

ORIGINAL ARTICLE

Mosquito vector diversity and abundance in southern Botswana, in a global context of emerging pathogen transmission

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Abstract

Background. The continued spread of infectious diseases by mosquitoes remains a formidable obstacle to the well-being of the people all over the world. Arboviruses are spread from one vertebrate host to another by vectors through intricate transmission cycles that involve the virus, the vertebrate host, and the vector. It is essential to acquire a better understanding of the current abundance and distribution of major vectors in order to adequately prepare for the possibility of arbovirus outbreaks. This is because the abundance and distribution of these major vectors determines the human populations that are at risk for the diseases that they transmit. The effects of climate change on the amount of mosquitoes and their ability to survive the seasons have had a substantial impact on the spread of diseases that are transmitted by vectors in many different parts of Botswana.

Methods. The purpose was to collect mosquito samples in Gaborone and the neighboring areas in southern Botswana, including border stations. We collected different stages of the mosquito from each place, raised them to maturity, and then identified them. Both morphological and genetic studies were utilized in order to successfully identify the organism. The species of *Culex* mosquitoes accounted for 88.3% of the 5177 mosquitoes that were collected and identified, whereas the species of *Aedes aegypti* and *Anopheles* mosquitoes accounted for 11.5% and 0.2% respectively.

Conclusions. These findings give entomological baseline data that will aid in the study of vectorial patterns and the estimation of future arboviral hazards provided by mosquitoes. Additionally, these findings document the diversity and abundance of mosquito species.

Keywords: *Aedes aegypti*, *Culex spp*, ovitrap, surveillance.

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INTRODUCTION

Mosquitoes play a major role in the complex transmission of numerous diseases to human hosts, resulting in substantial morbidity and mortality worldwide.^{1,2} Recent epidemics of arbovirus infections demonstrate the danger posed by other mosquito-borne diseases in addition to malaria. For instance, outbreaks of Zika virus in South and Central America in 2015, Cape Verde in 2016, and Angola in 2016-2017 led to high rates of congenital malformations in perinatally infected infants.³⁻⁵ Similar epidemics of Zika virus and other arboviruses are likely to emerge in other regions of the world where mosquito vectors, such as the *Aedes* species, are present. This is because global trade and climate change are altering mosquito distribution.⁴ Understanding the risk of the emergence or spread of mosquito-borne diseases of public health significance necessitates knowledge of the species composition, vector density, and seasonal variation of mosquito populations.

To date, entomological research in Botswana has focused on the mosquito species responsible for transmitting malaria and Rift Valley Fever in the malaria-endemic regions of the country's north.⁶⁻⁸ Limited data exists on the abundance and distribution of other possible mosquito vectors in the rest of the country. In fact, over the past five years, districts in the supposedly malaria-free southern region of the country have reported an increase in malaria cases.^{9,10} In southern Botswana, where urbanization has attracted the majority of the population, mosquito species surveillance is especially vital. In addition, the available data indicate that climate change has affected the abundance and seasonal survival of mosquitoes in numerous regions of Botswana, which has had a significant impact on the spread of vector-borne diseases.¹¹ As Botswana strives to eliminate malaria, it is necessary to increase awareness of the threat posed by other mosquito-borne diseases in order to guide ongoing targeted surveillance and vector control.¹⁰

The purpose of this study was to assess the species diversity and abundance of mosquitoes in Gaborone and the surrounding areas of southern Botswana. In addition to providing an early warning for impending

outbreaks in Botswana, the purpose of this project was to build the country's capacity for routine vector surveillance and risk modeling as part of a national preparedness plan.

MATERIALS AND METHODS

Ethical considerations

The study did not involve any clinical trials or human subjects; hence it did not have to go through the processes for consideration and approval by the University Research and Ethics Committee.

Vector surveillance sites

Longitudinal capture of mosquitoes in larval and adult stages was carried out at different sites (Figure 1) in Gaborone and surrounding areas, including border posts, in southern Botswana. The study was carried out from January 2017 to March 2018.

