx. pipiens* in sternal view; DAB, VAB: dorsal and ventral aedeagal bridges. AeS: aedeagal sclerite

Essentially, the male genitalia consist of the forceps (gonocoxite and gonostylus) and the aedeagus, a term used to describe collectively all the structures surrounding the opening of the male genital duct. Part of this aedeagus is called the phallosome, the structure used to separate members of the *Cx. pipiens* complex (Figure.3), and is the only component that we need to refer to in this module.

The pupa is of little value in mosquito identification so will not be considered here, but the larva is sometimes useful particularly in the case of *Culex* mosquitoes. Figure 4a is a drawing of the posterior of the *Culex* larva.
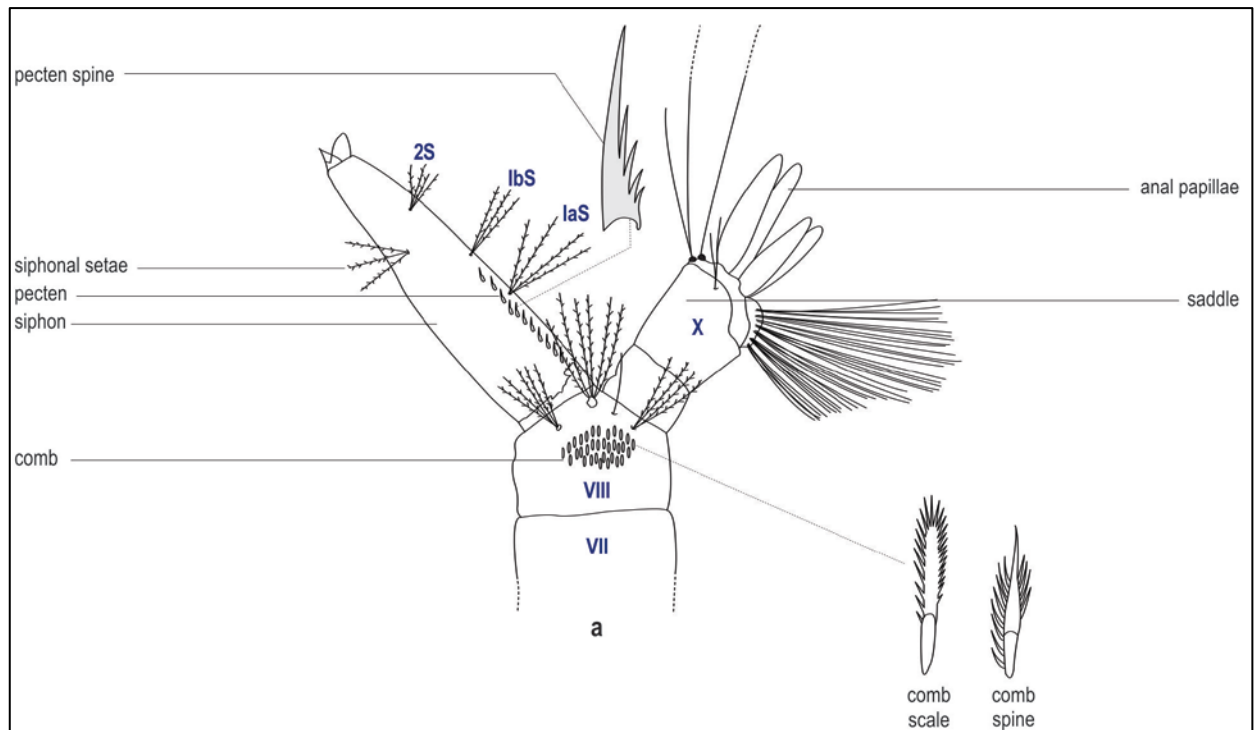
Figure 4a: Posterior of larval abdomen of *Culex quinquefasciatus*

These posterior portions of the abdomen - the modified segments VIII, IX and X are important taxonomically. The first 7 abdominal segments are similar in appearance and unspecialized (only part of VII is shown). Segment VIII is triangular bearing the comb and a long breathing tube, the siphon. Segment X, the anal

<u>segment</u> is highly modified and bears the dark sclerotized (covered in tanned chitin) <u>saddle</u>, 2 pairs of anal <u>papillae</u> and various setae. Segment IX is not visible as it is incorporated into segments VIII and X. The comb consists of rows of teeth that may actually be spines and/or scales depending on the species. A <u>scale</u> in the larva is a tooth which has a thin flattened rounded apex bearing a regular fringe, none of the apical denticles of which are markedly longer than the remainder. The siphon bears 2 important structures, the <u>pecten</u> and <u>siphonal setae</u> 1a-S, 1b-S and 2-S (subventral setal tufts). Usually the pecten teeth are spines and their number, shape and size are important for diagnosis, as is the length and number of branches of the siphonal setae.

Methods for rearing and preserving mosquitoes

A general overview of methods for rearing and preserving mosquitoes is given by Jupp (1996). Although it is possible to identify some species by means of a times 10 magnification hand lens, a dissecting microscope and a compound microscope are necessary. The dissecting stereomicroscope is used at magnifications from 7-100 times for preparation of pinned mounts of mosquitoes and making slide mounts of immature stages and genitalia. The final dissection and display of the male genitalia require a magnification of 80-100 times. Identification of pinned females or females lying in petri dishes ,from their external morphology, is done at magnifications of 7-50 times, while identification of larvae and male genitalia may require a magnification of up to 400 times with the compound microscope.

Standard abbreviations for genera and subgenera

The following are standard abbreviations (shown in parenthesis) for the genera and subgenera that are of veterinary-medical importance.

- Genera: *Aedes (Ae.), Ochlerotatus (Oc.), Culex (Cx.), Culiseta (Cs.), Coquillettidia (Cq.) Melanoconion (Mel.), Psorophora (Ps.)*
- Subgenera of *Aedes: Aedimorphus (Adm.), Neomelaniconion (Neo.), Stegomyia (Stg.)*
- Subgenera of *Ochlerotatus*: *Ochlerotatus (Och.), Culicelsa (Cul.)*
- Subgenus of *Culex*: *Culex (Cux.)*
- Subgenus of *Culiseta: Climacura (Cli.)*
- Subgenus of *Coquillettidia: Coquillettidia (Coq.)*

Identification of genera *Culex* and *Aedes/Ochlerotatus*

Keys for the identification of culicine genera are available for various countries e.g. the Afrotropical region (Service,1990), southern Africa (Jupp,1996), for North America (Darsie *et al,* 2004) and there is an internet facility developed by the European Mosquito Bulletin for identifying Italian mosquitoes that is being extended for the whole of Europe (go to: http://e-m-b.org/). These can be used by the reader who wishes to undertake a more comprehensive study of mosquito systematics. For the present purpose, the characteristics of the genus *Culex* are compared to the genera *Aedes - Ochlerotatus* and the distinction between *Aedes* and *Ochlerotatus* is also described. Figure.5 gives the 4 main differences between *Culex* and *Aedes - Ochlerotatus*.

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CHARACTER	CULEX	AEDES + OCHLEROTATUS
Tip of female abdomen	Blunt	Pointed
General body markings	Dull in colour	Usually bright and striking
Female: claws on fore and mid legs	Untoothed ("unarmed")	Usually clearly toothed ("armed")
Pulvillus (male and female)	Present (use high power of stereo microscope)	Vestigial

Figure 5: Differences between the genus *Culex* and the genera *Aedes-Ochlerotatus*

Examination of the claws should be made at high magnification under the stereomicroscope particularly to view the pulvilli and teeth (Fig. 5a and b).
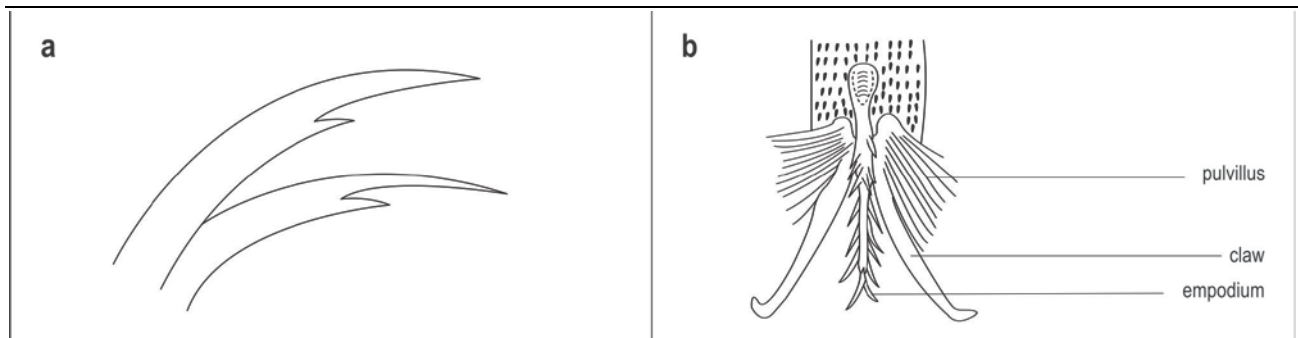
Figure 5a: Toothed ("armed") claws of female *Aedes/Ochlerotatus*

