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Emerging Infectious Disease: Ecological Niche Modeling and Molecular Identification of *Angiostrongylus cantonensis* in Rodents from Oklahoma and Louisiana

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Emerging Infectious Disease: Ecological Niche Modeling and Molecular Identification of *Angiostrongylus cantonensis* in rodents from Oklahoma and Louisiana

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Table of Contents

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Abstract

Emerging infectious diseases (EIDs) have devastating effects on wildlife. *Angiostrongylus cantonensis* is a zoonotic EID that causes eosinophilic meningitis in humans and neurological illnesses in wildlife. Because *A. cantonensis* has been documented worldwide and continues to spread, it is a clear example of an EID of potential pathogenicity to both humans and wildlife. The advent of modeling techniques to predict the geographic distribution of pathogens, in conjunction with modern molecular genetics, provides a unique opportunity to gain insight into the distribution of *A. cantonensis*, and evaluate methods of disease surveillance.

I used the modeling program Maxent in combination with IPCC bioclimatic variables to build an ENM to predict current and future distributions of *A. cantonensis*. I tested these predictions by sampling rodents in SE Oklahoma and Louisiana and analyzing tissues for the parasite using qPCR. Out of 34 samples identified as positive, sequencing analysis revealed only three definitive identifications, one from *Sigmodon hispidus* and two from *Rattus norvegicus*. The remaining 31 samples were classified as "false positives" by qPCR. Sequences from positive samples were compared to those on GenBank through BLAST with a match to *A. cantonensis*. Phylogenetic analysis confirmed relationships by comparing positive sample sequences to *A. cantonensis* and two closely related species, *Angiostrongylus vasorum* and *Angiostrongylus costaricensis*. All phylogenetic methods grouped positive samples and *A. cantonensis* with 100% confidence.

The projected future distribution for *A. cantonensis* indicates an overall decrease in suitable habitat and a range shift. The findings from this study alter our current perspective of *A. cantonensis* within the United States, and demonstrate the successful application of two important epidemiological techniques that may be applied more broadly to a variety of EIDs.

Introduction

In recent decades, there has been a resurgence of pathogenic infections within the United States and throughout the world (Daszak, Cunningham, & Hyatt, 2000). Although this resurgence may be a result of greater awareness and surveillance, there is a growing body of evidence suggesting that wildlife epidemics are increasing in prevalence (Dobson, & Foufopoulos, 2001; Jones et al., 2008). Moreover, as wildlife continues to be reservoirs of unknown microbes, additional pathogens will emerge. As these microbes and other pathogens (avian influenza, hendra virus, etc.) evolve and adapt to new hosts, the incidence and distribution of infectious diseases will likely expand into new areas, causing new disease threats and damaging economies (Cunningham, 2005).

Because of increased contact between pathogens, humans, domestic animals and wildlife (e.g., rise in trade and travel, climate change) the epidemiological perspective, and the means by which emerging infectious diseases (EIDs) are studied, is being altered. Human EIDs are classified as diseases that have increased in incidence, have been newly discovered, or are pathogens that are moving into new species, and/or new geographic ranges (Lederberg, Shope, & Oaks, 1992). A similar definition has been applied to EIDs in wildlife, which categorizes pathogens by novel or "emerging" characteristics as well as their epizootiology (Daszak et al., 2000). With the potential of devastating effects, including high fatality rates, these EIDs are a concern to all populations.