Larval collection

Mosquito larvae and pupae were collected from standing water sources such as small streams, water catchments and old tires using small dip nets, scoops and pipettes. Larvae were then reared until development into pupae in larval trays containing fresh water mixed with habitat water. Larvae were fed on ground cat food pellets. Pupae were transferred into disposable cups and placed in cloth mesh cages to emerge into adults. The insectary was maintained at $27 \pm 2^\circ\text{C}$ and $70 \pm 10\%$ relative humidity.

Ovitrap collection

Oviposition traps (Biogents AG[®]) made of black plastic cylindrical vases were filled with leaves, dry grass, twigs, and water to create an environment for mosquitoes to lay eggs. The ovitrap lid was smeared

Supplementary information The online version of this article ([Figures/Tables](#)) contains supplementary material, which is available to authorized users.

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with olive oil to trap flying adult mosquitoes as they exit the trap. The traps were left at sampling sites for 2 to 4 weeks, after which the larvae in the ovitraps were reared to adult stage in the insectary. Adult mosquitoes trapped on the smeared oil were carefully removed and placed in 1.5 tubes with silica gel.

Adult identification

Captured adult mosquitoes and the adults reared from larval habitats and ovitrap collections were killed in a -20°C freezer. All adults were stored in tubes with desiccating silica to absorb moisture before identification and further processing. The mosquitoes were identified morphologically to the species level under a stereoscopic microscope using published keys.¹² Morphological characteristics that were observed included pattern on the coastal area of the mosquito wing, patterns on the thorax, colour and patterns on the abdomen, legs and the general colour of the mosquito. For accurate identification, *Anopheles* mosquitoes were confirmed to the species level by multiplex PCR using specific primers.¹³

RESULTS

Mosquito species composition

Overall, a total of 5,177 mosquitoes identified as *Culex spp.*, *Aedes spp.*, and *Anopheles spp.* were collected from all the study sites. *Culex spp.* was the most abundant mosquito genus collected, accounting for 4,570 (88.3%), followed by *Aedes spp.* (n=593, 11.5%) and lastly *Anopheles spp.* (n=14, 0.2%).

The *Anopheles* mosquitoes were further identified to specific species level. All the *Anopheles* mosquitoes (n=14) from Kgope (n=10) and Oodi (n=4) belonged to the *Anopheles gambiae* complex. These were identified as *Anopheles quadriannulatus* and *Anopheles arabiensis* (Figure 2).

Distribution and density of the species from collection sites

Culex spp. was the most predominant genus in 12 out of a total of 19 study sites (Figure 3). From the 12 study sites predominated by *Culex spp.*, 10 study sites had 100% of the mosquitoes as *Culex spp.* while in Phitshane-Molopo and Ramotswa border

post (BP) had the genus accounting for 72.3% and 58.8% respectively. Study areas such as Lobatse BP, Modipane and Gabane were characterized by 100%, 83.1% and 78.8% of *Ae. aegypti*, respectively (Figure 5). *Anopheles spp.* was found in small numbers at only two sites, Oodi (Notwane River) (10; 0.5% of mosquitoes at that site) and Kgope (4; 5.2%). No one collection site had all three mosquito species. Actual numbers of mosquito species collected revealed large numbers of *Culex spp.* in Mochudi (862), Sikwane (873) and Oodi (783) (Table 1).

DISCUSSION

This study provides information on the diversity of mosquitoes in Gaborone and the surrounding areas of southern Botswana through a detailed profile of mosquito distribution. *Culex spp.* is the most numerous and widely distributed mosquito genus across all collection sites. The widespread presence of *Culex spp.* demonstrates the species' ability to successfully utilize a variety of natural and artificial breeding sites. Little is known about the ecology, composition, and distribution of *Culex* species in southern Africa, including Botswana, as most studies have centered on anophelines.^{14,15} Different *Culex* species are capable of transmitting to humans a variety of arbovirus infections, many of which are poorly understood.¹⁶ There is a need for additional molecular research to gain a better understanding of the *Culex* species that predominate at various locations.

