AHS Vector Surveillance in Thailand

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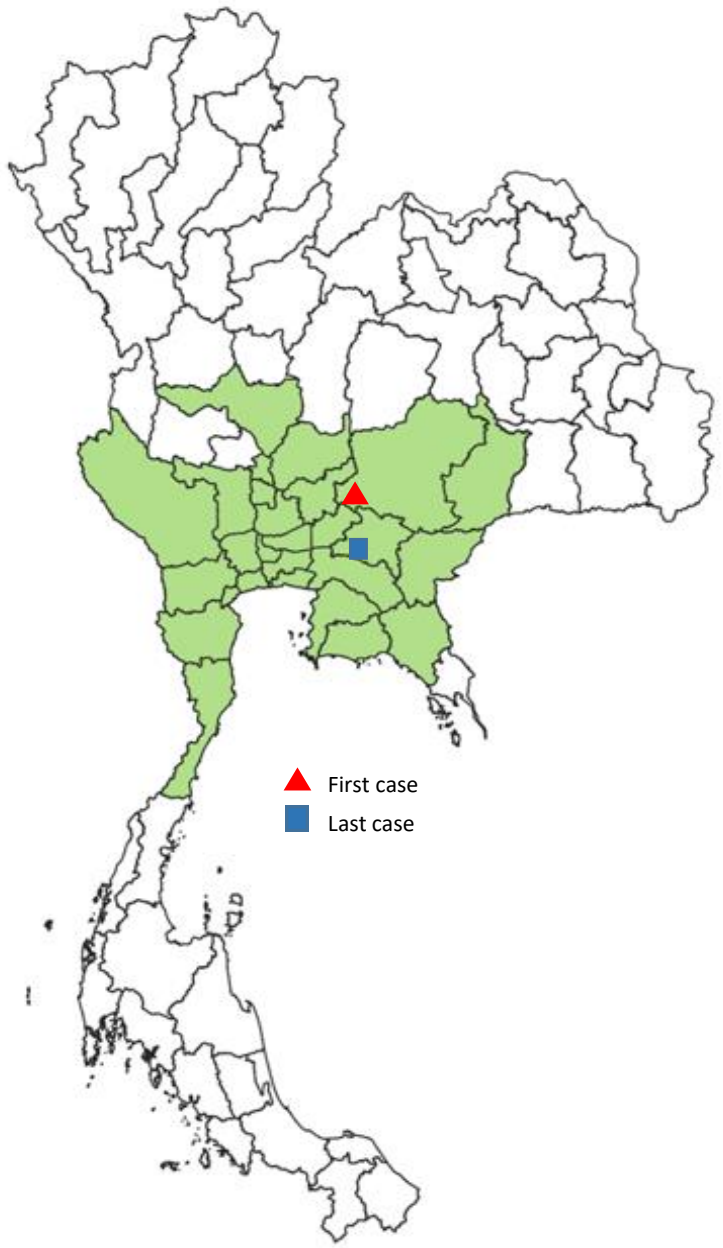
African Horse Sickness (AHS)

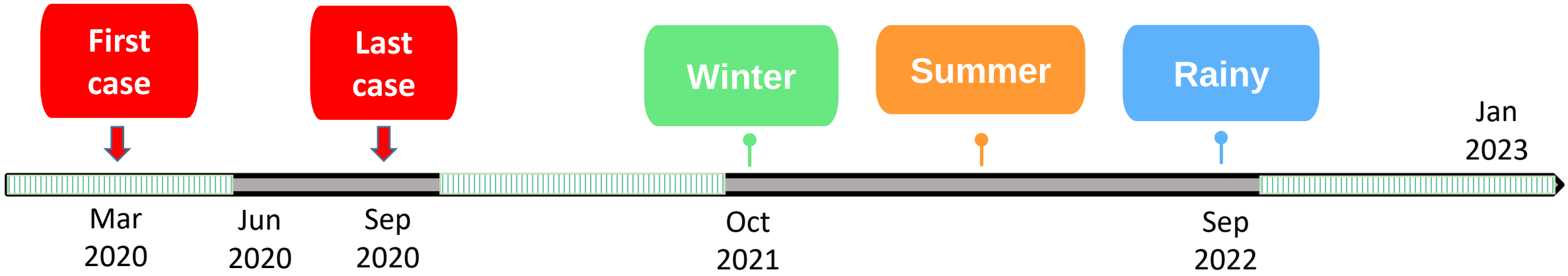
- AHS is affecting in the respiratory and circulatory function of all species of equids especially in horses.
- AHS is not directly transmissible but is known to be spread by insect vectors.
- *Culicoides* (midges) is a potential biological vector of AHS, however virus can also be transmitted by mosquitoes and ticks

AHS outbreak in Thailand



- The first confirmed cases of AHS were detected in March 2020 in Pakchong district, Nakornratchasrima province
- AHS spread throughout central part, lower northeastern part, western part, eastern part of Thailand, in total 17 provinces
- The last outbreak was recorded in September 2020 in Prachinburi province
- NIAH has provided diagnostic services during the AHS outbreak and surveillance period and is responsible for the vector surveillance, an important activity to prove absence of virus circulation

Vector surveillance timeline



Part I :
Short-term surveillance

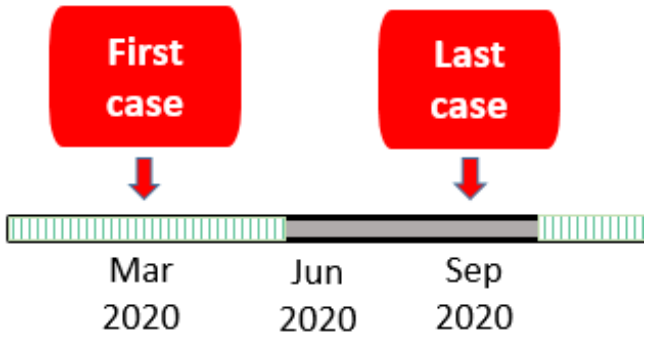
Part II :
Long-term surveillance

Overall objective

- Seasonal abundance, diversity and role of *Culicoides* spp. in African Horse Sickness transmission, Thailand