An estimated 60-75% of all EIDs are caused by zoonotic pathogens. The majority of these have wildlife origins (Taylor, Latham, & Woolhouse, 2001), representing the greatest threat of all EIDs to global health. Large-scale declines in multiple wildlife species have been attributed to EIDs. For example, epidemics such as phocine distemper have reduced seal

populations by 30%, some vulture populations have declined by up to 90%, and mass deaths in amphibian populations are all attributable to EIDs (Daszak, & Cunningham, 1999; Heidejorgensen, Harkonen, Dietz, & Thompson, 1992; Prakesh, 1999; Roelke-Parker et al., 1996). The increased potential for such pathogens to incite epidemics worldwide emphasizes the need for collaborative effort among specialists to address this growing concern (Ecker et al., 2005). By developing a thorough knowledge base of medically relevant, zoonotic microbes and pathogens, first responders to biological threats may better assist in identifying causative agents and dispersal vectors, developing plans of action, and implementing control and preventative measures, thereby decreasing the likelihood of an epidemic (Budowle et al., 2003). Moreover, by understanding a microorganism's means of dispersal and mode of infection, specialized, rapid identification and surveillance techniques developed for one species may be applied broadly to others. Specifically, the use of molecular genetic techniques and various assays for immediate identification of microorganisms, as well as predictive ecological models for the organisms' colonization and possible range expansion are critical in furthering microbial forensics as it applies to humans and wildlife health.

The nematode *Angiostrongylus cantonensis* is an emerging infectious parasite of global concern. Known as the rat-lung worm, *A. cantonensis* displays all of the characteristics of an EIDs as it continues to expand its geographic range and cause disease in humans and wildlife. Over the past 50 years, the parasite has spread from Southeast Asia to Africa, the South Pacific, the Caribbean, India, and recently to Australia and North America, with reports in Canada and Louisiana (Kliks & Palumbo, 1992). This rapid dispersion, coupled with adverse health effects such as eosinophilic meningitis (EM) in humans (Chikweto et al., 2009), and neurological abnormalities in wildlife (Kim, Stewart, Bauer, & Mitchell, 2002), emphasizes the need for a

better understanding of the ecology of *A. cantonensis*. Currently, there is little available evidence regarding the invasion patterns of *A. cantonensis* as it moves into new regions of the world, infecting new intermediate and definitive hosts and changing its methods of dispersal. Therefore, studies targeted at expanding this knowledge base will enhance our understanding of the epidemiology of *A. cantonensis* as an emerging infectious parasite and assist in developing broadly applicable predictive approaches to disease emergence and surveillance, whether in forensics or other relevant fields.

The objective of this study was to develop and assess the use of ecological niche modeling for the EIDs pathogen, *A. cantonensis,* by sampling rodent populations in areas where there is high (Louisiana) and low (Oklahoma) probability of suitable habitat. Thus, I provide an updated account of the distribution of *A. cantonensis* within Louisiana and Oklahoma as well as potential host species for the parasite. Furthermore, predictions under three IPCC climatic scenarios were used to determine the potential future distribution of the parasite. I implemented real-time PCR techniques, in conjunction with traditional parasitological methods to identify *A. cantonensis* in rat tissue and blood samples. The findings from my study evaluate the identification methods outlined by Qvarnstrom et al. (2010) and identify the potential for new surveying techniques for *A. cantonensis*.

Suitable Climatic Conditions for Tropical Pathogenic *Angiostrongylus cantonensis* Decline under Three Climate Change Scenarios.

ABSTRACT

Climate change is implicated in the alteration of the ranges of species worldwide. Such shifts in species distributions might potentially introduce parasites/pathogens, hosts, and vectors associated with infectious disease to new areas. The parasite *Angiostrongylus cantonensis* is an invasive, pathogenic species that causes eosinophilic meningitis in humans and neurological abnormalities in domestic animals and wildlife. Although native to southeastern Asia, it has now been reported from more than 30 countries worldwide. Given the health risks from the establishment of this species, it is important to describe areas with potentially favorable climate for the establishment of *A. cantonensis*, as well as areas where this pathogen might become established in the future. I used the program Maxent to develop an ecological niche model based on 66 localities obtained from published literature. I then modeled areas of potential *A. cantonensis* distribution as well as areas projected to have suitable climatic conditions under three climate change scenarios (A1b, A2, B2) by the 2050s and the 2080s. The best model contained three bioclimatic variables, including mean diurnal temperature range, minimum temperature of coldest month and precipitation of warmest quarter. Potentially suitable habitat for *A. cantonensis* was located worldwide in tropical and subtropical regions. Under all three climate change scenarios, the center of the projected distribution shifted away from the equator at a rate of 29–285 km per decade. However, the extent of areas with highly suitable habitat declined by 50.13–64.88% by the 2050s and 40.80–56.48% by the 2080s**.** These results conflict with previous studies which have generally found that the prevalence of tropical pathogens will