Aedes aegypti was the second-most common species of mosquito. These mosquitoes are competent vectors for numerous arboviruses that can cause severe human diseases, such as chikungunya, all four dengue serotypes, West Nile, yellow fever, and Zika.^{17,18} *Aedes aegypti* is predominantly anthropophilic, as it is an aggressive daytime biter that readily enters homes to rest and feed.^{19,20} To oviposit, it seeks out artificial containers such as tanks, discarded tires, and buckets.²⁰ This breeding behavior allows *Ae. aegypti* to spread via human-assisted transportation of used tires, machinery, and other goods. Ports of entry are therefore crucial areas for vector surveillance. *Ae. aegypti* was predominant at the Lobatse border post and in smaller numbers at

two other border posts, Ramotswa and Phitshane-Molopo, but was not found at the other three border posts surveyed. In addition, *Ae. aegypti* was discovered in two hotels in Gaborone. *Aedes aegypti* comprised 10 (3.1%) of the 326 mosquitoes collected at Hotel A; the remaining mosquitoes were *Culex* species. *Aedes aegypti* comprised all (100%) of the five mosquitoes collected at Hotel B. The presence of potential vectors in areas frequented by travelers poses a risk of disease introduction into new regions. A competent mosquito may feed on an infected person during the infectious period, resulting in a local outbreak²¹ and the spread of mosquito vectors to other areas. A 2019 collection formally documented the presence of *Ae. aegypti* in Palapye, central Botswana, demonstrating that *Ae. aegypti* are not restricted to the south of the country.²² Importantly, neither our results nor those of Buxton et al.²² are the first reports of *Ae. aegypti* in Botswana. During the summers of 1949 and 1952, *Ae. aegypti* and other *Aedes* species (*simpsoni*, *vittatus*, *metallicus*, *luteocephalus*, *unilineatus*, and *pseudonigeria*) were documented in Francistown.²³ The circulation of *Ae. Aegypti* in Botswana and neighboring countries was also confirmed by a 1956 study.²⁴ *Aedes aegypti* surveys in Botswana have not been published in the last 60 years, with the exception of a few recent works^{7,14} in which other species of the genus *Aedes* were identified. To evaluate the presence and density of *Aedes spp.*, techniques for collecting diurnal mosquitoes must be utilized.

This study did not identify any *Aedes albopictus* mosquitoes, an additional important arbovirus transmission vector. In areas with limited availability or quality of food, interspecific larval competition largely determines the abundance of different mosquito species. In these conditions, *Aedes albopictus* takes advantage of *Aedes aegypti*.²⁵ However, both the egg and adult stages of *Ae. albopictus* are more sensitive than those of *Ae. aegypti* to the low humidity and high temperatures that occur during dry periods.²⁶ These environmental conditions may have reduced competition between the two species and may explain why *Ae. albopictus* is absent from the sampled areas.

Due to the risk of malaria transmission, the presence of *An. quadriannulatus* and *An. arabiensis* at

established breeding sites in Oodi (Kgatleng District) and Kgope (Kweneng District), albeit in small numbers, warrants further investigation. Malaria vector *An. arabiensis* is known to inhabit malaria-endemic northern Botswana. Central and southern Botswana, which are not typically susceptible to malaria, experienced outbreaks in 2017. Malaria cases have been reported in Kgatleng and Kweneng districts, and the malaria vector *An. arabiensis* has been identified. As a result, residents of these districts are now at risk of contracting the disease if malaria becomes localized or fully established.²⁷ To prevent further spread of *An. arabiensis* in these regions, active entomological surveillance and vector control must be implemented.

Non-*Culex* species were randomly distributed across the sampled areas and were rare overall, possibly as a result of the sampling techniques employed in this study. Diverse mosquito species are captured with drastically different efficiencies by various trapping techniques.^{28,29} Therefore, these results may underestimate the presence of *Ae. aegypti* or *Anopheles spp.* at the sampled locations. Continual monitoring is required to reevaluate the mosquito species composition at these locations over time.

