

3rd OIE webinar on African horse sickness:

AHS' insect vectors

20 April 2020

Questions & Answers

CULICOIDES HABITAT/BEHAVIOR

- Does day/night impact on the behaviour of *Culicoides*? Are some of genus day biters?

Yes most *Culicoides* have increased activity at dusk and dawn, some may be active before dusk or after dawn and some even have day long activity, but none of these have been implicated to date as vectors of AHSV. In cold climates, species that are normally active at dusk may become active before dusk in cooler seasons to exploit the warmer temperature. Data from temperate and tropical areas show that there is great variations in the circadian rhythm in relation to daily temperatures and meteorological conditions, and seasons.

- What kinds of habitats are common to *Culicoides*? Any specific conditions?

Culicoides is a large genus of more than 1500 species. A relatively small proportion of these are associated with arbovirus transmission so we'll confine our comments to those species as comments relating to the entire genus will be too broad. *Culicoides* larvae are associated with a semi-aquatic organically enriched environment. Larvae are very vulnerable to dehydration. The larvae of livestock feeding species mostly live in wet soil, animal dung or rotting vegetation; the two requirements are an environment rich in organic matter and high water content. Adult resting sites are uncertain, but as they also dehydrate quite quickly, they are likely to rest in the humid, shady, cooler areas such as in vegetation¹ during the day. Populations need 4 factors to survive; suitable temperature, larval habitat, source of blood meals and suitable microhabitat for adults to rest.

- What is breeding ecology of *Culicoides*?

Some species of *Culicoides* form swarms that are visible during the evening and may be associated with hosts. Others mate facultatively.

- How many blood meals would a *Culicoides* usually take in a day?

Usually just one blood meal every 3-5 days (depending on temperature). They just need a minute volume of blood. As they are small they are rarely disturbed from the host. Afterwards they find a humid, cool place to digest and can produce a batch of eggs from this single blood meal.

- What is the volume of rainfall is the best for increasing the *Culicoides* sp. (AHS vector) (mm/month)?

Broad relationships have been drawn between the severity of monsoons and outbreaks of AHSV and related viruses, but these are not specific enough to provide an exact prediction on rainfall. Heavy rain is not favourable to midges (disrupts adult flying capacity and can damage larval habitats). Ideal conditions for midges: warm temperature, low wind speed and high humidity

¹ *C. brevitarsis* has been documented as resting in vegetation (grass) during the day.

- How high can *Culicoides* species fly? If we fence the area with cypermethrin and without a roof will it work if the flies can't fly high?
In one published study, Calvete et al 2010, used cypermethrin-impregnated canvas barrier of 2,6 meters without a roof. Midges were trapped inside, but in reduced numbers. This barrier screen is better than nothing, but would not eliminate risk if used alone.

ROLE OF CULICOIDES IN AHSV TRANSMISSION

- What is the reason behind selecting *Culicoides* as the major vector of AHSV and why not other insects like mosquitoes?
Culicoides have been implicated through transmission experiments, abundance near horses and through isolations of virus made from field populations to a much greater degree than mosquitoes.
- Do blood-sucking insects have transstadial / transovarial transmission?
While this is true of several mosquito-borne viruses, no vertical transmission of AHSV has been demonstrated to date.
- Is there any other viruses that could be transmitted by vertical transmission of *Culicoides*?
There is evidence for transovarial transmission of Schmallenberg virus in Europe, but virus has never been isolated from these populations of *Culicoides*. This transmission probably has limited impact on the disease epidemiology.