Fig 5b: Tip of 5th tarsomere of *Culex* in ventral aspect

Ochlerotatus and *Aedes* can only be separated by the female and male genitalia (Figure.5c-f).
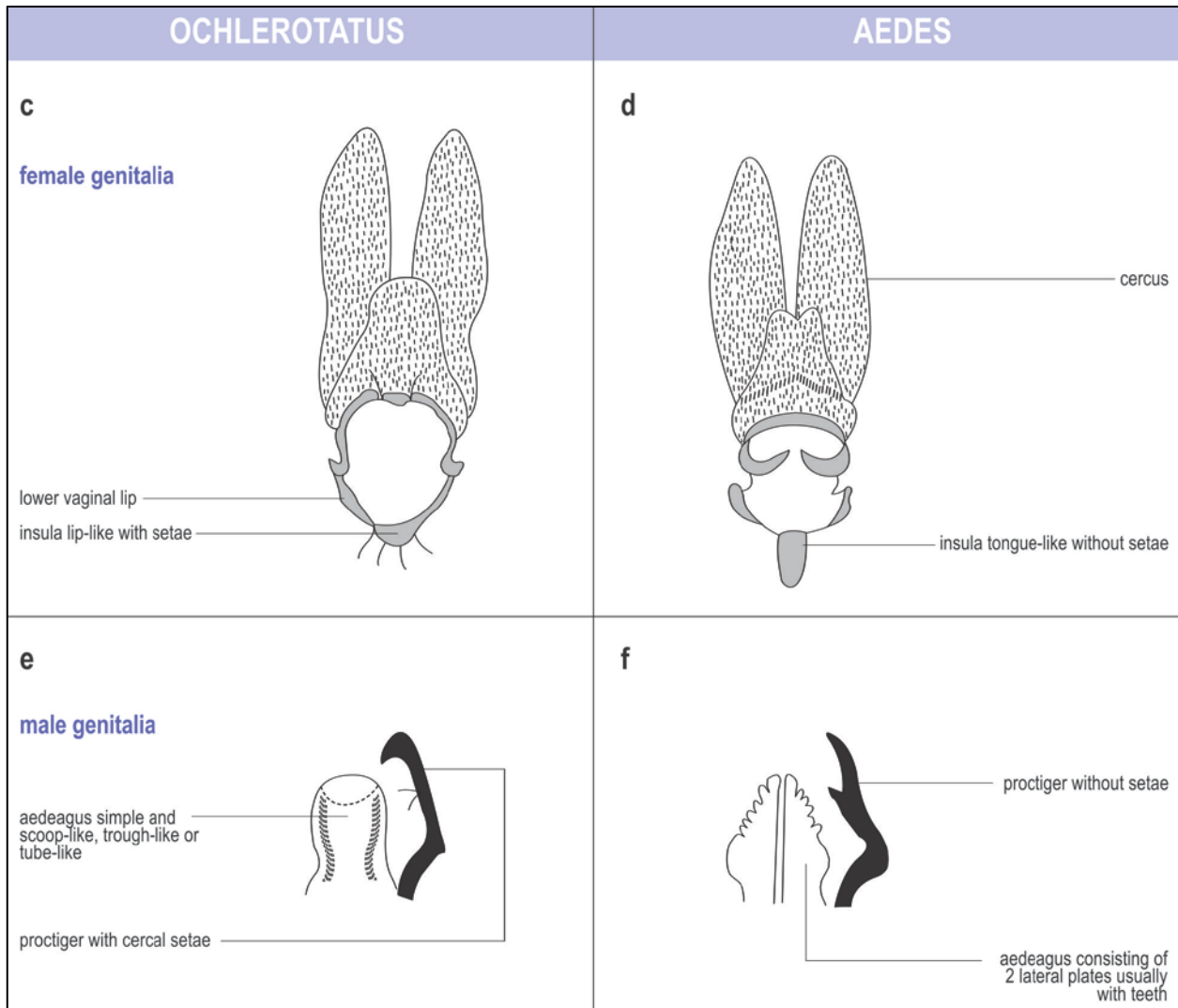
Figure 5c-f: Separation of *Ochlerotatus* and *Aedes* by their genitalia.

However, many of the species belonging to one or other of these genera have characteristic markings, several of which will be considered below. In the larval stage *Culex* may be readily distinguished from *Aedes - Ochlerotatus* (Figure 4a & b). The siphon is long in *Culex* and short in *Aedes - Ochlerotatus*. Furthermore, the latter have only one setal tuft on the siphon and both the siphon and saddle is darker due to a deeper melanization.
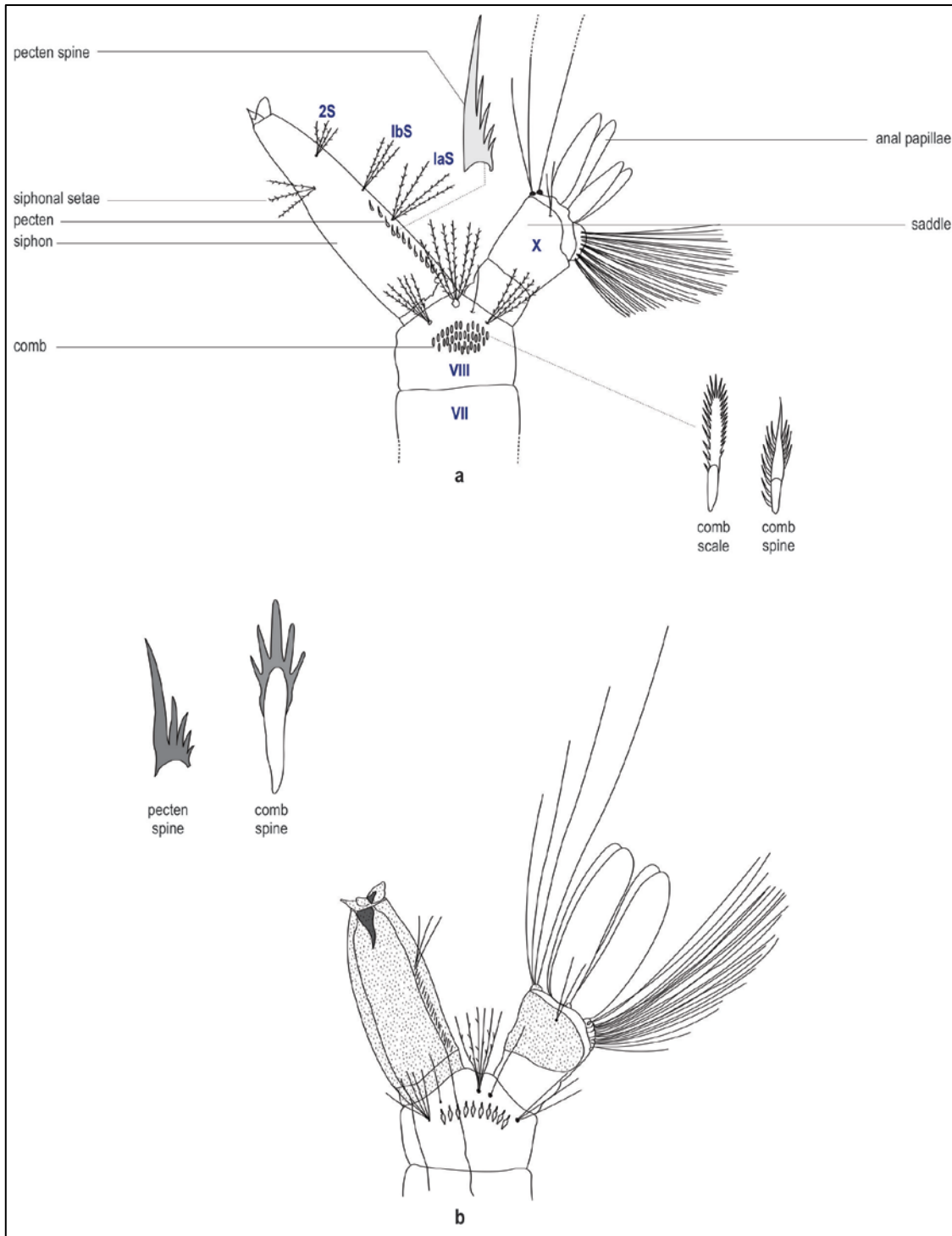
Figure 4a&b: Posterior of larval abdomen of *Culex quinquefasciatus* (a) and *Aedes aegypti* (b)

Identification of some vector species

Several species of *Culex* vectors are indistinguishable or difficult to distinguish in the female but their male genitalia are diagnostic and often their 4th stage larvae as well. Among these are *Cx.pipiens, Cx. quinquefasciatus* (Figure.6) and *Cx.restuans*.

