# *Water and Health in the Americas*

## IANAS Water Program


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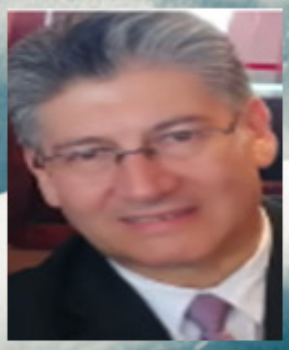

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# AGUA DEL PLANETA

Agua del planeta
cuánta falta
nos haces hoy,
cada gota tuya
hace que
renazca la vida.

Qué harían las flores sin ti.
Qué harían los mares, océanos,
lagos y ríos sin ti,
qué haríamos todos sin ti,
qué haría nuestro cuerpo sin ti.

Agua es vida,
agua es paz,
agua es salud,
agua es energía,
agua es esperanza.

Eres más que todo el
oro o el diamante del mundo.

Agua del planeta
tú no tienes precio,
por ti sola vales,
por ti sola nos das
salud, vida y existencia.

By the Peruvian poet Dr. Ciro Maguiña Vargas
May 24, 2023

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# PREFACE

Water is among the most critical resources provided by the Earth's environmental endowment. Life as we know it cannot exist without water. The production of food and fiber requires water either through precipitation or irrigation or both. Water is also needed for many industrial processes. And, water is crucial in providing environmental services. Recently we have noticed it is key to aid in confronting health threats as pandemics. Water is not always available in the desired quantities and qualities at the time and place of use. This is a primary manifestation of water scarcity where scarcity means that the amount of water (or other goods and services) that people want exceeds the available supply. Water scarcity is expected to intensify throughout the Americas in the future. Among the causes of this are increasing population, continuing economic growth, deteriorating water quality, overexploitation of ground water and climate change. In some instances, scarcity will become severe unless it is attended to. This means that effective management of water will be essential to ensure the availability of those resources to support human health, nutrition, economic performance and the environment1.

The Water Program of the Inter-American Network of Academies of Sciences (IANAS) was created to promote water science accessible to society in different forms. In 2019 a Strategy Plan was developed by focal points representing their country academies of which all are water experts in different fields. The plan was elaborated as a guide aimed to orientate a strategy for the upcoming future in confronting the most prominent and actual water problems to contribute to science orientated to better water management in the Americas

Having as referent the mission of the IANAS Water Program, which is to foster the cooperation between Academies of Sciences in the Americas; to build scientific capacity in higher education, governmental and private sectors; to generate and disseminate the best credible scientific information on water for society in order to promote public understanding of science and its role; and to provide an independent source of scientific advice and knowledge for effective policy making on water management for sustainable development and an informed public, a series of webinars on Water and Health were developed by the Program.

This publication compiles the presentations of various experts in the water and health phenomenon who addressed the successes, achievements, failures, and new challenges of the control, elimination and eradication of water related maladies in the Americas.

*1 IANAS Water Program

# Water-washed pathogens and diseases

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# *Water and Health: Water-washed diseases*

Martin S Forde, ScD
St. George's University, Grenada, W.I.

**Abstract**

The Bradley system of categorizing water-related diseases, based on environmental transmission routes, is a very effective tool to help identify the most impactful types of interventions for reducing disease incidence. This classification system identifies four main water-related disease transmission routes: water-borne, water-washed, water-based, and water-related insect vector. This paper focuses on water-washed (also referred to as water-scarce or water-access) transmitted diseases, diseases transmitted by a lack of clean water leading to inadequate hygiene conditions and practices. Unlike the other categories of water-related diseases, in water-washed transmitted diseases water assists with the prevention of disease transmission rather than as a vehicle for the transmitting of pathogens. The prime intervention, therefore, to mitigating this class of water-related diseases would be to augment the availability of water which in turn would lead to an improvement in sanitation and hygiene conditions.

## Introduction

The link between water and human health is well established. Given its importance in determining human health and wellbeing, several attempts were made to classify the various ways water impacts on human health (Bartram and Hunter, 2015). One such system is the Bradley Classification system developed by David Bradley (White, Bradley, & White, 1972), who proposed four classifications of disease transmission routes: waterborne, water-washed, water-based, and water-related insect vector (**Table 1**).

The Bradley Classification system categorizes water-related diseases based on their environmental transmission routes. This differs from the approach more commonly seen in medical texts which typically classifies diseases based on the taxonomic or clinical characteristic of the pathogen (Thompson and Cairncross, 2002). A key advantage of the Bradley classification system is that by focusing on environmental factors aimed to promote and propagate water-related diseases, it readily helps identify the types of interventions most impactful in reducing the incidence of these diseases.

**Table 1** The Bradley Classification of Water-Related Diseases

| Transmission Route | Mechanism | Disease group | Water-related disease examples |
|---|---|---|---|
| Water-**borne** | The pathogen (e.g., pathogenic bacteria or viruses) is in the water that is ingested | Fecal-oral | Cholera, typhoid, amoebic and bacillary dysentery, and other diarrheal diseases. |
| Water-**washed**<br>Water-**scarce**<br>Water-**access** | Person-to-person transmission because of a lack of access to clean water for hygiene | Skin and eye infections | Scabies, tuberculosis, trachoma, and flea-, lice-, and tickborne diseases in addition to most waterborne diseases |
| Water-**based** | Transmission via an aquatic intermediated host (e.g., snail) | Water-based | Dracunculiasis, schistosomiasis, and some other helminths |
| Water-related **insect vector** | Transmitted by insect vectors which breed in water | Water-related insect vector | Dengue, filariasis, malaria, onchocerciasis, and yellow fever |

*Source*: Modified from Cairncross and Feachem 1993

Water-washed (also referred to as water-scarce or water-access) transmitted diseases are favored by inadequate hygiene conditions and practices. White et al. (1972) formally defined water washed infections as 'those whose incidence or severity can be reduced by augmenting the availability of water without improving its quality' (p 162).

The fundamental mechanism by which water-washed infections are transmitted is due to poor sanitation and hygiene arising from a lack of adequate water supplies. Unlike the other categories of water-related diseases, in water-washed transmitted diseases water assists with the prevention of disease transmission rather than as a vehicle for the transmitting of pathogens. Transmission of water-washed diseases are interrupted by improving hygiene conditions which thus requires an improvement in the quantity of water available to effect efficacious hygiene behaviors and practices.

It should be noted there is a significant overlap in water-related diseases that are both water-borne transmitted and those water-washed transmitted (Cairncross & Valdmanis, 2006). Practically all potentially water-borne infections transmitted by the fecal-oral route can also be potentially transmitted by other means such as contaminated fingers, food, fomites, field crops, other fluids, and flies, all of which are also water-washed mediated routes (**Figure 1**). There are, however, several infections of the skin and eyes such as scabies and trachoma (Chlamydia trachomatis) where transmission is person to person and hygiene plays an important role in prevention and therefore, be considered solely water-washed transmitted diseases (Cairncross & Valdmanis, 2006).

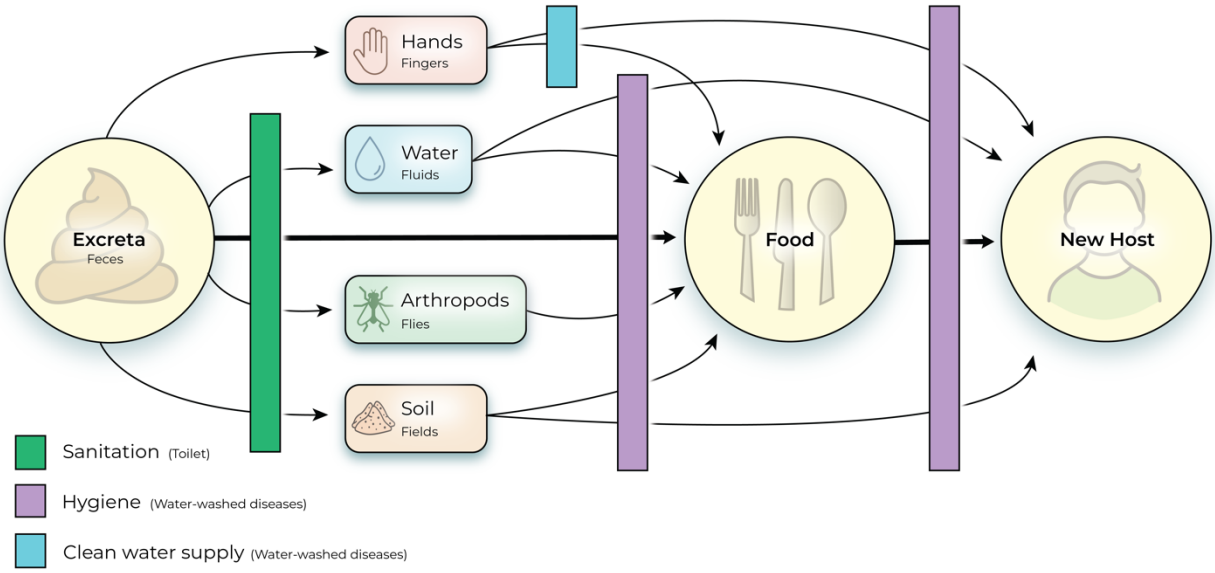


**Figure 1** The Fecal-oral disease transmission diagram, popularly referred to as the 'F diagram', using original terminology of Wagner and Lanoix (1958) showing the different fecal-oral transmission routes, and possible barriers to prevent excreta-related pathogens from finding a new host. It is and depicted with water as Fluids, arthropods as 'Flies', hands as 'Fingers' and sometimes with the addition of Fomites.

The risk of being infected by a water-washed disease largely depends on the availability of sufficient quantities of water. For example, having a ready and easy access to an adequate supply of water can affect transmission of COVID-19 by reducing the pathogenic load picked up by one's hands in environments where this virus is prevalent (Ong'ech et al., 2021). When incorporated with other non-pharmaceutical methods, the washing of hands with soap, which implies access to enough clean water, when also coupled with the maintaining of good personal hygiene, is identified as being one of the most important ways to curb the spread of this (and other) highly infectious disease (Global Water Partnership-Caribbean, 2020). Stocks et al. (2014) showed the importance of a readily available water supply in trachoma elimination as reflected in their Surgery, Antibiotics, Facial cleanliness, and Environment (SAFE) strategy.

The prevalence of water-washed diseases can also be impacted by providing education to improve Water Sanitation and Hygiene (WASH) practices and behaviors. WASH education has been singled out as a key intervention needed to lessen the disease burden caused by COVID-19 especially in resource limited com-

munities (Ong'ech et al., 2021). Improving WASH services is pivotal to ensuring effective prevention and control (IPC) practices.

There are still other variables linking access to water and human health being studied to determine which interventions are likely to make the biggest impacts. Several studies have found the time/distance to access water and location of water source ('on plot' or 'in dwelling') show a complex relationship to health outcomes (Bradley & Bartram, 2013; Cronk et al., 2015; Evans, et al., 2013).

**Conclusion**

Of the four environmentally transmitted water-related diseases, water-washed diseases are caused by the absence of adequate water supplies rather than because of its presence. Adequate hygiene and sanitation cannot be achieved in the absence of a ready supply of clean water. Hence, any goals that are aimed at ensuring maximum health for all must also ensure that this essential commodity is always made available to all.

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# WATER-WASHED PARASITES

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## Introduction

In some areas of many developing countries, the lack of clean water access in adequate amounts is associated with different parasitic infections and diseases. The improvement of water sanitation and hygienic measures could prevent the death of around two million children under 5 years old every year, typically associated with the diarrhea caused by protozoal diseases [1].

Water-washed parasites are commonly transmitted by: (a) direct skin contact between individuals due to poor sanitation and hygiene, (b) oral contact with soiled fingers (e.g., contaminated hands whilst eating), or (c) direct skin contact with infested soil.

**Water-washed parasites transmitted by direct skin contact between individuals due to poor sanitation and hygiene - Ectoparasites**

Sarcoptes scabiei: These parasites are transmitted by close skin contact with an infected person, including sexual contact. Fertilized females burrow under the skin and causes itching, especially at night. Producing erythematous papules and burrows mostly in interdigital areas, wrists, axillae, periumbilical areas, buttocks, and genitalia (Figure 1).

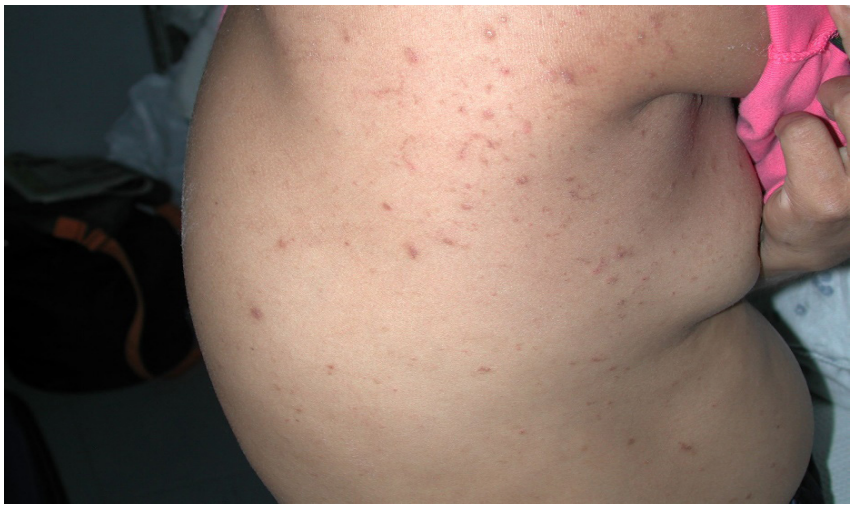

**Figure 1.** Small papular lesions and scratches due to scabies (Picture Dr Legua archives).

Diagnosis can be made by identifying the parasite present in skin scrapings. For treatment, a single dose of ivermectin 200 mcg/kg PO (by mouth) is recommended, repeated after 7-14 days, or topical application on the skin, except the face, of 5% permethrin overnight and repeated after 7-14 days. Washing or ironing of clothes, vacuuming floors, chairs, couches, and treatment for the whole family should be done [2].

Pediculus humanus capitis: Transmission is via direct head-to-head contact with an infected person. The parasite feeds off blood on the scalp and may result in pruritus, excoriations, regional lymphadenopathy, and anemia. Lice can also be infected with, and therefore, transmit other pathogens such as Staphylococcus spp., Streptococcus spp., and Borrelia spp.. Diagnosis is easily made by finding lice or nits on hair shafts [3]. For treatment, topical permethrin 1% lotion or cream should be applied on the head and repeated after 7 days; or ivermectin 0.5% lotion can also be used [4].

Pediculus humanus humanus: Transmission is via close contact with an infested person, sharing clothes, and bedding. Lice feed off human blood, while its eggs are found on clothing. It may cause generalized itching. Lesions may present as excoriations due to scratching, eczematous patches, or papular urticaria, and may be found especially in the neck and trunk. Scratching of lesions may cause secondary bacterial infections. Anemia and eosinophilia in cases with body lice have also been described. Body lice can be vectors for several pathogens (Bartonella quintana, Borrelia recurrentis, Rickettsia prowazekii). Once detached from

the host, the parasite will die within 3-5 days. Treatment is mainly through washing the body, clothes, and bedlinen with soap and hot water and hot drying of clothes and linen [3].
Phthirus pubis: These ectoparasites are transmitted via close physical contact and shared garments. The infestation usually involves pubic hair but may involve any area of the body with hair, including the eyebrows and eyelashes. It produces localized pruritus and, on examination, blue-gray macules or erythematous papules may be seen. Diagnosis is made by direct observation of lice on hair shafts. For treatment permethrin 1% lotion or cream, or ivermectin 0.5% lotion may be used; oral ivermectin is also effective [3].

**Water-washed parasites transmitted through oral contact with soiled fingers due to poor sanitation and hygiene – Protozoa and helminths**

The cysts or eggs of these parasites are already infective when eliminated in human feces.
Entamoeba histolytica: Is transmitted by ingestion of its cysts. The infection may be asymptomatic or may cause diarrhea, colitis with ulcers, appendicitis, or a colonic ameboma. Colonic ulcers have a well-defined undermined margin and the mucosa between ulcers appears normal (Figure 2). Microscopically ulcers are described as flask shaped. Extraintestinal locations may cause a liver abscess (Figure 3), lung involvement, and perianal skin ulceration [5].

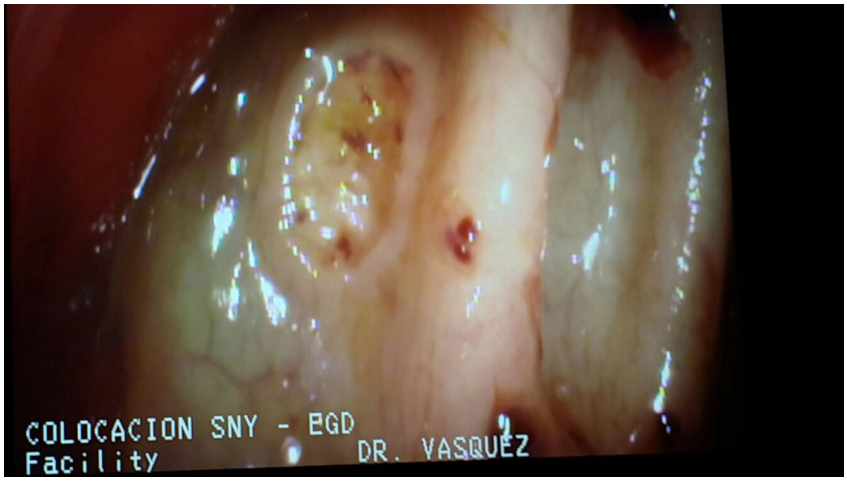


**Figure 2.** Typical amebiasis ulcer with well-defined margins and normal appearing mucosa between ulcers (Picture Drs Legua/Vasquez archives).

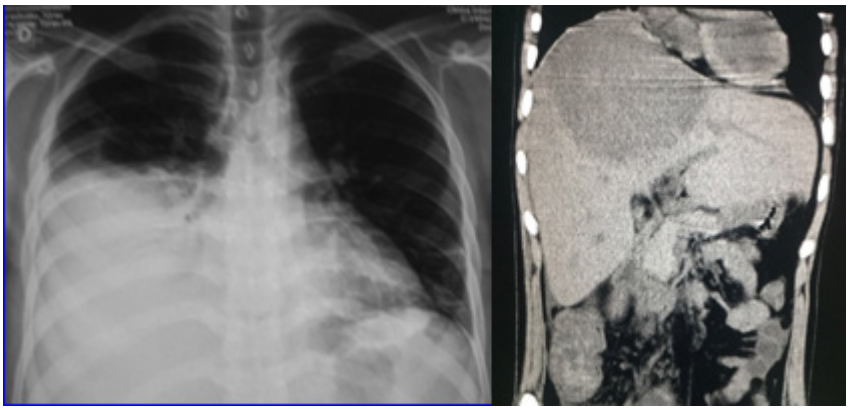

**Figure 3.** Amoebic liver abscess (Picture Dr Legua archives).

Diagnosis is made by finding the cysts in the involved tissue or the trophozoites in the stools (with diahrrea). For treatment metronidazole 500-750 mg PO every 8 hours for 7-10 days or tinidazole 2 g PO once daily for 3 days are recommended [4].
Giardia lamblia: This infection may be asymptomatic or produce anorexia, diarrhea, nausea, vomiting, abdominal distention, malabsorption, or loss of weight [2]. Diagnosis is made by finding the cysts or trophozoites in the stools. It may be treated with tinidazole 2g PO single dose, metronidazole 250 mg PO every 8 hours for 5-7 days, or nitazoxanide 500 mg twice a day for 3 days [4].
Balantidium coli: This parasite´s definitive host are pigs. Infection can be asymptomatic or cause chronic

diarrhea, abdominal pain, dysentery, anorexia, nausea, or weight loss. In severe cases may cause fulminant colitis with ulcers, intestinal perforation, hemorrhage, and shock (Figure 4).

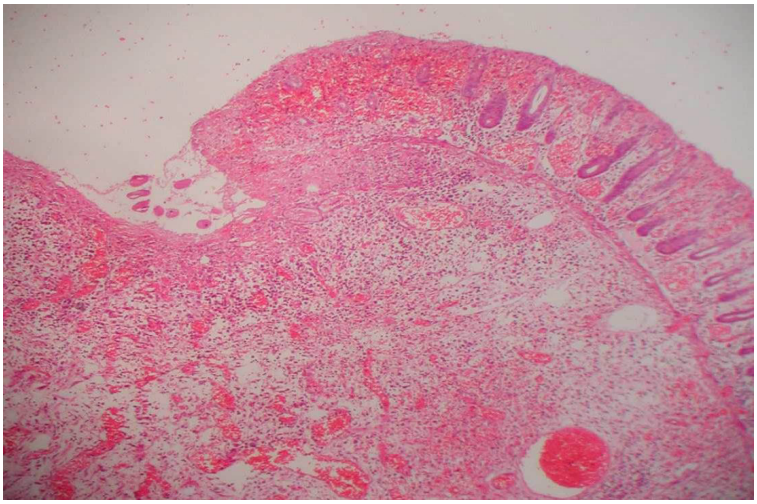

**Figure 4.** Biopsy of colonic ulcer showing *Balantidium coli* parasites within it (Picture Dr Legua archives).

Diagnosis is made by finding the trophozoite or cyst in stool. Recommended treatment is tetracycline 500 mg PO every 6 hours for 10 days or metronidazole 750 mg PO every 8 hours for 10 days [6].
Blastocystis hominis: This parasite, found in stools, usually does not produce disease, and requires no therapy, but in immunosuppressed hosts it may cause diarrhea, anorexia, nausea, or fatigue. These patients can be treated with metronidazole 750 mg PO every 8 hours for 5-10 days [6].
Enterobius vermicularis: These worms, known as pinworms, produce intense perianal and perineal nocturnal itching. In female children, it may cause vaginal itching and leukorrhea. Pinworms may migrate into the uterus, fallopian tubes, peritoneal cavity, or bladder. Pinworm eggs can be found attached to adhesive tape applied to the perianal area first thing in the morning. Treatment should be given to all the household contacts with either a single dose of albendazole 400 mg PO or mebendazole 100 mg PO. Therapy should be repeated after two weeks [7] (Figure 5).

**Figure 5.** Picture of adult *Enterobius vermicularis* in a petri dish (Picture Dr Legua archives).

Hymenolepis nana: Persons with heavy infections (i.e., more than 1000 worms) may experience anorexia, epigastric pain, nausea, vomiting, diarrhea, headache, dizziness, weakness, weight loss, irritability, restlessness, restless sleep, pruritus, or urticaria. Diagnosis is made by finding the eggs in the stool [8]. Therapy is with praziquantel 25 mg/kg in a single dose [4], preferentially repeated after 10 days, or with niclosamide 2g PO daily for 7 days [4].
Cysticercus cellulosae: Cysticercus cellulosae is the larval stage of Taenia solium, which is acquired by the ingestion of T. solium eggs. Clinical manifestations most commonly occur with involvement of the central

nervous system (i.e., neurocysticercosis) and may produce seizures, headache, focal deficits, intracranial hypertension, cognitive decline, or psychiatric manifestations. Antiparasitic therapy, usually with albendazole plus praziquantel with dexamethasone varies according with the clinical presentation [9] (Figure 6).

