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Presence of Paragonimus species Within the Secondary Crustacean Hosts in Bogotá, Colombia

Gillian Phillips

ABSTRACT

PRESENCE OF *PARAGONIMUS* SPECIES WITHIN THE SECONDARY CRUSTACEAN HOSTS IN COLOMBIA

By

Gillian Phillips

DATE

19 November 2015

Paragonimus spp. are parasites that infect many populations worldwide. It is predicted that infection rates within Asia reach ten to fifteen percent of the total population. Three largest areas of possible infection are Asia, Central and South America as well as Africa where the total population at risk is estimated to be 293 million people. Ingestion of raw or undercooked crustaceans are the source of infection to mammals. The crustaceans *Neostrengeria macropa* and *Procambarus clarkii* in Bogotá, Colombia were collected from local markets, pet stores and waterways. Dissection for presence of parasites is imperative to estimate the prevalence of crustacean infection by lung flukes. The preliminary findings show, pending laboratory confirmation, that the native crab species, *N. macropa*, has a prevalence of 17.2% infection. Invasive crayfish species, *P. clarkii* has a prevalence of 36.4% from both captive and field capture samples. While the prevalence estimated within this study is lower than compared to previous research in other cities of Colombia, there may be a number of factors that contribute to the difference in prevalence including: collecting season, overall low rainfall, temperature, altitude and the El Niño Southern Oscillation.

PRESENCE OF *PARAGONIMUS* SPECIES WITHIN THE SECONDARY CRUSTACEAN HOSTS IN COLOMBIA.

by

Gillian Phillips

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APPROVAL PAGE

PRESENCE OF *PARAGONIMUS* SPECIES WITHIN THE SECONDARY INTERMEDIATE HOSTS IN COLOMBIA

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Author's Statement Page

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Performing laboratory test and assisting veterinarians with exams, radiographs, and restraint.
Patient Care Specialist- June 2013- January 2014, Full-time
Monitoring the health of animals boarding in kennel as well as assisting treatment staff with care and procedures.

United States Fish and Wildlife Service
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Surveying and trapping from Peachtree creek for presence of Crayfish and other wildlife.

Connecticut Veterinary Medical Diagnostic Laboratory

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Chapter I

Introduction

Paragonimus spp. is a trematode parasite that infect human populations worldwide. It is considered a neglected tropical disease due to the lack of knowledge of human prevalence across the world. Based on current knowledge, an estimated 293 million people are at risk of infection worldwide. Paragonimiasis is an infection of *Paragonimus* spp. which manifests as chronic inflammation within mammalian lungs (Toledo, Esteban, & Fried, 2012). Clinical presentation of this parasite can mimic tuberculosis on chest radiographs, which is also endemic in the same areas and vulnerable populations. The cost of chemotherapy to treat paragonimaisis is simple and more cost effective to treat compared with tuberculosis (Pan American Health Organization, 2011; "WHO Initiative to Estimate the Global Burden of Foodborne Diseases," 2007). Not only is food safety education needed in endemic areas, but proper diagnosis and treatment is required to reduce global lung fluke infection.

Purpose of Study

With urban development, agricultural development, domestication and industrialization the need to understand human animal interactions as it pertains to infectious disease has increased (Reperant, Cornaglia, & Osterhaus, 2012). WHO has implemented an initiative to estimate the Global Burden of Foodborne Diseases, which includes *Paragonimus* spp. as a foodborne parasite, in order to gain better understanding of human infection ("WHO Initiative to Estimate the Global Burden of Foodborne Diseases," 2007). The purpose of this study is to determine the prevalence rate of *Paragonimus* spp. in secondary crustacean hosts of Bogotá, Colombia. Information collected will be used to estimate risk as well as predict future trends in lung fluke infections.

Research Questions

What is the prevalence of *Paragonimus* spp. within secondary intermediate hosts in Bogotá, Colombia?

What is the risk related to human populations as the definitive host of *Paragonimus* spp.?

What is the geographic distribution of secondary crustacean hosts?

What is the geographic distribution of *Paragonimus* spp.?

What cultural practices or socioeconomic status factors may lead to consumption of raw crustaceans?

Chapter II

Paragonimiasis

Lung flukes are trematode parasites that have affected human populations for centuries. Testing from a 400-year-old archaeological site in Korea revealed in cysts seropositive to *Paragonimus westermani*, which is the same species that is endemic to that area today. It was also a one hundred percent match to the contemporary genetic makeup. Presence of ova were found in the lung, feces, intestine and liver of the individual (Shin et al., 2012).

