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Mosquito ecology of the Northeastern parts of South Africa

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Make today matter



Sub-family mosquito classification



MOSQUITO FACTS



Mosquito Life Cycle

Culicine <u>female mosquitoes</u>, depending on species, can <u>produce between 30-300 eggs per</u> <u>blood meal</u>;

- All larvae and pupae require water in which to develop and will emerge as quickly as 3 days after hatching in some species but typically need 7-14 days, depending on species and water temperature.
- Mosquito <u>larvae feed on yeasts</u>, <u>bacteria</u>, <u>protozoa and other microorganisms</u> associated with submerged substrates, as well as organic particles found on or in the water column.
 - All mosquito pupae are aquatic, and comma shaped. <u>Pupae do not feed</u> but spend most of their time at the water surface taking in air through respiratory trumpets.

Mosquitoes in South Africa

- Historically in the country a large number of studies were published during the 1950's and 1970's
- Ae. aegypti is widespread in the tropical and subtropical areas in eastern South Africa (Muspratt, 1956)
- Ae. albopictus was reported in 1990, entering in SA in used tire imported from Japan on 3 separated occasions (Cornel, 1991)
- Sylvatic Aedes species: CHIKV in Limpopo province, northern KZN: The primary vectors were Ae. furcifer and possibly also Ae. cordellieri.



Distribution Ae. aegypti (Muspratt, 1956)



Zoonotic Arbo and Respiratory virus Research Program, CVZ One Health Strategy for Arbovirus in South Africa

SENTINEL ANIMALS: Bird Fatalities Neurological signs in horses livestock and wildlife Abortion, death in young animals: VHF signs

VECTOR SURVEILLANCE Tick;*culicoides*; nosquitoes; *sandflies* HUMAN SYNDROMIC SURVEILLANCE : Febrile; arthralgia; rash neurological; VHF







- Climate data was obtained from permanent stations of the South African Weather Service (SAWS)



MOSQUITO COLLECTION AND HANDLING

CDC light trap



BG-Sentinel Trap









STUDY AREA:

- Gauteng, Limpopo,
 Mpumalanga, North West, and
 KwaZulu-Natal.
- Since 2014, an entomological surveillance program has been conducted monthly.
- Supplementary collections were made in *ad-hoc* sites following detection of arboviral cases in animal or human hosts.



Sitor	Trans Events	Nighte	Trap Type	ΝΔρ	N Ao		NMa	Other	Total
Siles		Nights	set*	N All.	NAC.	N CA.	N /MG.	genera	Mosq.
Sentinel Sites									
Peri-urban									
Boschkop	353	76	1, 2, 3	294	664	3,153	1	1	4,113
Kyalami	338	75	1, 2, 3	1,793	577	6,314	3	49	8,736
Conservation									
Marakele	487	92	1, 2, 3	3,213	2,939	5,234	166	108	11,660
Lapalala	568	98	1, 2, 3	9,484	2,522	2,468	477	1724	16,675
Ad-hoc Sites									
				Urban					
Pretoria North	23	7	2, 3	4	217	49	0	0	270
Matikwane	9	2	1, 2, 3	72	345	194	0	4	615
Peri-urban									
Benoni	31	5	1, 2, 3	165	348	677	0	1	1,191
Roodeplaat	15	2	1, 2, 3	42	103	118	26	9	298
Vulpro	12	2	1, 2, 3	0	342	148	0	0	490
Rural									
Mnisi	171	45	1, 2, 3	821	864	2,309	1,042	9	5,045
Jozini	47	12	2, 3	4,332	2,348	2,229	2,041	4	10,954
Hectorspruit	9	3	1, 2, 3	97	285	904	159	25	1,470
Cork	9	3	1, 2, 3	2	19	35	0	3	59
Maluleke	9	3	1, 2, 3	20	37	148	11	5	221
Welverdiend	9	3	1, 2, 3	71	16	286	2	0	375
Conservation									
Knp Shingwedzi	25	5	1, 2, 3	693	118	336	15	9	1,171
Knp Skukuza	27	5	1, 2, 3	235	53	190	40	1	519
Knp Satara	9	3	1, 2, 3	34	172	86	2	1	295
Knp Malelane	9	3	1, 2, 3	99	65	167	1	0	332
Knp Punda Maria	9	3	1, 2, 3	23	3	86	1	1	114
Total	2,169	3		21,494	12,037	25,131	3,987	1,954	64,603
*1. Mosquito net trap, 2. BG – Sentinel trap, 3. Centers for Disease Control and Prevention miniature light traps									
An.: Anopheles: Ae.: Aedes: Cx.: Culex: Ma.: Mansonia: N: Total number collected: Mosa.: Mosauito: Knp: Kruger National Park.									