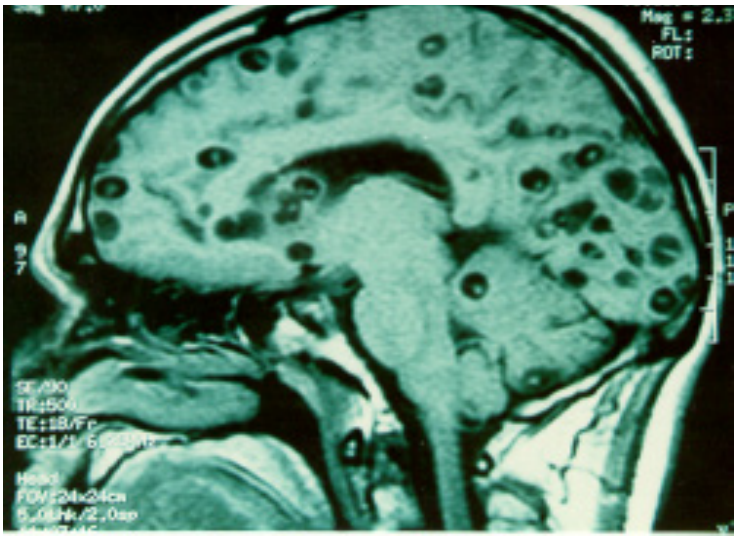

**Figure 6.** Multiple cysticercoids with identifiable scolex in the brain parenchyma, cerebellum, and lateral ventricle (Picture Dr Legua archives).

**Water-washed parasites transmitted through direct skin contact with infested soil due to poor sanitation and hygiene – Helminths**

These parasites develop to the infective stage in the ground after infectious people defecate on it.

Ancylostoma duodenale and Necator americanus: Infection is acquired when infective filariform larvae in the soil or sand penetrate through the skin when walking barefoot or lying on the ground. The parasite finds its way to the intestine and may produce vague abdominal complaints, epigastric pain, abdominal distention, malnutrition, oedema, or eosinophilia, but the most important consequence is iron deficiency anemia (due to blood sucking), and impaired development in children. The infection is diagnosed by finding the eggs in the stool and therapy may be with albendazole 400mg PO single dose or mebendazole 100mg PO twice a day for 3 days [10].

Strongyloides stercoralis: This infection is also acquired by infective filariform larvae penetrating through the skin and reaching the intestine. It may cause anorexia, chronic diarrhea, malabsorption, abdominal distention, cachexia, ascites, growth retardation in children, cutaneous larva currens, and eosinophilia. This parasite has the possibility of causing an internal autoinfection cycle which, most commonly in immunosuppressed individuals, may produce hyper infection (high parasite burden) and disseminated infection when the parasite involves organs outside of its normal life cycle [11]. Diagnosis is made by finding the rhabditiform larvae in the stool and can also be made by serology or DNA detection by polymerase chain reaction [12]. The drug of choice for this infection is ivermectin 200 mcg/kg/day PO for 2 days; another option is albendazole 400 mg PO twice a day for 7 days [4].

Conclusions

Clean water access in adequate amounts is a key element for hygiene and prevention of different parasitic infections and diseases. The improvement of water sanitation and hygienic measures could prevent millions of deaths worldwide, which mainly affect children and are typically associated with the diarrhea caused by protozoal diseases.

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# Water-borne pathogens and diseases

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# PRESENT DATA AND FUTURE PROSPECTS FOR ENVIRONMENTAL SURVEILLANCE OF WATERBORNE VIRUSES AND WATER-RELATED VIRAL DISEASES

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**ABSTRACT**

Diseases related to lack of basic sanitation are a problem still affecting the population globally. Within these diseases, those causing viral gastroenteritis are of major concern. These diseases cause public health and governments to suffer harm, damages, and losses, affecting the country's economy. Innovative methodologies developed over the last years allow effective detection and monitoring of enteric viruses from different water and environmental matrices. Current methods used for viral detection from water matrices have some disadvantages, potentially interfering with a more accurate diagnosis. Therefore, new techniques are being developed to meet current needs and reduce the disadvantages of existing methodologies. Among these techniques are next-generation sequencing and microfluidics technology. Despite the improvement and the emergence of new viral detection techniques, there are still many challenges to overcome, not only regarding monitoring, but also management and treatment of water and sewage.

## 1. Overview of damage induced by waterborne diseases

Water is an essential element for life and public health. However, water-related illnesses and consequent deaths continue to be a global issue in both developed and developing countries (ISLAM et al., 2020). Thus, ensuring drinking water is safe is a crucial challenge for public health worldwide. The transmission of water-related diseases, both direct and indirectly, can occur through contact with different sources of contaminated water such as drinking, recreational, irrigation, and groundwater (NWABOR et al., 2016; BONADONNA and LA ROSA, 2019; CHEN et al, 2021). Different pathogens can be responsible for these infections including fungi, bacteria, protozoa, metazoa, and viruses. Countless outbreaks are reported due to contact or ingestion of contaminated water, most of which have viruses as their causative agents. Viruses are not able to replicate outside the host cells, so their presence in aquatic environments is primarily due to contamination by human or animal excreta (NWABOR et al., 2016; BONADONNA and LA ROSA, 2019; ZAHEDI et al., 2021). Enteric viruses, termed those that infect and replicate in the gastrointestinal tract, are excreted in high concentrations in the feces of individuals following even asymptomatic infections. Therefore, their cycle could be maintained whenever water bodies are not properly protected against contamination, and these viruses are quite often present in human populations and are mainly associated with waterborne infections (CARRATALÀ et al., 2013; CARTER, 2005; KHORA, 2018).

The unavailability of safe water sources, lack of adequate sewage treatment, and climatic and environmental factors may interfere with real availability of water for human populations and also may enhance the proliferation of disease vectors (cite?). These conditions harmed the economy of several countries once the transmission chains of diseases, and so followed outbreaks, epidemics, and pandemics have generated numerous expenses and losses for the governments and the general population (CISSÉ, 2019). To exemplify these losses, some data is provided in Table 1.

Table 1: Examples of expenses and losses for the government and the general population due to lack of sanitation and adequate water treatment.

| | Impact | Place | Reference |
|---|---|---|---|
| **Public health- related harm** | More than 2 billion people face serious health risks because basic water services are not available | Globally | Instituto Trata Brasil (2022) |
| | Children in conflict settings are three times more likely to die from waterborne diseases than from violence | Globally | Instituto Trata Brasil (2022) |
| | 3.5 million people die from problems related to inadequate water supply each year | Globally | Instituto Trata Brasil (2022) |
| | Diarrhea kills 2,195 children a day and claims more victims than AIDS, malaria, and measles combined | Globally | Instituto Trata Brasil (2022) |
| | 4% of the population (25.5 million people) suffered from diarrhea in 2015, of which 60% were children under 5 years of age | Globally | Instituto Trata Brasil (2022) |
| | 38% of healthcare facilities in 54 countries do not have access to basic water sources, and about 20% of them do not have a primary sanitation infrastructure | Globally | Instituto Trata Brasil (2022) |
| | Nearly 400,000 work-related deaths occur each year due to diseases, the main cause of which is the consumption of poor-quality water, as well as poor sanitation and hygiene | Globally | WWAP (2016) |
| | There were more than 167,000 hospitalizations and 1,898 deaths from waterborne diseases in 2020 | Brazil | Instituto Trata Brasil (2022) |
| **Economic losses and impacts** | For every US$1 invested in sanitation, estimates a return of almost six times, considering lower health costs, increased productivity, and fewer premature deaths | Globally | World Health Organization (WHO) |
| | More than 70 million expenses were spent on hospitalizations due to waterborne diseases in the Unified Health System (SUS) in 2020 | Brazil | Instituto Trata Brasil (2022) |
| | The total economy with the improvement of the health conditions between 2004 and 2016 was BRL 1.7 billion, which resulted in an annual gain of BRL 134 million | Brazil | DATASUS (2020); Instituto Trata Brasil (2022) |

## 2. An overview of current methods of enteric viruses concentration and detection, examples from Southern Brazil

Effective detection methods to evaluate viruses and other pathogens in water sources are critical for accurate water quality monitoring. In addition, detection methods help make decisions about the infrastructure of water distribution systems, choosing the best water treatment, and preventing outbreaks (GIRONES et al.,2010; SAXENA et al., 2014; RAMÍREZ-CASTILLO et al., 2015). Currently, there is a wide range of possible approaches for viral analysis in environmental matrices. After determining the environment, the method selection varies according to the studied target. However, whatever method chosen must be sufficiently robust, sensitive, specific, reproducible, and, if possible, save cost, time, resources, and labor (GIRONES et al.,2010; SAXENA et al., 2014; RAMÍREZ-CASTILLO et al., 2015). The methodological challenges for environmental virology are the physical differences between the main groups of viruses and low viral concentration in a large volume of water. To mitigate for these challenges and the presence of inhibitors present in polluted waters from domestic and industrial sewage, the samples are enriched and concentrated (IKNER et al., 2012; JAKUB and PETRA, 2021).

The research virology group, Molecular Microbiology Laboratory, at Feevale University in Rio Grande do Sul, Brazil has been studying the presence of enteric viruses in different environmental matrices over the last years. The sections below describe some studies and methodologies used.

## 2.1. Water concentration methods

Even though enteric viruses are released into water in large quantities, viral particles present in these environments are usually in low concentrations, often requiring use of large volume samples (LODDER et al., 2010). Therefore, viral analysis in water matrices needs a concentration step before detection (HAMZA; BIBBY, 2019; LODDER et al., 2010). Numerous concentration methodologies have been developed; yet, it is difficult to standardize a single protocol for different types of water and enteric viral species (HARAMOTO et al., 2018). Among those methods of virus concentration from water samples, the concentration by adsorption-elution with negative membranes, the ultracentrifugation, and the Immunomagnetic Separation (IMS) are highlighted.

The concentration by adsorption-elution with negative membranes method has a high viral recovery rate and robust evidence of reliability. (DALLA VECCHIA et al., 2015). In samples contaminated by only one viral species, the recovery rate was 89% to 125%, whereas when the sample had more than one viral species, the recovery rate was 23 to 164%. HAdV-5 exhibited greater than 100% recovery when tested with human viruses and other AdVs, while BAdV and CAV-2 were not detected (DALLA VECCHIA et al., 2015). This method continues is used with samples of surface, underground, recreational, and drinking water (STAGGEMEIER, et al. 2015; DALLA VECCHIA et al., 2015; LUZ et al. 2015; GULARTE et al. 2017; SANTOS et al., 2015).

Currently, the concentration by adsorption-elution method has been replaced by ultracentrifugation, a robust method able to achieve high detection rates and viral diversity compared to unconcentrated samples. This was demonstrated by Girardi et al. (2017), who compared the presence of different species of human and animal AdV by nested PCR followed by DNA sequencing to identify the species in raw and concentrated waters by ultracentrifugation (Table 2) (Girardi et al, 2017). This method presents realiable results, and when compared to the adsorption-elution method is faster and less laborious. In addition, it does not require external PCR inhibitor substances, which can generate false-negative results. Therefore, it has been used to concentrate water in different water matrices such as surface, underground, recreational and potable waters (STAGGEMEIER, et al., 2017; PETEFFI et al., 2018; GULARTE et al., 2019; ROVERI et al., 2020; DEMOLINER et al. 2021)

Table 2: Comparison of the detection of different viral species in 55 concentrated and unconcentrated samples of recreational freshwater.

| Virus | Concentrated samples | Unconcentrated samples |
|---|---|---|
| HAdV-F | 16% | 12,7% |
| HAdV-D | 10% | 1,8% |
| HAdV-C | 7,2% | 10,9% |
| HAdV-E | 3,6% | 0% |
| MAdV | 3,6% | 0% |
| BAdV | 1,8% | 0% |
| Positive total samples | 43,6% | 23,6% |

Adapted from Girardi et al. 2017

The IMS technique is a concentration protocol using magnetic particles bonded to a specific antibody for binding with the target pathogen. This tool allows the formation of an antibody-antigen complex, with the target pathogen being concentrated in a much smaller final volume than the initial one (HAMZA; BIBBY, 2019; RODRÍGUEZ et al., 2009). In water samples, HAdV-C was detected in 60% (using monoclonal antibody) and 47% (polyclonal) by IMS-qPCR, compared with 13% when samples were concentrated by ultracentrifugation. HAdV-F was positive in 27% of samples by IMS-qPCR (polyclonal) and ultracentrifugation and 20% by IMS-qPCR (monoclonal). Gularte et al in this study, suggested that by comparing these different approaches, it was possible to notice IMS-qPCR demonstrated a higher number of samples positive for HAdV and provided a higher rate of genomic copy detection as well (Gularte et al., 2021).

## 2.2. Bioaccumulators

Bioaccumulators are organisms that can accumulate pathogens (e.g., enteric viruses and bacteria) present in their environment (BEURET et al., 2003). A study in Southern Brazil showed qPCR detection of AdV was 91.7% in water samples from coastal lagoons, while the presence of this same virus was found in 35% of the pink shrimp (Farfantepenaeus paulensis) (LUZ et al., 2015). In other words, poor water management resulted in food contamination; therefore, the consumption of contaminated undercooked shrimp constituted a serious health risk (STENTIFORD et al., 2009). Also, if adenoviruses, pointing for fecal contamination, may be found in these samples, then other chemical and biological pollutants might be present as well.

Furthermore, it is important to consider the role of freshwater snails, molusks and schrimp as organisms to assess the presence of enteric viruses in water matrices. Thus, HAdV was detected by qPCR in snails and water collected from wetlands of the Dos Sinos River Basin (Brazil). Fifty-three percent of water samples, 31% of gastropod hemolymph samples, and 16% of tissue samples from the same organism were positive for HAdV (GULARTE et al., 2017). Another organism extensively studied is bivalves, which are filter-feeders, in other words, their feeding occurs through the process of filtering particles present in the aquatic environment. Bivalves, such as oysters, have been associated with disease outbreaks, as they can accumulate pathogenic viruses of human origin, and are often consumed raw or undercooked, thus increasing the risk of contamination (RIGOTTO et al., 2010). Gularte et al. (2019) evaluated the presence of enteric viruses in different environmental matrices on the northern coast of Southern Brazil. The detection rate of HAdV-C by qPCR and viral isolation was 26% of the total samples. Of the positive samples, 26% were HAdV positive in bivalve samples by qPCR and 29% by viral isolation showing the presence of the infectious virus. These studies demonstrate bioaccumulating organisms can help in diagnosing fecal contamination as well as the risk in consuming these organisms from contaminated aquatic environments, becoming aquatic environmental microbiology sensors.

## 2.3. Viral infectivity

Among the challenges when using the molecular PCR technique are the presence of inhibitors in environmental samples and the recovery of nucleic acids (GREEN and LEWIS, 1999). Furthermore, the data obtained in these assays do not reveal whether the viruses are are capable of causing infection. Consequently, the use of cell culture combined with PCR (ICC-PCR) is an approach used to overcome most of the aforementioned drawbacks (REYNOLDS 2004). Detection depends on an initial biological amplification of the viral nucleic acid, followed by amplification via PCR (REYNOLDS et al., 1996).

Using the ICC-qPCR method, Demoliner et al. (2021) detected infectious adenoviruses in 12% of the groundwater samples and in 18% of surface waters, that were considered negative following analyses using qPCR, multiplex qPCR, and nested PCR. In Brazil, ICC-qPCR is used to analyze both seawater and freshwater used for recreational purposes (Rio de Janeiro and Rio Grande do Sul [RS]) as well as freshwaters (RS) . Gularte et al. (2019) studied the infectivity of HAdV for different environmental matrices on the northern coast of Southern Brazil (RS). Gularte et al. (2019) found the infectivity of HAdV from freshwater (RS) and unconcentrated samples performed better for this type of analysis. Overall, 26% of all samples showed infectivity for HAdV-C. Specifically, 37% of the sea surface microlayer samples were positive, followed by 29% of bivalve, 17% of the seawater, 12% of sediment, and 5% of air sentinel. Staggemeier et al. (2017), evaluated the infectivity of HAdV-C in sand and seawater samples from sites in Rio de Janeiro used during the Summer Olympics (2016) and by tourists. In this way, infectious HAdV was detected in samples of water (17%) and sand (25.8%).

The low viability rate found in different studies does not necessarily represent a low risk of infection. Sometimes it is necessary to increase the number of passages in the cell, as already demonstrated by an increase in the number of infectious samples from the first passage (20% of infectious samples) to the fifth passage (80% of infectious samples) in cells (GULARTE et al., 2019). Another point that can generate false-negative results for viral infectivity is the fact that viruses from the environment are wild, and thus, may not adapt to cell culture (DEMOLINER, et al., 2021).

## 2.4. Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) is a method used to estimate the probability of an infection after the individual is exposed to some pathogenic microorganism present in different matrices (HAAS et al., 1999). Procedures that use QMRA to assess risk are more frequently used in recreational water samples, but they can be used in other kinds of samples such as food, air, and soil.

To estimate health risks associated with exposure to water used for recreational purposes, Girardi et al. (2019) conducted a study applying QMRA in Arroio Belo (Rio Grande do Sul, Brazil). For the E. coli concentrations, lower infection risks were estimated (8.58E-05 to 2.17E-03). On the other hand, the HAdV-F concentrations were associated with a higher risk of infection in 9.99E-01, and for group C, 1.29E-01 to 9.99E-01. Also, Gularte et al. (2019) applied QMRA to estimate infection due to HAdV-C and F and E. coli associated with exposure by swimming in beaches of southern Brazil from seawater, sediment, sea surface microlayer, bivalves, and air. The QMRA estimated the maximum daily and annual risks (9.99E-01) in almost all samples, while in the E. coli analyses, the superior result obtained was 1.01E-01, indicating high infection risks associated with exposure to beaches in the northern coast of Rio Grande do Sul.

In general, both the results of water samples and other environmental matrices showed only

the commonly used bacterial evaluation does not match the real probability of the risk of infection that recreational users are exposed to in these environments.

## 3. Future applications of environmental virology

As discussed in the previous topics, different viral detection methods have been used, such as molecular and cell culture methods. Both methods have advantages and disadvantages and the choice of one or the other varies according to the purpose of the study. Given this, new methodologies have been developed to compensate for the disadvantages of the tools currently used (OGORZALY et al., 2015). Among the approaches recently employed in the analysis of environmental samples, next-generation sequencing (NGS) and Microfluidics technology are highlighted.

Advances in NGS platform technologies and the rapid development of bioinformatics and computational tools, in addition to allowing the sequencing of a heterogeneous mixture of genetic materials, with high sensitivity, offer new opportunities for surveillance of emerging infectious diseases (DATTA et al., 2015; KWOK et al., 2020). Furthermore, these platforms function in a sequence-independent manner, making them ideal tools for new virus discovery, showing potential for timely detection of rare or emerging infectious etiologies, as well as surveillance of food- and water-borne viruses (DATTA et al., 2015).

Microfluidics technology has emerged in recent years as a tool for detecting biological material in fluidic samples (CHATURVEDI; GORTHI, 2017; CHEN et al., 2017). This methodology plays an important role in the field of water quality assessment since it is possible to reduce the sample amount in automate devices (JAYWANT & ARIF, 2019), providing subsidies for the development of biosensors, as a fast, selective, portable, sensitive, and easy to handle approach, which contributes to the application in the area of pathogens detection in aquatic environmental (JAYWANT & ARIF, 2019; KUMAR et al., 2018). So far, some studies have already demonstrated the ability of IMS to improve viral detection in water samples (HARAMOTO et al., 2010; YANG et al., 2011; GULARTE et al., 2021). As described above, Gularte et al. (2021) established the use of IMS as a concentration method for the detection of HAdV-C and -F in water samples showing important advantages of IMS when combined with qPCR. Furthermore, the possibility of integrating this approach as an enrichment step on microfluidic platforms may be an important stage in the development of new sensors. Since biosensors will be the next generation of technologies to improve the detection of pathogens in aquatic environments in an easy and precise real-time manner (GULARTE et al., 2021), the IMS combined with microfluidic technology for the production of a "lab-on-a-chip" could be a promising way to achieve rapid response and in situ detection of enteric viruses as was initially developed for the detection of bacteria responsible for food contamination (AHMED et al., 2016).

## 4. Conclusions

Even though the technologies of molecular detection have improved over the last decades, there are challenges to overcome especially for the detection of enteric viruses in water samples. For this reason, it is important to continue improving these technologies to achieve an easy, fast, precise, and cheap; therefore, a method that will facilite the introduction of these markers into the countries' legislation for the monitoring an evaluation of water safety.

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# *Contamination of water with antibiotics and antimicrobial resistant bacteria in developing countries*

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## Abstract

The spread of antimicrobial-resistant (AMR) bacteria is a major threat to the treatment of infectious diseases. The abuse of antibiotics is significantly related to the emergence and spread of AMR bacteria among communities. Antibiotic contamination of the environment, especially in water, apart from livestock and their products, is an important factor in the emergence of outbreaks and spread of AMR bacteria. A study on the antibiotic use in Vietnam's rural communities revealed β-lactam antibiotics were the most prescribed antibiotics in humans, yet livestock were more likely to be treated with colistin. The study on residual antibiotics and AMR genes in the aquatic environmental found the presence of mostly sulfonamide antibiotics and extended-spectrum β-lactamase-producing genes. Interestingly, more than 60% of the people in the communities possessed AMR bacteria in their digestive tracts. Moreover, 30–60% of the retail meat available in the area was contaminated with AMR bacteria. A wide dissemination of colistin-resistant bacteria, one of the most notable AMR bacteria, was also observed among residents of the community.

This chapter constitutes a compilation of the results of various studies carried out by Yamamoto Y et al and these indicate the severity of the spread of AMR bacteria in developing countries, and how antibiotic contaminated aquatic environments may play an important role on it.

## Introduction

The emergence of antimicrobial-resistant (AMR) bacteria, particularly multidrug-resistant (MDR) bacteria, continues to increase the number of intractable infections due to the limited availability of suitable antibiotics for treatment (Fig. 1). The overuse of antibiotics in the agricultural and medical care sectors contributed to this major public health threat. In addition, the spread of AMR bacteria worldwide increased by the trans-border movement of people and globalization of agro-fishery products, leading to a global crisis.

