Chapter

Application of Infrared Techniques for Characterisation of Vector-Borne Disease Vectors

Maggy Sikulu-Lord and Rafael Maciel-de-Freitas

Abstract

The interest of the entomological community in the application of the Near Infrared (NIR) and Mid Infrared (MIR) spectroscopy techniques for characterisation of insect species has seen a dramatic increase over the last decade. In this chapter, we discuss the application of the Near infrared spectroscopy technique for characterising insects including mosquitoes, triatomine bugs, Culicoides and houseflies under varying environmental and experimental conditions. We focus our discussion on the recent progress made in the application of the NIRS technique to predict the age, species and infection status of mosquito vectors of malaria and arbovirus infections relative to traditional tools such as dissections and molecular techniques and how the tool could be applied in vector surveillance programs to determine disease hotspots and direct current and future interventions. Lastly, we briefly discuss the application of MIR spectroscopy technique for age grading, species identification, blood meal identification and as a potential diagnostic tool for malaria parasites.

Keywords: surveillance, malaria, arbovirus, vector-borne disease, dengue, disease transmission, Zika, chikungunya, age-grading, taxonomic identification

1. Introduction

Entomological and epidemiological surveillance can be broadly described as the continuous monitoring of the transmission potential of vectors and human cases in a given area, respectively. It is an information-based activity involving the collection, analysis, interpretation and appropriate presentation of large volumes of data originating from a variety of sources to monitor the presence of pathogens in a population in a timely manner [1, 2]. Teams with a strong background in entomology, epidemiology, demography, climatology, cartography, statistics and modelling contribute to this exercise creating a multidisciplinary surveillance system to help understand spatiotemporal features of disease transmission [3, 4]. Since one of the most relevant characteristics of surveillance is to inform where and when the risk of disease transmission is higher, it can trigger targeted application of appropriate interventions. Therefore, timely surveillance could help mitigate disease transmission in endemic settings [5, 6].

Surveillance of insects of medical and veterinary importance is a crucial undertaking required to assess the effectiveness of interventions targeting those insects. For example, prevention of mosquito-borne diseases including malaria and arboviruses such as Zika, dengue, chikungunya and yellow fever, rely on vector control interventions such as insecticide-treated bed nets, indoor residual spraying or the use of repellents as protective measures against infective bites [7]. The effectiveness of these interventions can be determined by assessing changes in insect's behaviour. For example, mosquitoes can change their biting and resting behaviour to avoid contact with insecticide-treated surfaces [8]. Moreover, a change in species composition might mean interventions that normally work against an existing species are no longer effective against the new species [9]. This means routine surveillance to monitor such changes among insect vector populations is a crucial determinant of the effectiveness of interventions.

Estimating survival of insect populations such as mosquitoes to determine how long they have lived is one way of assessing the effectiveness of an intervention. Another method for assessing the effectiveness of interventions against insect populations involves determining the species composition of the target population. This is traditionally achieved through amplification of DNA of the target insect using molecular techniques such as Polymerase Chain Reaction [10]. Effectiveness of interventions can also be assessed by determining whether the target insect population is infected with pathogens such as parasites or viruses. The life cycle of insects that transmit pathogens involves 1) adult emergence from pupae, 2) mating, 3) feeding including blood and sugar feeding, 4) laying eggs and repeating steps 3–4 until the insect eventually dies. When the insect feeds on an infected host, the pathogens infect the midgut of the disease vector where they undergo a period of development known as the extrinsic incubation period (EIP). The EIP usually ranges from 4 to 18 days depending on the insect and the environmental factors such as temperature [11, 12]. Mature pathogens migrate to the salivary glands of the insect and can be inoculated into a host during the next cycle of feeding. PCR [13, 14], enzyme-linked immunosorbent assay [15–17] or dissection coupled with microscopy [18] are the conventional ways of differentiating infected from uninfected insects. The effectiveness of more recent interventions where mosquitoes infected with an intracellular endosymbiont bacteria known as *Wolbachia* [19, 20] to block pathogen transmission [21, 22], are assessed by the number of insects that have maternally acquired the bacteria by PCR or quantitative PCR [23].

Traditional age grading, species identification and infection detection techniques can be time-consuming and costly for large-scale surveillance. These limitations have led to the development of alternative techniques including the application of the near-infrared (NIR) and the Mid-infrared (NIR) spectroscopy. The next sections of the book chapter will discuss the recent applications of the two techniques as alternative techniques for age grading, species identification, infection detection and blood meal analysis of mosquitoes and other insects of medical and veterinary importance.