Figure 6: *Culex quinquefasciatus*

In *Cx. pipiens* the ventral arm of the inner division of the male phallosome does not extend laterally of the dorsal arm of the outer division and the apex of the dorsal arm is truncate (See Figure.3b) while in *Cx. quinquefasciatus* the ventral arm of phallosome extends laterally of the dorsal arm and the apex of the dorsal arm is pointed (Figure.3d). Figure.3e shows how this difference in the ventral arm can be measured and expresed as a DV/D index.
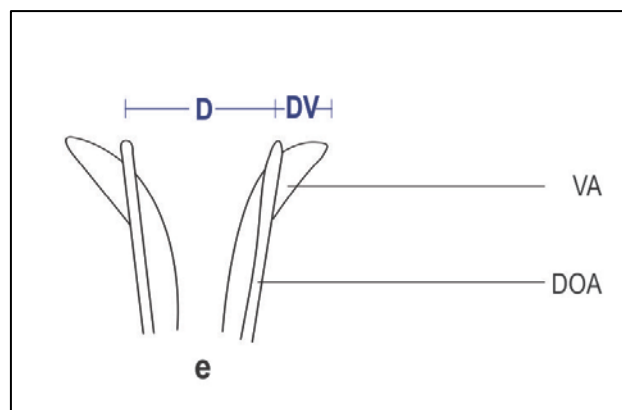
Figure 3e: Diagramme of ventral arm (VA) and dorsal arm (DOA) of male phallosome plate of *pipiens* complex to show measurements made for the DV/D ratio (after Sunderaraman, 1949)

In South African populations, the female wing of *Cx. pipiens* has cross vein index 1.4 or greater while that of *Cx. quinquefasciatus* is usually 1.3 or less (Figure. 3f). Additionally, the male maxillary palps have a setal hair index of about 0.4 for *Cx. pipiens* but 0.2 for *Cx.quinquefasciatus* (Figure.3g).
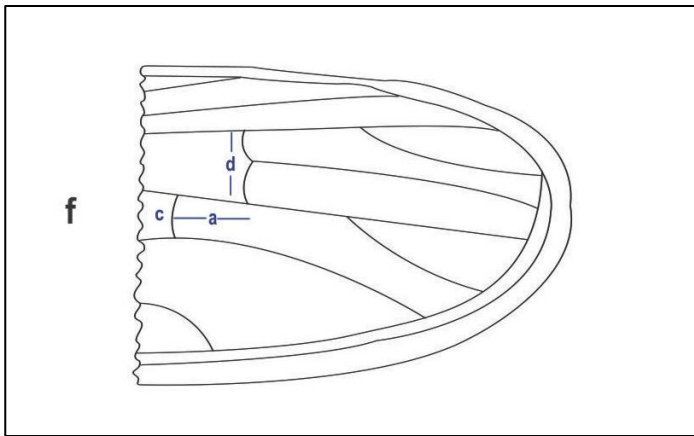
Figure 3f: Female wing of *Cx. pipiens* complex to show cross vein index $\frac{c+d}{a}$ (Jupp, 1978)
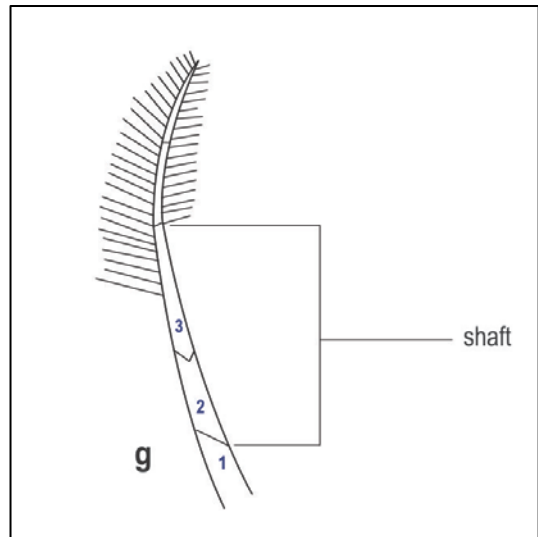
Figure 3g: Male maxillary palp of *Cx. pipiens* complex, the setae (hair) index is the proportion of shaft (segments 2 and 3) bearing setae (Jupp, 1978)

Cx. pipiens and *Cx.quinquefasciatus* can also be distinguished in the larva according to the number of branches on the most basal subventral setae 1aS of the siphon: 1-3 branches (*pipiens*) and 4-11 branches (*quinquefasciatus*). The above-mentioned *Culex* species do not have striking markings on their legs but *Cx. theileri* (Figure.7) has a longtitudinal white line extending the entire length of femora and tibiae.

Figure 7: *Culex theileri*

This can be compared to *Cx.univittatus* (Figure.8) where the ornamentation of the legs differs, notably the hind femur where there is a dark dorsal line but no dark ventral line so that no white longitudinal line exists as in *Cx. theileri*.

Figure 8: *Culex univittatus*

Ae (Neo.) mcintoshi (Figure.9) is characterized by striking bright yellow lateral bands on its scutum while these bands are pale yellow in *Ae.circumluteolus*.
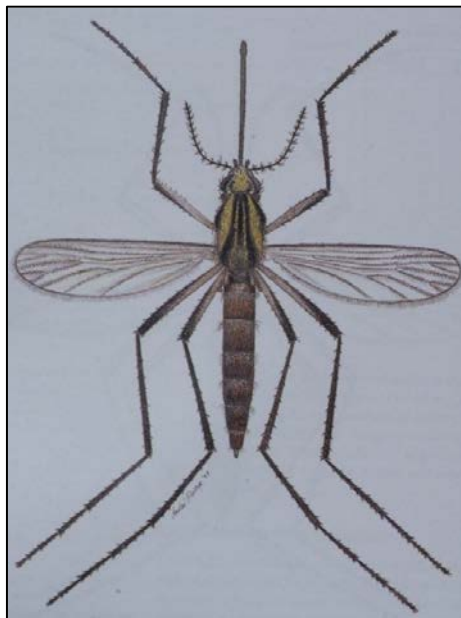
Figure 9: *Aedes mcintoshi*

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However, where 2 or more species of these *Aedes Neomelaniconion* occur together other features would have to be examined to distinguish them (see Jupp, 1996). *Ochlerotatus (Och.) juppi* (Figure.10) has a black integument clothed profusely with beige and white scales and some coppery coloured scales on its scutum; the tarsomeres have white basal bands. While the wing of *Oc. Juppi* is almost entirely dark, that of the closely related *Oc. (Ochl) caballus* is profusely speckled with pale scales on the veins.
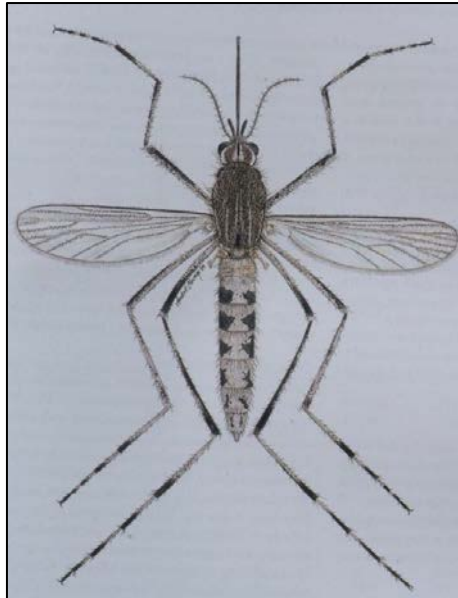
Figure 10: *Ochlerotatus juppi*

BIOLOGY

This account of mosquito biology only gives an overview for those genera and species that are vectors of the arboviruses infecting livestock. For a comprehensive treatment of the subject see Clements (1992 &1999). Mosquitoes deposit their eggs (oviposition) either on the water surface or on a moist substrate depending on the genus. In the latter case the eggs must be inundated before hatching will occur. After 2-3 days the eggs hatch to produce the first instar larvae. Culicine larvae feed on microorganisms on the bottom and grow larger through a series of 3 moults to become mature 4th stage larvae. These larvae moult to the pupal stage which does not feed. After 2-3 days the adult mosquito emerges from the pupa on the water surface (emergence).