Paragonimus spp. ova presents about the size of a coffee bean. Trematodes are a group of parasites that are flatworms of the Platyhelminthe phylum. This group of parasites target a wide range of tissues within the human body and those of other mammals (Bogitsh, Carter, & Oeltmann, 2013). The estimated prevalence of individuals infected with *Paragonimus* spp. equates to 20 million worldwide. The majority of infections are comprised of *P. westermani* in Asia, where population infection rates are estimated to reach five to ten percent of the total population (Davidson, 2005). In addition, worldwide there are an estimated forty *Paragonimus* spp. that infect humans (Restrepo et al., 2007). As of 2011, an estimated 293 million people are at risk of infection in three geographic regions: Asia (China, Japan), where 195 million people are at risk in China alone, (Liu, Wei, Liu, Yang, & Zhang, 2008), South and Central America (Ecuador, Peru, Costa Rica, and Colombia), and Africa (Toledo et al., 2012).

This parasite has three separate hosts within its life cycle. According to Bogitsh (2013), The definitive mammalian host excretes eggs in sputum or are swallowed and passed through stool where introduction to freshwater sources occurs. Upon introduction to the aquatic environment, the unembryonated eggs become embryonated. Hatching miracidia seek to inhabit snails through penetration of the foot, which serve as the first intermediate host. After undergoing several developmental stages, including miracidia, sporocysts, and rediae, the cercariae exit the snail. From the snail, this stage is released into waters where crustaceans are the secondary intermediate host. This next host relationship allows the parasite to encyst and transform to metacercariae. The secondary hosts are then infective to reservoir and definitive hosts which are mammals that ingest raw or undercooked crustaceans. Once in the definitive host, the parasites excyst in the duodenum, penetrate through intestinal lining, peritoneum and diaphragm to reach the most common destination, the lungs. Within the lungs encapsulation signifies maturation into adults where 4-6 adults encyst and sexual reproduction occurs. The cysts formed are connective tissue and often ulcerate, giving an uneven appearance on radiographs. The lifecycle is continued when excretion of oocytes are coughed up in sputum or swallowed and passed in feces (Bogitsh et al., 2013).

Within lung cavities, cysts are formed, measuring between half and one and a half centimeters in diameter which attachs to bronchial epithelium (Restrepo et al., 2007). Depending on length of infection or high parasite load, the development of coughing with brown sputum can occur (Davidson, 2005). When fibrous cysts rupture or ulcerate, eggs are then coughed up (Bogitsh et al., 2013). Clinical symptoms can also include fever, coughing blood, pain associated with the lungs, shortness of breath, and recurrent bacterial pneumonia. Those chronically infected can develop pleural effusion and abscesses of the lung (Kim et al., 2011).

Approximately twenty percent of individuals with paragonimiasis are asymptomatic. Acute infection presents two to fifteen days after ingestion of raw or undercooked crustaceans and may experience diarrhea and abdominal pain associated with intestinal perforation. As the metacercariae migrate to the lungs chest pain, dyspnea, cough, malaise and sweats can occur as the fluke seeks the lung where fever and high eosinophilia occurs. Chronic infections include a persistent cough, blood tinged sputum, chest pain and dyspnea (Bogitsh et al., 2013). Development of pleural effusion and recurrent secondary bacterial pneumonia can also be noted in long lasting infections (Davidson, 2005).

A definitive host of a parasite is a host in which sexual reproduction occurs during the course of the life cycle. *Paragonimus* spp. are zoonotic and have a wide range of mammalian hosts that they can inhabit. Documented cases of lung flukes have been noted in cats, dogs, foxes, wolves, tigers, lions, leopards and civits (Aka, Adoubryn, Rondelaud, & Dreyfuss, 2008). These hosts allow for maturation of metacercariae and continuation of the life cycle by passing mature oocysts in feces (Liu et al., 2008). A study conducted was conducted by Kirino et al. (2009) to identify both a source and definitive host of *P. westermani* in Japan. Wild boars are a source of infection if eaten raw. Boar hunting dogs in this study either ate boar meat or scraps or were fed a diet that did not consist of boar. In total 65.6% of dogs were seropositive, via serum ELISA testing, for *P. westermani*. All seropositive dogs were fed wild boar meat while the dogs that did not eat boar were all seronegative (Kirino, Nakano, Doanh, Nawa, & Horii, 2009) Ectopic or extrapulmonary sites where *Paragonimus* spp. are found include brain, lymph nodes, heart, abdomen (Cho, Lee, Lee, Lee, & Lee, 2011), skin (Lee, Kim, Moon, & Lee, 2012) and nervous system. Death can occur from heavy parasite load and from cardiac or cerebral infiltration. Cysts in the brain increase intracranial pressure and can cause epilepsy and paralysis in some patients (Bogitsh et al., 2013) that can calcify in late infections (Restrepo et al., 2007). Cranial infection is the most commonly documented form as well as the most deadly (Kim et al., 2011).