Part I. Vector surveillance during the outbreak period

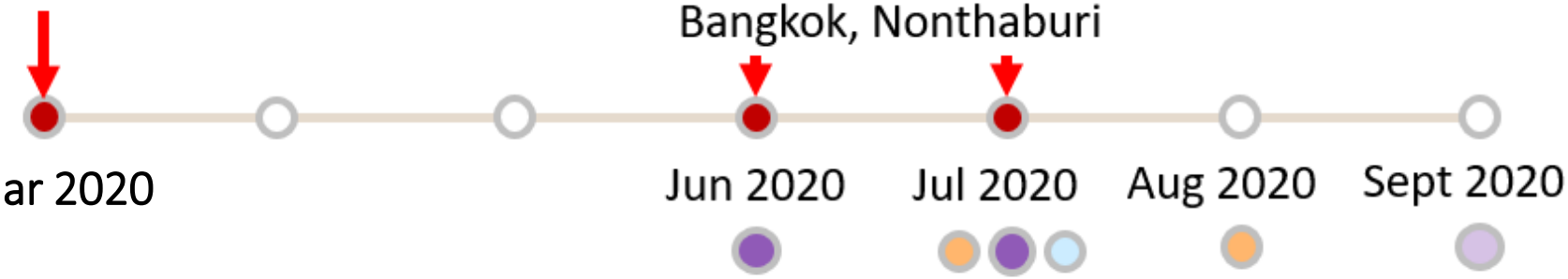
- Vector surveillance was performed shortly after the emergence of AHS in Thailand to identify AHS potential vectors and to check them for virus load
- The survey was carried out during June to September 2020 in outbreak areas, around zebra farms, and near stables with vaccinated horses.



Vector surveillance during the AHS outbreak

Outbreak
in Nakhon Ratchasima & Saraburi

Outbreak
in Chachoengsao, Pathum Thani,
Bangkok, Nonthaburi



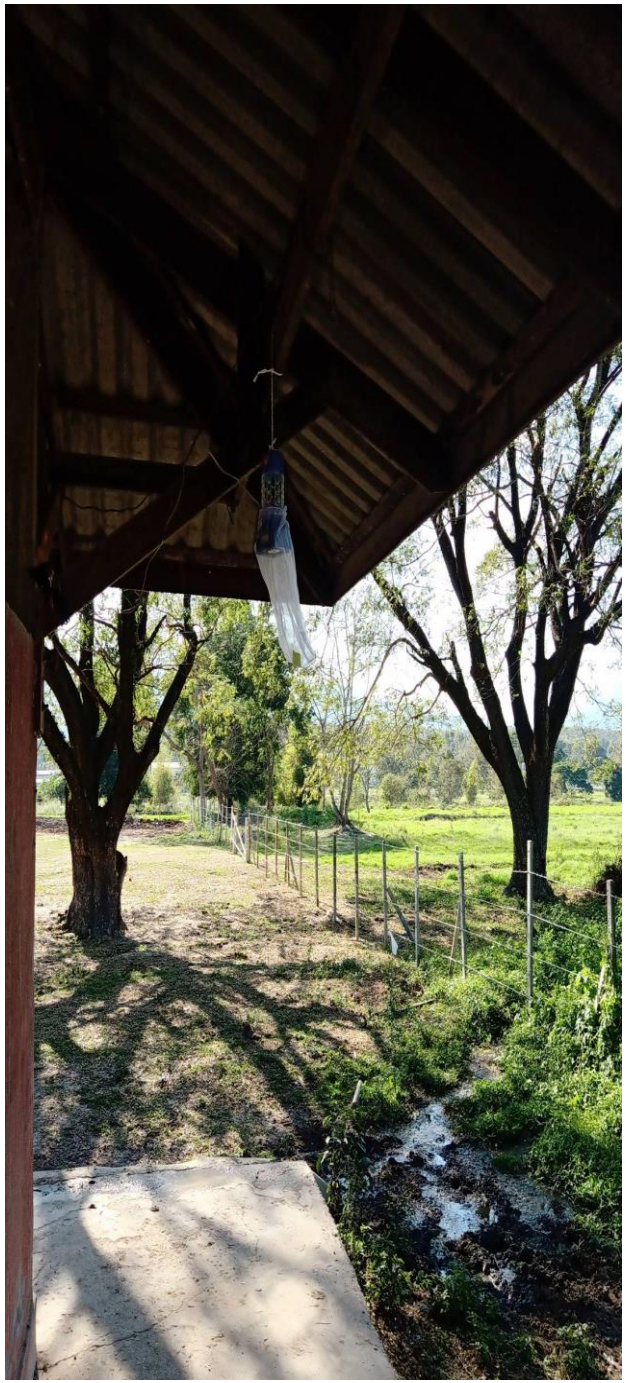
Surveillance areas

- outbreak areas
  - Nakhon Ratchasima and Saraburi
  - Chachoengsao, Pathum Thani, Bangkok and Nonthaburi
- zebra farms
  - Chachoengsao and Prachinburi
- a horse vaccinated area
  - Nakhon Nayok

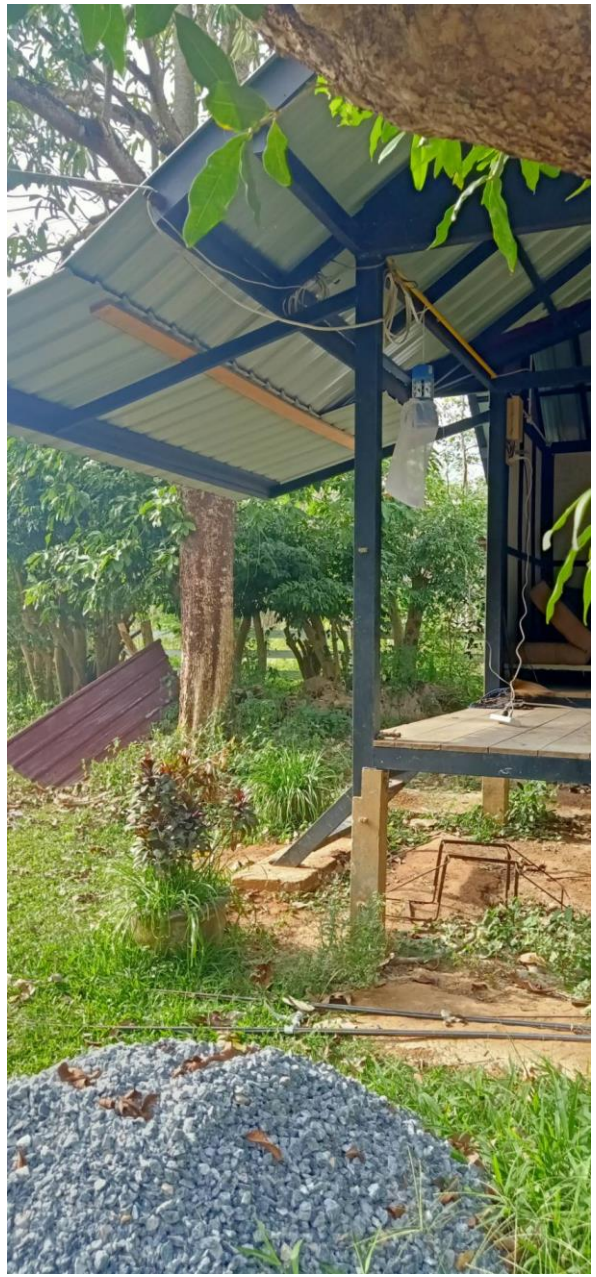
Trapping location

- Five traps were placed in each farm: two inside the stable and three in surrounding environment