CULICOIDES PRESENT IN ASIA

- In Asian Countries that *C. imicola* is absent, that is the most potential vector for AHS in Asia?
See Glenn Bellis' slide 5 – 2 important aspects to consider:
 - knowing the species that are the most abundant species biting horses is needed.
 - There seems to be a correlation between species transmitting AHS with species transmitting other orbiviruses
 - *Culicoides* are present throughout the world where horses are present (except New Zealand and Hawaii) and we cannot exclude any species of transmitting the disease but the above 2 factors can help to identify species that have the potential to play a role
- Reported species in Thailand: see the Thepparat et al 2015 paper

BEST WAY TO DIFFERENTIATE CULICOIDES

- What is the easiest way to identify different spp of *Culicoides*? is there any identification keys available?
 - List of references and contacts to be made available. www.gnatwork.ac.uk / @the_gnatwork
 - Many species can be reliably identified while still in ethanol but others require dissection, mounting onto microscope slides & examination under high magnification: This is a very difficult task requiring a lot of expertise and time
 - DNA identification is possible for only small numbers of species as it is expensive and slow. Many species in Asia do not have publically available DNA sequence data which reduces the usefulness of this technique.

COLLECTION OF MIDGES

- Why midges need to be collected in a solution?

When you collect midges in mosquito traps, they are dried and mixed together with many other insects. *Culicoides* are very small and fragile and dried specimens shrivel and are much more difficult to identify. Collecting them in solution (water + drops of soap or directly into ethanol if not too warm) produces much better specimens and it is much easier to remove them from other insects in the trap. Also, adding soap/ detergent to the water reduces surface tension so the midges will sink to the bottom of the solution and not fly away from the surface. A lot of published references exist.

- Can sucking tube be used for collecting *Culicoides*?

Presuming a sucking tube is an aspirator, aspirating midges directly from the horse, is indeed way to determine which species bite horses and to assess insecticide effect. This method is labour intensive and is neither accurate for collecting abundance information nor logistically feasible for surveillance.

- To detect AHSV in insects, do we need them to take blood from AHSV-affected horses?

Detection of AHSV in *Culicoides* needs to be carried out where the blood meal has already been digested and replication of the virus has occurred in the midge. Selecting these individual midges is time consuming as you need to identify the species, then separate the females to identify the parous specimens that have taken and digested a blood meal previously. Males and nulliparous females have not yet taken a blood meal so have not been exposed to virus and will not be infected. Engorged females must not be used because they could hold the virus in the fresh blood meal but might not be able to re-transmit. This is more a research activity and is not appropriate for surveillance. For vector competence studies in the lab, considering you have a *Culicoides* colony, you will need to have them fed on an infected blood meal made in the lab, or from a known viraemic horse.

- Where are OVI traps available?

Only 2 suppliers: Onderstepoort Veterinary Institute (OVI) in South Africa and Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM) Italy. They can be used on car battery or regular power. Research institutes in Asia may have some stock. Although considered as the reference trap, OVI traps are expensive (400 euros/unit) and heavy. One can think using other trap types considering that abundance and species diversity might not be comparable with other places using the OVI traps. In Australia, the Bioquip modified CDC trap is regularly used. Traps based on the CDC design are also used in Asia as well.

- How can horse industry enterprises get OVI traps and assistance in identifying *Culicoides* trapped therein?

Collaboration from the different stakeholders is welcome. The important point here is the reason why you want to collect midges, and how this can be linked to the risk of AHS transmission. It is important to be part of a national/regional protocol for midges surveillance. The bottleneck is the identification of samples which require coordination among the partners, long term training of dedicated staff to morphologically identify the specimens and specific database to manage the results. It is a regional problem that would benefit from regional coordination

PROTOCOL FOR MIDGES CONTROL

- Do you have a specific protocol for *Culicoides* control? How to control *Culicoides* breeding? Any biological control method for *Culicoides*?

Both stabling and insecticide/repellent application are recommended. In addition, stable interiors can be sprayed and also bedding if housing a viraemic horse. Transport should also be sprayed prior to use. No biological control methods are implementable in the field; some methods were tried in the past but haven't been effective in stopping the transmission and impacted the environment. But you could identify sources of favourable habitat in a specific environment and try to act on them (e.g. eliminating leaking water troughs creating a muddy environment).

INSECTICIDES & REPELLENTS

- What is the most effective insecticides for *Culicoides*? How often should they be used?