Transmission is restricted to areas where the primary and secondary hosts coexist in the same areas (Toledo et al., 2012). Snail genera known to transmit miracidium include

Semisulcospira, Tarebi, and *Brotia*. The parasite completes its maturation stages, which takes eleven weeks, and emerges into the water or on land to enter a freshwater crustacean. The crustacean can eat it or the exoskeleton can be penetrated by the cercariae cuticular stylet (Bogitsh et al., 2013). Another required component for infection is an area where improper preparation of seafood occurs through undercooked, raw, pickled (Toledo et al., 2012) or smoking (Singh, Sugiyama, Lepcha, & Khanna, 2014). Improper food handling through use of unsanitary utensils and work surfaces may also contaminate food with metacercariae (Kim et al., 2011).

Cultural customs and food habits contribute to infection rates among endemic areas. 'Kejang' is a traditional Korean dish in which freshwater crabs are soaked in soybean sauce then served without any heat applied. Persons of this region depict both buying crabs from local markets as well as gathering from local mountainous streams (Cho et al., 2011). In Cameroon, the practice of using raw crabs as a treatment for infertility in women is declining but still present (Kum & Nchinda, 1982). There, preparations include cooking crabs over hot ashes where doneness is determined by the changing carapace color, which is not a good indication of adequate intestinal temperature. This is insufficient to kill the metacercariae, yielding undercooked crabs (Moyou-Somo, Kefie-Arrey, Dreyfuss, & Dumas, 2003). In Ecuador, raw crabs are consumed for reduction of fever as well as a "cure" for hangovers (Vieira, Blankespoor, Cooper, & Guderian, 1992). Aside from cultural influences, areas with limited economic development where lack of food education, poverty and remoteness of health care services influence the prevalence of the disease in endemic areas (Narain et al., 2015). In some areas of Nigeria, food is scarce and raw crabs are eaten (Aka et al., 2008).

Extra-pulmonary infections have been noted in the abdominal wall, intestine, heart, lymph nodes, liver and urinary tract. Cranial infections are the most common and deadly, and can involve symptoms such as nausea, headache, sensory deficits, epilepsy and paralysis. Symptoms can vary in intensity, duration, number of reinfections as well as host immune function (Liu et al., 2008).

Diagnosis is primarily done through an ova and parasite exam of sputum or feces. It takes two to three months post-infection for this test to become positive and in chronic infections there may be periods of time when no ova are shed. Immunological testing is done through enzyme-linked immunosorbent assay (ELISA), dot immunogold filtration assay (DIFGA), and immunofluorescent antibody assay (IFA), which are sensitive testing measures but not available in rural areas yet. The intradermal skin test is effective, like the tuberculin skin test, to identify persons who have been exposed to *Paragonimus* before. However, this does not distinguish between present and previously cleared infections (Liu et al., 2008). Diagnostic imaging like radiographs, computerized tomography (CT), magnetic resonance imaging (MRI) may lead to increased differential diagnosis list such as: pneumonia, metastasis or tuberculosis. Biopsy of the tissue or previously discussed diagnostics can be performed for identification (Kim et al., 2011).

Treatment for paragonimaisis recommended by the WHO includes triclabendazole in one or two doses given in one day or praziquantel given three times a day over three days. Treatment of all confirmed cases is necessary to clear infection. It is recommended to treat all suspected cases in endemic areas. Mass drug administration is recommended yearly for clustered cases ("WHO Initiative to Estimate the Global Burden of Foodborne Diseases," 2007). Compliant treatment with praziquantal is successful in complete clearance of parasites in greater than ninety percent of cases. However, resistance to both medications has been documented primarily in Asia (Kyung et al., 2011).

Prevention of paragonimaisis is to ensure adequate cooking of crustaceans prior to consumption. Educational programs to educate consumers about proper food handling and safety are necessary to limit transmission of this parasite. Other prevention methods could include waste treatment of definitive host species in freshwater waterways. Mass drug administration has also been used in endemic areas to reduce the human parasite load. Lastly, some endemic areas are looking to control snail populations as it is a crucial step to the life cycle of this trematode (Liu et al., 2008). Such educational programs to reduce rates of paragonimiasis have also been successfully conducted in India. Narain et al. (2015) showed improvement in total prevalence rates through focus groups and interviews to determine knowledge then provide education services with charts and photographs to aid in presentation (Narain et al., 2015).

Colombia Research

Paragonimus caliensis was first described in Colombia in *Didelphis marsupialsis* and *Philander opossum*, two species of possum with metacercariae were found in the hepatopancreas of crabs (Little, 1968). The first recorded human case in the region was in Peru of *P. caliensis* in 1910, which is the likely species found in Colombia. Morphologically it has branched lobes of the ovaries that are the intermediate of long unbranched lobes. The encysted metacercariae is larger than other species (Little, 1968). Within South America there are other countries where *Paragonimus* spp is present. In Ecuador, Colombia's neighbor, 42.6% of the 1,043 crabs dissected contained metacercariae identified as *Paragonimus mexicanus* (Vieira et al., 1992). *P. mexicanus* has also been documented in Venezuela by Diaz and colleagues (2011), described two areas with the crustacean prevalence in Sucre at 4.07% and in Montes at 17.98% although the crab metacercariae burden was higher in Sucre than Montes (Diaz, Marin, Gomez, Prieto, & Ojeda, 2011).