Sample collection

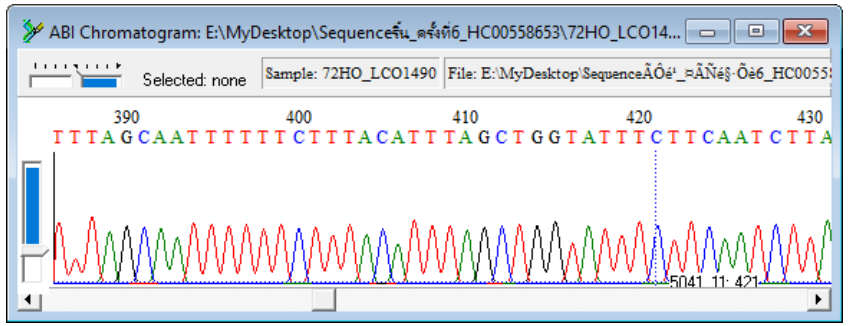


Sample collection



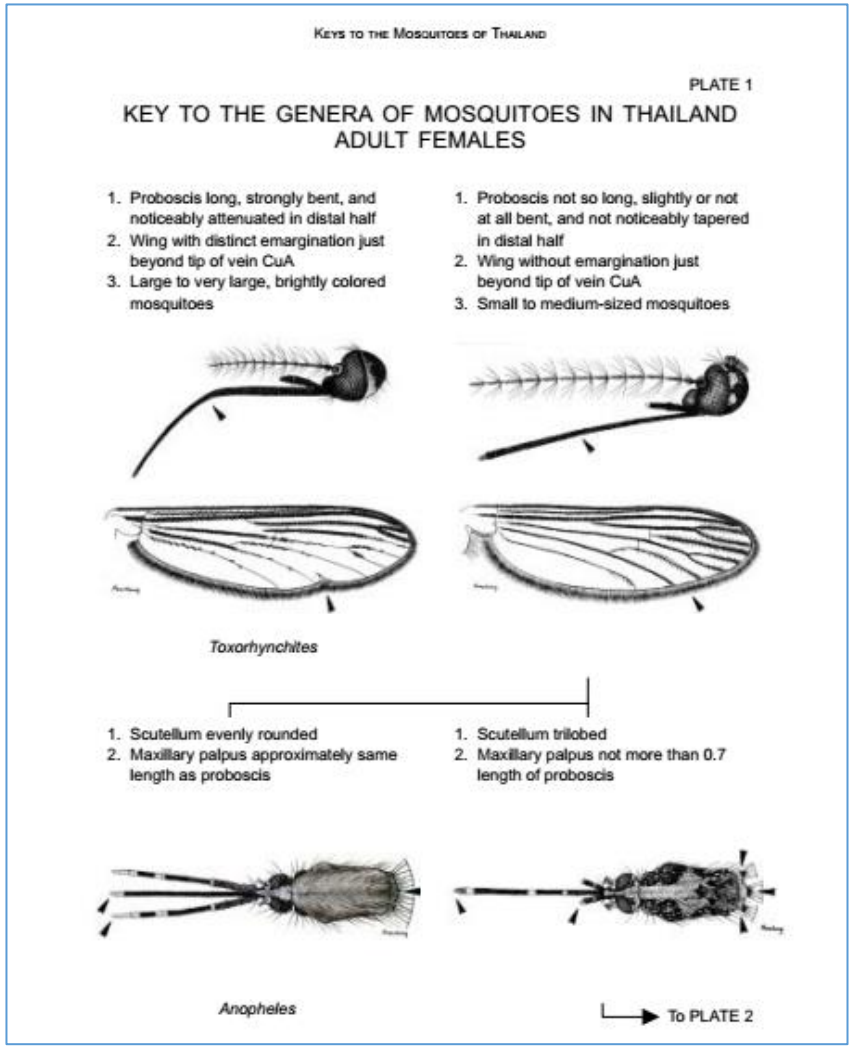
Laboratory methods: *Culicoides* Species identification

- *Culicoides* spp. morphological identification using Dichotomous key characterized by wing dark and pale spots patterns (Wirth and Hubert, 1989; Dyce et al., 2007)
- Representative individual of each species were identified using DNA Barcoding (Folmer et al. 1994)



Laboratory methods: Mosquitoes genus identification

- Mosquitoes genus were identified using Dichotomous key characterized by maxillary palp, Proboscis, Prespiracular and Postspiracular setae, etc. (Rattanarithikul et al., 2005)



KEYS TO THE MOSQUITOES OF THAILAND

PLATE 1

KEY TO THE GENERA OF MOSQUITOES IN THAILAND
ADULT FEMALES

1. Proboscis long, strongly bent, and noticeably attenuated in distal half
2. Wing with distinct emargination just beyond tip of vein CuA
3. Large to very large, brightly colored mosquitoes

1. Proboscis not so long, slightly or not at all bent, and not noticeably tapered in distal half
2. Wing without emargination just beyond tip of vein CuA
3. Small to medium-sized mosquitoes

Toxorhynchites

1. Scutellum evenly rounded
2. Maxillary palpus approximately same length as proboscis

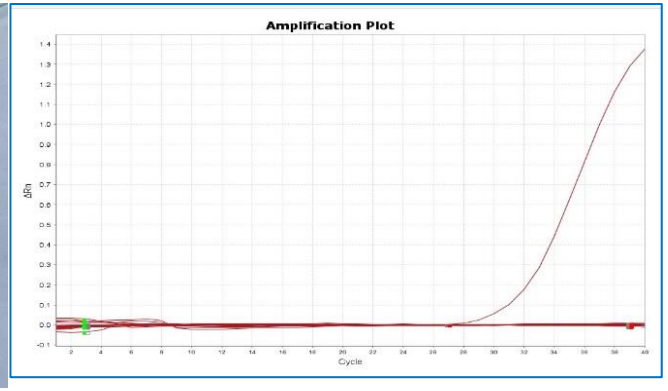
1. Scutellum trilobed
2. Maxillary palpus not more than 0.7 length of proboscis

Anopheles

To PLATE 2

Laboratory methods: Detection of AHS RNA virus

- Detection of AHS RNA virus in *Culicoides* samples were performed by real-time RT-PCR
- - 30 pooled samples per farm
- 25 insect vectors (same species) per one pool



Laboratory: Taxonomic identification & Real-time PCR

Results

- More than 10 *Culicoides* species were observed in this surveillance.
  - *C. oxystoma*
  - *C. imicola*
  - *C. innoxius*
  - *C. huffi*
  - *C. peregrinus*
  - *C. clavipalpis*
  - *C. gutifer*
  - *C. actoni*
  - *Culicoides* in subgenus *Avaritia*
  - *Culicoides* in subgenus *Trithecoides*
- *C. imicola,* a proven vector of AHS was observed in every farm.
- *C. oxystoma* was the most abundant species in majority of farms studied (Nakhon Ratchasima, Saraburi, Chachoengsao, Bangkok and Nonthaburi provinces).