A video of this life cycle for *Culex* mosquitoes can be watched on YouTube: http://www.youtube.com/watch?v=wFfO7f8Vr9c

The adults may disperse to a spot away from the water before mating and seeking a blood-meal (dispersal). Both sexes imbibe nectar from flowers or plant sap from stems or leaves but the female will normally have to blood-feed before she can develop eggs. *Culex molestus* can develop eggs without a blood-meal (autogeny). Other species are anautogenous – they develop eggs after a blood-meal which is obtained either from a feral or domestic vertebrate or from humans. Both sexes will seek out a moist sheltered place to rest (resting) while they await futher mating and the digestion of the blood-meal to form mature eggs. Resting

usually occurs on the ground in vegetation, or in animal burrows, pits or caves, although some species rest inside buildings. The species considered below mostly belong to the genera *Culex, Aedes* and *Ochlerotatus* but 3 other genera are also mentioned.

Genera *Aedes* and *Ochlerotatus*

In these genera the eggs are laid singly (Figure.13) on a moist substrate and providing the humidity is adequate in the microhabitat they can survive for a long period, even years, until the rains come or the snow melts and they are submerged. Instalment hatching, a mechanism whereby a portion of the eggs hatch after the first submersion and a portion after the second submersion and so on is characteristic and favours survival. In Africa, the genus *Ochlerotatus* and the aedine subgenera *Neomelaniconion* and *Aedimorphus* contain the floodwater mosquitoes which oviposit in grassland when it becomes inundated after heavy rain that is at the edges of pans (dambos), vleis, dams and rivers.

Figure 13: *Aedes* eggs

The eggs can survive a few centimetres down in moist soil for a number of years until the habitat is reflooded after heavy rain. The hosts of *Ochlerotatus* and *Aedes* on farmland are sheep, goats, cattle and humans. Biting occurs throughout the day with peaks usually occurring just before sunrise and just after sunset (the crepuscular periods). Thus the egg of *Ochlerotatus* and *Aedes* provides a mechanism whereby these mosquitoes can overwinter or aestivate through dry periods. There is no evidence that diapause occurs in eggs in Africa. In North America with its extreme winters the eggs of *Ochlerotatus* species, including *Oc.canadensis* behave in a somewhat similar way to African species. Many of the eggs deposited during the summer will subsequently survive the winter under the snow and hatch when it melts in spring. However, they survive the low winter temperatures by entering the dormant state of diapause which requires an increase in day length and temperature to be ended.

Genus *Culex*

Mosquitoes in the subgenus *Culex* all oviposit in permanent and to a lesser extent temporary ground water. A raft containing 200-300 eggs is deposited on the water surface (Figure.14). Such eggs cannot resist drying. Virtually all aquatic sites will be utilized by one species or another although certain species prefer particular habitats. For example, in South Africa, *Cx. univittatus* prefers temporary to semi-permanent accumulations of rain water over grass, usually in pools and ditches and *Cx.theileri* exploits all permanent water. The domesticated *Cx. quinquefasciatus* is an exception in that it prefers to oviposit in artificial containers which are rich in organic matter. In general, members of the subgenus *Culex* in all countries feed

mainly at night on birds (ornithophilic) and to lesser extent mammals. However, there are certain species from various countries that have also been recorded as biting both humans and horses namely *Cx.perexiguus*, *Cx.modestus, Cx.molestus* and *Cx.salinarius* while *Cx.tarsalis* also feeds on jack rabbits. *Cx.univittatus* is only a moderate feeder on humans and horses on the South African highveld. *Cx.(Cux.)* species in Europe and North America overwinter as hibernating adults (diapausing) while in the milder winter on the South African highveld they do this as quiescent larvae which may accelerate their development leading to adult emergence in mild spells. Members of the subgenus *Melaniconion* from tropical and subtropical America are extremely difficult to identify but it is known that several species bite rodents and will also feed on horses and humans when these become available. Biting occurs usually at night and during the crepuscular periods but certain species are daytime feeders.

Figure 14: *Culex* egg raft

Other genera

Culiseta melanura from the eastern USA deposits up to 300 eggs singly on to the water surface in the deep recesses of acid swamps. It overwinters as larvae and adult females are almost exclusively ornithophilic.

Coquillettidia perturbans,also in the eastern USA, deposits egg rafts in swamps with aquatic vegetation but when the eggs hatch the larvae and later the pupae attach to the stems and roots of the water plants by means of their especially modified siphons and trumpets. Hence they obtain air through the airenchyma cells in the plant. They remain attached except briefly for moulting and finally the pupa detaches and swims to the surface to allow the adult to emerge. This mosquito has a broad host preference feeding on birds, horses and humans.

Psorophora species from tropical and subtropical America deposit single eggs on moist soil in the same manner as *Aedes* and *Ochlerotatus* mosquitoes and similarly will bite both humans and livestock including horses.

ARBOVIRUS TRANSMISSION CYCLES

Arboviruses (arthropod-borne viruses) multiply in both vertebrates and arthropod vectors. The viruses produce viraemia in vertebrates to infect the arthropod vectors – mosquitoes in the cases dealt with here. The salivary glands of the mosquitoes must become infected so they can secrete virus in their saliva to infect further vertebrates. RVF virus is a *Phlebovirus*, WN and WSL are *Flaviviruses* while EEE, WEE and VEE viruses are all *Alphaviruses*. There are similarities in the ecology and life cycles of WN, EEE, and WEE viruses and between RVF and WSL viruses while VEE stands alone.

Arbovirus in a mosquito vector

The path of multiplication of virus within a mosquito that is a competent vector is shown in Figure.15. When such a mosquito feeds on a viraemic vertebrate, blood containing virus particles is drawn up into the foregut by the pharyngeal pump and then moves to the posterior midgut where virus multiplies in the interior epithelial lining. After a number of days, virus passes through the midgut wall into the haemocoel (mosquitoes have an open blood system) and after a further interval reaches the salivary glands which become infected. When the mosquito feeds again, virus particles pass with the saliva into the new susceptible vertebrate to infect it. Vector competence experiments can be done to determine whether a particular mosquito species is such a vector. A group of mosquitoes are fed an infective blood-meal and then after an interval will be allowed to feed on susceptible animals individually. Subsequently, each mosquito can be tested for infectivity and each animal can be tested to see whether it had become infected. If the percentage of mosquitoes becoming infected is high (high infectivity rate) and the percentage of infected mosquitoes successfully transmitting virus to the animals is also high (high transmission rate) that mosquito is a competent vector. The results of such laboratory experiments taken together with the frequency of virus isolations/detections made in field collected mosquitoes are evidence to incriminate a particular mosquito as a vector. Other evidence for this is the prevalence, distribution and ecological/biological characteristics of that species, particularly during an outbreak of viral disease.
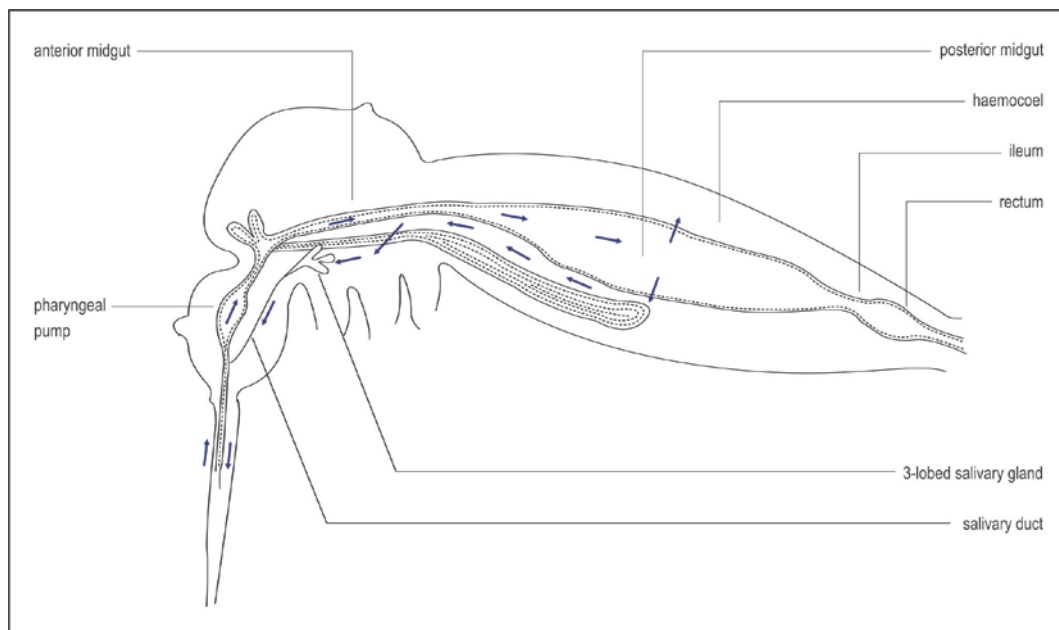
Figure 15: Longitudinal section of a mosquito vector to show the pathway of arbovirus infection

Rift Valley fever virus (RVFV)