A study to identify the prevalence of *Paragonimus* spp. within nine counties of Antioquia was conducted by Uruburu and colleagues (2008). Of the 52 brachyuran crabs collected, 42 had metacercariae present that were identified as *Paragonimus* spp. This yielded a prevalence rate of 80.8% within Antioquia, Colombia (Uruburu, Granada, & Velasquez, 2008). Based on another study conducted of 27 crabs collected near Medellin, the prevalence rate was 55.5% (Casas, Gomez, Valencia, Salazar, & Velásquez, 2008). Vélez and colleagues researched three hosts within Embrá Native Indian lands in Urrao, Antioquia, Colombia (2003). Within Hydrobiidae snails, they observed that only 1.6% of the 120 snails collected contained cercariae, however, 225 cercariae hatched from each snail primarily in morning and afternoon. This period is important, as the secondary crab hosts are most active during this period. 22 of the 44 crabs collected, *Hypolobocera bouvieri monticola* and *Hypolobocera emberarum*, yielded metacercariae with a prevalence of 50% (Vélez, Velásquez, & Vélez, 2003).

A drink named berraquillo is sold in Colombia, which is prepared with raw egg, fruit and a live freshwater (in Bogotá, *Neostregenria macropa*) crab blended into a smoothie and consumed as an aphrodisiac. This is one potential source of infection as well as other possible concerns about rural population consumption (Casas, Gómez, Valencia, Salazar, & Velásquez, 2008). Vélez and colleagues noted consumption of raw crabs in the Native Embera Indian community in Urrao, Antioquia, Colombia (Vélez et al., 2003).

A targeted educational program by Casas and colleagues (2008) was conducted for children and teenagers about food safety to improve the awareness of parasites within their community. This program used drawings and photographs to inform youth in Fuente Clara, Antioquia, Colombia about the life cycle, risk, clinical signs and symptoms as well as possible infection routes. This educational workshop with the community and academic institution also discussed the national resources in the waterways of their neighborhood (Casas, Gomez, et al., 2008). Based on the aforementioned study, a program to inform school children about the presence of *Paragonimus* spp. within aquatic ecosystems should also be implemented in the Medellin River Basin (Arias, Salazar, Casas, Henao, & Velasquez, 2011).

Tuberculosis

Diagnosis of paragonimiasis can be confused with tuberculosis as both present with patchy consolidation of lung parenchyma in bronchial cavities on radiographs. Lesions from lung flukes are typically less apical, or lower, than tuberculosis cavities within the lung chest visualized by radiography or other imaging diagnostics. However, this description is subjective and not a concrete differential diagnostic description. Eggs can be seen in sputum, not acid-fast bacilli. Serological testing can be used for diagnosis. Prazinquantel at appropriate dosage is effective for treatment (Davidson, 2005).

Colombia spends approximately 1000 USD per case of tuberculosis treatment per patient and changes with healthcare costs. Total estimated cost to control tuberculosis for Colombia is estimated to be 17.3 million dollars and has been increasing annually since 2007. Colombia has an incidence rate of six percent annually for tuberculosis and one and a half percent for multi-drug resistant tuberculosis. The overall prevalence rate of tuberculosis is sixty-five percent. Testing potential cases is not a barrier to treatment but better notification of disease is necessary, as only 17% of cases all cases of tuberculosis were notified after diagnosis. Treatment conversion of smear tests, from positive to negative, was seventy-seven percent (Pan American Health Organization, 2011).

Aquatic Vectors

Aquatic crustaceans are a significant portion of seafood consumed annually. Aquaculture of crustaceans is a part of global food industry. The need for creating safe, ecologically-sound and in demand products is necessary to ensure food security (Grant D. Stentiford, 2012). As of 2012, annual production of aquatic animals exceeded 10 metric tons of total food production globally (G. D. Stentiford et al., 2012). While crustaceans make up a small percentage of total aquatic products consumed annually, it still comprises one billion in total fishery cost yearly (FAO, 2009). Latin America produces a large amount of the total exported crustaceans to developed countries, with most of the aquaculture of crustaceans comprised of shrimp. With increasing demand and intensification of aquaculture and fewer overall farms on average, there are more than three billion dollars lost annually due to infections. (G. D. Stentiford et al., 2012). In addition, this does not include native fishing in streams and rivers.