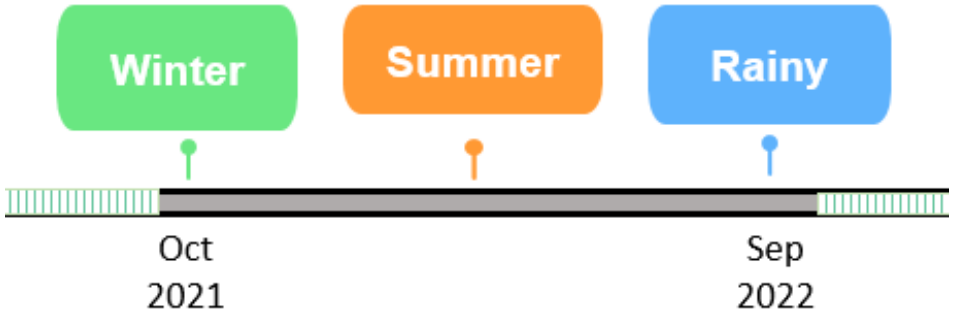
AHSV detection in *Culicoides* midges

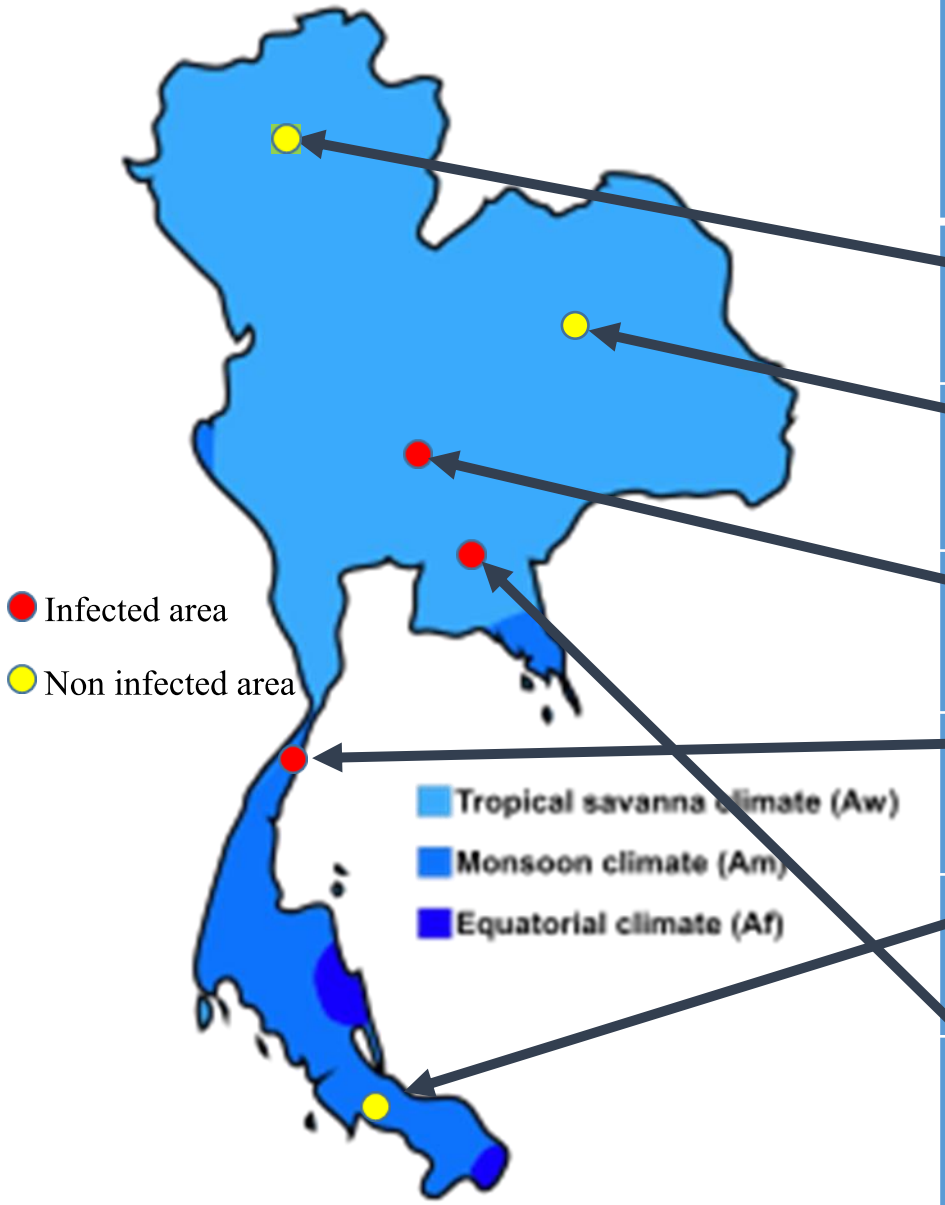
- All 449 pools from horse farms (6,754 *Culicoides* midges) were tested negative.
- Viral RNA detection from midge pools collected from zebra farms

Farms	Collection date	*Culicoides* spp.	Pool size	Number of pools tested	Viral RNA detected by RT-PCR (pools)
Chachoengsao	9-10 Jul 2020	*C. oxystoma*	25	11	0
Chachoengsao	9-10 Jul 2020	*C. oxystoma*	21	1	1
Prachinburi	16-17 Jul 2020	*C. imicola*	25	2	2
Prachinburi	16-17 Jul 2020	*C. imicola*	7	1	0
Prachinburi	16-17 Jul 2020	*C. imicola* (engorged)	1	7	6
Prachinburi	16-17 Jul 2020	*C. oxystoma*	25	9	5
Prachinburi	16-17 Jul 2020	*C. oxystoma* (engorged)	1	10	8