2. Application of NIRS for characterisation of insects

2.1 Age grading

Age grading involves the assessment of insect vectors to determine how long they have survived. For example, malaria and arbovirus transmitting mosquitoes are

only infectious to the host if they are at least 10–12 days old post emergence due to the period required by the pathogen to develop within the mosquito. Estimating age of mosquitoes is therefore crucial as it determines the efficacy of interventions that target survival of those insects. Traditionally, the age of mosquitoes is determined by dissecting their ovaries to determine whether they have previously laid eggs [24] or how many times they have laid eggs [25, 26]. Those that have laid eggs are presumed to successfully have been blood-fed on a host therefore they are considered older and potentially infectious compared to those without an egg-laying history which is normally regarded as young or harmless mosquitoes. Here we will discuss how infrared has been used as an alternative age grading technique for malaria and arbovirus transmitting mosquitoes as well as for fruitflies, houseflies and culicoides.

2.1.1 Houseflies

Houseflies are mechanical vectors of more than 100 diseases caused by viruses, bacteria, parasites and fungus to humans and animals. NIR was first applied to predict the age of houseflies in 2000 [27]. The technique was compared against pteridine fluorescence technique. NIR correctly classified houseflies with a predictive accuracy ranging from 89 to 91% as either 1 day or 10-day-old flies depending on whether the head or the whole body was scanned and whether they were scanned fresh or preserved in ethanol for 2 days. Wavelengths responsible for differentiating young from old flies were identified as 850–950, 1125–1225, 1350–1450, and 1650–1700 nm. These wavelengths correspond to the first, second, and third CH absorption overtones of CH3, CH2, and CH groups and the first combinatioof n CH absorption (**Figure 1**).

2.1.2 Mosquitoes

Mosquitoes were first age graded using NIR in 2009 by Mayagaya and colleagues using ASD NIR spectrometers (ASD, Inc., Boulder, Colorado), [28]. The authors showed that NIR could predict the age of the main African malaria vector *Anopheles gambiae* and *Anopheles arabiensis* reared in the lab with an accuracy of 90% into < 7 or ≥ 7 days age groups. NIR was also used to predict the age of male mosquitoes in the same study. Since then, NIR has been used to predict the age of the two African

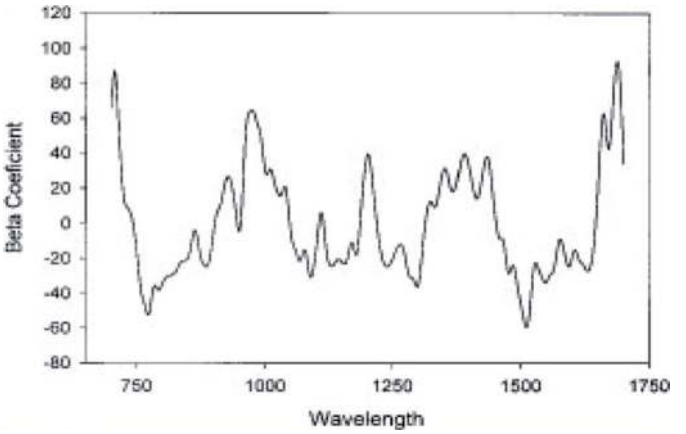

Figure 1.
Regression coefficient graph for age grading houseflies using NIR showing absorption bands in the 1st, 2nd, 3rd overtone regions and combination region [27].

malaria mosquitoes from varying environmental conditions including those reared in a semi-field system SFS [29], those exposed to pyrethroids [30], and more recently to predict the age of the primary vector of arboviruses *Aedes aegypti* [31] and the secondary vector *Aedes albopictus* [32] in the lab with similar predictive accuracy as the malaria vector. Across all species, NIR accurately differentiated very young (1–4 days) old mosquitoes from very old >15 days old mosquitoes but overlaps were observed for middle age groups. This could be due to minimal age-related changes occurring among the middle-aged (5–10 days old) mosquitoes. Recent advances in machine learning techniques have reduced this overlap to produce age prediction models with on a finer scale [33, 34]. NIR age prediction seems to correlate with a number of changes occurring within a mosquito as it undergoes the ageing process such as changes in cuticular hydrocarbons [35], changes in protein expression levels [36, 37] and changes in transcriptional profiles [38] all of which have previously been linked to the ageing process of a mosquito. **Figure 2** shows regression coefficients used to predict the age of female *An. gambiae* [28] and female *Ae. aegypti* [31] mosquitoes with the most informative peaks seen from 1100 nm to 1900 nm for both species.

2.1.3 Culicoides

The ability of NIR QualitySpec Pro spectrometer (ASD, Inc., Boulder) to predict the age of female *Culicoides sonorensis* the blood-feeding species of Ceratopogonidae commonly referred to as biting midges, a vector of bluetongue and other arboviruses was first demonstrated by Reeves and colleagues in 2010 [39]. Three colonies namely VanRyan (VR), Ausma (AU) and AK were used. Using PLS to train models, NIR differentiated younger midges that were 1–3 days old from older midges that were 9–12 days with an overlap observed between adjacent age groups. Younger age groups (1–3 days old) midges were generally predicted as older and older midges (12 days old) were generally predicted as younger (**Figure 3**).