increase during the 21st century. It is likely that *A. cantonensis* will continue to expand its current range in the near future due to introductions and host expansion, whereas climate change will reduce the total geographic area of most suitable climatic conditions during the coming decades.

INTRODUCTION

Changes in the distribution and phenology of many organisms were observed as the earth warmed by 0.6 ± 0.2 °C during the 20th century [1]–[4]. Since 1945, warming of the earth has been greater than any other time during the past 1,000 years [5]. Changing climate is predicted to drive 11% to 58% of vertebrate, invertebrate, and plant species to extinction by 2050 [6], and is also expected to promote expansion and/or geographic shift of tropical diseases into temperate areas [7]. Consequently, there is an urgent need to examine and model how climate change might alter infectious disease emergence within human, domestic, and wild animal populations worldwide [8].

Ecological niche modeling (ENM) predicts the fundamental and realized niche of species by relating point occurrence data of species to environmental factors [9],[10]. These models are useful in predicting the geographic range in which a species might be found, but are limited by the exclusion of detailed environmental characteristics (e.g. biotic interactions, heterogeneous landscapes). Maximum Entropy (Maxent) modeling uses environmental conditions and species presence only data to accurately estimate the distribution of a species [11]. By predicting the entire geographic range in which a species might occur, the fundamental niche of an organism is not limited by its realized niche. This approach can assess the relative importance of specific environmental factors to a species distribution, locate areas of current suitable habitat, and project changes in a species distribution over time [11].

Epidemiology uses a multifaceted approach to monitor, predict and prevent disease outbreaks. ENM is a valuable epidemiological tool because it determines the functional geographic responses of parasites and pathogens to climate change, both proximate and future. Recent studies have incorporated ENM to assess the potential impacts of climate change on infectious diseases vectors, reservoirs and/or pathogens (e.g. leishmaniasis, monkeypox, Chagas' disease, malaria and blastomycosis) [12]–[18].

Angiostrongylus (Parastrongylus) cantonensis is a parasitic nematode and a cause of the reemerging zoonotic disease, human eosinophilic meningitis, as well as neurological abnormalities in wildlife and domestic animals [19],[20]. Definitive and intermediate hosts for the parasite include rats and mollusks, respectively [21],[22]. Humans and other mammals are incidental hosts that become infected upon consumption of the third-stage larvae. Infection primarily occurs by consuming raw or undercooked mollusks or other infected paratenic hosts (e.g. freshwater prawns, frogs, monitor lizards) [21],[23],[24].

Angiostrongylus cantonensis was first documented in Guangzhou (Canton), China in 1935 [25]. In the past 50 years, the parasite has spread from Southeast Asia to over 30 countries worldwide [26],[27]. There have been more than 2,800 cases of *A. cantonensis* infection in humans worldwide with 116 cases involving U.S. citizens [27], as well as numerous infections in other animals. Given the rapid dispersal of the parasite and the health implications for humans and wildlife, there is a need to determine the potential distribution of *A. cantonensis*.

To my knowledge, no global model for the current and potential distribution of *A. cantonensis* has been published. Although Lv et al. [22] published a comprehensive distribution of *A. cantonensis* within China, their model did not examine the potential distribution worldwide. The aim of this study was to use Maxent modeling to determine the maximum range distribution

for the parasite globally and predict the potential future distribution of *A. cantonensis* under Intergovernmental Panel on Climate Change (IPCC) climate change scenarios.