Part 2. Longitudinal vector study during the surveillance period

- Vector surveillance for AHS was part of the surveillance plan that was established after the last clinical case to prove the absence of virus circulation
- It was carried out at five regions of horse locations and two zebra farms, both in infected and non-infected areas during October 2021 to September 2022
- Sample collections were carried out three times a year according to the three seasons (winter, summer, rainy)

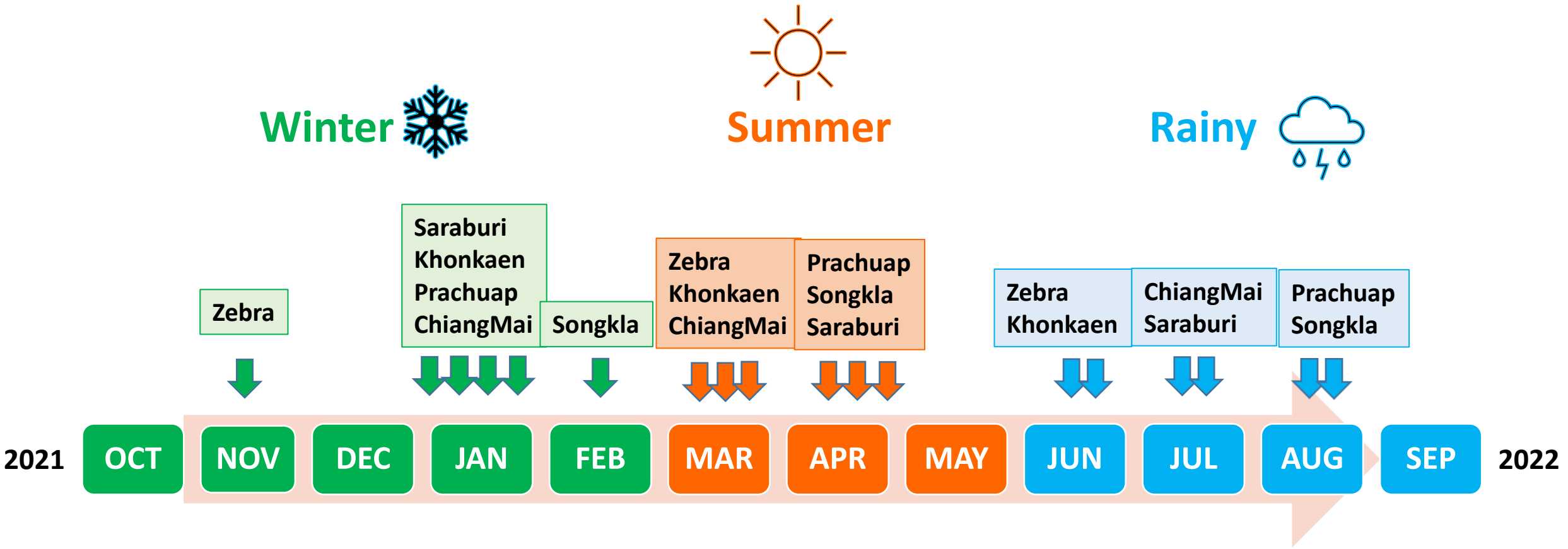




Area	Region	Climate zone	Sample number (Traps)		
			Winter (16Oct -15Feb)	Summer (16Feb -15May)	Rainny (16May -15Oct)
1	Northern (ChiangMai province)	Tropical savanna	15	15	15
2	Northeastern (Khonkaen province)	Tropical savanna	15	15	15
3	Central (Saraburi province)	Tropical savanna	15	15	15
4	Upper Southern (Prachuapkhirikhan province)	Monsoon	15	15	15
5	Lower Southern (Songkla province)	Monsoon	15	15	15
6	Zebra farms (Prachinburi and Chachoengsao province)	Tropical savanna	10	10	10
Total			85	85	85

Sample collection timeline:

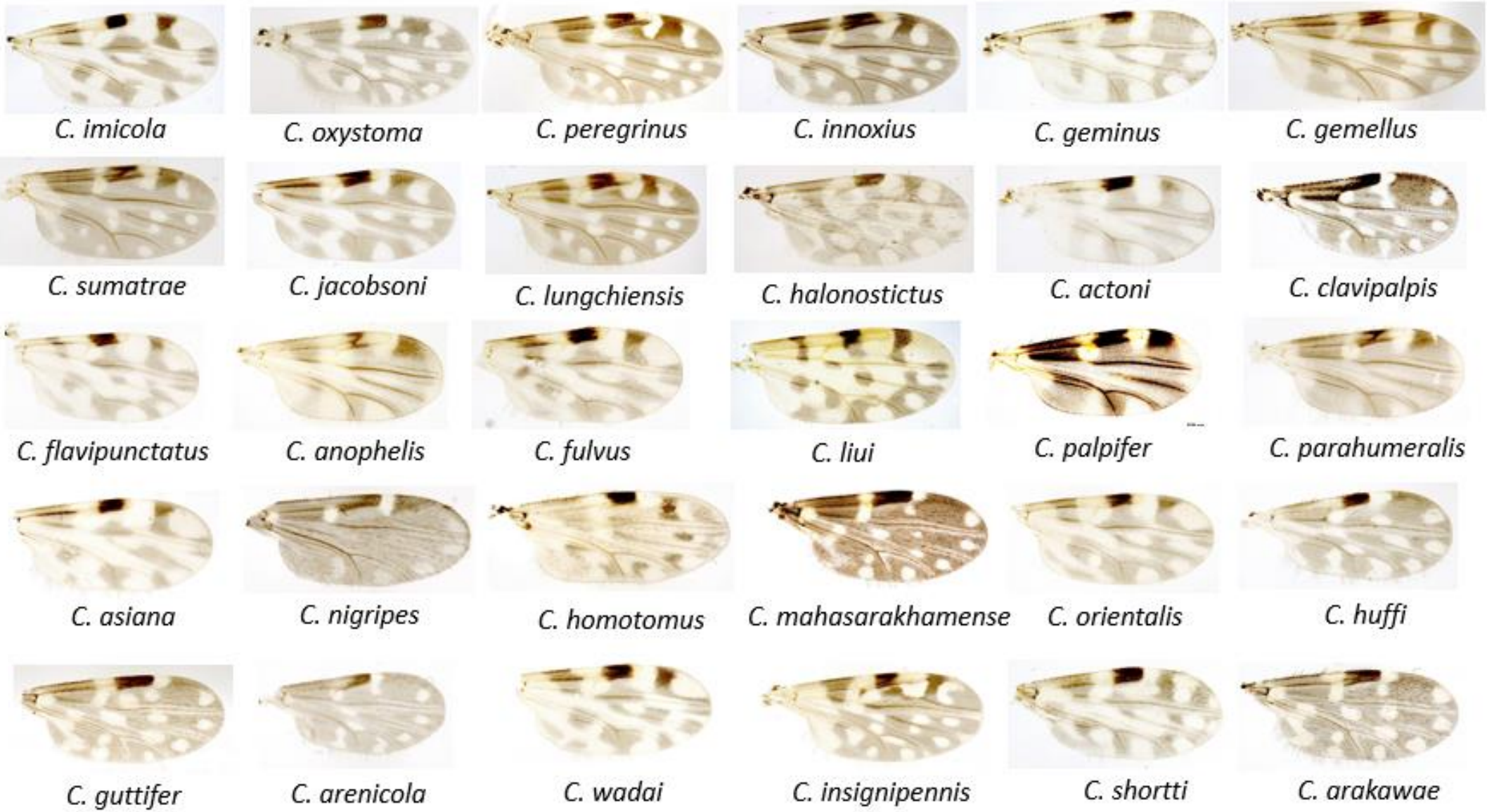
- Sample collection was done in 6 areas and 3 seasons