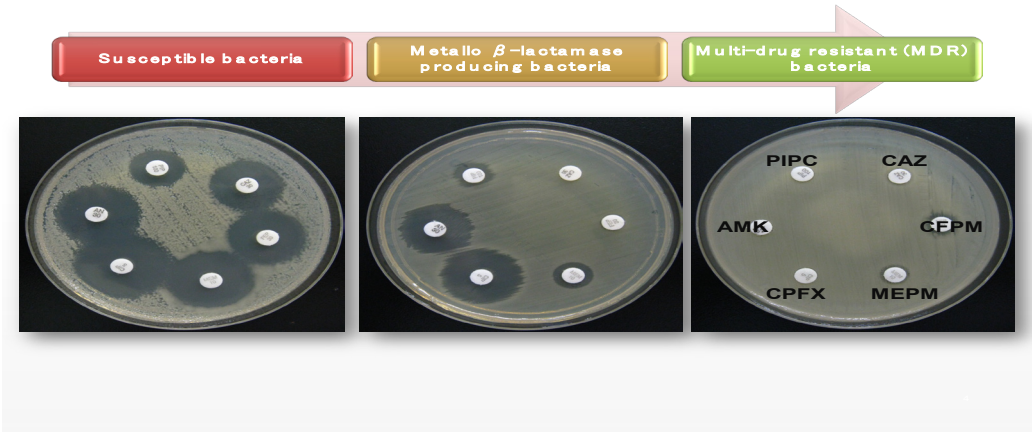



Figure 1. Examples of antibiotic-resistant (AMR) bacterial phenotypes. Sensitivity test for various antimicrobial agents. PIPC, piperacillin (Penicillin); AMK, amikacin (Aminoglycoside); CPFX, ciprofloxacin (Quinolone); MEPM, meropenem (Carbapenem); CFPM, cefepime (Cephem); CAZ, ceftazidime (Cephem).

A previous study revealed extended-spectrum β-lactamase (ESBL)-producing bacteria, a type of AMR bacteria spreading rapidly in recent years [1], were isolated from 37–53% of the fecal samples of residents in rural areas of developing countries [2]. Another study showed 61% of healthy residents in Thailand were carriers of ESBL-producing bacteria [3]. Thus, developing countries have a higher prevalence and spread of these bacteria than developed countries, such as Japan, with only 6.4% of its healthy residents being carriers [4]. Although ESBL-producing bacteria are not pathogenic, their AMR genes can be transferred to pathogenic bacteria and may induce intractable infections [1]. Therefore, ESBL-producing bacteria can be regarded as an emerging global public health threat.

**1. Field study in a developing country**

The rural communities of Thai Binh and Can Tho in Vietnam were chosen as study sites to examine the spread of AMR bacteria in a developing country (Fig. 2). These two sites were chosen because, like most rural areas in Asian countries, many households in both communities had livestock in their backyards for self-consumption; and therefore, preliminary studies of these communities showed their residents with a high prevalence of AMR bacteria [2].


Figure 2. Study sites in Vietnam

**2. Antibiotic usage in the community**

To assess the use of antibiotics in the study area, antibiotics prescribed to the residents by the health care center of the area for an eight-month period in 2014 were investigated [5].

The results revealed more than 80% of antibiotics consumed by the residents were β-lactams (Table 1). The per capita dose was unknown in this study, but there was a marked bias towards a specific class of antibiotics, the β-lactams.

Table 1. Supply of antibiotics to humans in the study area of Vietnam.

| Type of antibiotics | Times of supply | Percentage |
|---|---|---|
| β-lactum | 3,728 | 87.5% |
| ST-mixture | 244 | 5.7% |
| Aminoglycosides | 108 | 2.5% |
| Quinolone | 104 | 2.4% |
| Tetracycline | 4 | 0.1% |
| Others | 48 | 1.1% |
| Total | 4,260 | 100.0% |

To assess the dosage of antibiotics administered to domestic animals, specifically chickens, the study used data from the rural veterinary drug store to examine antibiotics prescribed in the area for a ten-month period in 2014 [5]. The data from the rural veterinary drug store were obtained via interviews. The types of antibiotics prescribed to chickens were significantly different from those prescribed to humans. The prescription for chickens mostly consisted of colistin combination treatments (Table 2).

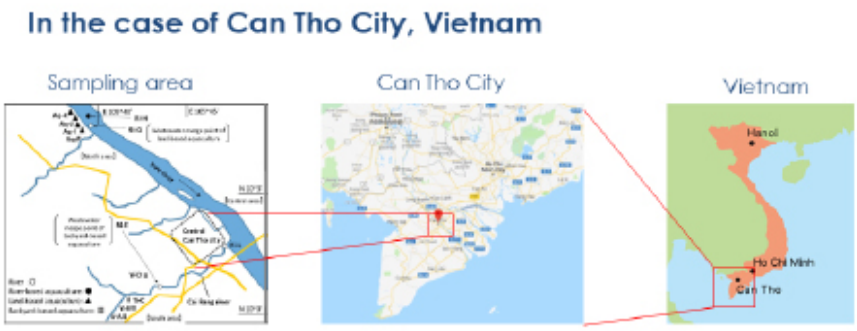
Table 2. Supply of antibiotics to livestock (chickens) in the study area of Vietnam

| Type of antibiotics | Times of supply | Percentage |
|---|---|---|
| Colistin | 289 | 34.5% |
| Colistin+Quinolone | 154 | 18.4% |
| Colistin+Ampicilin | 84 | 10.0% |
| Colistin+Trimetprim | 41 | 4.9% |
| Tylosin+Oxytetracyclin | 4 | 0.5% |
| Tylosin+Gentamycin | 48 | 5.7% |
| Tiamulin hydrogen fumarate | 118 | 14.1% |
| Sulphadimerazine+Diaveridine | 58 | 6.9% |
| Sulphaganidine+Neomycine | 41 | 4.9% |
| Total | 837 | 100.0% |

Colistin, also known as polymyxin E, was discovered by a Japanese scientist in 1949. This antibiotic is one of the few treatment options for intractable infectious diseases caused by MDR Gram-negative bacteria, including carbapenem-resistant bacteria [6]. Colistin is also used in agriculture, particularly in China, since the 1980s. Moreover, it is widely used in the livestock and fishery sectors regularly as a growth promoter, rather than being used as a therapeutic or preventive agent. However, in 2016, China banned the use of colistin for livestock growth promotion.

**3. Residual antibiotics in environmental water**

Environmental routes for AMR dissemination are receiving increased attention. Aquatic environments act as reservoirs or sources of AMR bacteria, antibiotic residues, and AMR genes. Therefore, it is imperative to identify the role of contaminated water on AMR dissemination. To assess the quality of the environmental water in rural areas in Vietnam, the freshwater systems of the Mekong Delta in Can Tho City were chosen as the study site [7]. Twelve water samples in which the residual antibiotics and AMR genes were determined (Fig. 3).




Sampling sites in Can Tho city and its suburbs. Four backyard-based aquaculture sites (V-A, V-B, V-C, and V-D), four land-based aquaculture sites (Aq-F, Aq-I, Aq-J, and Aq-K), and four river sites (Ri-E, Ri-G, Ri-K) including one river-based aqua-culture site (Ri-H).

Figure 3. Residual antibiotics in the environmental water

The study detected 28 residual antibiotics by screening with liquid chromatography with tandem mass spectrometry (LC-MS/MS). As shown in Table 3, some antibiotics (sulfamethoxazole, SMX; trimethoprim, TMP; sulfadimidine, SDD; sulfadiazine, SDZ; and cephalexin, CEX) were detected in all the analyzed water samples, but their concentrations and types varied. Among these, SDD showed a high residual concentration of 260 ng/mL. Only CEX was detected within the β-lactam class of antibiotics in the environmental water, even though β-lactam antibiotics were widely consumed by the residents (Table 1).

Table 3. Levels of residual antibiotics and AMR genes in environmental waters

| Sample site | Residual antibiotics (ng/L) | | | | | AMR genes (relative copy number/ng DNA) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | sulfonamide | | | | β-lactam | Sulfonamide | ESBL | | | Tetracycline |
| | SMX | TMP | SDD | SDZ | CEX | *sul* | *CTX-M* | *SHV* | *TEM* | *tet* |
| Household backyard water (4) | (2/4) 11-47 | (1/4) 2.8 | (2/4) 19-260 | (3/4) 6.6-110 | (2/4) 14-130 | (3/4) 4.4-360 | (3/4) 3.4-1200 | (2/4) 99-320 | (2/4) 7.3-8800 | (2/4) 59-440 |
| River water (4) | (4/4) 42-93 | (4/4) 1.0-2.8 | (1/4) 18 | (2/4) 0.3-2.8 | (1/4) | (4/4) 5.3-2400 | (4/4) 50-2900 | (4/4) 5.6-330 | ((4/4) 66-3900 | (4/4) 1.4-43000 |
| Land-based aquaculture water (4) | (4/4) 68-140 | (4/4) 1.5-3.3 | (4/4) 14-19 | (0/4) | (3/4) 6.0 | (3/4) 2.7-1900 | (3/4) 2.4-910 | ((3/4) 32-42 | ((3/4) 320-890 | ((3/4) 12-57000 |

The numbers in parentheses indicate the ratio of number of samples positive for residual antibiotics or AMR genes to the total number of samples analyzed. A total of 28 antibiotics were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Unlike residual antibiotics, most of the AMR genes detected in the environmental water were β-lactam-resistant genes, such as ESBL-producing genes (blaCTX-M, blaSHV, and blaTEM). The detection of AMR genes indicates the presence of AMR bacteria carrying those genes.

These results showed that at least some antibiotics were present in the aquatic environment, but their concentrations were not high enough to induce emergence of AMR bacteria, except for SDD. Interestingly, high concentrations of SDD and the corresponding sul gene were detected in water samples obtained from backyard-based aquaculture sites. Since sulfa-related drugs are antimicrobial agents that are stable in aqueous environments, these results reflect the physicochemical properties of these antibiotics. In addition, the sites

examined were located next to a pig farm, and pig waste was discharged directly into these sites which may explain the high levels of SDD and sul gene at the study site.

**4. Degradation of antimicrobial agents in the environment [8].**

Low levels of antibiotic residues in the environment have previously been detected, usually in the ng/L to µg/L range [9, 10]. Such low levels do not inhibit bacterial growth, but instead may contribute to the occurrence of AMR bacteria through various mechanisms [11]. Therefore, the aquatic environment is a potential reservoir of AMR bacteria.

Ampicillin (ABPC), belonging to the β-lactam class of antibiotics, is widely used in aquaculture because of its broad-spectrum applicability and low cost, and it is extensively used in Vietnam as well. However, this antibiotic was not detected in any of the environmental water samples tested in this study.

ABPC degrades to 2-hydroxy-3-phenylpyrazine (HPP) and other compounds, as shown in Figure 4. More than half of the ABPC concentrations had degraded in a few days, even under laboratory conditions (Fig. 5).

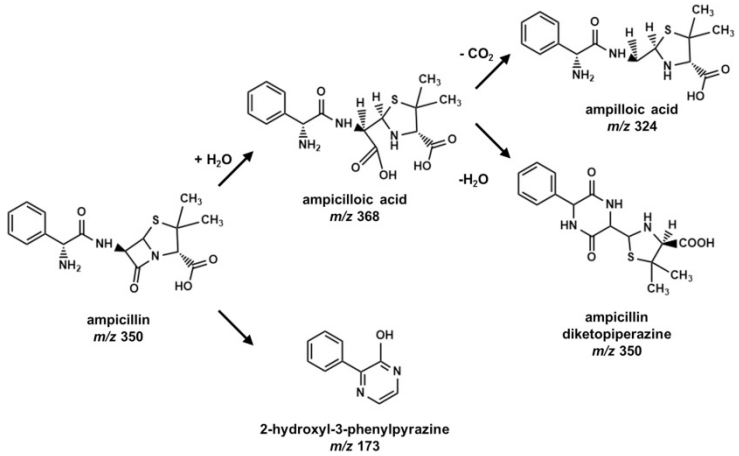



Figure 4. Degradation pathway of ampicillin in aqueous medium.

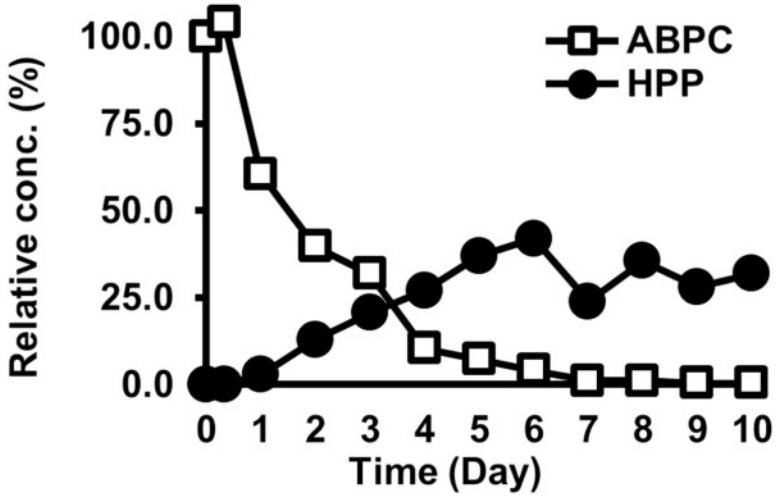



Figure 5. Time course of ampicillin (ABPC) degradation.
HPP (2-hydroxy-3-phenylpyrazine)

The results of this study showed HPP residues were detected in more than half of the environmental water samples collected from the areas examined (Fig. 6), but the concentrations varied among the examined areas. The presence of HPP in environmental water indicates ABPC degradation. Therefore, contamination of the environmental water with ABPC could be presumed, even though ABPC was not detected.

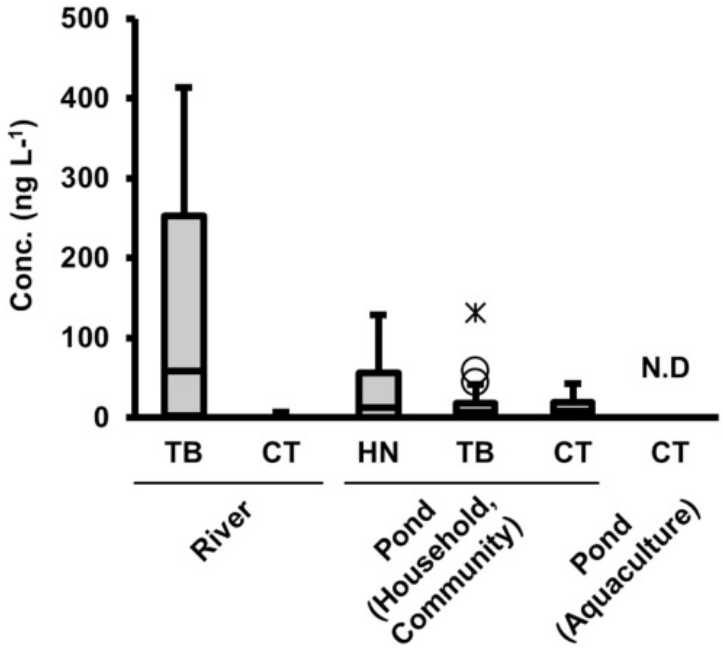



Figure 6. Tukey boxplot of the concentration of HPP residues in environmental samples. TB, Thai Binh; CT, Can Tho; HN, Ha Noi; N.D., not detected.

**5. Dissemination of AMR bacteria**

The dissemination of AMR bacteria in Vietnam was assessed by examining the prevalence of ESBL-producing bacteria in stool samples from the residents, as well as comparing it with the data from other Asian countries, including Japan. As shown in Table 4, more than 60% of the residents in Asian countries, except Japan, are carriers and have ESBL-producing bacteria in their digestive tracts. These ESBL-bacteria are not pathogenic; therefore, they may not cause any immediate health problems, but they pose a significant health risk in the future.

Table 4. Current status of ESBL-producing bacteria in community residents in Asian countries

[a], Nakayama et al., Infect Drug Resis, 8:1-9, 2015.
[b], Luvsansharav, et al., J Antimicro Chemo, 67:1769-1774, 2012.
[c], Luvsansharav, et al., J Infect Chemo, 17:722-725, 2011.

| | Vietnam[a] | Thailand[b] | Laos[a] | Japan[c] |
|---|---|---|---|---|
| Sampling | June, 2013 | Nov., 2010 | Nov., 2012 | 2009-2010 |
| Participants | 198 | 417 | 57 | 218 |
| ESBL positive | 101(51%) | 289 (69.3%) | 41 (71.9%) | 14 (6.4%) |

The transmission of AMR bacteria in the community through food, particularly animal products, is considered one of the most prevalent factors. Therefore, we examined the presence of AMR bacteria in retail meats and shrimps available in local markets of these communities. As shown in Table 5, many of these retail meats and shrimps were contaminated with ESBL-producing Escherichia coli.

Table 5. Status of ESBL-producing Escherichia coli in retail meat and shrimps in a local market in Vietnam

| Food type | Collection site | No of samples tested | No. of ESBL-*E. coli* isolated |
|---|---|---|---|
| Poultry | A | 143 | 84 (58.7%) |
| | B | 60 | 53 (88.3%) |
| | Total | 206 | 137 (66.5%) |
| Pork | A | 147 | 47 (32.0%) |
| | B | 69 | 35 (50.7%) |
| | Total | 216 | 82 (37.9%) |
| Shrimp | A | 60 | 11 (18.3%) |

Samples were collected in (A) 2013 in Nha Trang and (B) 2014 in Thai Binh.
A, Le et al., Foodborne Pathogens and Disease, 12:719–725, 2015.
B, Le et al., Int J Food Contamination, 2:17, 2015.

As colistin is widely used for the prevention of bacterial infections among livestock in Vietnam, we studied the prevalence of colistin-resistant bacteria in community residents. The results revealed a surprisingly high dissemination of colistin-resistant E. coli in the fecal microbiota of the residents of a rural community in Vietnam (Table 6) [12]. Additionally, it became clear most of the isolates carried the mobile colistin resistance gene mcr. On the contrary, in Japan, the dissemination of colistin-resistant E. coli was nil as per the assessment carried out by Yamamoto et al [12]. Similarly, in Europe, only a small percentage of healthy people carry colistin-resistant bacteria.

| No. of households | Range of participants per household | Ave. No. of participants per household | Total No. of participants | Age range (yr) | Median age (yr) | Males |
|---|---|---|---|---|---|---|
| 36 | 1-6 | 2.7 | 98 | 2-81 | 46 | 44 (44.9%) |

| No. of specimens tested (1 per participant) | No. of positive culture specimens on CL-CHROM* | No. of *E. coli* isolates that grew on CL-CHROM | No. of colistin-resistant *E. coli* Isolates MIC, ≧ 8 μg/mL | No. of *mcr* (+) *E. coli* isolates | *mcr* status | | |
|---|---|---|---|---|---|---|---|
| | | | | | *mcr-1* | *mcr-3* | *mcr-1/3* |
| 98 | 88 (89.8%) | 83 (84.7%) | 70 (71.4%) | 69 | 65 | 3 | 1 |

Table 6. Detection of colistin-resistant *E. coli* in residents of the study area
*CL-CHROM, CHROMagar® COL-APSE;MIC, minimal inhibitory concentration.

## 6. Conclusion

The dissemination of AMR bacteria in communities in developing countries is a serious public health threat. This epidemic is observed not only in humans, but also in livestock (and subsequently, retail meat) and freshwater systems, such as household backyards, rivers, and land-based aquaculture waters. Considering the significance of environmental water in the livelihood of a community, its role in the emergence of AMR bacteria and as a reservoir of AMR genes is significant.

Therefore, appropriate regulations on antibiotic use and their enforcement are required. Monitoring of environmental residual antibiotics and AMR bacteria is also necessary for effective public health measures to inhibit the spread of AMR bacteria. In this regard, the development of point-of-care tests for the detection of AMR bacteria and residual antibiotics in the environment, including water, is a necessity.

**Acknowledgement**

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# Presence of Antibiotics in Water Resources and the Developing Bacterial Resistance in Humans

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**Abstract**

Emerging contaminants present in water resources are causing complex impacts on human health and aquatic ecosystems. Input of antibiotics into water resources has created special risks due to evolving bacterial resistance bringing a reduction in their therapeutic potencial. The main contamination sources identified in studies are pharmaceutical industries, animal husbandry facilities, acuaculture, and domestic wastewaters. Wastewater treatment plants act as a special reservoir for antibiotics and antibiotic-resistant genes (ARGs), as traditional treatment processes are deficient in removing these contaminants. Rivers and lakes have different hydrological characteristics which affect the accumulation of antibiotics in water and sediments. The global contamination of antibiotics and antibiotic-resistant genes warrants more research for an improved regulation system to control these emerging contaminants.

Introduction

Globally there is growing concern for the availability and security of future human water consumption. Similarly, there are worries about the sustainability of ecosystems and their ability to supply vital industries (e.g., domestic, agriculture, industry, among others) (Singh,R., 2019). The global decline in water quality "is strongly also affecting the availability of water" (IANAS, 2021) as well as water contamination (microbiology, chemical and pharmaceutical contaminants), drought phenomena and climate changes in seasonal cycles. Water quality protection involves complex measures such as integrated management of watersheds to regulate contaminants flowing into water resources from different source sites. As one group has noted:

> *"This is especially true today as new and previously unknown contaminants emerge, often before their impacts on water and its uses can be adequately evaluated. Moreover, it is now understood the protection of ecosystems and the production of environmental services require careful management of water quality". (IANAS-IAP, 2021).*

Historically, the development of water quality problems began in the 19th century, but was largely limited to fecal waste, organic pollution, and nontreated sewage. Between 1940 and 2000 water quality problems became considerably more complicated due to the increase of processes involving metals, eutrophication with potential for development of cyanotoxins, nitrates, lixiviates from solid waste deposition, salinization, and acidification of groundwater (Vammen, K., 2021). Today, there are new types of problems involving emerging pollutants. The increase in global use of antibiotics in recent decades, are causing impacts on the quality of water resources. Van Boeckel et al. (2015) reported that their use increased by 35% in the first decade of the current century. This growth in use and expanded distribution in water resources has promoted the development of antibiotic resistance, now resulting in a major problem for human health. Antibiotic-resistant pathogens are emerging and distributed in human and animal populations worldwide. In the future, the problem is expected to be even more severe if regulation measures are not adapted to align with results of scientific research.

## *Antibiotics in different aquatic environments. Consequences for Human Health and Aquatic Biological Communities.*

Aquatic environments are important ecosystems susceptible to the accumulation of antibiotics. Rivers and lakes have different mechanisms of accumulation according to their hydrologic properties. In rivers, concentrations of contaminants in sediments decrease downstream from the source of pollution. In contrast,

lakes tend to accumulate pollutants according to the specific residence time of the water. In most countries, there is increased research concerning discharge of antibiotics into rivers. For example, in 2019 the World Economic Forum reported on a global study involving 72 countries' river samples, where concentrations of antibiotics exceeding the safe levels up to 300 times were found.(University of York, 2019).

This wide environmental contamination with antibiotics leads to the development and distribution of pathogens with antibiotic resistance causing serious impacts on human health. This can be further showcased with wastewaters which have a high frequency of resistant bacteria and are a breeding medium for the proliferation of resistant bacteria, especially to tetracycline and sulfonamides; thus, reducing the the therapeutic potential of those antibacterial agents (Singh et al., 2019).