2.1.4 Fruit flies; drosophila

Aw and colleagues have demonstrated the capacity of NIR spectroscopy technique (ASD, Inc., Boulder Colarado) to predict the age of male and female *Drosophila melanogaster* and *Drosophila simulans* using laboratory-reared colonies [40]. Training sets developed from *D. melanogaste*r Alst and *D. simulans* Hw strains with seven age groups

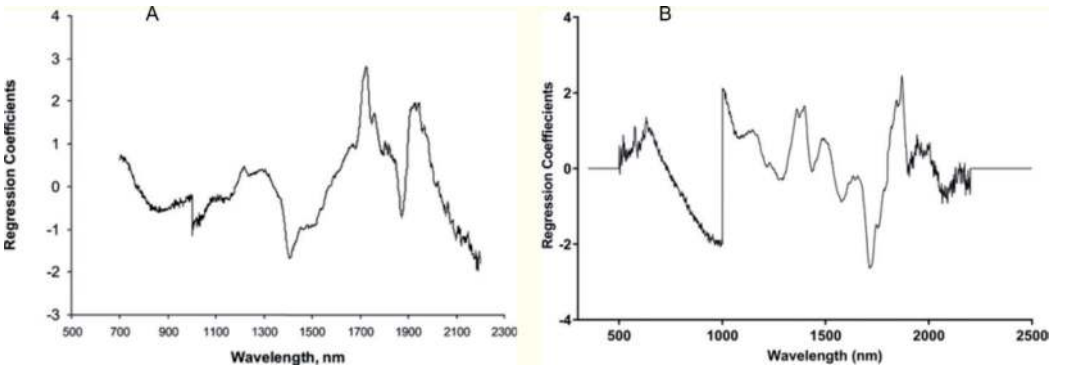

Figure 2.
Regression coefficients for predicting (A) the age of An. gambiae (Ifakara strain) and (B) Ae. aegypti (Australia strain).

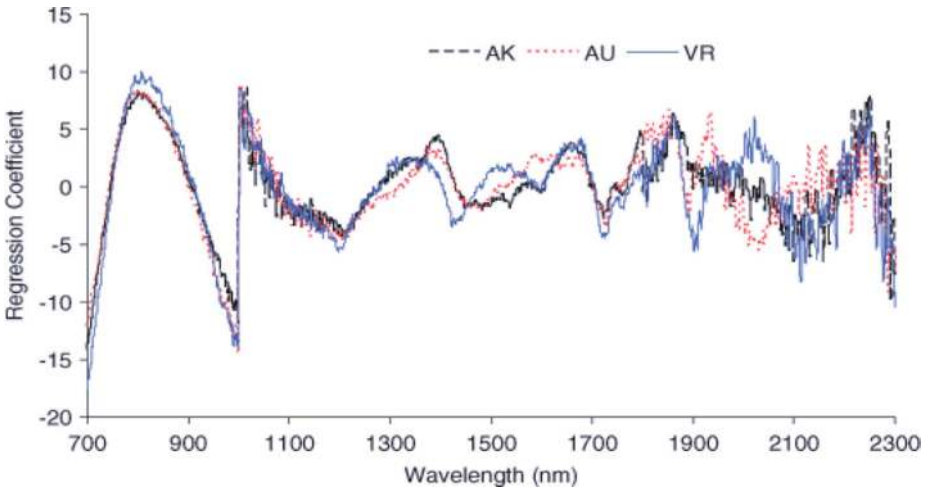

Figure 3.
Regression coefficients used to predict the age of thye three colonies of Culicoides sonorensis [39].

(1,5, 9, 13, 17, 21 and 25 days old) were used to predict independent sets of *D. melanogaste*r Dah and *D. simulans* Ky strains at three age groups (5, 13 and 21 days old) into three age groups (<9, 9–18 and > 18 days old). Generally, females were predicted with higher accuracy than males. The prediction accuracy of females that were 5, 13 and 21 days old was 88,86 and 56%, respectively for *D. simulans* Ky and 62, 83 and 84% for *D. melanogaster* Dah whereas the accuracy for predicting the age of males that were 5, 13 and 21 days old was 78, 82 and 37%, respectively for *D. simulans* Ky and 62, 83 and 84% for *D. melanogaster* Dah. Like mosquitoes, female flies were generally predicted more accurately into two groups < 9 (72–88% accuracy) and > 9 days old (88–98% accuracy) than into three groups most probably due to the increasing age-associated changes as the flies got older.