Species of *Aedes (Neomelaniconion), Ae.(Aedimorphus), Ochlerotatus (Ochlerotatus)* and *Culex (Culex)* have been implicated as vectors of RVFV in several African countries and in Saudi Arabia- the only country outside Africa (see section on Distribution of Vectors). Most of the evidence for the transmission cycle comes from work done in South and East Africa (Jupp, 2004). The inter-epidemic period between outbreaks of the virus usually lasts for years and there is some evidence to indicate that the virus survives either in infected mosquito eggs buried in the soil and/or through ongoing viral transmission among livestock without illness in certain foci where a hyperendemic state exists. A diagram of the transmission cycle is shown in Figure.16. When the rains arrive and the eggs of the floodwater mosquitoes hatch in, for example, the panveld of the inland plateau of South Africa, vast populations of *Ae.(Neo.) mcintoshi* and *Oc. (Och.) juppi/caballus* mosquitoes develop which may include some females infected by vertical or <u>transovarial transmission</u> from the parental females present in the previous generation. These mosquitoes when they bite the domestic livestock will infect them initiating an epidemic transmission cycle. After water has been standing in farmland for a while, *Cx.(Cux.) theileri* will oviposit and populations of these long-lived mosquitoes will also join the epidemic cycle.
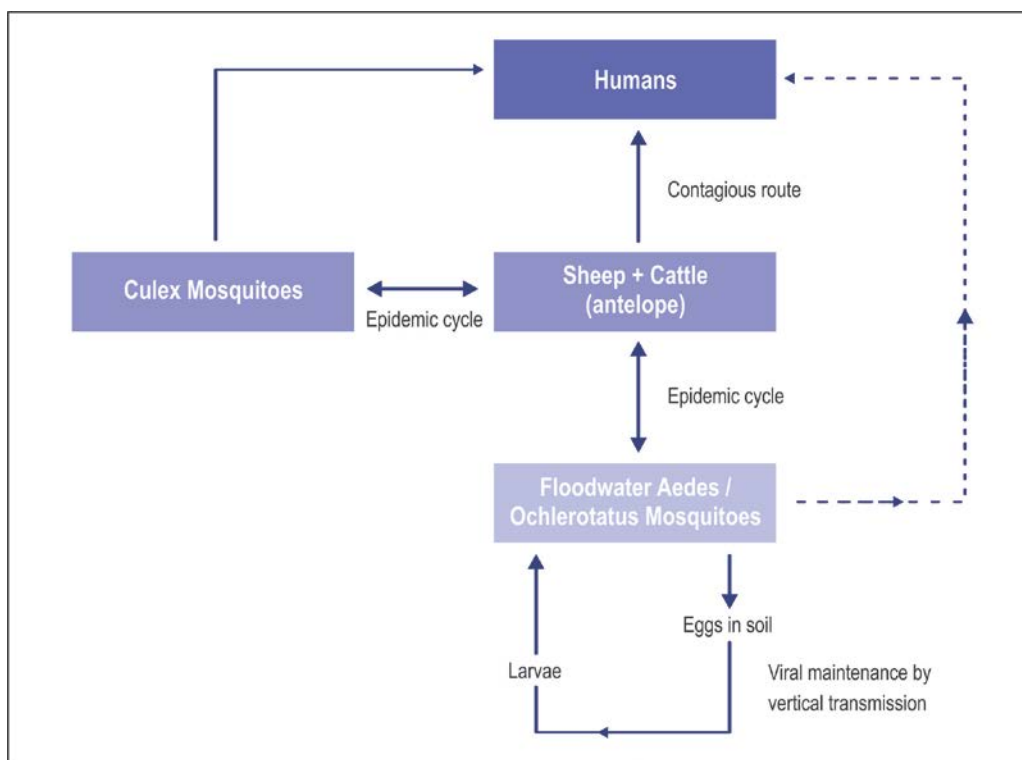
Figure 16: Transmission cycles of Rift Valley fever virus in southern Africa and Kenya; viral maintenance through the dry season is thought to be by vertical (transovarial) transmission by *Aedes/Ochlerotatus* mosquitoes

Humans occasionally become infected by mosquito bite but more frequently by the contagious route from undertaking post mortems without gloves. Figure.17 shows a typical flooded pan with thick sedge in the panveld of the Free State Province of South Africa, 2 net-traps and a light trap can be seen at the margins of the flooded area.

Arthropod vectors → Mosquitoes

Figure 17: Flooded pan with thick sedge, breeding place of floodwater *Aedes* & *Ochlerotatus* in the South African panveld of the Inland Plateau

Large numbers of floodwater *Aedes* and *Ochlerotatus* were collected at this breeding site. Normal <u>biological transmission</u> of the virus where virus multiplies in the mosquito is augmented by <u>mechanical transmission</u> during epidemics when mosquitoes and other biting flies spread the virus passively by multiple or interrupted feeding. This is possible because virus survives on the mouth parts for at least half an hour. RVFV is probably spread to new areas during an epidemic by movement of infected livestock, wind-driven flights of infected mosquitoes and perhaps the dispersal of infected *Aedes* or *Ochlerotatus* eggs on the feet of wetland birds.

Wesselsbron virus (WSLV)

This virus is widely distributed in Africa and there is one report of its isolation in Thailand from mosquitoes that needs confirmation. Evidence from work done in South Africa, Zimbabwe and some West African countries (Jupp, 2004), suggests that only floodwater *Aedes* and *Ochlerotatus* are involved in the epidemic cycle with livestock. In South Africa these are *Ae.(Neo.) circumluteolus* (Kwa Zulu-Natal coastal lowlands), *Ae.(Neo.) mcintoshi/luridus* and *Oc.(Och.) juppi/caballus* (South African Inland plateau). Although further quantitative vector competence tests are needed, preliminary laboratory experiments showed that the virus could be transmitted by *Ae. circumluteolus* and *Ae. caballus* s.l. but not *Cx.theileri.* It appears therefore that WSL virus is probably maintained during the dry season by means of vertical transmission by these species in a similar way to RVFV.

West Nile virus (WNV)

Only *Culex (Culex)* species have been implicated as vectors of WNV in Africa, Israel and Europe while in the USA, besides these mosquitoes, there are other species including *Ae. (Adm.) vexans vexans* and *Oc. (Och.) triseriatus* which act as link vectors to humans and horses. In South Africa many years of research has been undertaken on this virus in the highveld and Karoo regions (Inland plateau) (Jupp, 2001). A maintenance or endemic cycle has been shown to occur in the summer in which *Cx. (Cux.) univittatus* feeds on several species of wild birds, the primary vertebrate hosts of the virus. Human and equine infection depends entirely on this vector acquiring infection from birds and is therefore closely associated with avian infection. As

humans and horses are poorly viraemic after infection with the virus they cannot significantly infect mosquitoes so would usually represent a dead end in the transmission cycle. On the Highveld of South Africa *Cx.univittatus* has a low feeding rate on humans which tends to limit human infection. However, when climatic conditions- heavy rain and higher than usual temperatures – have favoured mosquito breeding and virus multiplication within the vectors, there have been significant outbreaks of the virus.