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- > The most common genus collected was Culex (38.90%, N = 25,131)followed by <u>Anopheles</u> (33.27%, N = 21,494),<u>Aedes</u> (18.63%, N 12,037), Mansonia (6.17%, N = 3,987) and other genera combined (3.03%, N = 1,954, Uranotaenia, Aedeomyia, Ficalbia, Coquillettidia, Mimomyia, Culiseta and Eretmapodites).
- Culex, Aedes, Anopheles, and Mansonia were the most medically and veterinary important genera collected.



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- Fourteen Aedes species which are recognized or suspected vectors of mosquito-borne viruses in southern Africa were collected.
- The most abundant potential vector collected throughout the study was the <u>flood-water species</u> <u>Ae. mcintoshi</u> which occurred in high numbers in Midlevel locations such as Marakele and Lapalala (Limpopo Province at conservation sites)
- <u>Aedes aegypti</u> occurred in all sites but in <u>low</u> <u>numbers</u>.
- Species from the <u>dentatus group</u>, including Ae. dentatus, Ae. cumminsii and Ae. Pachyurus; and leesoni group, Ae. juppi and Ae. unidentatus were common in the Highveld (Gauteng province at peri-urban sites)





- > The population structure and size of Aedes species fluctuated with season during the study period.
- An overall trend was evident with Aedes mosquitoes mean per trap night being lower in the drier months (June to October) than during the wetter months (November to mid-April). The population peaks appeared to correlate with rainfall events and the highest mean temperatures.



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- Engorged Aedes females comprised less than 1% of the adults collected.
- 112 freshly blood fed females were caught between January 2014 and May 2018. The specimens tested represented 16 Aedes species.
- 64/112 (54,46%) were successfully identified for blood meal origin which showed 62/64 (96,88%) mammal and 2/64 (3,12%) avian host species

Aedes species	Location	Host Blood Meal				
Ae. aegypti	Kyalami, KNP-SHI	Domestic cat (Felis silvestris catus), Northern puffback (Dryocopus gambensis) *				
Ae. aurovenatus	KNP-SHI	African buffalo (Syncerus caffer)				
Ae. circumluteolus	Jozini	Cattle (Bos taurus)				
Ae. dentatus	Kyalami	Horse (Equus caballus)				
Ae. dentatus group	Benoni	Cattle (Bos taurus)				
Ae. durbanensis	Jozini	Cattle (Bos taurus), goat (Capra hircus), sheep (Ovies aries)				
Ae. eritreae/karooensis	Vulpro	Bushbuck (Tragelaphus scriptus), nyala (Tragelaphus angasii), human (Homo sapiens)				
Ae. fowleri	Lapalala	White Rhinoceros (Ceratotherium simum), impala (Aepyceros melampus)				
Ae. hirsutus	Marakele, KNP-Mal, KNP-Sat	White Rhinoceros, blue wildebeest (Connochaetes taurinus), African buffalo, impala				
Ae. lessoni/alboventralis	KNP-SHI	African buffalo, bushbuck, impala, kudu (Tragelaphus strepsiceros)				
Ae. mcintoshi	Mnisi, Lapalala, Marakele, Vulpro	Cattle, hippopotamus (Hippopotamus amphibious), white rhinoceros, human, kudu, bushbuck *, Cape vulture (Gyps coprotheres), Blue wildebeest				
Ae. ochraceus	KNP-Sat and KNP-SHI	African buffalo, impala				
Ae. pachyurus	Benoni, Roodplaat, Kyalami	Cattle, human, goat, common duiker (Sylvicapra grimmia), domestic dog (Canis lupus) *				
Ae. quasiunivittatus	Boshckop, Mnisi, Lapalala, KNP-Mal	Sheep, cattle, hippopotamus, white rhinoceros				
Ae. vittatus	Mnisi, Lapalala	Cattle, human, waterbuck (Kobus ellipsiprymnus)				
Aedes spp	Mnisi, Marakele	Impala				
Ae: Aedes; KNP: Kruger National Park; SHI: Shingwedzi, Mal: Malelane; Sat: Satara						

RESULTS – DNA Barcoding

A total of a 52 COI sequences were generated in this study from 21 Aedes species belonging to nine subgenera: Stegomyia (4 Ae. aegypti, 2 Ae. simpsoni, 1 Ae. ledgeri), Aedimorphus (1 Ae. hirustus, 2 Ae. vexans, 3 Ae. fowleri, 4 Ae. cumminsii, 1 Ae. pachyurus, 5 Ae. eritreae, 3 Ae. durbanensis, 1 Ae. quasiunivittatus), Catageiomyia (5 Ae. microstictus), Neomelaniconion (2 Ae. aurovenatus, 4 Ae. mcintoshi, 1 Ae. circumluteolus, 3 Ae. unidentatus), Fredwardsius (2 Ae. vittatus), Ochlerotatus (3 Ae. juppi), Mucidus (2 Ae. sudanensis), Albuginosus (2 Ae. haworthi), and Diceromyia (1 Ae. furcifer).