RESULTS

The best model (i.e. the model with the lowest small sample corrected variant of Akaike's Information Criterion (AICc) score) included three environmental variables; mean diurnal temperature range (BIO 2), minimum temperature of coldest month (BIO 6) and precipitation of warmest quarter (BIO 18; Table 2). The area under the curve (AUC) was 0.947 ± 0.031 for this model. Figure 4 displays suitability in response to the three variables. Areas that were predicted to have suitability > 50% had a mean diurnal temperature range of 5.13–8.76° C, a minimum temperature of the coldest month of 14.79–32.17° C, and precipitation of the warmest quarter of 438.37–2,224.20 mm (Fig. 4). Areas with > 50% suitability were found primarily in tropical areas (Fig. 5), including the reported native range in southeast Asia.

Areas with suitable climatic conditions for *A. cantonensis* are predicted to decline by the 2050s and the 2080s under all three scenarios (Fig. 6). Currently, 6,023,577 km² are highly suitable (i.e. >50% chance of suitability). However, by the 2050s the amount of highly suitable habitat is expected to decrease to 3,819,402–5,397,195 km², with only 50.13–64.88% of the area in common with the current model (Table 3). By the 2080s, the area of highly suitable habitat will further decline in the A1B and B2A scenarios to 3,653,894 km² and 5,122,298 km², respectively (Table 3). The two scenarios had only 40.80–56.48% of the area shared with the current model (Table 3). However, the A2A scenario predicts a slight increase in area (from 4,492,529 km² to 4,524,055 km²) over the 30 year period from the 2050s–2080s with 46.41% of the area in common with the current model (Table 3). The centroid (geometric center of the species range) for the northern hemisphere will shift northeast to east-northeast 745–984 km by

the 2050s, a rate of 149–197 km per decade (Table 4). By the 2080s, the centroid will shift further to the northeast by 1,061–2,532 km, a rate of 134–316 km per decade. In the southern hemisphere, the centroid will shift south 145–148 km by the 2050s for A1B and A2A scenarios, at a rate of 29–30 km per decade (Table 4). By the 2050s, under the B2A scenario the centroid will shift 442 km west-southwest, a rate of 88 km per decade (Table 4). By the 2080s, the centroid will shift south to east-southeast 147–890 km, a rate of 18–111 km per decade (Table 4).

DISCUSSION

The effects of global climate change are hypothesized to result in direct disease range expansions (via pathogens spread) and indirect expansions (via reservoirs, hosts, or vector range expansions). This will increase the frequency of disease outbreaks and expand the pool of at risk populations [16],[28],[29]. Hales et al. [30], predicts an increase in land area compatible for Dengue fever transmission by 2085, with 50–60% of the world's population at risk. Within North America, leishmaniasis reservoirs and vectors are predicted to undergo a range expansion northward, leading to greater human exposure [18]. However, an emerging picture of the effects of global climate change on disease is that an increase in habitat suitability in one area will be counterbalanced by decreased suitability elsewhere, leading to a range shift or reduction [4],[31]. Though the proximate expansion of *A. cantonensis* into new suitable regions continues via introduction of definitive and intermediate hosts, my findings predict an ultimate decline of up to 39% in the area of suitable bioclimatic habitat by the 2080s.

The global model for the present distribution of *A. cantonensis* predicts that the most suitable habitat is located near the equator in tropical to subtropical regions. Three bioclimatic variables were found to contribute the most to predicting the potential distribution of the parasite: minimum temperature of the coldest month, minimum diurnal temperature range, and