2.2 Species identification

Species identification is crucial for vector control programs as it determines whether existing interventions are effective against all or selected vector species in the target area. A change in mosquito feeding behaviour that is from indoors to

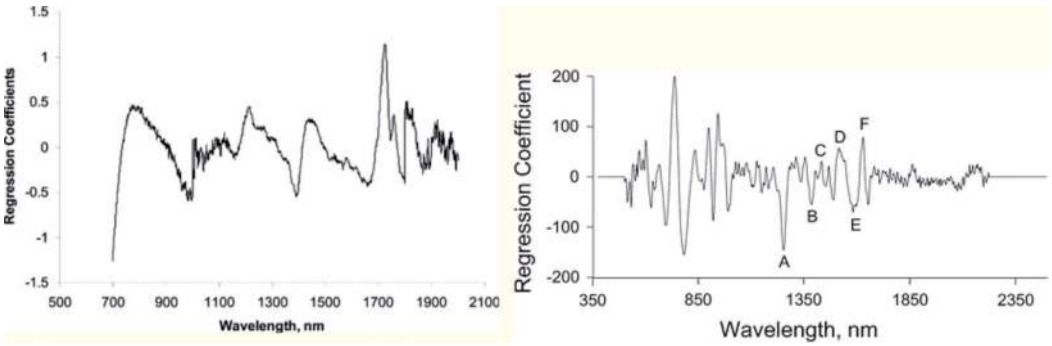

Figure 4.
Regression coefficients for determining the species of (A) female An. gambiae s.s. and An. arabiensis mosquitoes when using 10 partial least squares regression factors [28], and (B) D. melanogaster and D. simulans with important peaks shown with letters A-E [40] .

outdoors can lead to significant impacts on the efficacy of existing interventions. That means interventions targeting indoor biting mosquitoes such as bed nets or indoor residual sprays would no longer be effective [9]. Secondly, different species may require different control interventions depending on their breeding, biting or resting behaviour. We discuss here the application of NIR for differentiating cryptic species of mosquitoes and Drosophila.

NIR has been used to differentiate two insect species that would otherwise rely on molecular techniques for differentiation. The major African malaria mosquito *An. gambiae* and *An. arabiensis* are morphologically identical species. NIR has been used to differentiate these two species from laboratory colonies, semi field colonies and from the wild with predictive accuracies of 90% [28, 29] achieved for both laboratory and field collected samples. NIR has also been used to differentiate Drosophila species, *D melanogaster* and *D simulans* with 94% and 82% accuracy, respectively (**Figure 4**) [40].

2.3 Infection detection

One of the fields in which Infrared techniques have recently been successfully applied is the detection of infection in intact insects [41]. This is particularly important in detecting disease hotspots during an outbreak. For infection detection, the NIRS technique has been shown to be simple, cost-effective and rapid relative to molecular techniques such as PCR. In recently published work, NIRS was reported to be 18 times faster and 110 cheaper than RT-qPCR for Zika detection in *Ae. aegypti* mosquitoes [42]. Therefore, facilitating timely entomo-virological surveillance to reduce outbreaks in human population [43, 44].

2.3.1 NIRS detection of arboviruses in mosquitoes

The term arbovirus is an acronym for arthropod-borne virus, that is, virus transmitted through the bite of infected vectors. Arboviruses transmitted to humans by mosquitoes belonging to the family Togaviridae for example, Chikungunya and Ross River viruses or to family Flaviviridae for example, Dengue, Zika, Yellow Fever, Japanese Encephalitis, St. Louis, West Nile viruses etc. [45–47]. With the exception of the African Swine Fever Virus, arboviruses have ribonucleic acid (RNA) in their genome with a positive sense single-stranded RNA (+ssRNA), or a negative sense single-stranded RNA (-ssRNA) [48].

For arboviruses to be transmitted to humans, they require a period of development with an insect. The mosquito cycle begins when a mosquito ingests blood from an infected host during the viremia period and ends with the inoculation of the virus, into a susceptible host. However, an extrinsic incubation period (EIP) is required, whereby an intense viral replication in different organs and tissues of the insect occurs, including the reproductive system, possibly infecting unborn offspring [49]. Depending on the virus type and environmental temperature, viruses and parasites take 5–14 days from the ingestion of an infectious blood meal to reach the salivary glands of mosquitoes [50].

Traditionally, the diagnosis of arbovirus infection in mosquitoes is based on the use of molecular-based techniques such as polymerase chain reaction (PCR), and quantitative PCR (qPCR) [51–54]. However, these techniques are not suitable for large-scale surveillance due to the time, cost and expertise required to process samples. Thus, the development of new techniques to improve surveillance using a fast, reliable and affordable identification of pathogens in disease vectors is an urgent need [41, 55–57].

The development of spectroscopy techniques to detect arbovirus infection has been a growing field of research in the last 5 years. The majority of published results demonstrate the application of NIRS for the detection of arboviruses such as dengue, Zika and chikungunya in intact laboratory-reared *Aedes aegypti* mosquitões [41, 42, 58]. The first attempt to detect arboviruses in mosquito vectors using NIRS was published in 2018 by Fernandes and colleagues who showed that Zika virus (ZIKV) in laboratory-reared *Ae. aegypti* mosquitoes could be detected by simply shining infrared light on the head/thorax or the abdomen of female mosquitoes and using machine learning techniques to differentiate infected from uninfected samples. ZIKV was originally an understudied virus restricted to certain regions of Africa. However, around 2010, it started to spread to other parts of the world, and it was declared a public health emergency of international concern in 2015 following its association with microcephaly in newborns and Guillain-Barré syndrome in adults in South America.