In Colombia, *Neostrengeria macopa* is a freshwater brachyuran crab native to the mountainous region around Bogotá, Colombia. *N. macropa* is found between 2200 and 2900 meters within the Magdalena watershed, Santa Fe de Bogotá, and the Sabana (plain)Bogotá of the Cundinamarca Department. This species is considered of least concern under the IUCN Red list of threatened species with the only threats being water pollution and habitat loss and degradation. (IUCN, 2008). However, this population may become vulnerable due to indiscriminate trapping for consumption (Campos & Pedraza, 2008).

Procambarus clarkii is native to Southern Central United States as well as Northeastern Mexico. Due to aquaculture introduction of this species, it has invaded Africa, Asia, and Europe, as well as Central and South America. This species is extremely adaptive to freshwater ecosystems. Traits such as tolerance of extreme environments, r-selective reproduction, behavioral flexibility and ability as a predator and successful competitor with similar niched species make it a successful invader. Negative effects of invasion in freshwater include burrowing activities within riverbanks creating more turbid waters. Additionally, feeding habits of this species have the ability to over consume various types of food such as detritus, sediments, animal material and plant material. The wide range of food sources allow it a large range of possible habitats (Gherardi, 2006).

Only areas where both definitive hosts as well as intermediate hosts will complete the life cycle of *Paragonimus* spp. Upstream hosts, or definitive hosts, drive patterns of downstream, or intermediate hosts. There is a positive association between the the magnitude of upstream and downstream infection of intermediate hosts (Hechinger & Lafferty, 2005), (Robert Poulin & Lagrue, 2015). Without one species in the chain, the lifecycle will not be completed. Population control methods such as mollusk population reduction can limit disease progression (Wang, Feng, Sithithaworn, Feng, & Petney, 2011).

Concerns with Climate Change – Induced Range Shifts

Projected changes in arthropod vectors, like ticks and mosquitos, include geographic range shifts, rate of development, survival, reproduction of vectors, reservoirs, and the pathogens they carry. Additionally, changes in the biting rates of infected vectors, prevalence of infection in reservoirs populations as well as increased likelihood of transmission from human contact are of concern. In Europe there is evidence of an ability for liver flukes, another trematode, to adapt to environmental changes to persist at higher altitudes. Mild winters and high summer rainfalls create an environment that is ideal for parasite intermediate hosts, and therefore that stage of parasite. Farming practices may aid in transmission between livestock species, which could additionally increase infection rates in the future (Mackenzie, Jeggo, & Daszak, 2013, pgs 155-9). Similar patterns for altitude and other habitat adaptation for *Paragonimus* spp. may arise, as they are both trematodes with similar lifecycles.

Currently, Colombia is affected by El Niño Southern Oscillation changes that bring warm and dry climates with it. This is a natural cycle of the ocean-atmosphere system observed in the Pacific, which creates periods of two- to seven-year changes in annual variability of climate. Biological effects of ENSO include effects on survival and reproduction as well as distribution of organisms at many trophic levels of the ecosystem. For example, the thermocline results in reduction in the nutricline such that phytoplankton production falls and affects all species which directly or indirectly rely on this as a food source. While many of the effects are related to aquatic species, many of these populations are resilient and recover after the end of the ENSO if short lived. However, species that are more sensitive, such as coral, may not recover after larger changes in temperature. Birds in particular are affected by the lack of aquatic food sources (Fiedler, 2002). Shifts in avian geographic distribution may significantly be affected by climate change in areas like Colombia where species will shit into higher elevations, reduction in population sizes and fragmentation of geographic range reducing the overall population of birds. This would restrict the current diverse biota into two smaller and fragmented hotspots within the country (Velásquez-tibatá, Salaman, & Graham, 2013).

Identification of the effects of ENSO is useful for prediction of the effects of climate change within ecosystems because it has previously been used as a proxy of how these ecosystems may respond to future changes. Such predictions can include latitudinal range expansion, contraction of habitats or changes in species composition (Arntz, 2002). However, during an ENSO cycle in 2008, Oliva and colleagues surveyed mole crabs for *Profilicollis* spp. parasites and documented number of shore birds. The abundance and distribution of shore birds show a negative impact on prevalence effectively reducing the total number of parasite abundance in all hosts (Oliva, Barrios, Thatje, & Laudien, 2008).

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Chapter III

Manuscript

Introduction

Paragonimus spp. are infectious trematodes commonly referred to as lung flukes. Paragonimiasis, the infection of an individual with lung flukes, is estimated to have a prevalence of 20 million people worldwide (Davidson, 2005). As of 2011, an estimated 293 million are at risk of infection (Toledo et al., 2012). This infection is most prevalent in Asia where population infection rates are estimated to reach 5-10% of the total population. It is also endemic in India, Africa as well as Central and South America (Davidson, 2005). There have been few documented cases of infection in other areas of the world including the United States (Fried & Abruzzi, 2010).