There is growing evidence from studies worldwide that antibiotics cause changes in biological communities downstream from pharmaceutical factories, hospitals, wastewater treatment plants, runoff from agriculture, aquaculture installations, and more. Some suggest high concentrations of antibiotics can impact multiple levels of aquatic biological communities (e.g., algae, bacteria, fish embryos, among others). High concentrations of antibiotics can completely eliminate the growth of algae. (Lehman, R., 2018).

Antibiotics have biostatic and biotoxic effects on microbial populations causing the disappearance of key groups related to specific ecological interactions (Sagaseta de Ilurdoz; M. et al, 2022). This can also alter the composition of species in other communities with resulting changes in various levels of the trophic aquatic system such as algae, invertebrates (Daphnia), and vertebrates as embryos of zebrafish (Bielen et al., 2017). Furthermore, the mixture of pharmaceutical products can interact synergically, with certain combinations of antibiotics causing accumulative risk for aquatic ecosystems (Gonzalez-Pleiter et al., 2013; Yang et al., 2018). Certain antibiotics (oxytetracycline and ciprofloxacin) can form chemical complexes with heavy metals (e.g., copper, zinc, and cadmium), becoming increasingly toxic for bacteria such as Vibrio fisheri and algae such as Scenedesmus (Dickenson, A.W. et al., 2019). In water with high concentration of anitbiotics, fish embryos may suffer from development issues and overall survival.

*Presence and Sources of Antibiotic Pollution in Waterbodies*

Antibiotics and resistant bacteria have been found in the water of rivers, lakes, sediment of rivers and lakes, groundwater, and potable water (Martínez, 2008; Schwartz et al., 2003). Their distribution in sediments extend even to the Arctic zone (Tan et al., 2018).
An important source of antibiotics contributing to water was identified from the meat industry, where enormous quantities of antibiotics are used in animal farms and in bio-fertilizers. Importantly, it is estimated that antibiotic consumption in livestock and other meats (e.g., chicken, pigs, etc.) is much larger than in human consumption (Van Boeckel et al., 2014, 2015). Similarly, antibiotic pollution is also observed in aquatic ecosystems immersed in or close to aquaculture farms.

The effluents of wastewater treatment plants are also one of the principal sources of antibiotic contamination. Mixtures of different antibiotics enter wastewater treatment plants from domestic waters with human and animal excretion and later flow into receiving water bodies (measured in concentrations of ng/L) (Singh et al., 2019). Wastewater treatment plants are the main reservoir of resistant bacteria, antibiotic-resistant genes (ARGs), and mobile genetic elements such as plasmids or transposons (Alonso, A. (2001). These are all present in the final effluent and, therefore, potentially increase resistance in bacteria in the aquatic ecosystem of the receiving waterbody. In consequence, inefficient processes of treatment and high discharge of antibiotics lead to impacts on the aquatic ecosystems (Vammen, K. 2021). These sources and the process of flow into water bodies and their consequences for human, animal, and environmental health risk are illustrated in Figure 1.

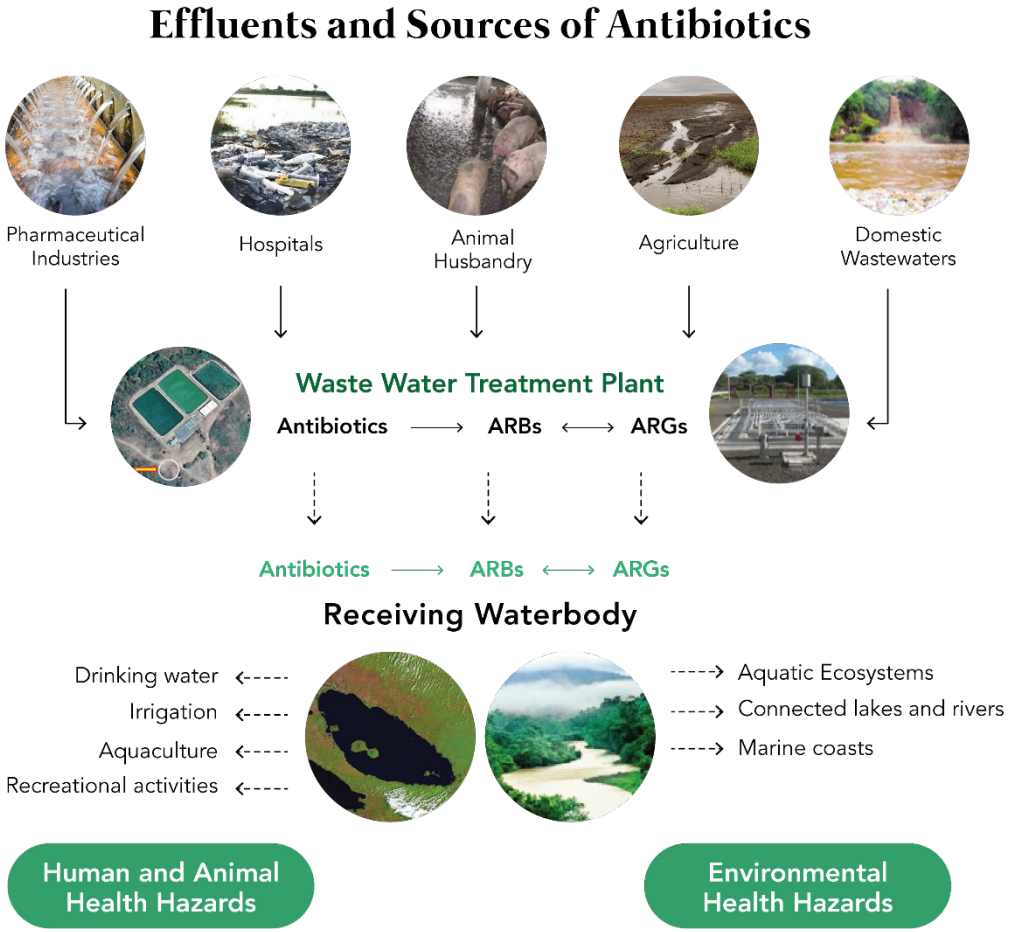


Figure 1. Sources and flow of antibiotics into water resources. (Elaborated by Ana Sofía Montenegro Salvatierra)

Conventional wastewater treatment and water purification processes are not effective in removing most emerging pollutants (UNESCO, 2017). In a review of studies on the elimination of antibiotics from wastewater treatment processes, Sagaseta de Ilurdoz, et al. (2022) found the most adequate methods are mostly biological methods such as: biological aerated filters, anaerobic digestors, biological activated carbon filters, and membrane technologies (e.g., Nanofiltration and Reverse Osmosis). Chemical treatments such as coagulation-flocculation and constructed wetlands brought under 80% removal results.

*Examples of Studies on the Distribution of Antibiotics and Resistant Bacteria in River Systems and Waste Water Plants Worldwide.*

In the last decade, researchers explored the source of this widespread distribution of antibiotics. Some examples of the findings are identified below:

- In *South Africa,* the degradation of river water quality due to antibiotics and resistant bacteria was found in urban effluents of waster waters. (Sibanda et al, 2015).
- In *Europe*, quantities of antibiotics and antibiotic-resistant genes (ARGs) were significantly higher in downstream waters from waste water treatment plants and from sources of agriculture and animal farms (Jechalke et al., 2014).
- In *North America*, one example is the River Poudre in Colorado where ARGs were found due to urban centers and agricultural areas (Zeba, 2005).
- In *Latin America and Caribbean,* the river La Paz in Bolivia receives urban runoff from the Andes where large areas under irrigation with river water are contaminated with multiple enteropathogenic bacteria resistant to antibiotics (Poma, V. et al 2016). In Cuba in Río Almendarias, antibiotics were found due to the presence of animal farms (Mocktar et al, 2009).
- In *Central America*, the Center for the Investigation of Environmental Contamination of University of Costa Rica (Centro de Investigación en Contaminación Ambiental-CICA) carried out studies in recent years on the presence of emerging contaminants including antibiotics from urban wastes

and areas with animal husbandry and swine farms (Ramirez-Morales 2020, 2021). Rivers with a strong urban influence from San José were studied and the antibiotics: cephalexin (25.9% detection frequency), ciprofloxacin (18.5%), ofloxacin (7,4%), azithromycin (3.7%), clarithromycin (3.7%) and trimethoprim (3.7%) were found (Ramirez-Morales, D., 2021).

- In *South China,* a study of four sewage treatment plants in the Pearl River Delta found most antibiotics were not removed and were detected in plant effluents. Antibiotics detected included ofloxacin, norfloxacin, roxithromycin, erythromycin and sulfamethoxazole (Xu.W., et al, 2007).

Lake Systems and Contamination with Antibiotics and Antibiotic-Resistant Genes (ARGs)

Lakes are the principal source of drinking water worldwide (McConnell and Abel, 2013) and are considered very susceptible to the accumulation of contaminants. Since lakes have high retention times, pollutants circulate for longer periods in the water and through different ecosystem compartments (Yang, Y., et al. 2018); therefore, leading to increased risks for aquatic biota and human health. More studies are needed to understand better the destiny of antibiotics and antibiotic-resistant genes (ARGs). As mentioned by Bengtsson-Palme and Larsson (2015) "antibiotics can accumulate in food webs and even more alarming, antibiotic-resistant genes (ARGs) can be transferred between environmental bacteria and human pathogens". Antibiotics detected in lakes are classified mainly into the following categories: sulfonamides, tetracyclines, quinolones, macrolides, lincosamides, and others (e.g.,β-lactam, quinoxalines, and polyether and amphenicol antibiotics). The detection frequency may be related to the degradation and adsorption mechanisms of antibiotics in lake water and sediments. For example, tetracyclines, fluoroquinolones, and macrolides have strong adsorption on particles in suspension and sediments (Huang et al., 2011; Kümmerer, 2009a,b).

Antibiotics and the development of resistance

The US Centers for Disease Control (CDC, 2019) created a comparison between the year an antibiotic was released after approval (initiating with Penicillin) and the year in which resistance was detected (Table 1).

Table 1. Examples of Development of Antibiotics and Year of Detected Resistance (Data Source: CDC, 2019)

| Antibiotic Released for use | | Resistance Microorganism Identified | Year |
|---|---|---|---|
| Penicillin | 1941 | Penicillin-resistant *Staphylococcus aureus*<br>Penicillin-resistant *Streptococcus pneumoniae*<br>Penicillanase-producing *Neisseria gonorrhoeae* | 1942<br>1967<br>1976 |
| Vancomycin | 1958 | Plasmid-mediated vancomycin-resistant *Enterococcus faecium*<br>Vancomycin-resistant *Staphylococcus aureus* | 1988<br>2002 |
| Amphoterizin B | 1959 | Amphotericin B-resistant *Candida auris* | 2016 |
| Fluconazole | 1990 | Fluconazole-Resistant *Candida* | 1988 |
| Caspofungin | 2001 | Caspofungin-Resistant *Candida* | 2004 |
| Daptomycin | 2003 | Daptomycin-Resistant methicillin-resistant *Staphylococcus aureus* | 2004 |
| Ceftazidime-avibactam | 2015 | Ceftazidime-avibactam-resistant KPC-producing *Klebsiella pneumoniae* | 2015 |

The above Table shows that the rate of development of new antibiotics in the last decades has slowed but the rate of the development of resistance after their release has increased. This could be explained by their wider distribution of water resources worldwide or better detection methods.

*Urgent Need for Information for the Development of Regulation for Antibiotics in Water Resources.*

It is still not completely clear how the evolution of antibiotic resistance functions but it is urgent to solve the massive entrance of antibiotics into water bodies which results precisely in their growing resistance

and therefore a high risk for human health. Moreover, studies involving monitoring and control of sources are needed in all continents to develop information systems with data on their distribution worldwide. As observed, the main source of this problem is waste production. For this reason it is fundamental to place more attention to the content of all wastes – liquid, solid, and their environmental paths. The effects of antibiotics in the different ecosystem compartments in water resources should also be a research priority. It is imperative to develop and apply pretreatment of wastewaters directly from hospitals, animal farms, aquaculture installations, and other sources before their release into the municipal sewage system. There is a need to develop technologies for improved efficiency in the removal of antibiotics and antibiotic-resistant genes in wastewater treatment plants and also in water purification processes especially considering the reduction in the therapeutic potential of key antibiotics for human health. This process involves strengthening scientific, technical, and policy capabilities to manage antibiotics and all emerging pollutants, thus, reducing impact on human health and environmental risks. The process of regulation means assuring "water quality and wastewater management, including safe reuse of wastewater and enhanced water and food security" (UNESCO, 2017). It is pertinent to remind and conscientize medical and pharmaceutical staff about the antibiotics' environmental impact and the emerging resistance along with the need to improve their rational use.

In conclusion, further research will aid in developing better regulation of effluents in pharmaceutical industries, hospitals, and lifestock industry. Regulations should target all sources including animal and aquaculture farms and wastewater treatment plants. In the case of antibiotics, it is imperative to have more information on distribution and toxicity levels which will serve as the basis for updating specific water and wastewater quality standards. Such regulation should establish limits or safe levels of antibiotics and resistant bacteria allowed to be released into water bodies. This regulation system is aimed toward protecting public health, water quality, and ecosystems.

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# Water-based pathogens and diseases

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# *Water-based diseases transmitted by Copepods*

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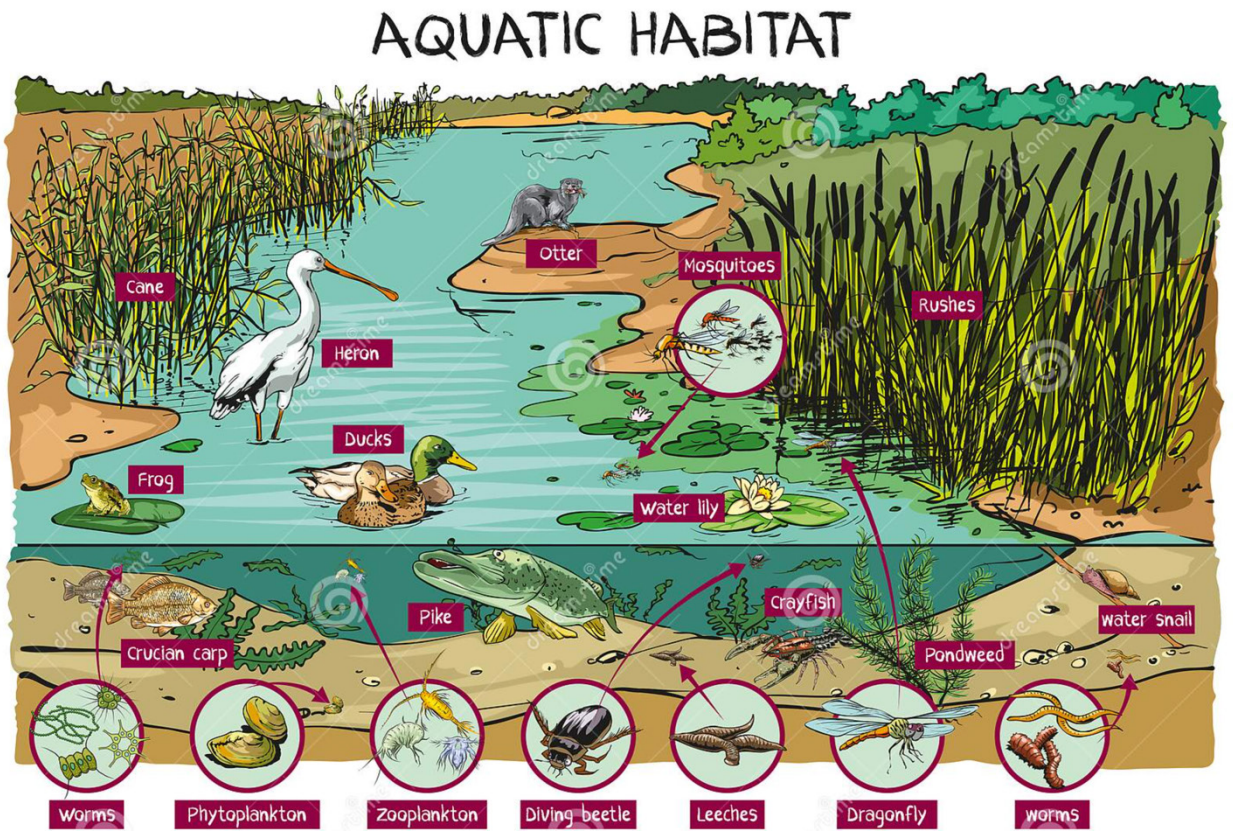
**Abstract**

Copepods are dominant multicellular animal components of the world's freshwater and marine ecosystems. They are sensitive indicators of local and global climate change, key aquatic ecosystem service providers, parasites, and predators of aquatic animals, as well as potential vectors of water related diseases. They can serve as vectors for viral, bacterial, and parasitic anthroponotic and zoonotic diseases. Most notably, copepods can be infected with bacteria or parasites capable to induce human diseases such as Vibrio spp. (e.g., V cholerae which causes Cholera) or Dracunculus spp. (e.g., Dracunculus medinensis which causes Guinea Worm), to name a few. Most of the Vibrio spp. are nonpathogenic and can develop a mutualistic-symbiotic relationship with aquatic flora and fauna such as algae, fished, shrimps, corals, crustaceans, and even zooplankton such as copepods. Water-based diseases related with Copepods spp. are incapacitating and devastating maladies not yet well studied and lack multidisciplinary approach interventions. This chapter provides an overview of one of the most relevant human diseases associated with Copepods – Cholera. As well as an update on copepods and their relevance with Guinea Worm eradication efforts.

Human diseases related to water need to be approached from a One Health perspective in which physicians, veterinarians, and environmentalists collaborate in a multidisciplinary endeavor to combat these maladies. Freshwater and marine aquatic environments can be complex ecosystems with exceptional interaction [Picture 1]




https://www.dreamstime.com/aquatic-habitat-pond-living-world-vector-cartoon-illustration-infographic-image237403832

Picture 1: Freshwater Aquatic habitat (Bron et al., 2011)

Copepods are dominant multicellular animal components of the world's freshwater and marine ecosystems (Bass et al., 2021). They are also sensitive indicators of local and global climate change, key aquatic ecosystem service providers, parasites, and predators of aquatic animals, as well as potential vectors of water related diseases (Bron et al., 2011). Copepods can be vectors for viruses, bacteria, and parasites (Bass, 2021); however, their association with human diseases is not well established and will be the focus of this chapter.

**Copepods as vectors of viral diseases**

Among animals, Copepods are reported to be involved in the transmission of viruses to marine fauna. For example, Argulid branchiurans may transmit spring viremia of carp virus as well as carp pox. While lymphocystis virus may possibly be transmitted to the dermis of its fish hosts by copepods and to the fish visceral organs by a Cymothoid isopod (Overstreet et al., 2009). Furthermore, copepods are implicated in transmitting infectious hematopoietic necrosis, infectious salmon anemia, and infectious pancreatic necrosis of the salmon (Oidtmann et al., 2018).

**Copepods as vectors of bacterial diseases: Cholera example in a Latin American context**

Most of Vibrio spp. are nonpathogenic and can develop a mutualistic-symbiotic relationship with aquatic flora and fauna such as algae, fished, shrimps, corals, crustaceans and even zooplankton such as copepods. Nevertheless, some of them can cause detrimental bacterial infection in fauna and are responsible for human disease. In humans, about a dozen Vibrio spp. are pathogenic (Vibrio cholera, Vibrio parahaemolyticus, and Vibrio vulnificus), causing human illnesses (Sampaio et al., 2022). Vibrio cholera, the pathogen responsible for Cholera, is a free-living aquatic bacterium and one of the main water-borne microorganisms causing disease in humans (Poirier et al., 2012). It can interact with and infect a variety of organisms, from amoeba to humans, including insects, crustaceans, and fish. This free-living aquatic bacterium can adapt to fresh, brackish, aquaculture, and marine waters. The ecology of this aquatic microorganism is complex showcasing its high genetic and phenotypic adaptability to the environment (Escudero & Mazel, 2017). Its environmental adaptability, the ever-changing global environment, and humans ecologically harmful behaviors, are helping V. cholera become once-again a challenge for human, animal, and environmental health (Sampaio et al., 2022). Copepods is regarded as a natural reservoir for V. cholera, but it is essential to explore copepods not only as reservoirs but as potential vectors for diseases such as Cholera.

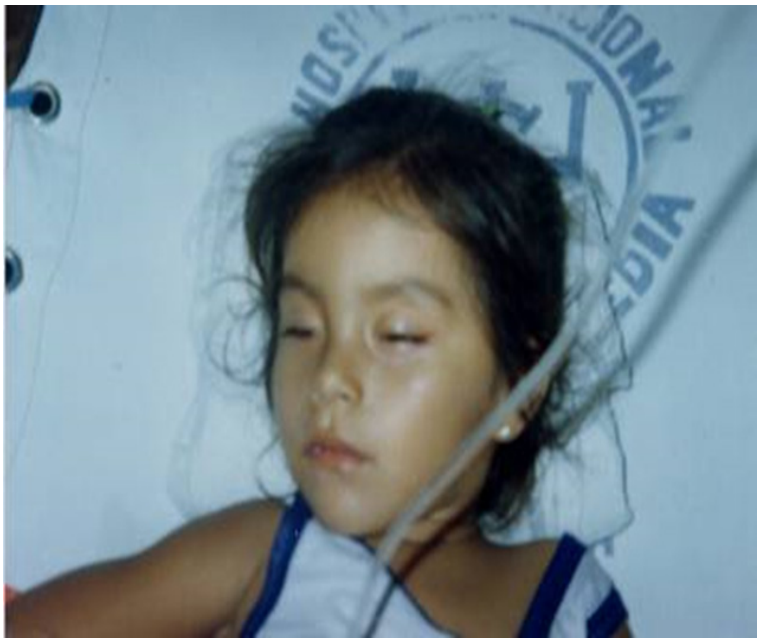
History of Cholera

The term cholera has ancient origins and is derived from the Greek words meaning "flow of bile". John Snow's epidemiological work during the London cholera epidemic of 1849 serves as the cornerstone of modern epidemiology. While Snow's work focused on stopping the cholera outbreak, the pathogen causing the illness was not identified until 1854 (Lippi & Gotuzzo, 2014), when Filippo Pacini coined the name Vibrio cholera when he observed the curved bacilliform from cholera patients (Carboni, 2021). In 1883, Robert Koch made the same discovery, confirming the presence of the vibrio in the intestines of corps during the autopsies and concluded it was linked to contaminated water supplies. . (LIPPI et al., 2016).

Cholera, now mostly endemic to certain regions of the world, had historically encompassed the globe in a series of pandemics. The cholera pandemics wreaked havoc on populations, with a case-fatality rate of 50%. Interestingly, with the invention of Oral Rehydration Salt (ORS) treatments, a treatment not targeting the pathogen itself but aimed at rehydrating the patient, cholera's case-fatality rate considerably dropped. However, its presence is still highly felt with recent outbreaks. The "Seventh pandemic" began in 1961 in the Celebes islands and spreading into Asia, Europe, and Africa by plains, vessels, ships, cars, horses, camels, etc. In January 1991 it arrived at the coast of Peru triggering explosive outbreaks in South and Central America. In October 2010 Haiti reported the presence of cholera in Hispaniola. More recently, prolonged cholera outbreaks have been reported in Angola, Ethiopia, Somalia, Sudan, and Vietnam (Mengel et al., 2014; Nguyen et al., 2017).