Using Partial Least Square regression models, independent samples of mosquitoes that were either infected with Zika or without Zika infection were predicted with 97.3% and 88.8% when head/thoraces were scanned and 88.8% when abdomens were scanned. The difference in accuracy between heads/thoraces and abdomen is most likely related to virus multiplication in the mosquito body. The highest accuracy of 99.6%, (N = 228) was observed when mosquito heads/thoraces were scanned 7 days post-infection (dpi), which could correlate with the duration of ZIKV EIP in *Ae. aegypti* mosquitoes [42]. Slightly lower prediction accuracy for mosquitoes scanned at 4 and 10 dpi indicates low viraemia in heads/thoraces following this incubation period.

Following successful demonstration that NIR can predict Zika in intact mosquitoes in laboratory-controlled assays, its accuracy in a more natural setting was tested. This involved infecting mosquitoes with ZIKV or chikungunya virus (CHIKV) and scanning the samples 14 dpi, that is, after the virus had sufficient time to complete its EIP within the mosquito. Mosquitoes were killed by aggressively shaking their cage to mimic natural death in the field whilest being in traps. The intention was to avoid the use of any chemical compound that could affect NIR spectra. Traditionally, surveillance systems for arboviruses vectors rely on checking mosquito adult traps once every week. Following the death of mosquitoes, they were individually added to a catching bag of a BG-Sentinel trap, a harsh microenvironment produced by the constantly blowing fan intended to retain trapped mosquitoes. Spectra were individually collected daily over a period of seven days. The findings of this study show that NIR can predict both ZIKV and CHIKV 7 days post their death with predictive accuracy >90% for both arboviruses (**Figure 5**) [58].

2.3.2 NIRS detection of Wolbachia in mosquitoes and drosophila

Wolbachia is a gram-negative intracellular symbiont bacterium that is found naturally infecting arthropods, such as insects, arachnids, crustaceans, and isopods [59–61]. In addition, they are also found in nematodes. This bacterium is present in 40–60% of species, including some mosquitoes such as *Culex quinquefasciatus*, *Astacus fluviatilis* and *Ae. albopictus* [62]. However, the main vectors of malaria and arboviruses; *Anopheles* and *Ae. aegypti* are not naturally infected by this bacterium.

The NIRS technique has been used to detect *Wolbachia* in *D. melanogaster* and *D. simulans*, both of which are natural hosts of *Wolbachia*. The average predictive accuracy was 87% and 92% for *D. melanogaster* and *D. simulans*, respectively [40]. Authors attributed their findings to spectral signatures related to either the presence and the concentration of lipopolysaccharide molecules or the physiological changes caused by

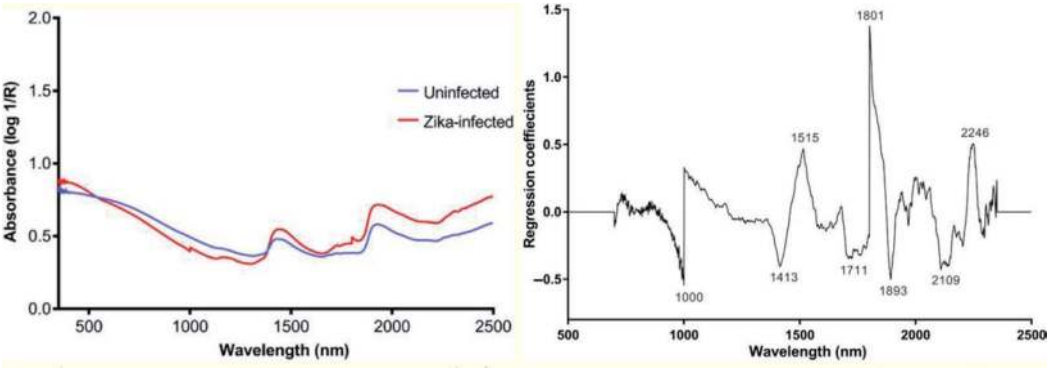

Figure 5.
Average raw spectra (left panel) and regression coefficients of Zika infected and uninfected mosquitoes. Fig adapted from [42].

this bacterium on *Wolbachia*-infected flies. *Wolbachia* is presented in high densities in insect bodies, which can be detected in germ line cells and non-reproductive host tissues [40, 63, 64]. For mosquitoes, NIRS has only been used to detect two species of *Wolbachia* in male and female *Ae. aegypti* [65]. NIRS differentiated females and males infected with *w*MelPop from uninfected ones with an overall accuracy of 96% and 87.5%, respectively whereas *w*Mel infected females and males were predicted with na accuracy of 92% and 89%, respectively. The higher accuracy in detecting *w*MelPop-infection in *Ae. aegypti* females might be due to their existence in higher densities following its transinfection in *Ae. aegypti* compared to *w*Mel. In addition, NIRS differentiated females and males infected with *w*Mel from those infected with *w*MelPop with an accuracy of 96.6% and 84.5%, respectively [65] and in a separate study to detect *w*Mel in mosquitoes 7 days post death (**Figure 6**) [58].