After ingestion, *Paragonimus* spp. will penetrate the GI tract and diaphragm and mature once in the lungs (Toledo et al., 2012). Within the lungs, cavities are formed in cysts, which connect with the bronchi. Depending on length of infection or high parasite load, then the development of coughing with brown sputum can occur. Clinical symptoms can also include fever, coughing blood, pain associated with the lungs, shortness of breath, and recurrent bacterial pneumonia. Those chronically infected can develop pleural effusion and abscesses of the lung (Davidson, 2005).

Transmission is restricted to areas where the primary intermediate mollusks as well as secondary intermediate crustacean hosts coexist in freshwater waterways. Infection occurs through improper preparation of crustaceans through undercooking, raw, pickled (Toledo et al., 2012), cultural preferences as well as in relation of socioeconomic status (Narain et al., 2015). Cultural traditions include Berraquillo in Colombia, a smoothie made from raw egg, fruit and a live crab blended to be used as an aphrodisiac. In Ecuador, raw crabs are consumed as a natural cure for fever as well as legend for the cure for a hangover (Vieira et al., 1992). Additional traditions such as 'Kejang' in Korea prepare crabs by placing them in soybean sauce without cooking (Cho et al., 2011). Raw crabs are consumed not only as a cultural custom but also as a female cure for infertility in Nigeria (Kum & Nchinda, 1982). When food is scarce, an increase in crab consumption in Nigeria has been frequently documented (Aka et al., 2008). Additionally, rural areas may influence preparation, food education as well as location of health care services to treat infected individuals (Narain et al., 2015).

Uruburu and colleagues (2008) completed a study to identify the prevalence of *Paragonimus* spp. within nine counties of Antioquia Department in Colombia. Of the 52 brachyuran crabs collected, 42 had metacercariae present that were identified as *Paragonimus* spp. This yielded a prevalence of 80.8% within Antioquia, Colombia (Uruburu et al., 2008). In another study, 27 crabs were collected near Medellin for detection of *Paragonimus* spp., for which the prevalence rate was 55.5% (Casas, Gomez, et al., 2008). Lastly, a study of indigenous Emberá lands within Antioquia resulted in a prevalence of 50% of crabs and 1.6% of snails (Vélez et al., 2003). While a literature search did not reveal human prevalence studies, information about the presence of disease among food sources and waterways is important to ascertain risk of a vulnerable population.

Tuberculosis in Colombia has been estimated to have an incidence of six percent of the total population and one and a half percent of multi-drug resistant form of tuberculosis (MDR-TB) per year. MDR-TB is mainly due to incomplete treatment of tuberculosis upon diagnosis on both individual and access to consistent treatment. While testing for TB in Colombia has risen in the overall population follow through is that main barrier to TB control. On average, it cost 1000

USD to treat one case of tuberculosis in Colombia with a budget of 17.3 million USD estimated to control tuberculosis in Colombia. WHO recommendations for non multi-drug resistant TB should be two months of the induction phase which includes isoniazid, rifampin, pyrazinamide, ethamutol with a continuation of isoniazid and rifampin for four months (Pan American Health Organization, 2011).

Misdiagnosis of lung flukes with TB is due to similar presentation on radiographs. Patchy consolidation of lung parenchyma are visible on X-rays however the lesions from lung flukes are typically less apical than TB lesions. However, this criterion is not diagnostic for differentiation of disease. Sputum testing in tuberculosis positive patients would be stained for acid fast bacilli can change the morphology of the eggs such as reducing the overall size hindering species identification. Eggs can be seen in sputum testing if appropriately stained (Zeibig, 2013). Serological testing, such as ELISA or IFA, can be used for diagnosis as well (Boland et al., 2011), but common and inexpensive methods have not been developed .

Treatment of lung flukes with Prazinquantel at 25 milligrams per kilogram three times daily for three days or the simpler triclabendazole at 10 milligrams per kilogram twice for one day is effective for treatment. In addition, there is better patient adherence to treatment compared to long courses of antibiotics. Additionally, Novartis Pharma AG and WHO have donated triclabendazole since 2010 with shipping cost when applied through the ministry of health ("WHO Initiative to Estimate the Global Burden of Foodborne Diseases," 2007).

Neostregenria macropa is a brachyuran crab native to the Bogotá mountain range of Colombia. The native range is from 2200 to 2900 meters above sea level. This crab inhabits freshwater tributaries and waterways of the Río Bogotá. While *N. macropa* is not currently listed as a vulnerable species on the IUCN Red List of threatened species (2008) it may become listed in the future. According to the IUCN it is threatened by human related habitat loss and water pollution (IUCN, 2008) as well as local trapping (Campos & Pedraza, 2008).