Figure.18 shows the feral cycle in Africa described above, in Egypt *Cx. perexiguus*, a mosquito taxonomically close to *Cx.univittatus,* is the vector. As can be seen in Figure.18, the endemic cycles in France, other parts of Europe and the USA are all similar with various *Culex(Cux.)* species feeding on wild birds. In every case humans and horses are a dead end in the cycles.The ecology of the virus in eastern Europe ,such as Romania ,differs from Africa in that *Cx.molestus,* a member of the *Cx.pipiens* complex of mosquitoes, feeds on domestic birds in an urban epidemic cycle that is linked to the feral endemic cycle. Also, while hibernating *Culex* mosquitoes do not occur in Africa, both European and North American mosquitoes hibernate which is thought to allow the virus to pass through the winter in infected hibernating (diapausing) females. In New York and the eastern USA where WNV has recently become endemic, various other mosquitoes act as link vectors to carry virus from the endemic cycle to infect humans and horses. These mosquitoes include *Cx.salanarius* and various *Aedes* and *Ochlerotatus* species. In the mid-western and western USA *Cx.tarsalis* is the endemic vector, while *Cx.quinquefasciatus* fulfills this role in the south east. When this southern *Cx.quinquefasciatus* meets the northern *Cx.pipiens* hybrids are produced that feed on birds and mammals. Hence the number of human and equine infections can be higher in this hybridization zone.
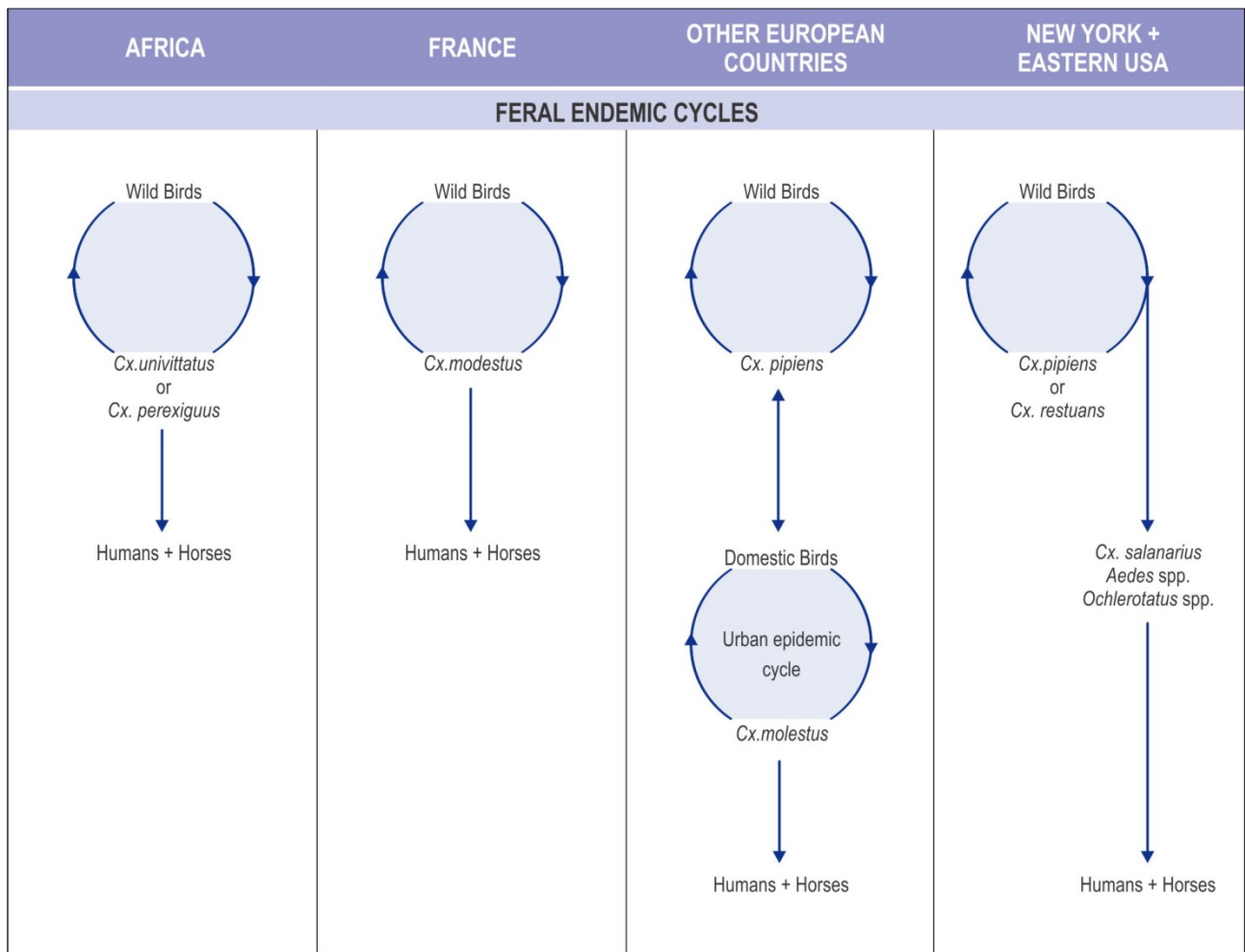
Figure 18: Transmission cycles of West Nile virus

Eastern, Western and Venezuelan equine encephalitis viruses (EEEV, WEEV & VEEV)

Referring to Figure.19, EEEV and WEEV both depend on mosquitoes feeding on wild birds in their endemic cycles viz *Culiseta melanura* and *Cx.tarsalis* respectively. EEEV in the eastern USA has several link vectors that are both ornithophilic and mammal feeders which enables them to transmit infection from wild birds to horses and humans. These are *Coquillettidia perturbans, Ae.vexans vexans* and *Oc.canadensis* among others. As horses develop a viraemia with EEEV in the laboratory sufficient to infect mosquitoes, it is probable that feral mosquitoes are infected in this way. In the case of WEEV in eastern and western USA, there is also an epidemic cycle in which *Oc.melanimon* feeds on jack rabbits and also transmits virus to horses and humans; *Cx.tarsalis* passes infection to these rabbits from the endemic cycle. It is possible that hibernating infected *Cq.melanura* and *Cx.tarsalis* mosquitoes are important for viral overwintering but the actual mechanism may be more complex than this. The ecology of VEEV is complex and probably varies among the different countries in the subtropical and tropical Americas. A number of *Cx. (melaniconion)* species feed not only on various wild rodents but also on humans and horses. These both develop viraemias high enough to infect further mosquitoes such as *Oc.taeniorhynchus* and various *Psorophora* species. Because of this characteristic as well as the fact that the virus can be transmitted experimentally by *Ae.*

Arthropod vectors → Mosquitoes

(Stg.) albopictus, it is the most likely of the 3 equine encephalitides viruses for possible accidental importation into Europe and establishment there. The ecology of VEEEV is further complicated by the existence of both endemic and epidemic subtypes of the virus.
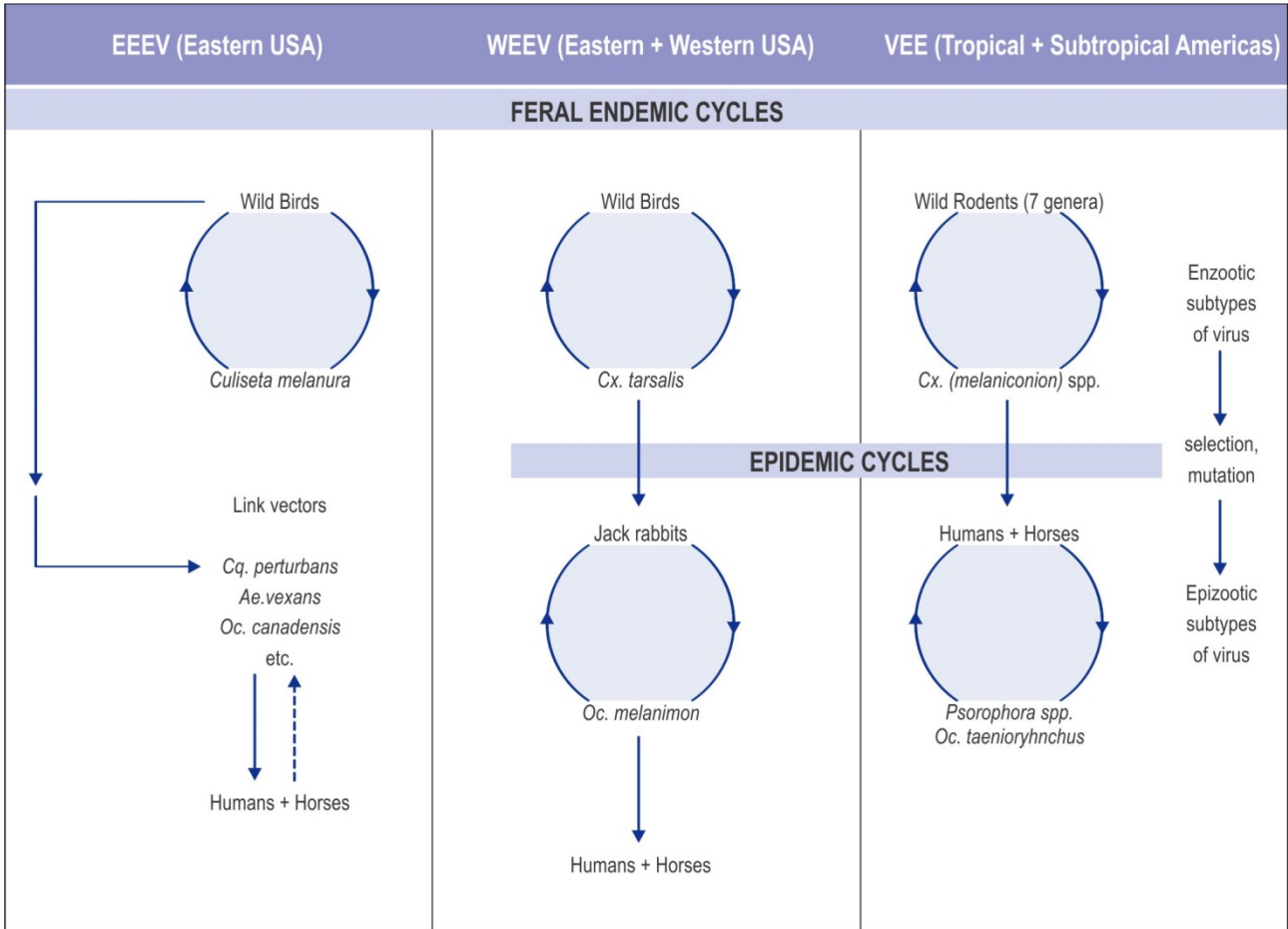
Figure 19: Simplified transmission cycles of Eastern, Western and Venezuelan Equine Encephalitis viruses (EEEV, WEEV, VEEV).