2.3.3 NIRS detection of plasmodium parasites in anopheles mosquitoes

Malaria is an infectious, febrile, potentially serious disease caused by protozoan parasites belonging to the genus Plasmodium transmitted to humans via bites of female infected Anopheles mosquitoes. A total of five species of Plasmodium can be transmitted to humans: *Plasmodium falciparum* is responsible for > 90% of reported annual cases and deaths, mostly in sub-Saharan Africa.

Malaria occurrence is widespread but has a higher prevalence in tropical and subtropical areas. In 2020, 240 million cases of malaria were estimated, resulting in an estimated 627,000 deaths with 95% of the cases and deaths in Sub-saharan Africa.

Esperanca and colleagues demonstrated for the first time that NIR could detect *Plasmodium berghei in laboratory-reared Anopheles stephensi* [66]. The average classification accuracy for differentiating infected from uninfected mosquitoes was 72%. However, NIRS was incapable of determining sporozoite intensity [66]. A more recent publication indicated the technique was capable of detecting oocyst and sporozoite-stage infections with 88% and 95% accuracy, respectively but the prediction accuracy was dependent on parasite concentration [67].

The latest manuscript reported findings on the investigation of the ability of NIRS to identify *P. falciparum* in *An. coluzzii* mosquitoes. Models were developed laboratory-reared infected mosquitoes by using blood from infected subjects. Overall, NIRS correctly classified 73% of mosquitoes as infected or uninfected and differentiated between uninfected and those with either oocysts or sporozoites with a 70% accuracy.

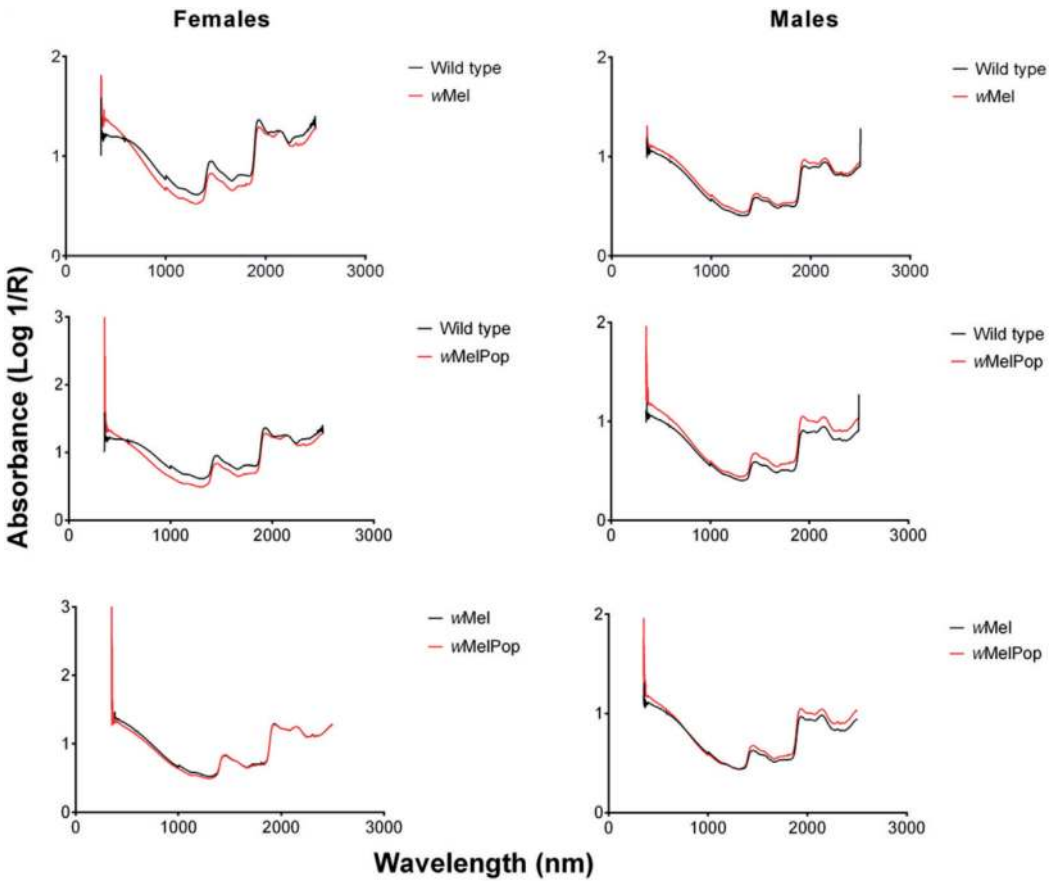

Figure 6.
Shows comparative average raw spectra of wild, wMel and wMelPop Wolbachia strains for female and male Ae. aegypti mosquitoes [65].