Results

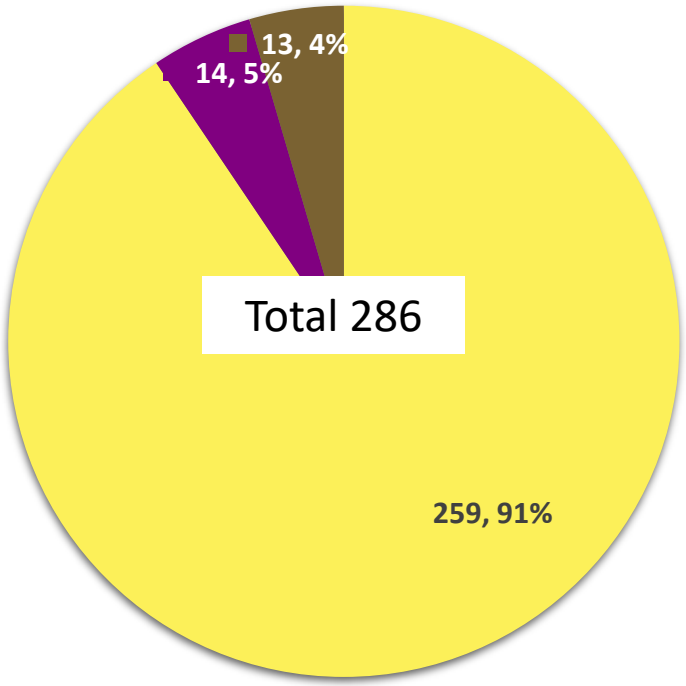
- Biting insects that we have found from this study included *Culicoides* spp. *Aedes* spp. *Anopheles* spp. and *Culex* spp.

Species of *Culicoides* found in this study



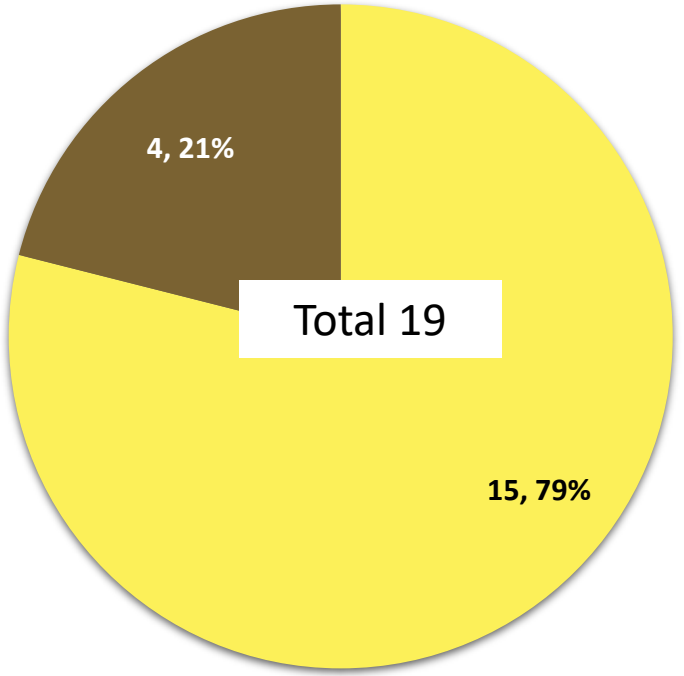
Prachinburi: Zebra farm (infected area)

Winter



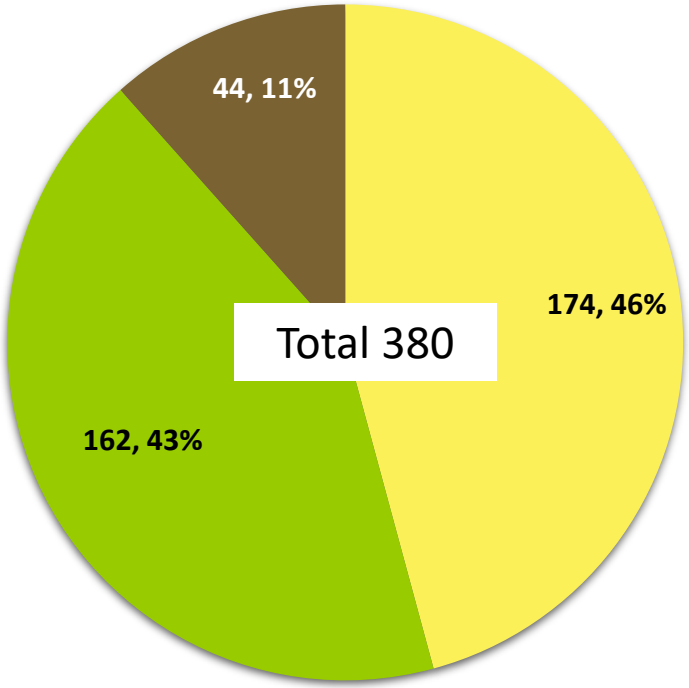
peregrinus | parahumeralis | others (5 species)

Summer



peregrinus | others (4 species)