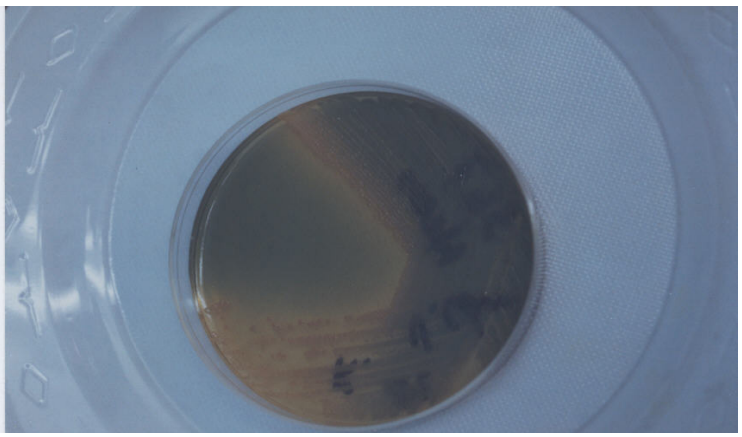
Clinical presentation

V. cholerae O1 and O139 cause clinical disease by secreting an enterotoxin that promotes secretion of fluids and electrolytes by the small intestine. When water is the vehicle of transmission, the infectious dose is $10^3$ to $10^6$ bacterium. The incubation period may vary from a few hours to five days (Baker-Austin et al., 2018). In moderate and severe cases, the patient looks anxious or sometimes obtunded, the eyes are sunken, the mucous membranes are dry, and the skin losses elasticity [Picture 4]. Blood tests reflect an isotonic dehydration. Severe cases may enter in an electrolyte imbalance. Acute renal failure is the most severe complication (Baker-Austin et al., 2018).



Picture 4: Severe case of cholera disease (Picture Dr Izurieta archives)

Clinical diagnosis is enough to start the rehydration treatment in cases of human cholera. Confirmation of cases can be done using dark field microscopy and culture in Thiosulfate-citrate-bile-salt-sucrose agar (TCBS) [Picture 5]. Confirmation is made with antisera O1 or O139. For the transportation of samples from the field to the laboratory it is advised to use alkaline peptone water. The rapid field test Sensitive Membrane Antigen Rapid Test (SMART) (produced in the District of Columbia, USA by New Horizons) can be used for quick diagnosis (Izurieta, 2006).



Picture 5: Culture of *V. cholera* in thiosulphate citrate bile salt sucrose (TCBS) (Picture Dr Izurieta archives).

As any other diarrheal disease, Cholera cases should be immediately placed under oral rehydration therapy (ORT), where between 90-95% of cases can be managed successfully. In severe dehydrated cases, intravenous rehydration with electrolytes should be administered. Since Cholera is a toxin mediated gastroenteric infection, antibiotics such as ciprofloxacin, tetracycline, azithromycin, and doxycycline can be used mainly to prevent transmission and replication of the vibrio (Carpenter, 1992). Vitamin A and Zinc are two key micronutrients to restore the gastrointestinal mucosa microanatomy and function [Picture 6].

Picture 6: Soup of carrots with a high Vitamin A content being administered to patients with acute diarrheal disease. (Picture Dr Izurieta archives)

*Risk factors for cholera disease*

Behavioral practices related to water, sanitation, and hygiene play an important role in disease transmission. An example of behavioral practices increasing the risk of cholera transmission is displayed in the picture below [picture 2]. Traditionally, Ecuadorian native communities (i.e., descendants of the Incas) have a cultural custom dictating a body must remain in the familial home for five days following death to facilitate visitation from family and friends. However, this tradition results in increased exposure of healthy community members to potentially infectious waste and material. Cholera is characterized by effortless diarrhea and post-mortem sphincter relaxation – passive expulsion of colonic contents at the time of death. This expulsion results in soakage of the clothes and sheets covering the deceased. These contaminated articles are likely handled by the family members who are also attending the community visitors. It is common for family members in the home of the deceased to serve visitors food, where they would likely cook and serve food potentially further exposing healthy community members to cholera. Consequently, increased mortality was observed in this native community funerals with a subsequent increase in cholera incidence (Malavade, 2011)

Picture 2: Having lunch with ancestors in the graveyard during the Day of the Dead (Picture Dr Izurieta archives)

Another cultural practice associated with cholera outbreaks is native cuisine. For example, ceviche (i.e., raw marinated sea food) became one of the main vehicles of transmission during the 1991 Latin America cholera epidemic. Similarly, to the practice mentioned in the previous paragraph, other cultural behavioral practices associated with Cholera transmission are burial ceremonies in which food and drinks are shared among wake attendees.

*Socioeconomic and ethnicity factors in the Andean region*

Due to water's importance for survival, most cities and towns in the world are located along water sheds. This is also true in developing and resource-limited countries. Yet, low-resourced countries are the most affected by V. cholera epidemics because of drinking water contamination and lack of knowledge regarding microbiological quality of water meant for human consumption. This was evident from a survey on urban drinking water where 14.7% of the water reservoirs in Burkina Faso had V cholera non-01 and non-0139 present (Kaboré et al., 2018).

During the 1991-1993 cholera pandemic, one of the main foci of dead and disease for the country of Ecuador was the city of Otavalo, home to an ancestral Inca community. For centuries, Pre-Columbian populations have lived on the shores of the Otavalo Lake, located north of the Ecuadorian Andes. The lake provides the community with a source of drinking water, fishing, and farming [Picture 3].

Picture 3: Freshwater and sea water can be environmental reservoirs for many infectious agents, like Vibrio cholerae. (Picture Dr Izurieta archives)

*Potential climatic impacts*

Vibrio spp. are identified as microorganisms impacting human, animal, and environmental health and their impact will be further enhanced by climate change (Vezzulli et al., 2013). Any change in climate and water temperature can theoretically affect the distribution and frequency of Cholera outbreaks since there is already a clear seasonal pattern of transmission. Furthermore, there is a pattern of periodicity associated with El Nino and The Monsoons climatic phenomenon further implying how changes in climate can possibly lead an increase in Cholera.

*Vibrio cholerae in the environment*

As alluded to in the previous section, environmental changes can influence the geographic distribution of Vibrio spp., community composition, predominance of certain species, and even the selection of virulent strains (Di et al., 2017; Jesser & Noble, 2018). More specifically,

> "...*Vibrio cholerae* the causative agent of cholera epidemics represents a paradigm for this process in that this organism evolved from environmental non-pathogenic strains by acquisition of virulence genes. The major virulence factors of V. cholerae, cholera toxin (CT) and toxin coregulated pilus (TCP) are encoded by a lysogenic bacteriophage. ..." (Faruke, 2012)

Figure 7: Phage injecting its genome into bacterial cell phage: Thomas Splettstoesser (www.scistyle.com), https://creativecommons.org/licenses/by-sa/3.0

Phages have also been shown to play a crucial role in modulating seasonal cholera epidemics. Thus, the complex array of natural phenomena driving the evolution of pathogenic V. cholerae includes, among other factors, phages that either participate in horizontal gene transfer or in a bactericidal selection process favoring the emergence of new clones of V. cholerae (Safa et al., 2020) [Figure 7]

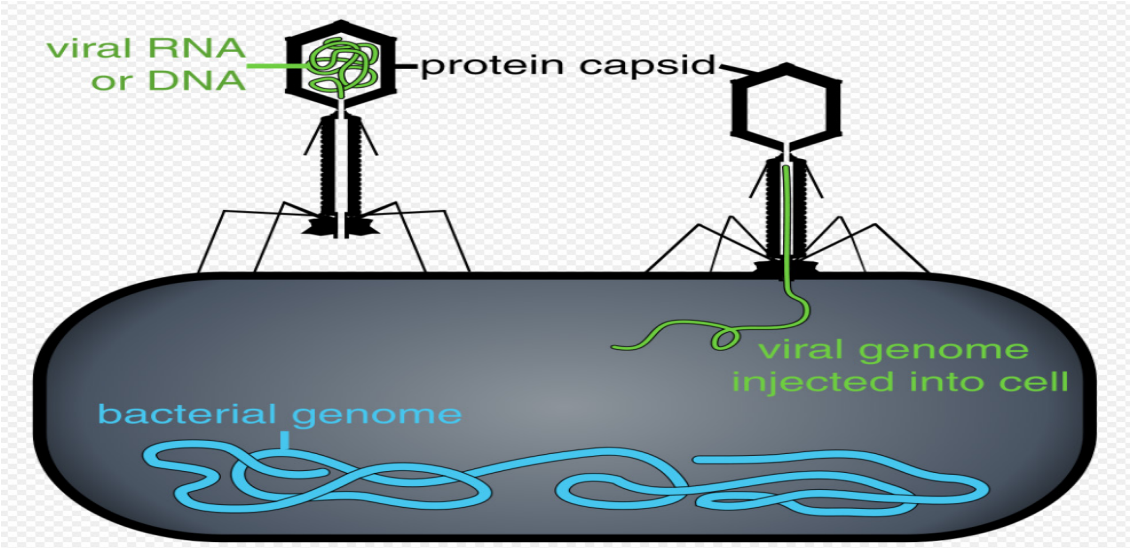
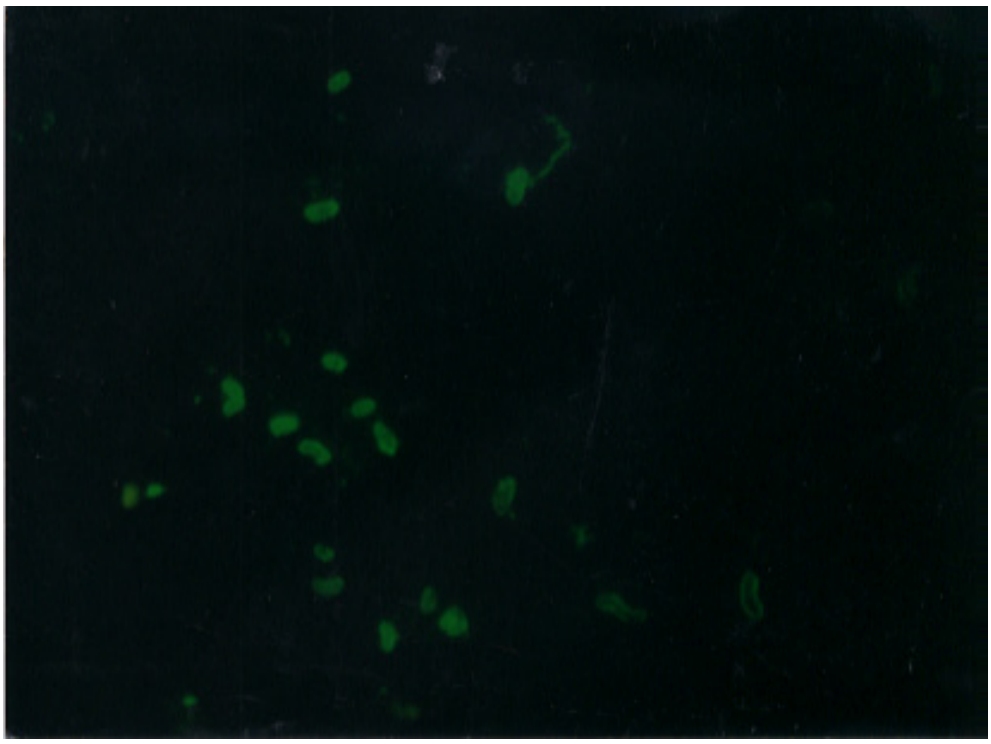



Figure 7: Phage injecting its genome into bacterial cell phage: Thomas Splettstoesser (www.scistyle.com), https://creativecommons.org/licenses/by-sa/3.0

Ecological studies established Vibrio spp. as member of a group of microorganisms necessitating an aquatic ecosystem. Studies on V. cholerae 01 found its survival depends on factors such as association with aquatic plants or animals, specific physiochemical conditions of the environment, and the presence of biofilm communities (Charles & Ryan, 2011; Sakib et al., 2018).

Environmental identification has been a challenge for decades. Clinical techniques for the diagnosis of V. cholerae have been unsuccessful due to the non-culturable pseudo coccus this microorganism can form under hostile environmental conditions. However, new microbiology techniques have been developed to detect V. cholera in environmental samples; these include immunofluorescence [Picture 8], RT-PCR, and sequencing (Martín Forde, 2019).



Picture 8: Immunofluorescence diagnosis of *V cholerae* in water samples (Picture Dr Izurieta archives)

*Cholera epidemiological control*

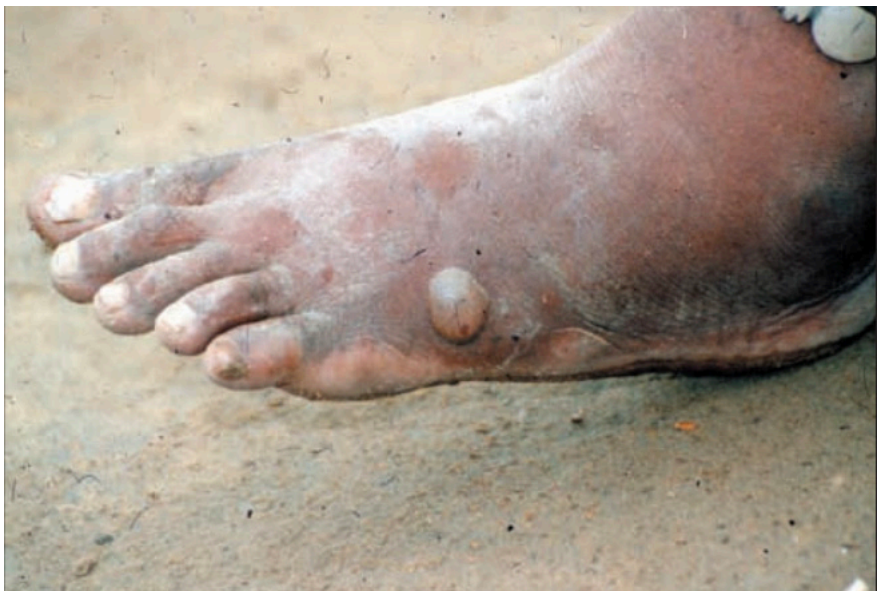
The control of cholera can be approached by the three levels of prevention. In the Primary Prevention and Control level the objective is to avoid people getting cholera through public health education, boiling of water, adding chlorine to water before drinking, avoiding outside food and drink, no feasting after death, and monitoring water sources and supply channels. In the Secondary Prevention and Control level the objective is early detection and treatment. Lastly, in the Tertiary Prevention Control level the objective is to avoid incapacities and deaths (Malavade et al., 2011).

Most recently, new safe and effective oral vaccines are available in developed and developing countries. Three vaccines are currently available: Vaxchora-r: FDA approved 16-18 years old, Dukoral-r: Classical (Inaba and Ogawa) and Sanchol-r: El Tor, killed vaccine plus subunit, two doses. Vaccines should be used as a complementary measure to oral rehydration, water, and sanitation (Wierzba, 2019).

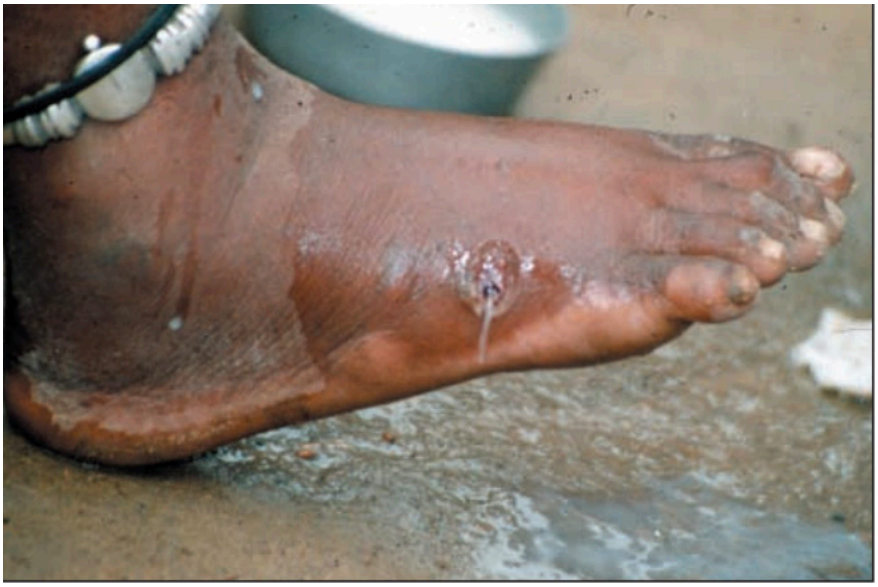
**Copepods as vectors of parasitic diseases**

Copepods play an important role as vectors in the transmission of some human parasitic diseases including Diphyllobothrium latum known as fish tapeworm and Dracunculus medinensis known as the Guinea worm. In the case of a fish tapeworm infection, the ova of Diphyllobothrium latum present in the aquatic environment are ingested by the copepods which are also ingested by fish. When the human host ingests raw or not well-cooked infected fish, the Diphyllobothrium latum ova hatches and the fish taenia develops in the intestine. Later, the eggs of Diphyllobothrium latum tapeworm are released into the environment by the feces of the human host. Limited information exist about the role copepods can play in the cycle of transmission of other water-related parasitic infections (e.g., Amphimerus spp). Yet, besides the human host, other fish-eating animals can play the role as definitive hosts including birds, seals, dogs, and felines (Romero-Alvarez et al., 2020).

Guinea worm or Dracunculiasis is a disease caused by the ingestion of the nematode worm Dracunculus medinensis which, for centuries, has been known as the "fiery serpent". It is usually transmitted to humans by copepods (water fleas: Cyclopidae: Cyclops and Mesocyclops) while drinking from contaminated surface water.



Picture 9: Blister caused by Guinea worm. The Carter Center/S. Fitzgerald (Carter-Center, 2022)



Picture 10: Guinea worm emerging from dermal lesion. The Carter Center/S. Fitzgerald (Carter-Center, 2022).

Historical evidence confirms the presence of Guinea worm in the Americas (Cairncross et al., 2002; Greenaway, 2004; Watts, 2007), where the disease was reported to occur in Cuba, Curacao, Grenada, St. Vincent, and Brazil but died out spontaneously (Cairncross et al., 2002; Greenaway, 2004; Watts, 2007). The World Health Organization (WHO) classified the following countries and territories with a possible history of endemic Dracunculosis: Brazil, Colombia, Cuba, the Dominican Republic, French Guiana, Grenada, Trinidad and Tobago, Jamaica, Guyana, Haiti, Mexico, and Surinam. In December of 1996, Cuba was certified as free of the disease followed by the remaining countries in 1997 and 1998 (WHO, 1999)1998</title><secondary-title>WEEKLY EPIDEMIOLOGICAL RECORD,</secondary-title><tertiary-title>chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://apps.who.int/iris/bitstream/handle/10665/230796/WER7419.PDF?sequence=1&isAllowed=y</tertiary-title></titles><periodical><full-title>WEEKLY EPIDEMIOLOGICAL RECORD,</full-title></periodical><volume>May 14 1999</volume><number>19</number><dates><year>1999</year></dates><publisher>WHO</publisher><urls></urls></record></Cite></EndNote>. The historical evidence of the presence of this parasitic disease in the Americas reinforces the feasibility of Dracunculosis eradication from the world.

In the late 1980s, there were some 3.5 million new cases each year in 20 countries considered endemic. However, by 2016 thanks to the incredible program success executed by the Carter Foundation, eradication seemed to be possible with 16 countries certified as free of guinea worm transmission. Four countries are so far unable to achieve the target of zero cases: Chad (8), Ethiopia (1), Mali (2) and South Sudan (4). Globally 54 human cases were reported in 2019, 28 in 2018, and 30 cases in 2017. In 2021 a total of 15 were reported worldwide. In this current year (up to April 30$^{th}$, 2022), just 2 cases have been reported in Chad, the other 5 African countries under close surveillance have reported 0 cases so far (Carter-Center, 2022)

However, a new challenge emerged for the eradication of this malady. Recent field studies reported the Dracunculosis agent may be maintained by dogs, cats, baboons, frogs, and small crustaceans known as copepods (Galán-Puchades, 2020; Garrett et al., 2020; Guagliardo et al., 2020; Wee et al., 2021). Therefore, it is suggested reducing copepod populations might be the most effective control method for this transmission route. There is, therefore, the possibility that fish, and particularly small fingerlings are acting as paratenic or transport hosts, whereby humans and dogs could acquire infection via consuming fish (Molyneux et al., 2020).

The global eradication program has been implemented based on the provision of safe water by the filtration of contaminated water and the construction and maintenance of boreholes equipped with pumps, as well as education to the communities (Aikhomu, 2000). The near eradication achievements of the Guinea worm program strategy are due to a combination of public health measures including safe water and case detection

and containment, regardless of any drug, vaccine or pre-patent live diagnostic (Boyce et al., 2020).

## Conclusion

Water-based diseases related with Copepods spp. continue being incapacitating and devastating maladies which have not been studied and intervened from a multidisciplinary One Health approach. Cholera is one of the worldwide maladies that demonstrated the dynamic interaction of the human host, the intermediate animal hosts and the aquatic environment in the reemergence of this pathogen. Great and encouraging achievements have been reported with the global eradication program of Guinea worm disease. It needs to be recognized that these eradication achievements are a combination of public health two control measures, safe water and case detection and containment, but further innovation in control measures are required regardless of drug, vaccine or prepatent live diagnostic. ,

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# Water-related vector-borne pathogens and diseases

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# *Carrion's Disease and the Re-emergence of Bartonella Species: through a climate change lens*

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**ABSTRACT**
The new Bartonella species are emerging zoonotic diseases and among them is Carrion disease, a disease caused by the blood-borne bacteria Bartonella bacilliformis. Historically, this disease is endemic in Peru, Colombia, and Ecuador. Since the 1980s it has resurged in Peru, in both endemic and new areas of the inter-Andean valleys and Amazon rainforest. This resurgence is mainly due to constant climatic changes caused by the El Niño phenomenon. However, intervention measures managed to control this disease in recent years. This bacterial disease is one of the few bacterial diseases transmitted by vectors of the Lutzomyia genus.

**Keywords:** Bartonella, Bartonella Infections, El Niño Phenomenon, Climate Change.

## INTRODUCTION

Global warming due to the increasing greenhouse effect over the last 50 years, has generated notable changes in the world's climate. This is mostly evident by the emergence of various phenomena such as record-breaking number of yearly hurricanes and typhoons. More specifically, the world is experiencing increased the "El Niño" phenomena, which produces droughts or floods in various regions of the world, which in turn causes a greater increase in vector-borne diseases such as malaria, dengue, zika, Carrion 's disease, leishmaniasis, etc., as well as water-associated diseases like cholera, leptospirosis, and campylobacteriosis (1).