Procambarus clarkii are not only invasive in the United States but also in Central and South America, Europe, Africa and Asia. Invasive species can drastically alter the natural food web and the effects are greater within freshwater ecosystems compared to terrestrial ecosystems (Sala et al., 2000)**.** Crayfish are long-lived crustaceans that can survive in high densities. Introduction of crayfish into other habitats has been accidental and intentional due to cultivation as a food source due to escaping from aquaculture productions. Once introduced into the native water system they feed on invertebrates, detritus, macrophytes and algae. Over time this can drastically alter the native food web by competition of native crustacean species and addition of crustaceans to predators (Gherardi, 2006).

Methods

Neostrengeria macropa were purchased from Paloquemao, a large market in Bogota, Colombia, but originated from streams around Gachancipá north of the city. *Procambarus clarkii* were purchased from a pet store as well as collected from the Club El Rincon de Bogotá golf course were collected.

Animals were placed into an ice bath or water chilled to 2° C as noted in the literature as the preferred methods of relaxation of crabs (Uruburu et al., 2008). After 10 minutes the specimens were measured, and cardiac tissue was removed to ensure cessation of life before continuation. Tissues of focus were heart, hepatopancreas, and gill as well as large deposits of muscle in claws and the abdomen of *P. clarkii* were placed on slides for examination with stereomicroscopy. From the literature, these tissues were documented as the most likely to contain metacercariae (Bogitsh et al., 2013; Little, 1968; Vélez et al., 2003).

Research of invertebrates is not required to have an IACUC protocol. Paperwork was submitted to AMSC IACUC for animal use off campus. Permission to conduct research on invertebrates granted through Universidad Nacional de Colombia, Instituto Ciencia de Naturales. Protocols for relaxation and dissection were found in the literature (Uruburu et al., 2008).

Wilcoxon Rank Sum was used to determine whether the prevalence is different from both zero and previous findings. This non-parametric test was conducted using the statistical package R, with the use of R Commander with CA-1 CRAN mirror. Wilcoxon Rank Sum was also performed on *N. macropa* for mu=0.555 and mu=0.808, the two previous prevalence estimates for Colombia. The prevalence of *Paragonimus* spp. within Bogotá (17.2%) is significantly different from the department Antioquia at 80.8% ($p=2.627^{-6}$) and Medellín 55.5% ($p=2.627^{-6}$).

Results

Table 1: Prevalence of Parasites Collected from Crustacean Hosts

	Paragonimus spp.	***Profilicollis spp.***
N. macropa		
Market	(5/29) 17.2%	(22/29) 75.9%
P. clarkii		
Market	0/8	0/8
Wild-caught	8/14 (57.1%)	0/14

N. macropa were purchased from Paloquemao market in Bogotá, Colombia. *P. clarkii* were purchased from a local pet store, market, or were wild-caught from Club El Rincon de Bogotá,. Both species were dissected with the same method and prevalence of both parasites recorded in Table 1.

Table 2: Wilcoxon Rank Sum of Parasite Prevalence

	Mu=0	**Mu=0.555**	**Mu=0.808**
N. macropa	p=0.03689	p=2.627e^{-6}	p=2.627e^{-6}
P. clarkii	p=0.005962	-	-

Paragonimus spp was also identified from both *N. macropa* and *P. clarkii* as shown in Table 1. Of the market purchased *N. macropa* five of the twenty-nine (17.2%) had metacercariae present in tissues. While none of the market purchased *P. clarkii* yielded metacercariae, eight of the fourteen (57.1%) yielded metacercariae.

I found *Profilicollis* spp. within the gills of twenty-two (75.9%) *N. macropa* purchased at market in Bogotá (Table 1). Literature searches yielded no previous documentation of this acanthocephalan parasite in Colombia. This parasite is commonly known as a thorny or spiny headed worm that attaches to the intestines of its definitive avian host with the proboscis (La Sala et al., 2013) and is passed from infected birds to water, where crabs are the sole intermediate hosts, and back to birds as they consume crabs (Goulding & Sarah Cohen, 2014).

Discussion

Paragonimus spp. infected *N. macropa* at a rate of 17.2% (Table 1) compared with 80.8% in Antioquia, 55% in Medellín, Antioquia and 50% in Urrao, Antioquia. This is of concern since the specimens from this study were purchased at a local market and therefore are meant to be consumption items. Added to cultural practices such as drinking berraquillo, an aphrodisiac that is made by blending raw eggs, fruit and a live freshwater crab, there is an increased risk of ingesting metacercariae. Food preparation is warm or cooked food, such as soup, however, there may still be a lack of food knowledge may be reflected in continued practice of berraquillo. Studies conducted by Casas and colleagues (2008), conducted interviews of local youth in Antioquia in conjunction with a university in order to ascertain food safety knowledge as it relates to crustaceans. The youth were not aware that there was risk associated with eating raw crabs (Casas, Gomez, et al., 2008).