For mesh: Cypermethrin 0,02% and above (many registered products); in one field study daily replenishment spraying of mesh kept the concentration at target levels. For application to horse: cypermethrin (insecticide); or rather DEET (repellent) –good data and safe if not above 15-17%.

- How often that we can use permethrin (Etofenprox) on horses?

There is no specific data on Etofenprox (pyrethroid) on horses. For permethrin, one study with 3.6% pour on showed slight non-significant reduction in midges caught in tent trap. Permethrin is not as potent as cypermethrin against *Culicoides*.

- Any adverse effects reported from 15% DEET in horses?

15% applied daily oiliness in the hair coat at the application site particularly. Other than that, and except if you treat a horse with particular sensitivity, there shouldn't be any problem with concentrations 15% or lower. Advice: to check that your horse is not sensitive, on Day 1 treat a small surface of the horse (e.g. 1 side of the neck) and look for reaction the next day.

- Any potential adverse effect of DEET on human?

DEET has a plasticizing effect that can damage some fabrics and surfaces. Recommend read human safety reviews "the normal use of DEET does not present a health concern to the general population, including children" <https://www.epa.gov/insect-repellents/deet>

- What are the risks of toxicity from the cypermethrin nets?

One Cypermethrin side effect in people is paraesthesia = tingling sensation (on nose or lips for example). Read the product label. Consult human safety reviews

- Have you observed permethrin resistance in midges?

No strong evidence of resistance in midges. In India, there was some weak signal suggesting possible resistance. Insecticide resistance has been proven in USA with organophosphate components. Spraying the environment could facilitate resistance, you should rather spray where we know *Culicoides* are.

- Is alphacypermethrin treated mesh is also helpful to prevent anopheles mosquitoes too? any study?

Meshes that stop *Culicoides* are likely to have a similar greater impact on mosquitoes due to their larger body size. However, resistance in mosquitoes may be present and is more clearly understood than in *Culicoides*.

- How often should the treated meshes be replaced?

Daily spraying of the mesh e.g. with >0.02% cypermethrin is recommended.

- How high can *Culicoides* species fly? If we fence the area with cypermethrin and without a roof will it work if the flies can't fly high?

In one published study, Calvete et al 2010, used cypermethrin-impregnated canvas barrier of 2.6 meters without a roof. Midges were trapped inside, but in reduced numbers. This barrier screen is better than nothing, but would not eliminate risk if used alone.

- How can we protect non AHS infected horses from *Culicoides* biting in the endemic area? (for example in Thailand)

General recommendation to reduce risk: Horses should be kept in a stable, protected with mesh treated with cypermethrin (>0.02%) at dusk and dawn and horses should be treated with DEET (not higher than 15%) every day. This would reduce, but not eliminate risk.

- For insecticide susceptibility test which type you recommend either wild type or lab reared *Culicoides* midges?

Field collected *Culicoides* can be used but suffer high mortality even in control populations.

- Are there any recommendations for *Culicoides* prevention measures for zebras?

Zebras can be viraemic up to 40 days and represent a high risk of disease transmission in areas where AHS is present. If a place is available, they could be safely sprayed with repellents or insecticides on a daily basis. The use of the Duncan applicator could be explored which is used to treat wildlife with insecticide in the field <https://duncanapplicator.com>

- What is the most sustainable solution to the arbovirus problems?

Efficacious vaccines; monitoring of global trade networks; collaborative and transparent working on control.

OTHER QUESTIONS

- Are movement of bedding and feed a risk pathway?

If an infected horse has been in the environment it is worth spraying bedding to make sure there are no midges left alive in there. Spraying of bedding may reduce theoretical risk, but not aware of specific data.

- What about donkey's skin?

Midges do not recognise hides as originating from animals and will not feed on them. This is also true of dead animals.

NETWORK

- May i know is there any network or forum that we can join or follow for more info, discussion on *Culicoides* spp and AHS after this informative webinar?

www.gnetwork.ac.uk / @the_gnetwork