Limitations

The presence of *Ae. aegypti* and *An. spp.* in previously unstudied regions demonstrates the importance of surveillance to identify areas of high-density infestation and periods of rising mosquito populations. In areas where the vector is no longer present, entomological surveillance is essential to detect new introductions before they become widespread and difficult to control, as demonstrated by this study. Monitoring insecticide resistance should be an integral part of any program that employs insecticides. Botswana requires frequent routine vector surveillance activities. Continuous research must be conducted for effective implementation.

CONCLUSIONS

This evaluation of mosquito species distribution in southern Botswana identified areas at risk for ar-

bovirus and malaria transmission, highlighting the significance of ongoing vector surveillance for public health preparedness. In an era of increasing globalization and climate change, countries that have made comparable progress in eradicating malaria must remain vigilant against the threat of mosquito-borne diseases in new regions.

INFORMATION

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Contributions. NM conceptualized writing of the manuscript. NM, KL and GMP contributed to manuscript writing and reference search. PR and TKK did data collecting, analysis, manuscript reviewing and reference search.

Conflict of interest. GMP is a member of the editorial board for this journal, NM, PR, TKK and KL declare no potential conflict of interests.

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Availability of data and materials. All data generated or analyzed during this study are included in this published article.

Ethical approval and consent to participate. The study did not involve any clinical trials or human subjects; hence it did not have to go through the

processes for consideration and approval by the University Research and Ethics Committee.

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MOSQUITO VECTOR DIVERSITY IN BOTSWANA

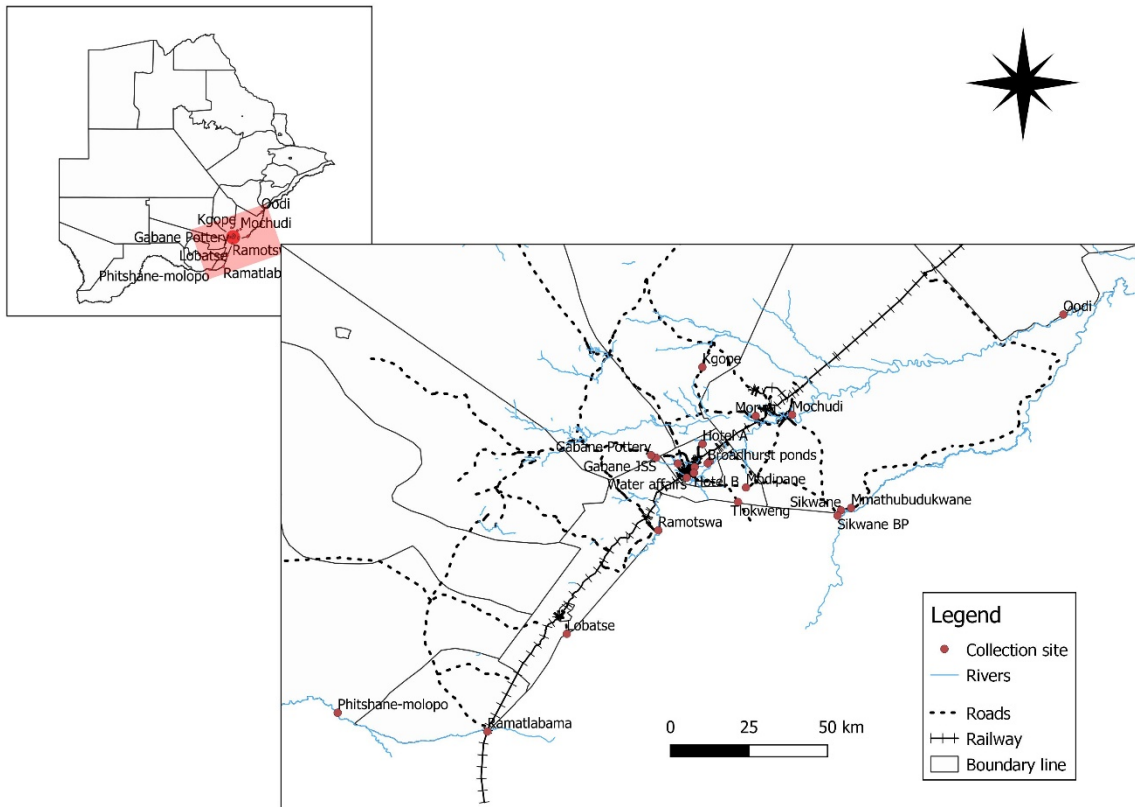


FIGURE 1: Map of Botswana showing collection sites in Gaborone and surrounding areas.