A parasite identified as *Profilicollis bullocki* was previously identified in Chile (Riquelme, George-Nascimento, & Balboa, 2006; Rojas & Ojeda, 2005; Zambrano & George-Nascimento, 2010) and *Profilicollis chasmagnathi* was found in Argentina (La Sala, Perez, Smits, & Martorelli, 2013). Within mole crabs, *Emerita analoga* of Chile, prevalence of the parasite in male and female crabs was 68.3 and 88.6 percent, respectively (Jerez & George-Nascimento, 2010). The distribution of this acanthocephalan species that frequently infects mole crabs runs from the western coast of Washington State, North America down to Chile in South America (Goulding & Sarah Cohen, 2014). Infection is facilitated through avian migration (Balboa et al., 2009). Many species of birds migrate August through October to seek out warmer weather and more secure food sources. The Franklin Gull (*Leucophaeus pipixcan)* is one example of bird that is common in Central Northern America that migrates to the Pacific Coast of South America for the winter (Elphick, 2007). This and other gulls follow the coastal lines that pass through the Tehuantepec Isthmus of Mexico where they can continue to South America (Villegas-Patraca & Herrera-Alsina, 2015). The species of bird from which this parasite transmission occurred is unknown as these crabs were purchased from market. However, Colombia is one of the richest countries in the world for bird diversity (Velásquez-tibatá et al., 2013) and further identification of this lifecycle may inform additional birds or additional migration patterns.

Altitudinal differences between Bogotá (2600 meters) and Medellín (1500 meters) may also contribute to differences in parasite prevalence. Higher overall human parasitemias were identified of apicomplexan (Clarke et al., 2004) and comorbid with helminthes parasites in Africa (Achidi et al., 2008) at lower altitudes. Other characteristics of lower altitude include warmer temperatures, flatter land, increased vector breeding (Achidi et al., 2008) as well as increased precipitation (Meléndez et al., 2014).

Temperature differences also influence parasite prevalence as trematodes have both free-living as well as host stages. However, it does not have an effect on cercariae development within or emergence from the mollusk host in number. The trematode showed an ability to withstand higher temperatures more favorably than mollusk host, although, as the host begins to become temperature intolerant then the cercarial output does begin to decline likely to host physiological status (R. Poulin, 2006). This is an example of the indirect influences of an aspect of climate on the obligate partner may diminish response to abiotic influences (Meléndez et al., 2014).

Collection of the crustaceans may have factored into prevalence found in Bogotá. Initially, the invasive *P. clarkii* were purchased from a pet store as access to wild crayfish was unattainable. After trapping of crayfish from a golf course that frequently floods during the wet season from the Río Bogotá were the only crayfish that contained metacercariae. Additionally, collection during June is considered winter and the dry season. Due to the multiple hosts necessary for transmission of this parasite may be different during the wet season during December.

The El Niño Southern Oscillation (ENSO) is a two to seven year change in climate of the Pacific Ocean-atmosphere system that results in a warmer and drier climate. ENSO has great influence over population dynamics, species distribution, diversity, reproduction, behavior as well as water and thermal temperatures in South America (Oliva et al., 2008). The period during data collection was during and ENSO cycle and may have influenced the prevalence rates.

Effects of ENSO on current populations have been used as a proxy for climate change prediction modeling (Arntz, 2002). The cycles of ENSO may aid in projection of changing endemic parasite rates of humans.

Conclusions

Previous research on *Paragonimus* spp. indicates that infection rates are higher in other cities within Colombia. First and foremost, the altitude and temperature differences can play a part in disease transmission by impacting the prevalence intermediate hosts in the life cycle, notably the snail. Other influences can include the season collected as well as presence of drought including the El Niño Southern Oscillation (ENSO). Additional collection of specimens in the wet season as well as from the wild would aid in the strengthening of this project in the future.

Due to cultural customs and areas of poverty and food insecurity crabs and invasive crayfish may continue to be consumed raw. Educational programs within freshwater systems as well as about berraquillo are necessary to reduce the spread of infection. Due to animal reservoirs elimination of this parasite is unlikely, making educational programs the primary prevention method for disease reduction.

Profilicollis spp., while well documented in Chile, has not previously been identified in any Colombian crab species. This parasite is transmitted from birds into water sources then back to birds as they consume crab populations. While little is known about the pathogenesis of this parasite it has mostly been identified as a highly seasonal parasite. As birds are known to pass through Colombia on their seasonal migration routes, long-distance dispersal of this parasite is probable.

Future directions for this research will be to expand *N. macropa* collections to wild caught crabs and snail species. Additional considerations for future studies include collecting in the wet season when hosts are more likely to cohabitate. Identification of bird species in the areas where crabs were collected for transmission of acanthocephalans is necessary to complete the picture of the life cycle. Lastly, a more comprehensive view of total prevalence within Colombia is necessary, particularly in regions thought to be endemic for the parasite such as Chocó. Continued research can help predict future trends of parasite prevalence in order to provide targeted educational programs.

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