However, those models were unable to predict infection in wild-caught mosquitoes [68]. This indicates that robust NIRS models for predicting infection in wild mosquitoes need to be developed from naturally infected mosquitoes.

2.3.4 NIRS detection of Trypanosoma cruzi in Triatomine sp.

Chagas disease (CD), or American trypanosomiasis, is an infectious disease that plagues the entire American continent, from the southern United States to Argentina and Chile. The WHO estimates that the number of infected individuals in Latin America is approximately 6 million people, with about 70 million living at risk of contracting the disease [69]. In recent decades, due to increase mobility between distant regions of the globe, CD has become a global health priority. The disease has become a problem in areas such as Europe and Japan, as a consequence of the immigration of infected individuals acting as blood or organ donors to non-endemic countries [70].

The etiological agent of CD is *Trypanosoma cruzi*, a flagellated protozoan of the order Kinetoplastida, family Trypanosomatidae, genus Trypanosoma, which is characterised by the existence of a flagellum and a single mitochondrion, where the kinetoplast - a specialised organelle containing the DNA - is located. *T. cruzi* is the only human trypanosome transmitted by the excreta of an invertebrate vector and is included in the section Stercoraria. The strains of the parasite circulating in nature,

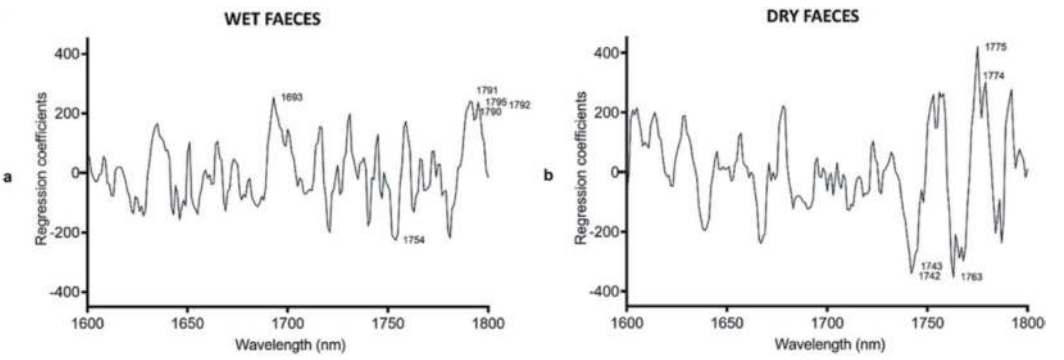

Figure 7.
Regression coefficients using 6 and 5 factors for (a) wet and (b) dry excreta, respectively, in the PLS model based on the NIR spectra in the 1600–1800-nm region for differentiating T. cruzi-infected from uninfected excreta. Adapted from [73].

during their life cycle, can infect different vertebrates (humans, domestic animals and other wild mammals) and invertebrate (triatomines) hosts. Throughout its life cycle, *T. cruzi* assumes morphologically and physiologically distinct evolutionary forms. These evolutionary forms are identified by the relative position of the kinetoplast in relation to the cell nucleus and the appearance of the flagellum, the main ones being: trypomastigote, amastigote and epimastigote [71].

The vectors of CD are insects of the Triatominae subfamily (Hemiptera: Reduviidae) and are popularly known as *barbeiros* (Brazil), *vinchuca* (Argentina, Bolivia and Chile), *chinchorro* (Ecuador), *chirimacha* (Peru), *chipo* (Venezuela) and kissing bug (USA). They are nocturnal insects a characteristic that helps their hemophagic behaviour, because they take advantage of the resting period of their hosts to perform their blood repast while reducing the chances of being predated. Currently, there are 150 described species of triatomines, all of which are considered potential vectors of CD. The main vector of CD in the Americas is *Triatoma infestans*, although other species such as *T. dimidiata*, *T. brasiliensis*, and *Rhodnius prolixus* among others, may be of high relevance in specific geographic areas [72].

NIRS has only been recently applied to detect *T. cruzi* in Triatomine species reared in the lab. It was 100% accurate for predicting *T. cruzi* infection in either the midgut or the rectum of *T. infestans*. Furthermore, the model developed for predicting infection in insect midgut could be applied for diagnosis of *T. cruzi* on the rectum and vice-versa. The authors also demonstrated that NIRS could accurately predict infection with 100% accuracy using either wet or dry samples [73]. **Figure 7** shows regression coefficients used to differentiate infected from uninfected excreta.