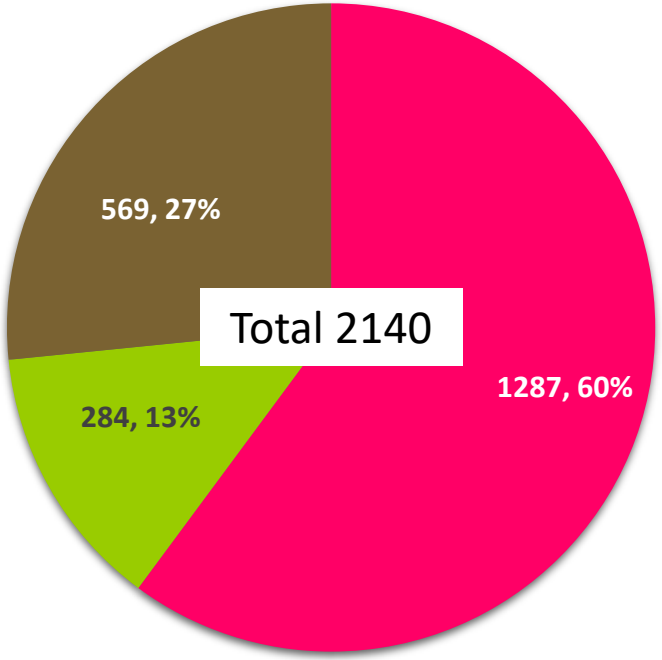
Rainy



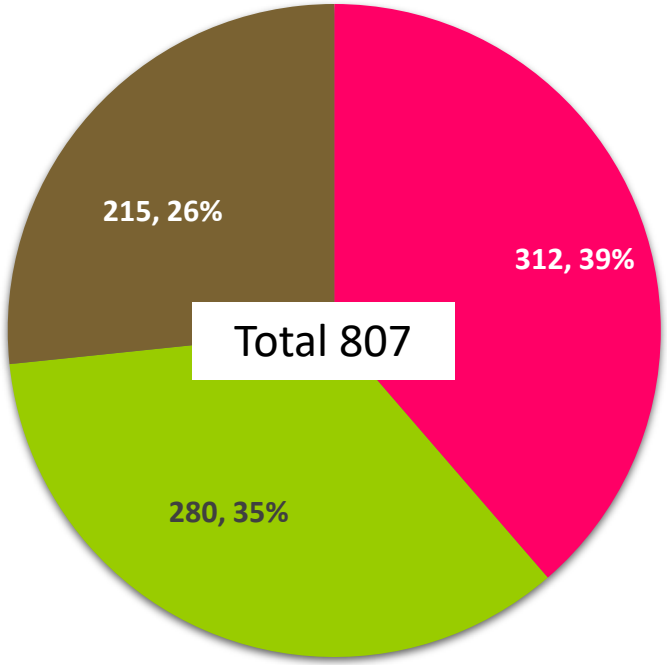
peregrinus | oxystoma | others (12 species)

Saraburi: 3 Horse farms (infected area)

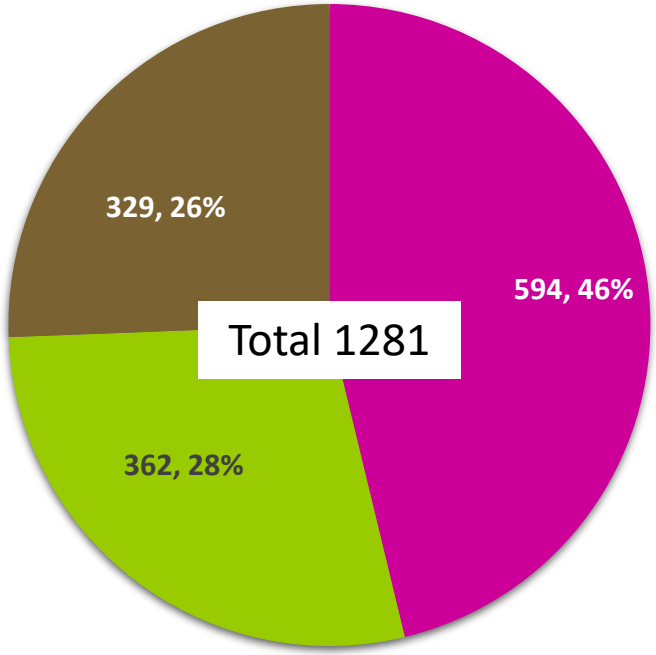
Winter



Summer

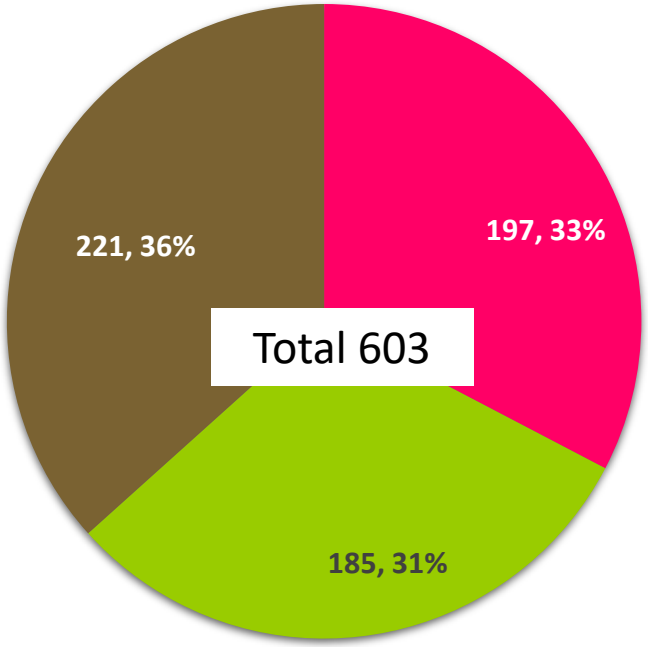


Rainy



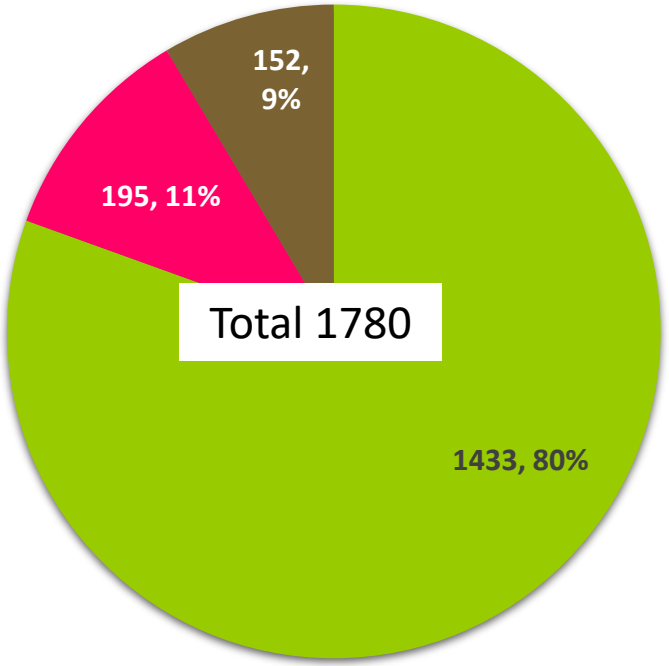
Khonkaen: 3 horse farms (non-infected area)