DISTRIBUTION OF MOSQUITO VECTORS BY VIRUS AND COUNTRY

The list (Table 1) is based on virus isolations often confirmed by vector competence experiments. The *Culex* species are all subgenus *Culex* except the one reference to *Cx. (Melaniconion)* spp.

Virus	Country	Mosquitoes
RVFV	South Africa (inland plateau)	*Ae.(Neo.) mcintoshi; Ae.(Och.) juppi / caballus; Cx.theileri*
	South Africa (KwaZulu-Natal coast)	*Ae.(Neo.) circumluteolus; Cx.zombaensis*
	Egypt	*Cx.pipiens*
	Kenya	*Ae.(Neo.) mcintoshi; Ae.(Adm.) ochraceus; Cx.antennatus; Cx.zombaensis*
	Senegal	*Ae.(Adm.) dalzieli; Ae.(Adm.)ochraceus; Ae.(Adm.) vexans arabiensis*
	Saudi Arabia	*Ae.(Adm.) vexans arabiensis; Cx.tritaeniorhynchus*
WNV	South Africa	*Cx. univittatus*
	Egypt	*Cx.perexiguus*
	Israel	*Cx.perexiguus; Cx.pipiens*
	Pakistan	*Cx.vishnui* complex
	India	*Cx.vishnui* complex*; Cx.quinquefasciatus (?)*
	France	*Cx.modestus*
	Europe	*Cx.pipiens; Cx.molestus*
	Eastern USA	*Cx.pipiens/Cx.restuans; Cx.salanarius; Ae.vexans vexans; Oc.(Och.) triseriatus*
	Mid-West & West	*Cx.tarsalis*
	South East, USA	*Cx.quinquefasciatus*
WSLV	Zimbabwe	*Ae.(Neo.) mcintoshi*
	South Africa (inland plateau)	*Ae.(Neo.) mcintoshi / Ae.(Neo.)luridus; Oc.(Och.) juppi / caballus*
	South Africa (KwaZulu-Natal coast)	*Ae.(Neo.) circumluteolus*
	Ivory Coast	*Ae.(Adm.) abnormalis* group*; Ae.(Adm.)dalzieli; Ae.(Adm.)tarsalis* group

Arthropod vectors → Mosquitoes

	Senegal	*Ae.(Adm.) dalzieli; Ae.(Adm.)minutus; Ae.(Adm.)vittatus*
	Central African Republic	*Ae.(Adm.) abnormalis* group*; Ae.(Adm.)tarsalis* group*; Ae.(Adm.)vittatus*
	Thailand	*Ae.(Adm.)mediolineatus; Ae.(Neo.)lineatopennis* (needs confirmation)
EEEV	Eastern USA	*Cs.(Cli.) melanura; Cq.(Coq.) perturbans; Ae.(Adm.) vexans vexans* *Oc.(Och.) canadensis*
WEEV	Eastern and Western USA	*Cx.tarsalis; Oc.melanimon*
VEEV	Tropical and subtropical Americas	*Cx. Melaniconion* spp; *Oc. (Culicelsa) taeniorhynchus; Psorophora* spp.

Table 1: RVFV=Rift Valley fever virus; WNV=West Nile virus; WSLV=Wesselsbron virus; EEEV, WEEV &VEEV= Eastern, Western & Venezuelan equine encephalitis viruses.

IMPORTANCE

Outbreaks of RVF and WSL are intermittent, usually with intervals lasting several years. Rift Valley fever is most severe in sheep, cattle and goats producing high mortality in new-born animals and abortion in pregnant animals (see Rift Valley fever). Thus there can be heavy stock losses. Wesselsbron virus also infects sheep, cattle and goats but is only important for the low mortalities that it causes in new-born lambs and kids. An epidemic of RVF with the resultant high loss of livestock has significant economic impact. Rift Valley fever was confined to Africa until an epidemic occurred in 2000 in Saudi Arabia. This indicates that it might spread further afield.

For a long time WN virus had only been known to cause disease in humans although it was never a major health problem. However, since the mid-1990s outbreaks have occurred in both humans and horses in Europe countries, North Africa, Russia, Israel and the USA. Historically the majority of equine infections were unapparent or mild but more recently severe disease has been reported related to more virulent strains of the virus. Hence horse breeders need to be alert to the potential danger of WN virus to their industry.

Epidemics of equine encephalitis due to EEE, WEE or VEE virus are now rare but when they occur they cause heavy mortality in horses and illness in humans that may lead to death.

It should be noted that exposure of livestock to extreme densities of uninfected mosquitoes can lead to a general health deterioration and reduction in milk yield. An example is the super- abundance of floodwater *Aedes* mosquitoes that occur after heavy rains in several parts of South Africa.

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CONTROL

Livestock housed in stables or barns can be protected from mosquito bites while it may be possible to move animals living outside away from areas where mosquitoes are particularly abundant. Alternatively, populations of the vector mosquitoes can be reduced either by chemical or bacterial insecticides or by the use of an insect hormonal growth regulator. Burning of particular vegetation on a farm may also help control mosquitoes in certain cases.

Protection of livestock in stables and barns

This is most relevant for the protection of stabled horses from mosquitoes carrying WN virus or the equine encephalitides viruses. Buildings should be screened and repellents can be applied to the animals, the repellents of choice being those containing DEET (diethyl toluamide). Further information on repellents can be found at http://www.cdc.gov (see 'CDC West Nile virus -what you need to know about mosquito repellents') and http://www.liquidfence.com/ (products for use on horses). The use of fans in stables can reduce mosquitoes feeding and turning off lights also deters these insects. Aquatic sites for larvae near barns and stables should be removed and also chickens and pigeons should not be allowed in or near these buildings as they can act as reservoirs of virus in the bird-mosquito viral transmission cycles. It may also be possible to reduce the biting populations in the vicinity by "removal" trapping with high powered traps baited with CO_2 and an octenol lure (see section on 'Collection Methods'). Livestock outside can be moved to higher ground away from marshes, dams and pans at least during the peak biting periods of the vectors concerned i.e. night, dawn and dusk. In the case of RVF and WSL viruses, the eggs of *Aedes* and *Ochlerotatus* vectors present in the soil in the dry season can be killed by burning the vegetation in selected places such as dry pans.

Chemical insecticide control

In many cases, e.g. on large farms with extensive aquatic areas producing mosquitoes, insecticidal control may not be feasible and vaccination of livestock together with the avoidance of areas with high mosquito densities would be advisable. Today, for larval control, the tendency is to use the more "environmentally friendly" and more effective bacterial insecticide BTI (see below) or the insect growth regulator Methoprene rather than chemical larvicides that cause environmental pollution. However, chemicals are still important for the control of adult mosquitoes. Milking sheds or stables can be sprayed with residual insecticides such as the organophosphates malathion, fenthion (Baytex) or fenitrothion (Sumithion). Other residuals are the carbamates (Sevin) and propoxur (Arprocarb). For wider adult control, a "knock down" pyrethroid such as resmithrin (Scourge) which degrades after 4 hours can be used for ULV (ultra-low volume) aerial or ground application.

Larval control with BTI

The bacterium *Bacillus thurigiensis israeliensis* (BTI) was discovered in Israel in 1976 and has since been developed and marketed as an effective bacterial insecticide which targets only mosquito larvae making it safe for the environment. BTI is available from Abbott laboratories or their subsidiary companies as briquets, pellets, granules or liquid under the trade names 'Bactimos' or 'Vectobac' which can be applied on the ground either by hand or with a machine or in the air by spray aircraft. 'Bactimos' briquets, for example, are a sustained release larvicide that provides effective control in aquatic larval sites for several weeks. BTI can be

applied to an existing larval site or a dry pan can be seeded with pellets or granules prior to the onset of the rains. Hence BTI would be useful for controlling floodwater *Aedes* in pans and vleis as well as those *Culex* species that occur in water containers or small ground pools.

Larval control with methoprene

Methopene is an insect juvenile hormone analogue which regulates insect growth. It kills 2nd, 3rd, and 4th larval instars but not pupae. As it only kills mosquito larvae and leaves all the other aquatic fauna unharmed it is environmentally safe. Because it kills the 4th instar larva not always killed by BTI it may be a superior larvicide. It is marketed as 'Altosid' by the Zoecon Corporation and is available as briquets, pellets, sand or liquid. Altosid XR briquets have been shown to release effective levels of methoprene for up to 150 days in small bodies of water, marshes and flood plains after one application. Altosid pellets release methoprene for up to 30 days and can be applied either on the ground or by air. Altosid liquid can be applied in a similar way. 'Altosand ' is a mixture of sand, Altosid liquid and silicon dioxide which is useful for applying by air over mosquito breeding sites which have dense vegetation or a canopy.