3. Mid infrared for predicting age, species and infection

MIR is a recently developed technique in the field of vector-borne diseases. For age grading mosquitoes, it has been demonstrated on the African malaria mosquitoes *An. gambiae* and *An. arabiensis* and *Aedes aegypti* mosquitoes. The capacity of MIR to predict the age of laboratory-reared mosquitoes was demonstrated by Jiménez and colleagues in 2019 [74] using Bruker Vertex 70 spectrometer (Bruker Corporation, Billerica, Massachusetts, USA). Here they showed that the age of young (1d and 15 d old mosquitoes) could be differentiated from the middle age groups. In their most recent study on age grading, they scaled up the sample size of Anopheles mosquitoes

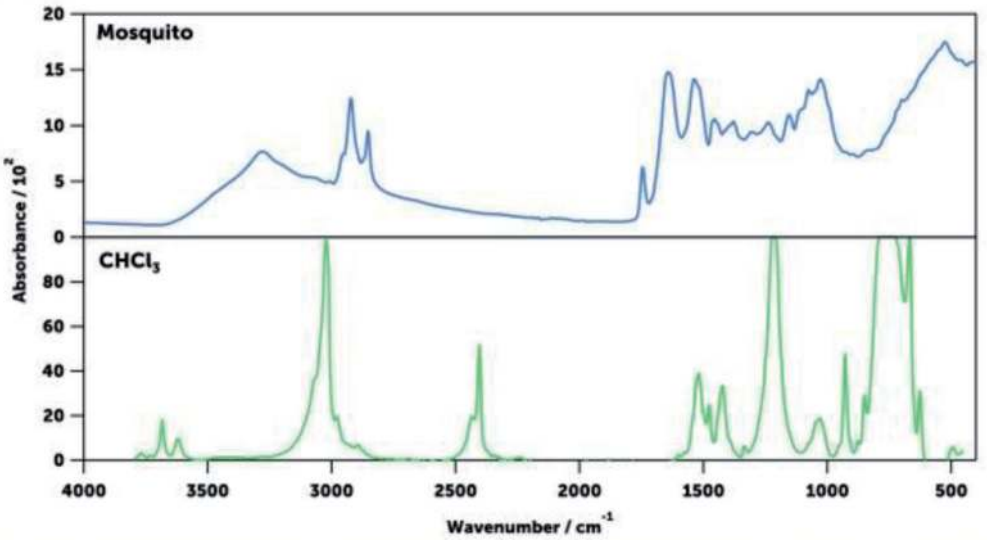

Figure 8.
MIR spectra of a mosquito and chloroform adapted from [74].

to 40,000 and demonstrated the technique's capacity to distinguish mosquitoes with varying egg laying history that is, those with 1–3 gonotrophic cycles [75]. In addition to age grading, MIR has been shown as a predictor of the source of host blood meal of blood-fed Anopheles mosquitoes [76]. This is important as it demonstrates whether a mosquito species prefers one host over the other or if it can feed on multiple hosts species. In addition to age grading and blood meal analysis, MIR can differentiate cryptic species of *An. gambiae* with a predictive accuracy of 90% for both laboratory and field-collected samples [74].

Other studies have reported on the capacity of MIR to detect infections such as *Wolbachia* in mosquitoes [77] as well as a diagnostic tool for Plasmodium parasites in blood samples from human subjects in Thailand [78] and Tanzania [79]. Overall, the findings of these studies demonstrate the potential of MIR technique for multiple applications in the field of mosquito-borne diseases. A raw spectrum of a mosquito collected by mid-infrared is shown in **Figure 8**.

4. Preservation techniques compatible with infrared techniques

Most vector control interventions conduct large scale studies in remote areas where thousands of samples are collected, stored and processed at a later stage in the laboratory. These samples are often stored in preservatives to ensure the DNA/RNA does not degrade prior to processing of the samples. For infrared techniques to be incorporated in such studies, the predictive accuracy of samples should not be affected by these preservatives. Three studies have demonstrated that a number of preservatives are compatible with these techniques including Silica gel, ethanol, Carnoy's solution, RNAlater solution and storage in 4 degrees [78–80]. The predictive accuracy of samples preserved by these techniques was observed from 1 to 52 weeks. Overall, preservation with the silica gel technique has been recommended for both MIR and NIR techniques for periods of 2–52 weeks [72]. RNAlater, storage in 4 degrees was also shown accurately for the longest period that the samples were stored

under that is 4 weeks [78]. Based on the nature of studies conducted for mosquito control programs, silica gel is the preferred preservation technique as it is associated with low cost and can used in the field under varying temperatures.

5. Conclusions

The application of both Near and mid-Infrared spectroscopy techniques for age, species and infection detection for malaria and arbovirus vectors is a relatively new area of research. Several studies have reported the potential of infrared techniques as alternative tools for vector surveillance and diagnostic tools. Relative to existing techniques, they are rapid and cost-effective and can easily be scaled up for programmatic use. However, there is very limited data on their applicability in the field. It is recommended that for both techniques to be field ready, robust models should be developed and validated using field collected samples from multiple settings. Secondly, none of the published studies provide evidence whether their ability to predict age, species or infection is based on the detection of specific chemical compounds or the actual pathogen presented in the mosquito/human samples. This needs to be established to improve the predictive accuracy of these techniques. Overall infrared spectroscopy techniques represent a big step forward in the field of vector-borne diseases and have potential to stop outbreaks in a timely manner through rapid identification of hotspots.

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Conflict of interest

The authors declare no conflict of interest.

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