In this review we present Carrion's Disease, historically a disease of Andean countries (Peru, Ecuador and Colombia). Carrion's Disease is produced by Bartonella bacilliformis, one of the few bacteria in the world transmitted by the bite of a vector from the genus Lutzomyia.

The genus Bartonella is currently considered one of the world's emerging diseases (2,3). Carrion 's disease, an emblematic disease within Peruvian medicine, also known as Peruvian Wart or Oroya Fever, was first described in 1905 by Alberto Barton (4). In 1914 the second Bartonella agent, B. quintana, was discovered to cause Trench Fever and in the 1990s was classified as an agent of bacillary angiomatosis, a dermal and visceral bleeding disease, described in patients with advanced AIDS (2,3). Later, despite being first described in 1953, it was not until 1993 that cat scratch disease was identified as a disease caused by one of its main agents: Bartonella henselae (5,6). More recently, in 2007 a tourist from the United States visiting Peru was acutely infected with B. Rochalimae – a new species of this pathogen (7). Similarly, in 2013 while studying patients in Peru with dermatological eruptive lesions suggestive of Peruvian wart, another novel species, Bartonella. ancashensis was identified (8). In fact, Bartonella. ancashensis is described as a mutation of Bartonella bacilliforimis.

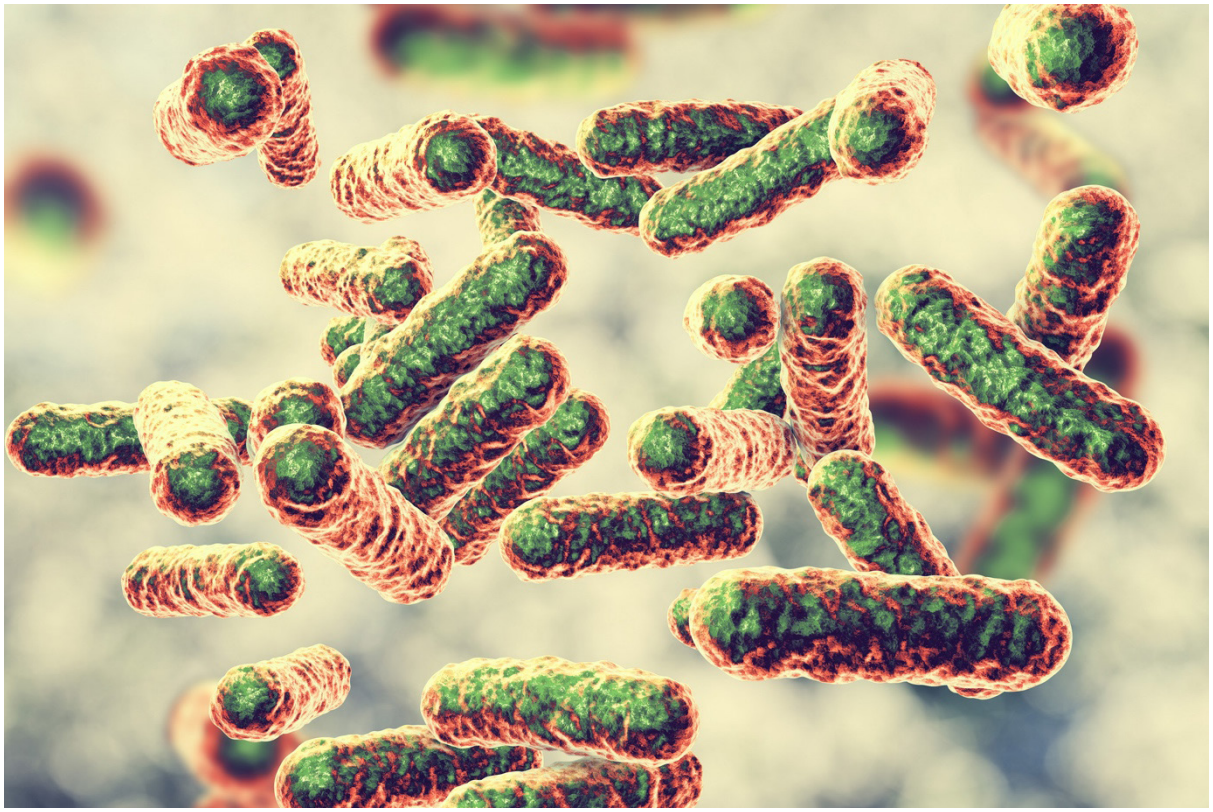

Figure 1 *Bartonella* spp. Bacteria (Dreamstime.com)

**Epidemiology**

Carrion's disease or Peruvian wart is mainly endemic in Peru, Ecuador, and Colombia (9, 10).

In Peru, this disease is transmitted mainly by the bite of Lutzomyia verrucarum female mosquitoes found between 5° and 13° 13' south latitude (11). In recent years, the disease was identified in new geographical areas, coinciding with the appearance of new Lutzomyia species such as L. maranonensis and L. robusta (12,13).

In relation to reservoirs, no wild or domestic animals are identified as reservoirs of endemic areas, but one study found 23% of people with the typical reddish eruptive forms of Peruvian warts had positive cultures and PCR results for Bartonella bacilliformis in their blood, compared to only 0.7% in asymptomatic people (12).

The reappearance of Carrion's disease, with its periodic outbreaks since the 1980s, in endemic and non-endemic areas, is caused by different factors such as population migrations, lack of disease control interventions, and especially climate changes. For example, in 1993 an outbreak of an unknown febrile illness in Cajamarca rainforest region (northern Peru), was reported for the first time among indigenous natives in this Amazonian region (15). Later more cases of patients fell ill with what was then discovered to be Carrion's disease, however, this time in new areas of the Peruvian rainforest (16, 17,18,19,20). Among the most important outbreaks, were those observed in 2001 to 2006 throughout Peru. During these outbreaks the Peruvian Office of Epidemiology reported more than 26,189 new cases between 2004 and 2006 in 16 provinces (20,21). Additionally, as alluded to at the beginning of this chapter, the increase in intensity of the "El Niño" phenomenon (14), as observed in the regions of Ancash and Cusco, Peru, raises concerns over the effects climate change has on future outbreaks.

**Clinical picture and therapy**

Carrion's disease classically has two typical forms, the first is an acute febrile anemia -hemolytic phase and the second is known as the chronic form with only the presence of reddish eruptive lesions throughout the body – known as Peruvian wart. Nevertheless, as with other infectious diseases, most of the affected

are asymptomatic, and depending on the severity, patients can develop mild or moderate to severe illness Typically, acute febrile symptoms are present with fever, headache, malaise, hyporexia, bone and muscle pain, progressive pallor, jaundice, and fatigue. Therefore, the differential diagnosis should include typhoid fever, acute brucellosis, leptospirosis, hepatitis viral, and dengue, among the main differential diagnosis diseases. An important characteristic to note is around weeks 2 and 3 after onset of discomfort the patient may experience various neurological, cardiovascular, and liver complications, among others. Furthermore, some patients present some coinfections by pathogens such as typhoid and nontyphoid salmonella, leptospirosis, malaria, neomocystosis, histoplasmosis, toxoplasmosis, and tuberculosis to known a few, further complicating the clinical evolution and prognosis of the case (22,23,24,25,26,27).

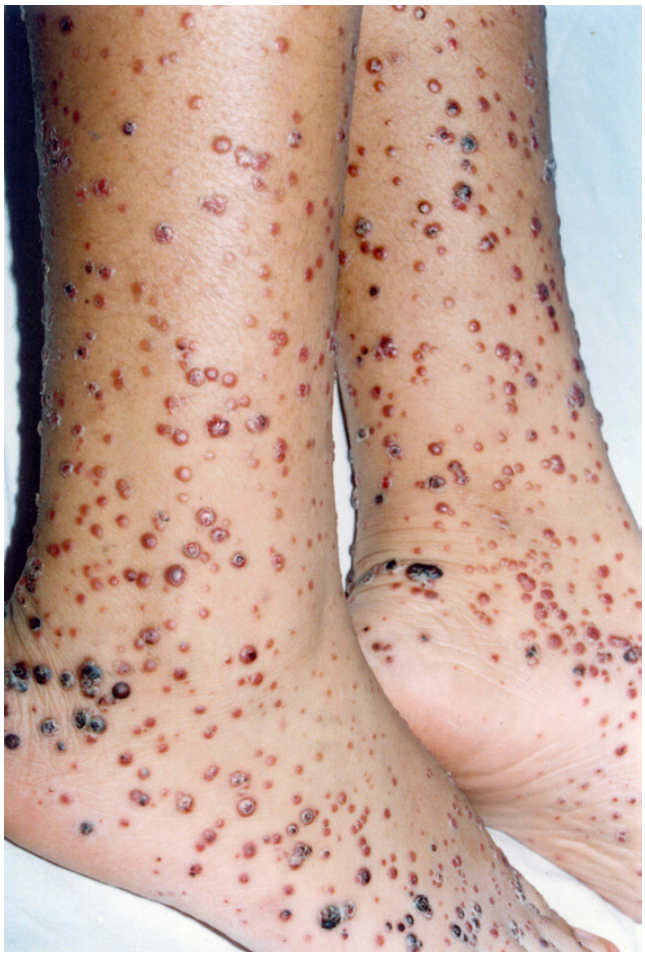

Figure 2: *Bartonella bacilliformis* warts in Carrion's disease patient (Dr Ciro Maguina's collection)

In relation to therapy, for the acute phase, penicillin and chloramphenicol has used successfully in the past; but, since the 1990s ciprofloxacin, amoxicillin plus clavulanate, and azithromycin are used for the eruptive phase (29). Currently, the case fatality rate in the acute phase is around 9%.
Disease control needs integrated interventions including economic investment for vector control, therapy for sick patient and possible reservoirs, improvement of housing, and permanent health education (30,31).

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# *Real time Unmanned Aerial Mapping and Treatment of Vector Mosquito Habitats for Implementing Real Time Macro and Micro Seek and Destroy in an peri-urban agro-ecosystem in Kwale District Kenya.*

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**Abstract**

Apparently, effective malaria vector control is being acutely limited by our abilities to accurately identify and treat breeding sites of Anopheles mosquitoes, the leading malarial transporting species. In Kenya, there are an estimated 3.5 million new clinical cases and 10,700 deaths each year Thirty-four percent of the malaria-endemic areas are reporting insecticide resistance to all four most common insecticides- 89% resistance to at least one insecticide class. We employed, a real-time vector control tool using a patent-pending technology which combined geospatial artificial intelligence (geo-AI), machine learning (ML) and deep learning (DL) algorithms, ecological sensing, and satellite data in an interactive smartphone application (app) to map breeding habitats of Anopheles, in an agro-pastureland village in Kwale County, Kenya. Recent advances in geo-AI technology and ML/DL algorithms (e.g., Randon Forest) have created a paradigm shift for many object-based applications. For instance, ML/DL based approaches have achieved a big leap forward over the previous state-of-the-art in UAV and satellite image classification, segmentation, and recognition. Image classification can involve assigning a class label to a drone sensed image, whereas object localization involves drawing a bounding box around one or more objects [e.g., potential seasonal hyper-productive, georeferenced, Anop heline larval habitat] in an image. In this experiment we combined these two ML/DL tasks in an interactive iOS, smartphone app. Our research employs satellite image/ and UAV video data analog processing based on geo-AI and ML/DL to significantly improve existing image/video processing techniques and applications for hyperspectral signature predictive mapping and treating unknown sentinel site, capture point,An. gambiae s.l., funestus s.s., arabiensis s.s. and merus in an village agro-apstureland ecosystem on th Coast of Kenya. First, we develop the real time tool using geo-AI and ML/DL obect-based recognition algorithms, in conjunction with an iOS smartphone dashboard application (app) for hyperspectral signature, mapping sentinel site, capture point, georeferenced, Anopheles, larval habitats across the entomological, intervention village site. We tested the scale-up of the app from a georeferenced, sentinel site, capture point to the District-level. We then employed, real time, control measures at reducing larval, vector density [Macro Seek & Destroy(S&D)] and blood parasite level [MicroS&D] in malaria treated and suspected intervened population in Kwale District using drone larviciding. We show that local vector control personnel can be trained to treat Anopheles habitats using real time drone larviciding [Macro S&D] at the sub-County pastureland villege level and other personel can be trained to treat suspected patients by implementing [Micro S&D] which can be subsequently scaled up to the District-level. Zero malaria prevalence is expected within eighteen months at the inertervention site.

**Key words:** Malaria, Geospatial, machine learning, deep learning, drones, Seek and Destroy, Kenya.

## 1. Introduction

Flying and biting female, mosquito vectors are responsible for causing the burden of diseases (malaria, dengue, Yellow Fever, etc) to humans, historically, successful mosquito larval habitat elimination programs have stopped transmission by reducing the flying-biting vector arthropod populations located in small well-defined areas in aggregated stages of development restricting their mobility [1]. For example, the optimal ecological situation is only found during the aquatic immature stages of the malaria mosquito Anopheles gambiae s.l. mosquito population, which is in direct contrast to the volumes of large wide-open

areas available and used by flying and biting adults. The operational purpose of any integrated vector management (IVM) program is to eliminate the vector using the most efficacious methods while preserving the natural ecology of the treated area. The control method must be cost effective regarding personnel, equipment, supplies and the amount of insecticide applied. The decision to initiate insecticide applications should be based on the location and identification of the habitat in which they are located. The killing tool of choice is optimal in areas where the targeted vector arthropod populations are the most concentrated in number, with limited ability to evade or escape. They are clustered most in their earliest developmental, aquatic larval stages [2].

Based on investigations of several species of Anopheline mosquito habitats in Cambodia, and East/West Africa, only one square meter of each 640 square meters of aquatic environment produces flying-biting Anopheles species [3]. Again, this is in contrast to flying adults which occupy an unrestricted open or even closed air space landscapes. We have used these known facts to develop a real time IVM program using a Red, Green and Blue (RGB), multispectral, multi-angular camera which allows for creating a fingerprint (i.e., signature) in ArcGIS Pro TM for determining the precise geolocation of vector/nuisance, mosquito, arthropod larvae in their most concentrated stages, in the aquatic habitat. Coupled with this capability, we have developed a means to use this signature to eco-cartographically locate other similar sites using geo-spatial artificial intelligence (geo-AI), machine learning (ML) and deep learning (DL) algorithms. The real-time system essentially has a built-in learning capability to geolocate similar habitat sites as the drone follows its preplanned flight pattern over an area [2].

Combining geo-AI and ML/DL algorithmic classifiers and ArcGIS in an interactive, dashboard configurable, web-friendly, smartphone application (app) can aid in optimally scaling up georeferenced, sentinel site capture point, hyperspectral signatures for predictively mapping unknown, georeferenceable, County-level, vector, mosquito, larval habitat, seasonal, abundance and distribution. For example, by employing real time, unmammed aerial vechile (UAV) or drone retrieved, capture point, sentinel site, wavelength, reflectance datasets of seasonal, imaged, land use land cover (LULC) classified, vector arthropod, larval habitat characteristics [e.g., water situation(turbid or clean, stagnant or running), substrate type, (e.g.,moist or dry) site type (man-made or natural), sunlight situation, site situation (transient or permanent, with or without vegetation) etc.] an interpolatble, Red Green and Blue (RGB), sentinel site, hyperspectral signature may be generated employing geo-AI, ML/DL technologies and ArcGIS infused into an iOS smartphone application (app). Interpolation predicts values for cells in a raster from a limited number of sample data points which used to predict unknown values for any geographic point data [4]. such as a georeferenced, sentinel site, RGB indexed. UAV imaged hyperspectral signature of a seasonal, Anopheline, aquatic, larval habitat. The hyperpectral signature is the variation of reflectance or emittance of a material with respect to wavelengths (i.e., reflectance/emittance as a function of RGB wavelength) [5].

We have created a large library of vector, mosquito larval habitat, seasonal habitat fingerprints (e.g., artificial water container habitats of vector mosquito Dengue Zika, Yellow Fever, etc., Aedes aegypti habitats, West Nile Virus vector Aedes albopictus and Culex quinfasciatus storm sewer breeding site foci, An. arabiensis rice tillers. etc.) which we used to geolocate the precise capture points where larvae are clustered in previously unknown habitats (e.g., man-made ponds, natural ponds, drainage ditches, irrigation canals, and uncultivated swamps). We have also added the appropriate means within a real-time IVM to deliver an environmentally friendly control tactic called, 'Seek and Destroy'; (S&D) where, local sub-County District-level vector control officers and trained village workers, bury UAV imaged, real time, predicted, field verified, small, household habitats; (e.g. man-made ditches, vechile ruts, hoofprints), or use geographic, spatiotemporal (henceforth geo-spatiotemporal) precisely targeted, drone larviciding of a previously unknown large seasonal breeding site (neglected wells, rock quarries, etc.) (i.e., Macro S&D). In 31 days post-Macro S&D intervention, there was zero vector density, indoor, adult, female, An.gambiae s.l.funestus s.l., and arabiensis s.s count as ascertained by pyrethrum spray catch (PSC) at an agro-pastureland, peri-urban intervention site in northern Uganda (Akonyibedo Village in Gulu District) [6]. After a mean average of 62 days, post implementation of Micro S&D, blood parasite levels revealed a mean 0 count in timely diagnosed suspected and treated malaria patients in the Ugandan, entomological, intervention site.

The Micro Seek and Destroy (S&D) component of an IVM intervention may be based on the implementation of a timely diagnosis and treatment of human malaria cases to halt Plasmodium spp. development into its sexual cycle interrupting its transmission from the human reservoir into the Anopheline vector. The hypothesis proposed for the Micro S&D component in this research experiment was that that timely malaria treatment is associated with low population parasitemia and lower malaria incidences in towns and cities. The rationale for this hypothesis is evidenced by the period of potential transmissibility which can be significantly shorter among early treated malaria patients in field studies [7]. In a study carried out by Reina in Esmeraldas, Ecuador, the percentage of patients with gametocytemia at diagnosis was much lower among early treatment patients when compared with late or extremely late treatment patients; therefore, reducing the possibility of parasite transmission in the communities. In Reina's study, early treatment parishes achieved 100% reduction, compared to 67% extremely late parishes and 25% among late treatment parishes [7]. This shows an evident and significant association between early malaria treatment and its incidence rates in the subsequent periods. In another study carried out in Colombia by Arango et al. (2004) [8] it was demonstrated that no patient had gametocytemia when diagnosis and treatment was made within the first 24 to 48 hours post-onset of symptoms.

Based on preliminary data, an added benefit of this unique hyperspectral signature habitat fingerprint system is that, it it may be scaled-up to locate with precision, seasonal hyperproductivem georeferenced, vector arthropod, habitat , capture point from sub-meter resolution satellite data allowing the ability to establish the distribution of all productive unknown, aquatic, vector breeding site, geolocations in a sub-County or District-level area. The expansion of habitat fingerprints over time due to seasonal or climatic changes expands the opportunity for planning the complex logistical requirements for real-time IVM implementation and assessments of transmission risks. Since such a real-time system provides the exact geolocation for mosquitoes, the environment incurs limited use of insecticides resulting in minimal disturbance to the ecology. Financial savings regarding personnel, supplies, equipment and insecticide [i.e.,in this pilot study we utilized liquid Bacillus thuringiensis serotype israelensis (Bti), larvicide products] costs can be expected. Furthermore, the probability of genetic resistance and environmental contamination will be considerably reduced [6].

In Kenya, there are an estimated 3.5 million new clinical cases and 10,700 deaths each year, [9] Thirty-four percent of the malaria-endemic areas are reporting insecticide resistance to all four most common insecticides- 89% resistance to at least one insecticide class [10]. Therefore, our objective in this interventional research was to develop and validate a new tool using geo-AI and ML/DL object-based algorithms operating on sentinel site, larval habitat, capture point, hyperspectral signatures in conjunction with an iOS dashboard app to geolocate and treat unknown active, Anopheline, aquatic, breeding sites in a selected agro-astureland peri-urban village sub-County, intervention site in Kwale district, Kenya [Macro S&D] followed by geo-spatiotemporal scaling-up, to the District level area expanse, across seasons.Secondly, the local, trained, District-level, vector control officers working with Kenya Medical Research Insistute (KEMRI) personel and University of South Florida (USF) collaborators evaluated and confirmed the reduction of malarial blood parasite prevalence and malarial deaths in the treated area [Micro S&D]. In order to determine efficacy of our drone larviciding intervention households were selected for participation in the human landing catches, pyrethrum spray, exit trap collections, and environmental measures [6].

## 2. **Methodoology**

**2.1 Study Site:** The study was conducted in South Coast Kenya in Kwale County. The study area has been previously described .Anopheles funestus s.l. and An. Gambiae s.l. are the main malaria vectors in the area [11]. They occur all year round, with peak season during the rainy season. In the area, 50% of households have universal ITN coverage (≤ 2 persons per ITN). Generally, for the Kenyan Coast an increase in malaria prevalence from 4 to 8% since 2010 has been reported. Sampling was conducted in two villages; Marigiza (Latitude – 4.443036, Longitude 39.461887) and Kidomaya (control village) (Latitude – 4.578639, Longitude 39.157574) which are about 50 km apart representing the Coastal plain and Coastal estuarine habitats, respectively. The two villages have received parallel distribution of LLINs through the National Malaria Control Programme by mass distribution campaigns held in 2006 and 2012.

Kwale county has a monsoon climate; it is hot and dry from January to April, with the coolest months being

June to August. Rainfall is divided into two seasons: short rains from October to December, and long rains from March to June/July. The country's average temperature is 24.2°C. In the coastal low lands, temperature ranges from 26.30°C to 26.60°C; in Shimba Hills, 25.00°C to 26.60°C , and in the hinterland, 24.60°C to 27.50°C. Annual rainfall varies between 400 and 1,680 mm.

### 2.2 Entomological Sampling

Prior to the onset of this study, all households in the intervention, urban and agro-village sites in Kwale, County were enumerated and mapped, which we used to generate a sampling frame for generating seasonal, An. gambiae s.l., funestus s.l., arabiensis s.s. and merus entomology surveys. All households enumerated during the survey was assigned a unique number. A random sample of 120 households were selected in the intervention and control site to generate a list of households to be approached for recruitment into the seasonal entomology surveys. From the list we selected households for participation in the human landing catches, pyrethrum spray, exit trap collections, and for determining environmental measures.

A separate list of random households were selected to generate a list of households to be approached for recruitment into the study being conducted under a separate protocol. Mosquitoes were sampled using miniature CDC light traps (Model 512; John W. Hock Company, Gainesville, Florida, USA) positioned 1m above the floor at the foot end of the bed where a person sleeps under an ITN. Traps were set at 19.00h and collected at 07.00h the following morning by KEMRI-KIlfi and local district vector control officers. If the trap was set up in the intended house, the trap was moved to the nearest similar house after obtaining written informed consent from the head of household or an adult household representative. If the occupant did not spend the night in the selected room or the trap was faulty, the data was excluded from the analysis. The number was determined, and the presence of long lasting impregnated nets (LLINs) was recorded. Each night approximately 12 traps were set for 4 nights in each week in each village. One hundred and twenty cohort study houses in the intervention and control villages were sampled every other week during the study.