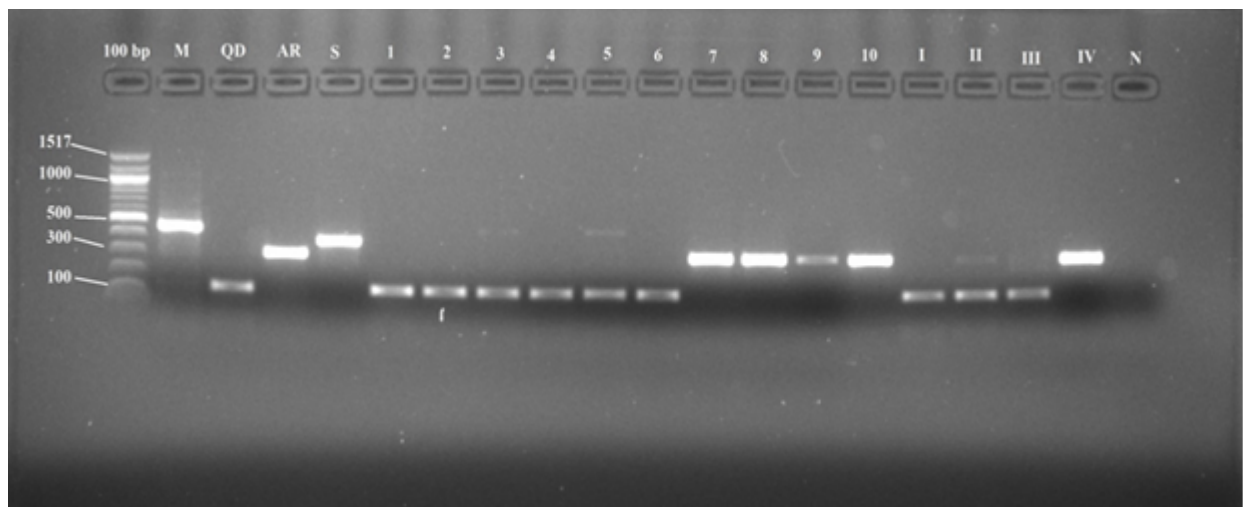


FIGURE 2: Molecular identification of *Anopheles* spp. from Kgope and Oodi. M- *Anopheles merus*; QD-*Anopheles quadriannulatus*; AR-*Anopheles arabiensis*; S- *Anopheles sensu stricto* 1-10- *Anopheles* (Kgope); I-IV-*Anopheles* (Oodi, Notwane River) and N-Negative control.

TABLE 1: Mosquito species density from different collection sites.

Collection Site	<i>Culex spp.</i>	<i>Aedes aegypti</i>	<i>Anopheles spp.</i>
University of Botswana	55	0	0
Water affairs (Gaborone)	357	1	0
Gaborone, extension 9	40	58	0
Gaborone, Block 5	11	0	0
Broadhurst ponds	6	0	0
Glen Valley	4	4	0
Gabane	17	63	0
Oodi (Notwane river)	783	0	4 (3 QD, 1 AR)
Morwa	29	142	0
Mochudi (Notwane river)	862	0	0
Mmathubudukwane	7	63	0
Sikwane	837	0	0
Modipane	14	69	0
Kgope	184	0	10 (6 QD, 4 AR)
Tlokweg	333	0	0
Sikwane Border post	319	0	0
Ramotswa Border post	30	21	0
Lobatse Border post	0	17	0
Ramatlabama Border post	0	0	0
Phitshane-Molopo Border post	366	140	0
Hotel A	316	10	0
Hotel B	0	5	0

QD-*Anopheles quadriannulatus*, AR-*Anopheles arabiensis*.