Winter



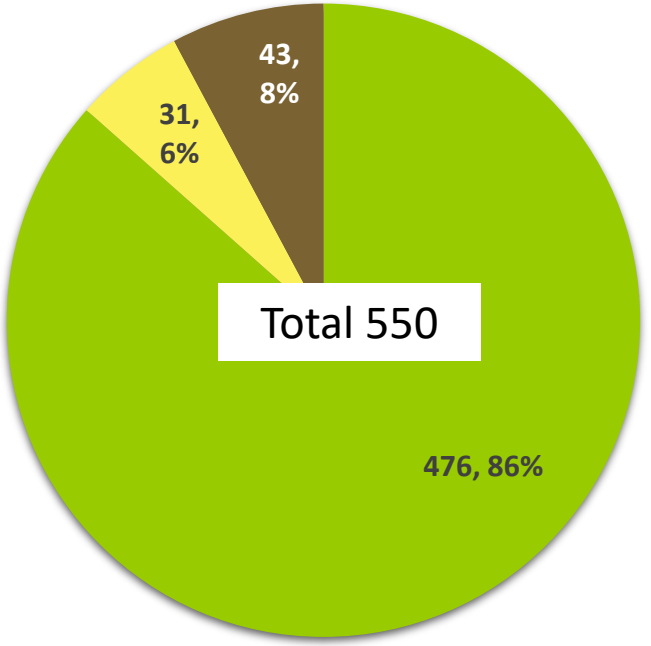
imicola | oxystoma | others (11 species)

Summer



oxystoma | imicola | others (17 species)

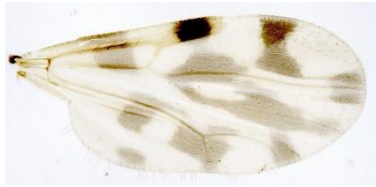
Rainy



oxystoma | peregrinus | others (10 species)

Species of *Culicoides* found from each area in this study

Zebra		Central	Upper Southern	Northern	Northeastern	Lower Southern
(Chachoengsao)	(Prachinburi)	(Saraburi)	(Prachuapkhirikhan)	(ChiangMai)	(Khonkaen)	(Songkla)
C. peregrinus	*C. peregrinus*	*C. imicola*	*C. oxystoma*	*C. oxystoma*	*C. oxystoma*	*C. oxystoma*
C. oxystoma	*C. oxystoma*	*C. oxystoma*	*C. imicola*	*C. orientalis*	*C. imicola*	*C. huffi*
C. imicola	*C. imicola*	*C. orientalis*	*C. huffi*	*C. imicola*	*C. peregrinus*	*C. peregrinus*
C. fulvus	*C. parahumeralis*	*C. peregrinus*	*C. palpifer*	*C. peregrinus*	*C. huffi*	*C. fulvus*
C. shortti	*C. anophelis*	*C. huffi*	*C. peregrinus*	*C. innoxius*	*C. innoxius*	*C. shortti*
C. parahumeralis	*C. arakawae*	*C. actoni*	*C. parahumeralis*	*C. shortti*	*C. nigripes*	*C. guttifer*
C. innoxius	*C. huffi*	*C. parahumeralis*	*C. guttifer*	*C. fulvus*	*C. guttifer*	*C. anophelis*
C. anophelis	*C. innoxius*	*C. innoxius*	*C. orientalis*	*C. palpifer*	*C. mahasarakhamense*	*C. mahasarakhamense*
C. orientalis	*C. orientalis*	*C. palpifer*	*C. fulvus*	*C. insignipennis*	*C. arakawae*	*C. palpifer*
C. palpifer	*C. lungchiensis*	*C. guttifer*	*C. innoxius*	*C. arakawae*	*C. parahumeralis*	*C. orientalis*
C. huffi	*C. shortti*	*Culicoides spp.*	*C. anophelis*	*C. sumatrae*	*C. actoni*	*C. innoxius*
C. actoni	*C. fulvus*	*C. mahasarakhamense*	*C. mahasarakhamense*	*C. guttifer*	*C. shortti*	*C. parahumeralis*
C. asiana	*C. palpifer*	*C. fulvus*	*C. asiana*	*C. jacobsoni*	*C. orientalis*	*C. flavipunctatus*
C. guttifer	*C. jacobsoni*	*C. halonostictus*	*C. flavipunctatus*	*C. parahumeralis*	*C. asiana*	*C. insignipennis*
C. lungchiensis		*C. jacobsoni*	*C. shortti*	*C. huffi*	*C. halonostictus*	Subgenus Avaritia
C. insignipennis		*C. sumatrae*	*C. actoni*	*C. anophelis*	*C. anophelis*	*C. asiana*
C. sumatrae		*C. shortti*	*Culicoides spp.*	*C. mahasarakhamense*	*C. homotomus*	*Culicoides spp.*
Culicoides spp.		*C. homotomus*	*C. clavipalpis*	*C. liui*	*C. sumatrae*	*C. imicola*
C. arakawae		*C. asiana*	*C. homotomus*	*C. asiana*	*Culicoides spp.*	*C. gemellus*
C. gemellus		*C. wadai*	*C. lungchiensis*	*C. gemellus*	*C. lungchiensis*	*C. jacobsoni*
		C. nigripes	*C. geminus*	*C. actoni*	*C. fulvus*	*C. arenicola*
		C. anophelis	*C. halonostictus*	*C. nigripes*	*C. clavipalpis*	*C. actoni*
		C. lungchiensis	*C. jacobsoni*	*C. lungchiensis*	*C. geminus*	*C. lungchiensis*
		C. arenicola	*C. nigripes*	*C. wadai*		*C. geminus*
		C. clavipalpis		*Culicoides spp.*		*C. sumatrae*
		C. insignipennis		*C. flavipunctatus*		
				Subgenus Avaritia		
				C. halonostictus		



C. imicola

C. oxystoma

C. peregrinus

C. huffi

C. orientalis

Detection of AHS virus in insect vector samples by real-time PCR

- 574 pools of 8,217 *Culicoides* midges and 11 pools of 142 mosquitoes such as *Aedes* spp. (16) *Anopheles* spp. (6) and *Culex* spp. (120) collected from infected-area have been tested
- No AHS RNA virus detected in all pools of insect samples

In conclusion

- The seasonal abundance and diversity of *Culicoides* species is enormous and seems to be related to location and host species
- No AHS RNA virus detected in all pools of insect samples which might prove absence of AHS virus circulation

Thank you

from NIAH vector team