Randomly selected houses in the intervention village village was sprayed using an aerosol of non-residual pyrethroids with a piperonyl butoxide synergist each month. These sprays were combined with exit traps placed over the windows of the houses to capture any escaping mosquitoes. In the site, 10 households were randomly selected for the spray collections from the entomology recruitment list generated from the enumeration database in the intervention and control village sites. The same 10 households were sampled one day every 4 weeks. Written informed consent from the head of household or an adult household representative was obtained prior to conducting the pyrethrum spray and exit trap collections.

Collection was taken place between 06.00-08.00h. The number of children and adults who slept in the house the previous night were determined and the presence of LLINs was recorded. White sheets were spread on the floor and over the furniture in the house. Two field workers, one inside the house and one outside, sprayed around the eaves with 0.025% pyrethrum emulsifiable concentrate with 0.1% PBO in kerosene. The vector control officer inside the house then sprayed the roof and walls. The house was closed for 10 minutes, after which the white sheets were brought outside (where there was sufficient light), and dead mosquitoes were collected from the sheets and transferred to the KEMRI–Kilifi laboratories on moist filter papers in Petri dishes for identification and processing. To collect house-leaving mosquitoes, window exit traps were set at 18.00h and collected between 06-07.00h the following morning. Mosquitoes from each trap were put into paper cups separately and transferred to the laboratory for processing. Mosquitoes were provided with sugar solution for 12 hours from the time of collection. Parity dissections were performed on 500 of each species each month at each site.

The entomological, intervention, agro-pastureland peri-urban, village site was surveyed for water bodies each month. Site coordinates were recorded using a Garmin e Trex 10 Worldwide Handheld GPS Navigator. Purposeful sampling was conducted to maximize the collection of the aquatic stages of mosquitoes using a 350-ml dipper (Clarke Mosquito Control Products, Roselle, IL, USA). At each georeferenced, potential, sentinel site, An. gambiae s.l., arabiensis s.s.funetus, and merus larval habitat, 10 dips were made in places likely to harbor mosquito larvae, such as around tufts of polluted submerged vegetation or artificial water containers. In extensive water bodies, dipping was carried out over a 100-m walk. Larvae was classified

either as Anophelines, Culicines or Aedes. Anopheles larvae which was stored in 100% ethanol, which was refreshed on reaching the laboratory. Randomly selected subsamples of Anopheline larvae/pupae selected during the routine mapping of the sentinel site area was subsequently identified by amplification of ribosomal DNA using polymerase chain reaction (PCR).

A molecular approach incorporating both PCR endpoint assay and sequencing of portions of the internal transcribed spacer 2 (ITS2) and cytochrome c oxidase subunit 1 (cox1) loci was used to confirm the identity of the Anopheles. The morphologically identified Anopheles confirmed molecularly was archived in KEMRI-Kilifi. Additionally, any observed Ae aegypti or Cx. quinquefasciatus larvae and pupae at many of the seasonal habitats was archived.

The depth of water of an aquatic, sentinel site, An. gambiae s.l., funestus s.s., arabiensis s.s. and merus larval habitat was measured from different places depending on the size of the habitat using a meter stick, and the average depth will be taken. The distance to the nearest homestead was measured using a tape measure for less than 100m and estimated if more than 100 m. Distances were categorized into four classes: (1) ≤ 100 m, (2) 101 to 200 m, (3) 201 to 300 m, (4) 301 to 400 m. Surface debris, presence of algae and emergent plant coverage was determined based on visual observation. Vegetation cover was visually observed and expressed as open (no vegetation), tree (for the presence of large trees within a range of 10–15 m where shade and foliage could reach), and shrub (woody plants smaller than a tree within 10–15 meters). Anopheline larval habitat perimeters were measured using a tape measure and classified as < 10 m, 10–100 m, and > 100 m. Habitat stability was expressed in terms of the length of time the habitat contained water after the rain. An An. gambiae s.l., funestus s.s., arabiensis s.s. or merus habitat was considered temporary if it held water for 2 weeks or less and permanent if it held water for more than 2 weeks after rain. Though larval sampling was taken on monthly basis, the village intervention study area wasinspected for the presence or absence of rain continuously. Turbidity was be measured by placing water samples in glass test tubes and holding them against a white background, and categorized into three levels: low, medium, and highly turbid. Light intensity was visually categorized as sunlit if the sentinel site,. An. gambiae s.l., funestus s.s., arabiensis s.s. and merus habitat received full sunlight that could occur throughout the day, otherwise the site was described as shaded. The substrate type was categorized as mud, stone if the pool was lined with stones that were large in size (rocks generally larger than 10 cm in diameter) and gravel when the stones weresmall in size but larger than sand. Water temperature was recorded using a water thermometer at the time of collection, and pH was measured using pH indicator paper. Rainfall of the intervention sites during the study period was obtained from the Kenyan National Meteorological Agency. Larval breeding habitats and a number of immature Anopheles mosquitoes sampled will be described using tables.

**2.3 Statistical Analyses:** Correlation analysis was used to investigate the relationship between pH, temperature, and water depth to the An. gambiae s.l., funestus s.s., arabiensis s.s. and merus and other measured, seasonal larval density. Anopheles larval density was determined as the number of larvae (early or late) divided by the number of dips taken from each larval habitat. Larval density was log-transformed log10 (x + 1) to improve the normality of distribution. Multiple regression analysis was used to identify the environmental variables associated with the occurrence of An. gambiae s.l., funestus s.s., arabiensis s.s. and merus. Mann–Whitney U test was used to compare samples with two variables; the presence of algae (presence or absence), habitat permanency (temporary or permanent), surface debris (present or absent), the intensity of light (sunlit or shaded), and water movement (still or flowing). Kruskal–Wallis H test was used to compare samples with more than two groups: water turbidity, water perimeter, distance to the nearest house, canopy cover, emergent plant coverage, habitat type, and substrate type. The Kruskal–Wallis test by ranks, Kruskal–Wallis H test, or one-way ANOVA on ranks is a non-parametric method for testing whether samples originate from the same distribution. It is used for comparing two or more independent samples of equal or different sample sizes [12]. These non-parametric tests were used to compare larval densities from sites with different An. gambiae s.l., funestus s.s., arabiensis s.s. and merus habitat characteristics.

Data was analyzed using IBM SPSS statistical for Windows (IBM corp., Armonk, NY), version 20.0. Values were considered significantly different if $p < 0.05$ for all the tests. A large number of specimens were collected from the different, seasonal, aquatic, larval habitat, sentinel sites, and from the different collection methods. All Anopheles were identified taxonomically to species level so as to identify immatures. To process

the mosquitoes, we implemented a systematic procedure for labeling and recording the specimens, which included the following information: 1) area where the Anopheline samples were collected, 2) house number (which was linked to GIS data), 3) method of collection, 4) date of collection, and 5) the serial number of the specimen. When processing the specimens, labels werebe written in pencil and placed with the relevant specimens in Eppendorf tubes, and similar information was recorded in a register for easy data entry and cross-checking.

### 2.4. **Drone Sampling**

UAV sensing surveys was carried out using a a Parrot Sequoia sensor (Parrot, France) with a Pika NUV2 hyperpsectral imagery collection. The flight plan was programmed with Pix4D Capture app in an iPad Mini 4 (Apple, California, US). We incorporatinged leading Edge technologies Leading Edge Aerial Technologies, Inc. which is located in Port Orange, FL, United States and is part of the Scientific Research and Development Services Industry. Mr Bill Reynolds the CEO of the company was our lead consultant on this project. Mr Reynold has worked on implementing a Macro S & D drone malaria mosquito program in Cambodia with Dr. Jacob **(**"Development of Habitat Signatures for Anopheles Mosquitoes in Cambodia." Agency: Bill & Melinda Gates Foundation Grant # OPP1171887 (6408-1102-00), $874,456, (.50FTE), 6/1/17- 9/30/18 (PI: Novak, Co-PI Jacob)).

For approximately 30 minutes, the drone was flown over the entomological, intervention site using the high end, radio-controlled, and camera-equipped for urban, agrovillage and rural pastureland explorations. The drone was integrated with handheld devices to greatly enhance its capabilities for aerial footage. The multangular drone camera within the kit recorded, stored, and managed the capture point, seasonal, georeferenced, sentinel site, An. gambiae s.l., funestus s.s., arabiensis s.s. and merus signature data in the drone dashboard spectral library. A copy of the sentinel site, Anopheline, larval habitat LULC data, and spectral imagery was stored in the on-flight computer and concurrently transmitted down to the ground stations via WiFi communication in real-time employing the cloud-based, DroneDeployTM platform.

Orthomosaic construction of all the seasonal, UAV sensed, An. gambiae s.l., funestus s.s., arabiensis s.s. and merus larval habitat, sentinel site data were conducted in the app. The photogrammetric processing (e.g., capture point, Anopheline, breeding site, aquatic foci reflectance, surface measurements based on photographs) was conducted in AgiSoft Photoscan Pro (https://www.agisoft.com). The resulting UAV imagery was imported into Photoscan where the data was used to construct an orthomosaic (i.e., georeferenced, overlapped, sentinel site, LULC gridded image with correction for topographic distortions) for the entomological, village intervention site. The position of the drone at the time of image capture for each georeferenced, seasonal, sentinel site, An. gambiae s.l., arabiensis s.s., funestus s,s, and merus larval habitat, photo was recorded automatically by the on-board GPS; hence the orthomosaic will be georeferenced without the need of Ground Control Points (GCPs).

The standard procedure we used photo-alignment (accuracy: highest; generic preselection active, reference preselection active; Keypoint limit: (1) 80,000; adaptive camera model fitting active); (2) dense cloud building (quality: high; depth filtering: aggressive); (3) elevation model (geographic projection using; resolution of 0.1 m and 0.02 m per pixel for acquiring the RGB and multispectral sentinel site signature images respectively; interpolation: extrapolated; all georeferenced, An. gambiae s.l., arabiensis s.s., funestus s,s, and merus larval habitat, capture point, seasonal, LULC classes to generate digital surface model); (4) orthomosaic building (input surface:; blending mode: mosaic; resolution of 0.1 m and 0.02 m per pixel for the multispectral, georeferenced, sentinel site, land cover, UAV, seasonal, images respectively. Once the drone signatures were captured, they were real-time transferred into the ArcGIS-AI Interface Kit™, in the app where a stochastic interpolation algorithm ran the signature over the entire entomological intervention site using commercial high-resolution satellite data (46cm Worldview-2, visible and NIR wavebands) to identify unknown, larval habitat, aquatic foci at the intervention sites. This real-time scaling up RBG hyperspectral sentinel site, signature, seasonal, An. gambiae s.l., funestus s.s aabiensis s.s. and merus larval habitat, grid-stratified, LULC mapping was based on convolutional neural network (CNN) algorithms being applied to

real-time georeferenced, capture point, sample, estimator UAV imaged datasets which included real time GPS indexed, component, sentinel site,video, analog data.

Our approach is based on a R-CNN embedded in an iOS interactive app. Region- based convolutional neural networks (R-CNN) are a family of machine learning models for computer vision and specifically object detection. We successfully merged a region proposal network (RPN) and Fast R-CNN [i.e., a machine learning classifier] within a dashboard, smartphone, interactive app to parsimoniously build on archived, datasets of real time, UAV sensed, georeferenced, capture point, seasonal, aquatic, An,gambiae s.l., funestus s.s., arabiensis s.s. and merus , larval habitat, RGB sentinel site, hyperspectral signatures by classifying, georeferenceable, gridded, LULC capture points (e.g., edges of streams and water puddles on drying streambeds, agropastureland ecosystem, community tap foci etc.) Mask RCNN [13] and the architecture of Faster R-CNN [14] were employed for identifying georeferenceable, seasonal, aquatic, An. gambiae s.l., arabiensis s.s., funestus s,s, and merus, larval habitat, sentinel site, RGB signatures which in this experiment were developed in two stages in the interactive, smartphone app. The first stage consisted of two networks, backbone (ResNet, VGG, Inception, etc.) and RPN. These networks ran once per, UAV sampled, sentinel site, capture point in the app, which subsequently rendered a set of region proposals [i.e., district-level, geolocations in a feature, signature, interpolated, probability map which contained a forecasted, breeding site, larval habitat, positive for Anopheles larvae/pupae and its' associated land cover]. In the second stage, the network in the app predicted bounding boxes and object class for each of the proposed regions obtained in stage1 (see Figure 1].

**Figure 1. Supervised classification of 7 UAV digital surface model gridded, LULC lasses identified in a drone image: open water, emergent aquatic vegetation, agro-pond, trees/ bushes, grass, bare soil, untarmacked roads/paths, and agriculture; the sentinel site**

UAV imaged Anopheline habitat delineated in r font (Jacob & Izurieta, Seek and Destroy Team).

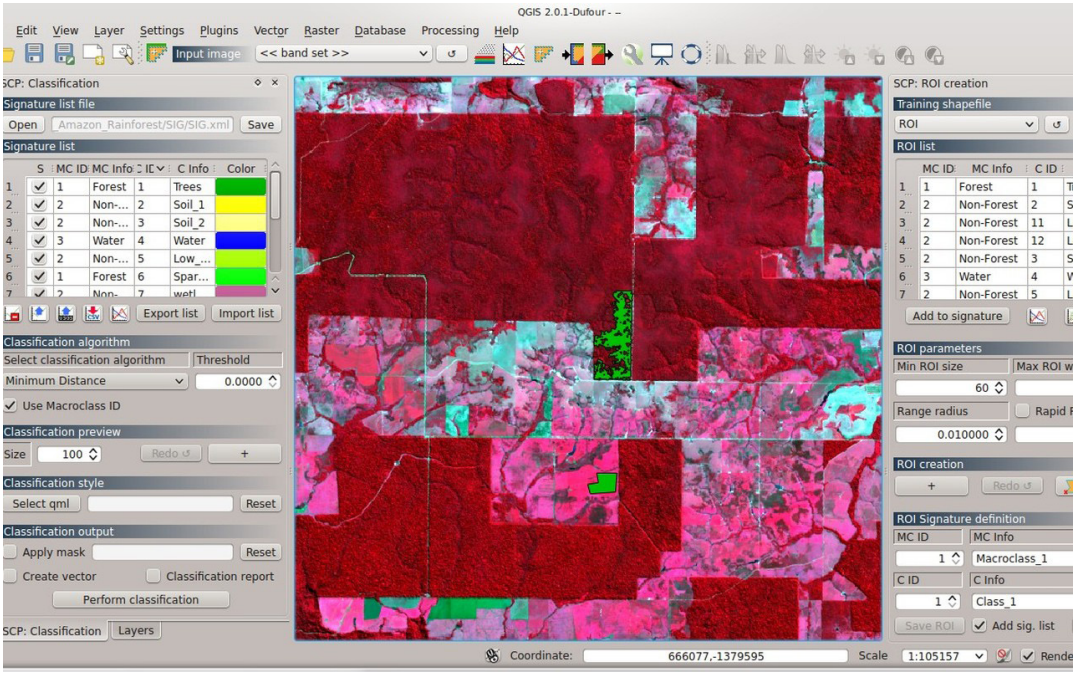
Each proposed gridded LULC region of the intervention site was of different size, whereas fully connected layers in the app network required fixed size vector to make robust predictions [e.g., exact GPS centroid coordinates of the capture point, sentinel site, An. gambiae s.l., arabiensis s.s., funestus s,s, or merus, breeding site, aquatic foci]. The size of these proposed regions was fixed in the app by employing the Region of Interest [RoI] pool method. RoI pooling solved the problem of fixed image size requirement for the sentinel

site, seasonal, object detection networking. Region of interest pooling (also known as RoI pooling) is an operation widely used in object detection tasks using convolutional neural networks [15].

**Figure 2 CNN signature model to detect RoI on the feature Bounding Boxes and object class of commercial road side** An. gambiae s.**l. habitats labelled by GPS coordinates in a Drone dashboard iOS app in Marigiza village (Jacob & Izurieta, Seek and Destroy Team)**

Leveraging USFs research team's expertise, a dashboard app interface and experiences was built employing the Unity game engine software and Vuforia 6 SDK. The Vuforia Area Target Creator application allowed us to easily generate an Area Target using a depth-enabled mobile device [iPads, and iPhones]. Vuforia is an augmented reality software development kit (SDK) for mobile devices that enables the creation of augmented reality applications [16]. This developer used computer vision technology to recognize real time, sentinel site, seasonal images and 3D objects. This image registration capability enabled us to position and orient virtual, An. gambiae s.l., arabiensis s.s., funestus s,s, and merus larval habitat objects, [e.g., canopy gap understory and midstory vegetation, vertical foliage distributions etc.] in relation to the sentinel site, breeding site, seasonal, aquatic foci when they were viewed through the drone camera of a mobile device. The virtual object tracked the position and orientation of the habitat image in real-time so that the viewer's perspective on the object corresponded with the perspective on the georeferenced, mosquito habitat target.

**Figure 3 The interactive smartphone iOS dashboard application with Google reference map of habitat locations based on GPS ground coordinates (Jacob & Izurieta, Seek and Destroy Team)**



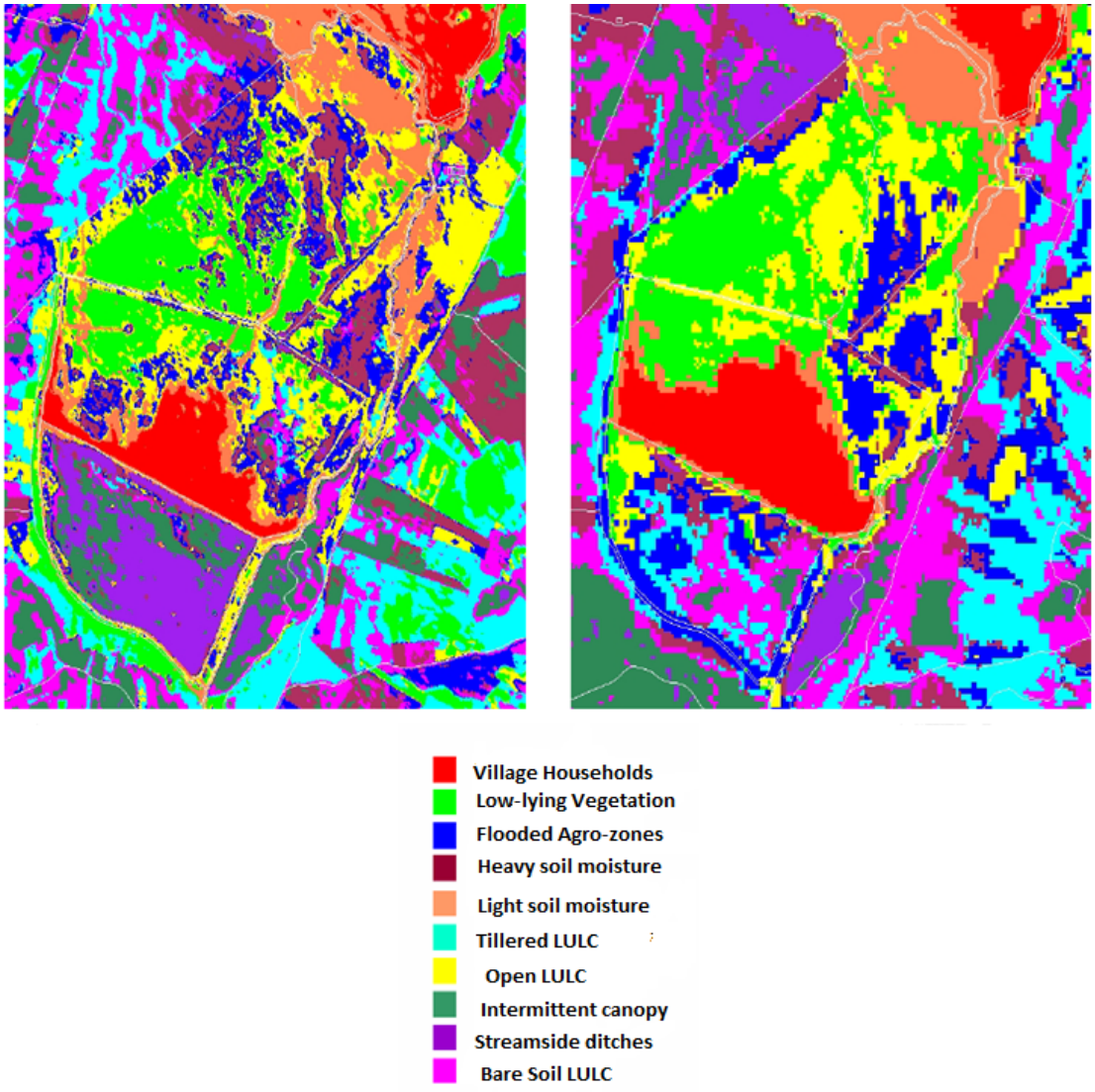

## 3. Results

A subset of the An. gambiae Complex larvae identified morphologically, was analysed initially using rDNA-PCR technique resulting in 68.22% (n = 1,290) An. gambiae s.s., 8.011% An. funestus s.l. 7.93% (n = 150) An. arabiensis and 15.84% (n = 451) An. merus. Multiple logistic regression model showed that emergent plants (p = 0.019), and floating debris (p = 0.038) were the best predictors of Anopheles larval abundance in these habitats. After the sentinel site hyperspectral signature wayward flights, seven videos with a total of 25 minutes were collected over multiple varying georeferenced, sentinel site, An. (gambiae s.l., funestus, s.s., arabiensis s.s., merus habitats. The total number of frames extracted was 1,064, with 88% of them containing at least one potential Anopheline larval habitat.

**Figure 4. Signnature imaged, sentinel site habitat types of** Anopheles gambiae **s.l. immatures that were sampled in a Maragizi agro-village in Kwale District, Kenya. A, Soil burrow pit dug to mine soil for wall construction of a house. B, Agro-pond dug to mine soil to make bricks. C,Hoof print. D, Grssy dich**

Real-time, interactive, web-based, geospatial analytical geoprocessing tools within the drone dashboard were employed to carry out inspections of reflectance, seasonal, landscape characteristics of potential, , An. gambiae s.l., arabiensis s.s., funestus s,s, and merus larval habitats using video analog, classified, seasonal, LULC data with georeferenced, sentinel site, capture point,RGB signatures previously obtained. These LULC types were labeled [Figure 5].