MOSQUITO VECTOR DIVERSITY IN BOTSWANA

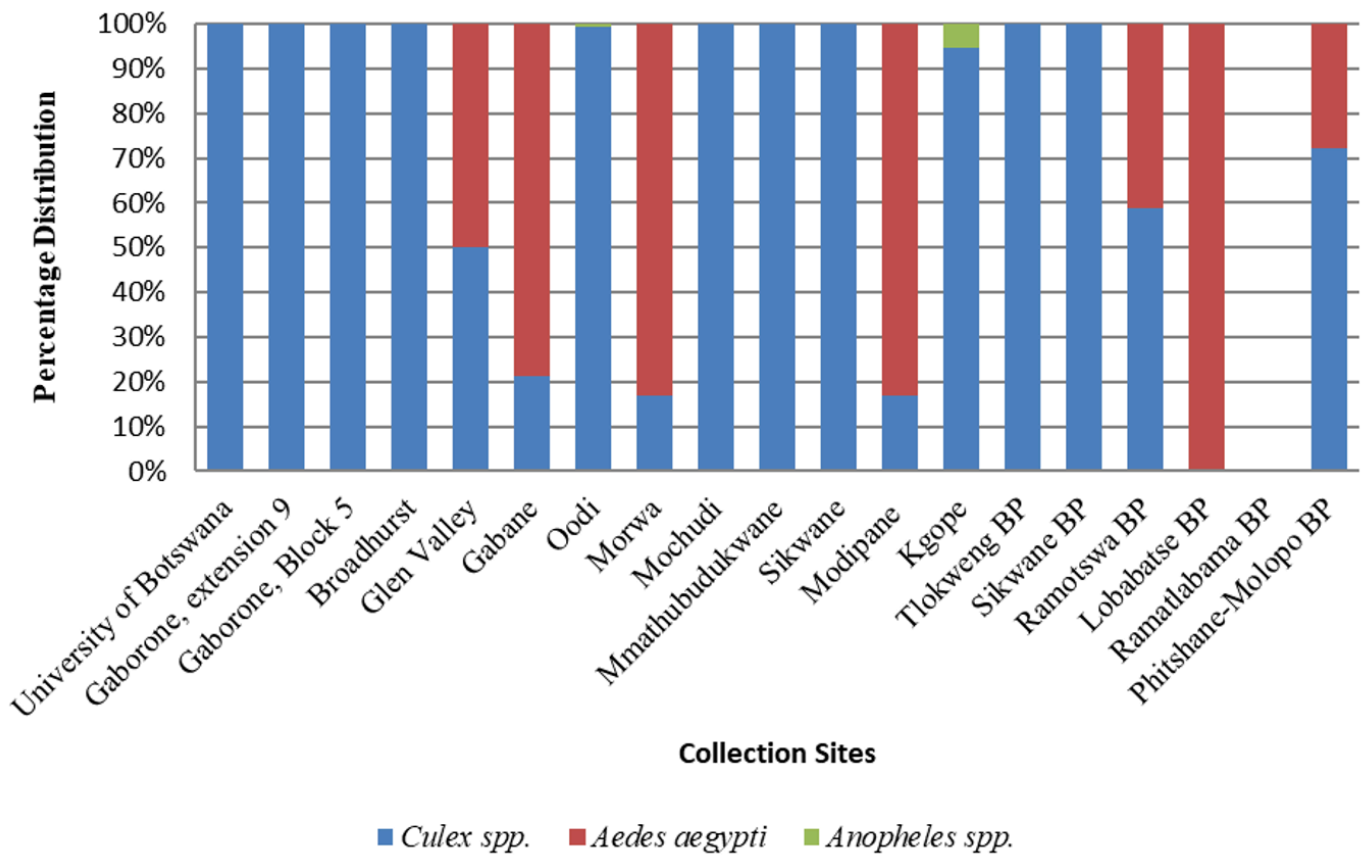


FIGURE 3: Distribution of mosquito species across the different collection sites.