- Species belonging to Aedimorphus subgenera were recovered in different clusters providing that this subgenus was not monophyletic.
- Sequences produced here from mosquitoes which were identified morphologically as Ae. cumminsii clustered with Ae. pachyurus (of the same group) and Ae. quasiunivittatus (of another group within the same subgenus) and did not cluster together with sequences from Ae. cumminsii from Kenya (KU187000.1, KU187001.1, and MK300225), Guinea (MN552300.1) and Senegal (MG242484).





Species belonging to the Neomelaniconion subgenus were divided in two clusters, a small one containing only Ae. aurovenatus while a larger one with no separation between Ae. unidentatus, Ae. mcintoshi, and Ae. circumluteolus.



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- A total of 25 pools (<u>25 Alphavirus positive homogenate pools</u> / 1,462 mosquito pools tested; 1.71%) were found to be positive for Alphavirus of which Sindbis (<u>15 SINV</u> positive pools / 25 Alphavirus positive pools; 60%) and MIDV (<u>7 MIDV</u> positive pools / 25; 28%) viruses were the most prevalent alphaviruses, while Ndumu virus (NUDV) was detected in just three pools (<u>3 NUDV</u> / 25; 12%)
- A total of 13 pools were found to be positive for SHUV
- No other Orthobunyavirus were detected in the study.
- Mansonia uniformis was the species that was found to have the greatest SHUV detection
- A total of <u>17 pools were positive for ISFVs</u>
- One pool of Aedes species were tested positive for <u>Alphavirus insect-specific collected in</u> <u>Lapalala</u>

- The maximum likelihood phylogeny based on the larger fragment (345 bp) on the nsP4 gene region confirmed that all SINV positive pools detected belonged to genotype 1 and clustered with an isolate from a horse detected in South Africa in 2014.
- All five MIDV positives were also confirmed as MIDV and clustered with previously positive horse strains isolated from South Africa and Zimbabwe.
- From all positive samples, 10 pools amplified the E gene region for MIDV (4 samples), SINV (5 samples), and NDUV (1 sample).



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Phylogenetic comparison of the alphavirus positive sequences identified in this study relative to 41 reference sequences of a 345 bp region of the nsP4 gene. The tree was constructed by employing the program MEGA 7, using the maximum likelihood method based on the Tamura3-parameter model and 1,000 bootstrap replicates

Five samples, a larger region of the S segment could be amplified to confirm the clustering with SHUV strains previously identified in horses and wildlife from South Africa.





Phylogenetic tree of SHUV positive detected based on 30 sequences and 328 base pairs of the nucleocapsid (S) segment gene. The tree was constructed with MEGA 7, using the Maximum Likelihood method and the Kimura 2-parameter model with 1,000 bootstrap replicates.

CONCLUSION

- The survey in this study was <u>limited geographically and in trap coverage</u>, however it was possible to gain sufficient understanding of the <u>diversity and relative abundance of Aedes</u> species in the areas sampled.
- COI sequences for nine Aftrotropical Aedes species, for which there were previously no sequences, were added to NCBI GenBank.
- These findings provide important evidence of presence of three Alphavirus species (MIDV, SINV, and NUDV) in South Africa. No CHKIV positive pools were identified during the study period although outbreaks have been reported in the past in northern KZN and eastern Limpopo Provinces.
- > We have demonstrated the presence of <u>SHUV in different mosquito species in SA</u> were neurological cases had previously been identified in animals and humans.



CONCLUSION

- Aedes durbanensis could be considered a suspect vector of alphaviruses in the KZN Province because of Alphaviruses detected in multiple pools as mentioned. They were the most abundant Aedes species collected in the Jozini site in the KZN Province and were shown to be feeding on livestock animals (cattle, goat, and sheep) in the site sampled
- Aedes dentatus group is another group that could play a role as a vector for alphaviruses, particularly in the Highveld region. They were the most abundant species collected in the Highveld in Gauteng Province. Horse derived blood was found in recently engorged individuals and this species tested positive for MIDV during an outbreak of MIDV on a horse farm.
- Mansonia species had the highest SHUV detection rate and was found to be positive in conservation and rural areas sampled. This genus was the fourth most common genera collected across the sites and were commonly tested positive for arboviruses. Previous studies showed that these species can feed readily on humans and animals. The result in this research could indicate that Mansonia species can have a potential epidemiological importance for SHUV in South Africa, and they could be a candidate bridging species between animals and humans.



RVFV in northern KZN

- Evidence of RVFV circulation in cattle and goats (Van den Bergh et al. 2019)
- Potential vectors: Ae. mcintoshi, Ae. circumluteolus
- Other possible vectors: Ae. durbanensis, Cx. tritaeniorhynchus, Cx theileri, and Cx. zombaensis, Cx. neavei, Cx. Poicilipes, and Cx pipiens



De We<u>t et al. 2013</u>





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Rift Valley fever virus transmission cycles



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