**Figure 5. Real time, UAV retrieved LULC classifications of a georeferenced, targeted sentinel site area in Margazi intervention agro-village pastureland ecosystem in Kwale District, Kenya (Jacob & Izurieta, Seek and Destroy Team)**



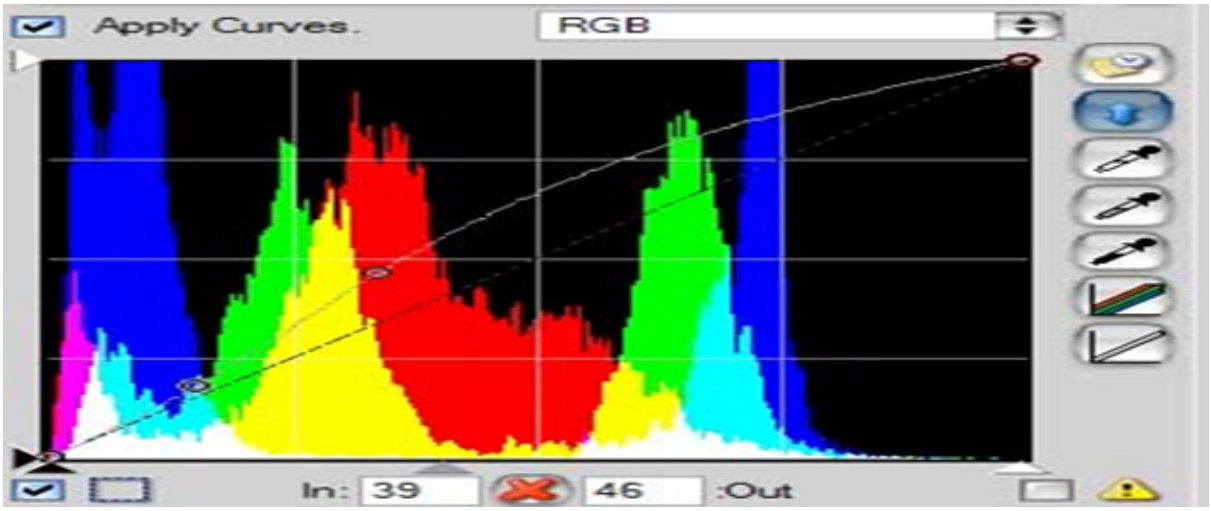

The data was subsequently exported in real-time to a handheld device (e.g., tablet, iPad, mobile phone); so that USF collaborators and KEMRI control personnel could view the multi-directional footage using a mobile Apple handheld devices (i-Pad) which provided GPS coordinates of all potential, seasonal, An. gambiae s.l., funestus s.s., arabiensis s.s. and merus breeding sites on classified LULC using sentinel site, capture point, geo-saptiotemporally interpolatable, georeferenced, larval habitat spectral signatures in the app.

Real-time, web-based, interactive, spatial analytical geoprocessing tools within the drone dashboard were employed to carry out inspections of signature reflectance, anomalous, seasonal, landscape characteristics of potential An.gambiae s.l., funetus, s.s. arabiensis s.s.and merus sentinel site, larval habitats, or potential intervention sites using video analog data with seasonal, breeding site, aquatic, capture point signatures obtained. Selected experimental larval habitat signatures were assembled in real-time from multiple experimental georeferenced capture points. The data was exported in real-time to a handheld device (e.g., tablet, iPad, mobile phone); so that personnel could view the multi-directional footage using mobile hand-

held devises (Apple I-tablet); with a dashboard geo-AI app providing GPS coordinates.

Spectral signatures of seasonal, georeferenced, capture point, georeferenced, An. gambiae s.l., arabiensis s.s. funestus s.s., merus breeding sites ranged around 400-1575nm but they were modified at some seasonal landscapes (flooded post-harvested paddy habitats, deforested cultivated swamp capture points etc.) [Figure 6]. Optical-geometric, sentinel site, reflectance, wavelength interactions were captured in the dashboard smartphone, iOS app whereby incident radiation was scattered in accordance with the 3-D structure of a seasonal, entomological sampled, sentinel site, georeferenced, Anopheles, larval habitat surface

**Figure 6. A comercial road sise ditch, georeferenced,m** An. gambiae s.l. **sentinel site, larval labitat RGB hyperspectral signature (Jacob & Izurieta, Seek and Destroy Team)**



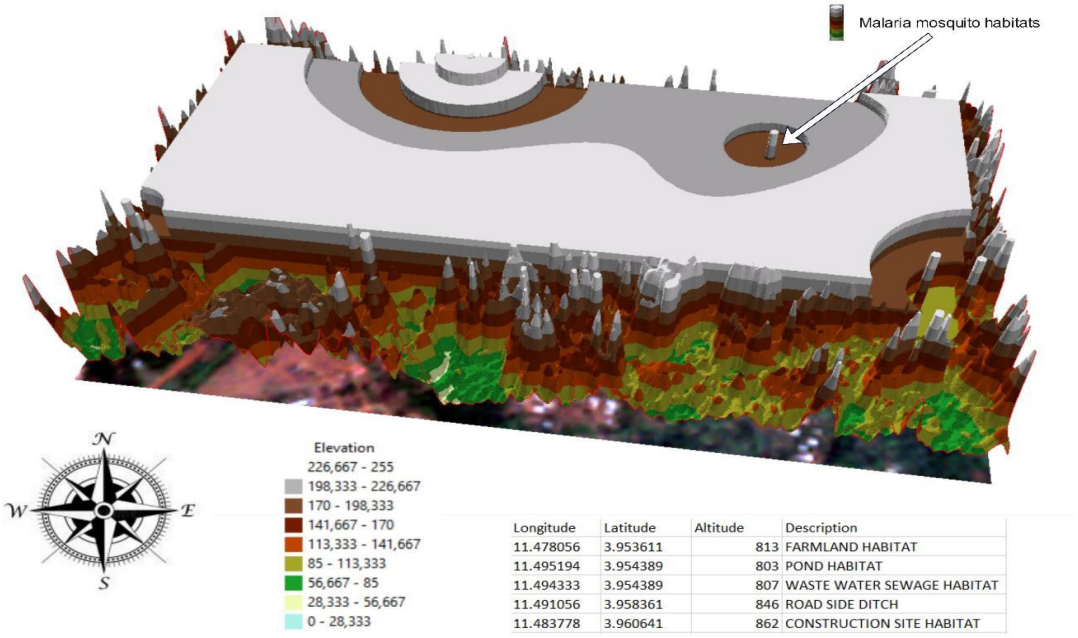

Additionally we utilized a handheld spectrometer to acquire precision full range, hyperspectral, reflectance measurements of multiple, georeferenced, sentinel site, seasonal, An. gambiae s.l., arabiensis s.s. funestus s.s., merus, larval habitats objects [ e.g., surface algae and or floating leaves on an urban storm sewer village agro-pond, or soil moisture in a hoof print or vechile rut habitat], designed around a radically streamlined cable-free workflow. ASD QualitySpec Trek is a general purpose, all-in-one, full-range, portable spectrometer that can be used to measure the visible and infrared regions (350-2500 nm) of georeferenced, entomological, larval habitat, seasonal, sentinel site, capture points. Other habitat objects were seasonally mapped

We then tested the scalability of the dashboard iOS app for detecting potential peri-domestic aquatic, seasonally georeferenceable habitats from drone video capture point in a sub-county geolocation [Marigizi village] in Kwale County, across seasons. Once the signatures were captured it was real-time transferred into the ArcGIS-AI Interface Kit™, in the app where an interpolation algorithm ran the signature over the entire district using commercial high-resolution satellite data (i.e., 46-centimeter WorldView -2 band visible and near-infra-red data) to identify previously unknown aquatic, An. gambiae s.l., arabiensis s.s. funestus s.s and merus breeding site, larval habitat, aquatic foci. This real-time, scale-up, interpolation hyperspectral signature mapping of the capture point, georeferenced Anopheline,sentinel site, larval habitats was based on the Faster R-CNN algorithm being applied to real-time imaged, seasonal, signature, entomological sample datasets which included the RGB component video. Drone images were analyzed to predict potential Anopheline larval habitats first in village and then throughout theentine County, using the smartphone iOS app [see Figure 8. We tested the scale-up of a georeferenced capture point by applying the previously discussed methods, including validation,

**Figure 7. Scaling up signature by kringing the RGB sentinel site signals draped over a 3-D,geospatial LULC map generated from World View 3 satellite data for targeting** Anopheles **habitats in Kwale District from an interpolated UAV imaged georeferenced capture point in Maragizi intervention village (Jacob & Izurieta, Seek and Destroy Team)**

By overlaying the natural flow lines derived from a Wv-2 3D-DEM with the reconstructed physical drains, a comparison of the flow direction and the orientation of the drains was achievable in a krige, hyperspectral, Anopheles habitat, signature layer generated in the interactive, geo-AI, smartphone dashboard with infused ML/DL object based algorithms [see Figure 9a]. Peak flows were calculated for each eco-cartographically, predictively, delineated watershed catchment geolocation as predicted by the UAV model and the results were used to check drains, for example, an standing water, agricultural ditch, sentinel site, capture point, An. gambiae, funestus, arabiensis s.s. or merus larval habitats. A combination of a DEM and rainfall-runoff model constructed within our interactive, iOS dashboard, improvse estimating runoff on partly urbanized watersheds and determined the size and orientation of these drains (potential, underground, water storage tank, Anophels, larval habitats) precisely, since we were using high resolution drone video data for sentinel site,geo-AI mapping the Kenyan County, intervention, study site. A regularly spaced raster grid of elevation values of sentinel site, habitat, georeferenced locations of seasonal, larval, breeding sites was also determined through surface classified, contour mapping, LULC classified, capture point, attribute features ( e.g., WorldView-2, 3-D, catchment watershed, slope coefficients of agro-ponds, rock pools and other sources of standing water) in the geo-AI-iOS platform [see Figure 9b). We generated a real time workflow in the app for interpreting 3D-DEM point clouds based on, ML and Random trees which derived domain specific semantics for geolocating, varying sentinel site, georeferenceable, An. gambiae, funestus, arabiensis s.s. or merus aquatic habitats with and without larvae/pupe and dry seasonal foci in the dashboard seasonally without having to re-create explicit spatial 3-D, hyperspectral signature, capture point, UAV models or explicit rule sets (i.e., we would simply overlay the larva/pupae signatures over the seasonal interpolated habitats).

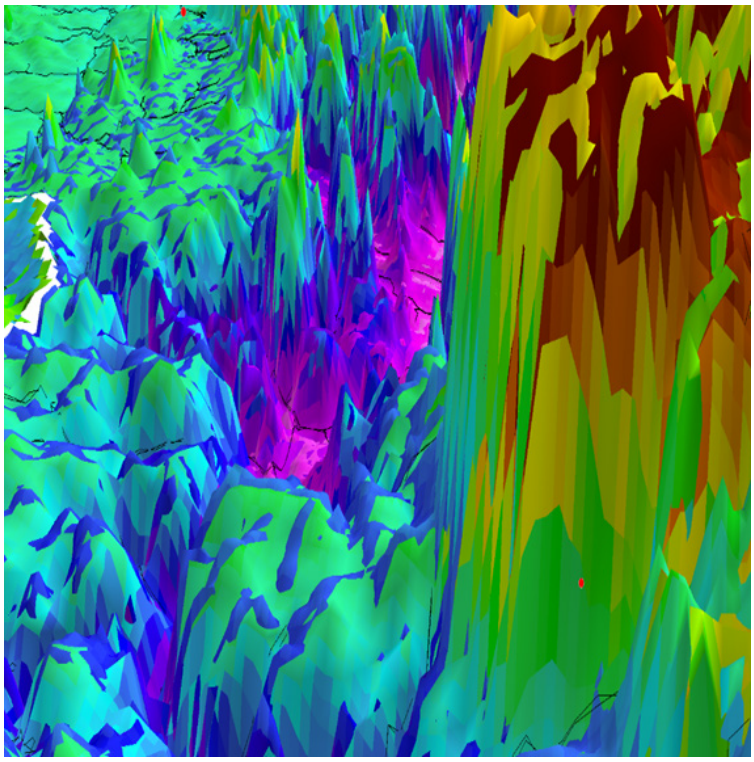
**Figure 8 a. 3-DEM delineating potential flood vulnerabile** Anopheles **habitat geolocations in the 3-D model created from the krige layer**




| Longitude | Latitude | Altitude | Description |
|---|---|---|---|
| 11.478056 | 3.953611 | 813 | FARMLAND HABITAT |
| 11.495194 | 3.954389 | 803 | POND HABITAT |
| 11.494333 | 3.954389 | 807 | WASTE WATER SEWAGE HABITAT |
| 11.491056 | 3.958361 | 846 | ROAD SIDE DITCH |
| 11.483778 | 3.960641 | 862 | CONSTRUCTION SITE HABITAT |

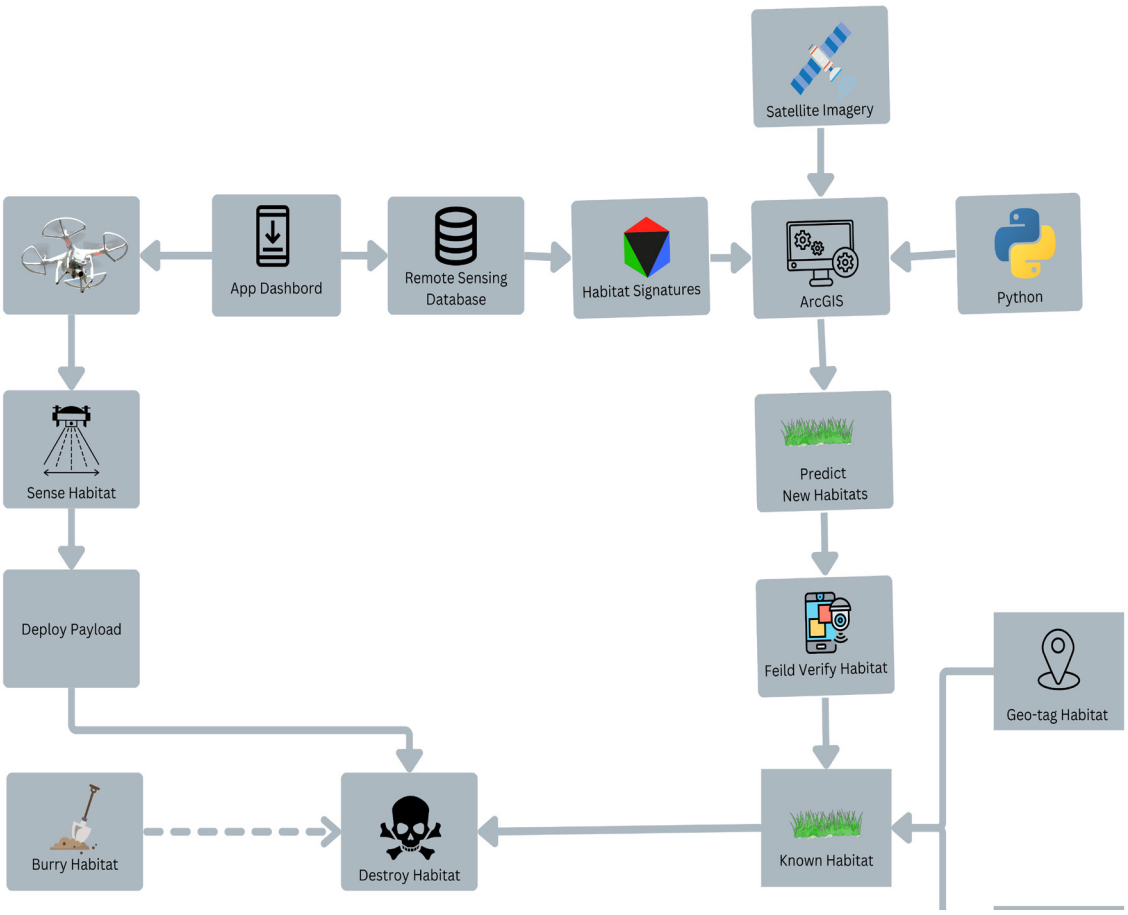
Combining an overlay of a 3-D DEM and the clustering and signature spectral analysis in ArcGIS Pro ™ in the app employing the resulted in a final optimal prediction of potential, georeferenceable, eco-geographic, seasonal, geo-spatiotemporal, interpolated ("kriged") capture point, sentinel site, larval habitat, aquatic foci geolocations present of immature, absent of the vector larve/pupae or dry foci along, y rural landscape LULC classified, entomological, intervention site [Figure 8]. The main rivers and streams in wale District are Marere, Mwaluganje and river Ramisi [14].

**Figure 9 Sentinel site, georeferenced** An. funestus **agro-pond habitat drone real time visualization (red font upper left corner) conducted using an oblique view in an iOS interactive app for reconstructing a synthetic visual image of the terrain in an urban –riceland intermitten geolocation in in Maragizi village**



We real time retrieved (hence, no lag time between seasonal, aquatic, Anopheles, larval habitat, mapping and treatment of foci) each georeferenced sentinel site, hyperspectral signature which was subsequently archived in the drone dashboard spectral library using the smartphone app. Each georeferenced, UAV sensed, capture point was inspected using a mobile field team (i.e., trained local village residents led by a vector control officer) on the same day the habitats were geo-AI signature mapped,geo-spatiotemporally forecasted and treated [i.e., Macro S&D].

**Figure 11. Real time UAV, signature** heles**Convolution neural network for assigning learnable weights to various habitat objects in capture point LULC imagesfor aerial sensed habitat mapping unknown habitats of** Anopheles **(Jacob & Izurieta, Seek and Destroy Team)**




Water bodies were identified in the drone sensed imagery, as well as ancillary information for implementing real time larval control activities [e.g., Macro S&D, which involved entirely burying breeding site, aquatic, Anopheles foci such as potholes, commercial roadside ditches, temporary rain pools, footprints, tire tracks and other household habitats with soil substrate]. The soil substrates were effective for approximately 150 days, but a secondary validation was applied employing the ArcGIS-AI dashboard app within 1 week of treatment. Our drones have a sensor-controlled drop-down appendage which was controlled by the iOS app, which aided in optimally targeting and treating exact geolocations of georeferenced, larger, breeding sites, [e.g., applying.05mg of Bti insecticide per inoculation to only the open sun lite exposed sides of a 10meter (m) x10m, rock pit, quarry, seasonal, Anopheline, aquatic, habitat foci where the larvae/pupae reside]. The real time control technique was extremely cost-effective as we applied only minimal amounts of the insecticide to the real time, drone mapped, field verified, remotely targeted, breeding sites [e.g., inoculations only on permanent or semi-permanent, uncanopied, pre-flooded tillers in a mature paddy field hence avoiding intermittent, dry post-harvested foci] with surgical precision as compared with non-real-time, drone applied, blanket treatment, insecticide spraying since we applied the Bti at a height less than a foot [i.e., 0304m] (no spillage, no droplet drift) above the targeted habitat which allowed implementing Macro S and D [Figure 12].

**Figure 11. Real time drone larviciding a urban riverine tributaries environments and agro-ecosystem ricleands (Jacob & Izurieta, Seek and Destroy Team)**

## 4. Discussion

We employed real-time, seasonal, UAV retrieved, capture point, datasets of hyperspectral imaged, LULC classified, sentinel site, georeferenced, An. gambiae s.l., arabiensis s.s. funetus s.l. and merus aquatic, larval habitat sentinel site, wavelength, reflectance, characteristics [e.g., water situation(turbid or clean, stagnant or running), substrate type, (e.g., moist or dry) site type (man-made or natural), sunlight situation, site situation (transient or permanent, with or without vegetation) etc.] for parsimoniously constructing an archive of seasonal, interpolatable, signatures using geo-AI and ML/DL learning technologies infused into the UAV-iOS app [6].

Thereafter we tested the scalability of the smartphone, dashboard app for detecting potential aquatic unknown County-level, sentinel site, An. gambiae s.l., arabiensis s.s. funetus s.l. and merus habitats from drone video using high-resolution Worldview-2 46 centimeter, gridded, [270 m x 270m] satellite data. Field validation revealed that of 65 predicted breeding site habitats, all contained Anopheles larvae/pupae revealing a sensitivity and specificity approaching 100% for pre-rain and dry season.We then implemented Macro and Micro S&D at the entomological, agro-pastureland intervention village site [2]

We continued to signature, drone seasonal, forecast map all treated sub-county, capture point, district-level, intervention sites every 7 -14 days to establish if new aquatic foci had occurred and treated those habitats. In so doing, we were able to ascertain valuable, district-level, seasonal, entomological information [e.g., georeferenced routes to a large, algae, matted cultivated, swamp habitat adjacent to an agro-pastureland village homestead population; precise drying temporal, sample frames of lagoons, transient poolsand flooded, man-made hole, sentinel sites, etc.] for optimal, real time, seasonal, drone vulnerability signature forecast mapping and treating Anopheles, capture point, breeding site, aquatic foci.

Advances in computer vision have made it possible to get credible intelligence from UAV and satellite imagery using geo-AI techniques such as Deep Learning in ArcGIS. For example, ArcGIS Pro allows the usage of machine learning classification [e.g. Random Forest (RF)] classification algorithm] methods to classify real time, sentinel site, vector arthropod, seasonal archived, UAV and/or remotely-sensed, RGB, signature imagery. Random Forest ensemble models are made of many decision trees using bootstrapping, random subsets of features, and average voting to make predictions [17-18].

Combining artificial intelligence (AI) machine learning classifiers and interpolative, ArcGIS [geo-AI] in an interactive, dashboard configurable, web-friendly, iOS, smartphone application (app) can aid in optimally

scaling up sentinel site capture points for predictively mapping unknown, County-level, sentinel site, An.gambiae s.l., arabiensis s.s. merus and fuenstus s.l., larval habitat, seasonal, occurrence, abundance and distribution. By employing real time, UAV retrieved, capture point, sentinel site, wavelength, reflectance datasets of seasonal, imaged, LULC classified, Anopheles larval habitat characteristics a georefereceable Red Green and Blue (RGB), signature may be generated employing geo-AI technologies infused into an iOS app

In conclusion the improved ability of multispectral sensors and the statistical and ML/DL computational geoprocessing tools in ArcGIS Pro in the iOS app provided an essential real time, sensing data resource for optimizing spatial data visualization of UAV imaged, real time, retrieved, seasonal, quantitative, thematic information, [e.g., marshy land cover of a georeferenced, semi-permanent, sentinel site, capture point, aquatic, An. funestus s.l., larval habitat,) employing a web-configurable, interactive, smartphone app. Subsequently we scaled-up the RGB indexed databases of seasonal, sentinel site signatures and the capture point's grid-stratifiable, LULC classifiable, habitat objects using geo-AI intelligence in the app for mapping unknown, , An. gambiae s.l., arabiensis s.s. funetus s.l. and merus County-level, aquatic, larval, breeding sites for implementing real time IVM. For example, one of the objectives of this research was to construct a real time UAV system where a query video is infused into an iOS for retrieval of a ranked list of visually similar, classified, land cover, grid-stratified, Anopheles habitats by GPS coordinates employing the interactive dashboard app which we did so successfully. In 42 days, post-Macro and Micro S&D intervention there was zero vector density, indoor, adult, female, Anopheles count as ascertained by PSC at the intervention site. After a mean average of 77 days, blood parasite levels revealed a mean 0 count in treated malaria patients

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