For an in-depth study of mosquito control or for reference on a particular aspect, the reader is referred to the recent book by Becker *et al.,* 2010.

COLLECTION METHODS

Reasons for collecting mosquitoes

- To determine which species are present in an area and their relative densities.
- To rear progeny from the collected mosquitoes for taxonomy or for establishing a laboratory colony or for vector competence tests with an arbovirus.
- Trapping methods using animal bait indicate the host preferences of different mosquitoes.
- The mosquitoes collected can be tested for the presence of arboviruses.
- Sometimes "removal trapping" can be done to reduce densities of biting mosquitoes (control).

Collecting with test tubes

A female mosquito searching for a blood-meal is attracted to the vertebrate host from a distance by the CO_2 and lactic acid exhaled by the animal. As it nears its host, the animal's movement, body odour and body heat will attract the insect further. Traps baited with an animal or CO_2 depend on such behaviour. Adult females can readily be collected as they come to bite humans by inverting a glass test tube (15mm diameter and 90mm long) over the probing mosquito and subsequently plugging the tube with cotton wool. Both males and females resting on a surface in houses, caves or in holes in the ground may be collected in the same manner, although a small net is an easier way to collect off vegetation while a Hausherr's electric aspirator is better for collecting off a surface. The advantage of obtaining individual specimens, each in a tube, is that they can be inspected with a hand lens immediately, often identified in this way and then transferred either to a killing bottle or to a small cage where they can be given a blood-meal if progeny are to be obtained. Such specimens are usually in good condition for identification, whereas some of the specimens collected in traps, especially those with suction motors, may be damaged.

Traps with suction: light traps and gravid traps

Many different traps have been designed including suction traps operated with various attractants such as light and/or CO_2 or animal bait such as pigeons, sheep or hamsters. Figure.11 shows the home-made NIV light trap (Jupp, 1986) which consists essentially of a cylinder containing a fan that sucks mosquitoes downwards into a cage.

Figure 11: NIV light trap

Attractants are a torch bulb mounted at the entrance to the cylinder together with a padded envelope containing 2kg of chopped dry ice. The trap is set overnight. Another trap depending on suction is the gravid trap that collects those females that contain eggs (Reiter, 1986). This means that they have already fed so a catch is more likely to include virus-positive mosquitoes. This trap is particularly suited to collect *Culex* to test for the presence of virus. In this trap mosquitoes attracted to a pan of hay infusion to oviposit are sucked upwards into a holding cage. Details on a range of light and gravid traps that can be purchased are available (http://johnwhock.com/). Suction traps can also be suspended under a cage containing sentinel pigeons so that mosquitoes attracted to the birds to feed are sucked downwards into collecting cages. These catches with any virus-positive mosquitoes they contain can be related to antibody conversions occurring in the sentinel birds in the case of a virus like WN.

The net-trap

Other traps depend upon animal bait or CO_2 without suction e.g. the net-trap shown in Figure.12, which is a useful method for sampling a large variety of species. This is a tent of mosquito netting measuring 2m long, 1.6m wide and 1.8m high, supported on poles and having a zip at one end to permit access. When set at

nightfall the sides are rolled up to provide a 15cm opening all round to allow entry of mosquitoes and 2.5kg of broken dry ice in a box or an animal such as a sheep are placed inside as bait. Early the next morning the sides are rolled down and the operator enters the tent, collects the mosquitoes with a battery operated aspirator and transfers them to small cages for return to the laboratory. The light trap and net-trap baited with CO_2 and the gravid trap are recommended for veterinary mosquito collections. A comprehensive review of collection methods is given by Silver (2008).

Figure 12: Net-trap

Collection of immature stages

No single collecting method will sample all the species present at a site so in conducting a mosquito faunal survey a wide variety of methods should be employed which include the collection of larvae and pupae. These immature stages can be collected with a small net or soup ladle "dipper". A dipper may be large and constructed from an enamel bowl of about 750ml capacity with a long handle so a large volume of water can be sampled from the bottom of a ground pool at each dip. Individual larvae or pupae can be sucked up from the water sample using a glass tube fitted with a large rubber bulb. As many different kinds of aquatic sites as possible should be sampled.

Removal trapping

Removal trapping has been done with some success using traps incorporating both a flow of CO_2 from a cylinder and chemical lures as attractants. Examples are the "Magnet trap" (http://www.mosquitomagnet.com/) that utilizes a cataytic combustion unit to convert propane into CO_2 and the ABC PRO trap (Cilek *et al., 2003*)

Killing and storage of mosquitoes

Adult mosquitoes can be killed by placing in a domestic freezer for 30 minutes or by using hydrogen cyanide (HCN) generated from potassium cyanide (KCN) crystals (killing bottle) or from calcium cyanide ($CaCN_2$) (Cyanogas) when cages of mosquitoes are to be killed. Specimens needed for critical taxonomic examination should be killed with HCN as freezing causes the legs to become unbendable. Such specimens should either be pinned (Jupp, 1996) or placed between layers of tissue in a small box. Adults needed for arbovirus testing can be killed in either of the above ways and then stored in liquid nitrogen or dry ice. Jupp (1996) has described how to make a killing bottle. Larvae for taxonomy should be killed by placing in hot water (60° C) and then transferring them into a mixture of glycerine (1 part) and 96% ethanol (9 parts) in a screw top bottle with exclusion of air bubbles.

FREQUENTLY ASKED QUESTIONS (FAQ'S)

1. **How does one distinguish mosquitoes from other flies that look very similar?**

   The main character to look for is the mosquito's long proboscis which is absent in chironomid midges and other non-biting flies. Furthermore, mosquitoes have a covering of flat (decumbent) scales and a fringe of scales on the posterior wing margin.

2. **Is it only the female mosquito that feeds on blood? If so, why doesn't the male need blood as well?**

   Yes, only females take blood-meals which they require to obtain protein for egg development. As the males don't deposit eggs, they don't require the protein. Both sexes feed on nectar from flowers and plant sap from stems and leaves.

3. **Do the same mosquitoes that carry human malaria also transmit viral diseases to livestock?**

   No, only certain *Anopheles* species (anophelines) carry the human malaria parasite and the arboviruses causing certain diseases in livestock are only carried by culicine mosquitoes.

4. **What is it that attracts mosquitoes to bite us and our livestock?**

   From a distance it is the CO_2 and lactic acid exhaled by humans and animals. Then as the insect becomes closer the host's movement, body temperature and body odour enable it to finally find its target.

5. **Is pouring oil on to the surface of water still used to control mosquito larvae?**

   Not really, although it might still be used for small accumulations of water e.g. in containers. Nowadays, the bacterial insecticide *Bacillus thurigiensis israeliensis* (BTI) or the insect hormone Methoprene is added to the water as pellets or briquets that release the active ingredient over a period of time.

6. **If *Culex* species bite birds in the West Nile virus transmission cycle, how do they penetrate all those feathers?**

Arthropod vectors → Mosquitoes

The mosquitoes go for the area around the eyes, the feet and the thinner down feathers on the breast and abdomen.

7. Why is West Nile virus so widely spread in several continents while Rift Valley fever virus and Wesselsbron virus are almost confined to Africa?

Being an avian virus, it has probably spread from south to north by migrating birds that were chronically infected. However, importation into North America is thought to have been by an infected mosquito arriving from Israel in an aircraft as birds do not migrate from west to east.

8. What is the simplest way for a person with no special knowledge of mosquitoes to collect mosquitoes biting cattle and to send them to a laboratory for identification?

An ordinary test tube (15mm diameter and 90mm long) can be inverted over a mosquito as it alights and starts probing. Such tubes can then be placed in a domestic freezer for 30 minutes to kill the insects. After this the dead mosquitoes should be emptied onto layers of tissue paper in a plastic box. The specimens would be between layers of tissue paper so that they do not move around but are not packed too tightly which would damage them.

9. Why is it that in South Africa human infections of WSL virus on farms are very rare during an outbreak in sheep, while the number of sheep infected is high?

Mosquito vectors of this virus are confined to the floodwater *Aedes* and *Ochlerotatus*, which tend to keep to the vegetation in or near the pans, dams or vleis. Usually the farmer doesn't enter these areas, particularly at dawn or dusk, so he doesn't expose himself to bites from these vectors.

10. What would be the most effective way for a farmer to protect his sheep from becoming infected by RVF if this disease was active in his area?

Probably to immunize his flocks against the virus and to move them away from the mosquito breeding sites (pans, vleis, marshes and dams) particularly at dawn and dusk.

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