precipitation of the warmest quarter. Under all IPCC climatic scenarios, my models predict a shift in the distribution of suitable habitat for *A. cantonensis* in the Northern and Southern hemispheres. Under the A1b, A2A, and B2A climatic scenarios, a shift in the parasite's distribution is expected to occur north and east in the Northern hemisphere by the 2050s (range = 149–197 km per decade) and continuing through the 2080s (range = 133–316 km per decade). The shift in the parasite's distribution in the Southern hemisphere under all climatic scenarios was predicted to occur southward by the 2050s (range = 29–88 km per decade) and through the 2080s (range = 18–111 km per decade). Although there have been no endemic reports of *A. cantonensis* within Europe, all three models suggest an increase in suitable habitat for *A. cantonensis* within Europe while simultaneously showing an overall decline in global suitability. This potential range shift into Europe is most likely due to a predicted increase in the minimum temperature of the coldest month, which demonstrates the need for additional monitoring programs within Europe. These programs should include long term surveying or screening for the parasite within definitive, paratenic, and intermediate hosts. Furthermore, an increase in public health programs targeted at awareness of the parasite and its transmission will be essential in deterring an increase in human infection.

Temperature and precipitation are environmental variables that significantly influence the distribution of *A. cantonensis*. Because temperature plays a critical role in influencing biological processes [4] it will likely have a significant impact on pathogens, infectious disease hosts, vectors and reservoirs. As global temperatures rise (IPCC), there is increased potential for vector-borne diseases and pathogens to spread and/or increase in severity [36],[32]–[34]. Increases in temperature can speed the rate of development for some malarial protozoa, increasing the risk of transmission from mosquito to host [35]. However, the positive association

between temperature and pathogen transmission might be offset by a pathogen's total bioclimatic requirements for survival. If such requirements are not met, host, vector, and/or pathogen mortality might increase. Similarly, increased temperatures might initially further the spread and occurrence of *A. cantonensis*. However, with an expected temperature increase of 1.4°C–5.8°C from 1990 to 2100 [36] in areas where the bio-climatic norm exceeds an ecologically critical threshold temperature, resources needed to support parasitic growth and reproduction may become increasingly limited [4],[37]. Such demands could restrict the parasites' distribution to areas with sufficient resources, potentially limiting disease incidence.

Global climate change is expected to increase the risk of intense precipitation and increased humidity in some regions, whereas other regions will experience extreme drought [36]. The effects of climatic variability in precipitation might induce the emergence of diseases in new areas or intensify infection rates of endemic pathogens. In several cases, disease occurrence has been demonstrated to be positively [33],[38]–[41] associated with rainfall. Alternatively, regions experiencing drought might negatively impact pathogen viability. Many parasites having intermediate hosts, such as *A. cantonensis*, require moist or wet environments for development and survival. Without sufficient precipitation, the distribution of the parasite might become more restricted, thereby decreasing the risk of transmission.

As *A. cantonensis* continues to spread, health complications in both humans and wildlife are expected to increase. Following introduction into a new area, *A. cantonensis* quickly infects and causes illness in humans, domestic animals and wildlife [19],[42]–[44]. Infected humans are often hospitalized with eosinophilic meningitis, and might also experience extraocular muscular paralysis [45]. In wildlife, *A. cantonensis* can cause a variety of symptoms (e.g. lethargy, limb paralysis) due to neurological invasion and might result in death [19],[42],[46]–[50]. To deter

future outbreaks, *A. cantonensis* monitoring programs should be established worldwide, evaluating known definitive, intermediate and paratenic hosts and other wildlife. In addition, increase public awareness of the parasite and the means by which it is transmitted, may lead to a lower incidence of infection.

These results provide the first global perspective of high risk areas for *A. cantonensis* colonization. The methodology employed here has been applied broadly to other studies on global climate change. More recently the application of ENM in evaluating disease distribution, risks and spread [12],[13],[17],[18],[51],[52] has proven useful. By identifying and documenting information (e.g. distributions, ontogenetic requirements) on known hosts (e.g. *Rattus* sp., molluscs) and conducting field surveys for the parasite, future studies might provide a much improved and conservative representation of *A. cantonensis'* current and future range.

METHODS

Maxent was used to model the current and projected distribution of *A. cantonensis*. Documented occurrences of *A. cantonensis* were collected from published records. Records of the parasite in endemic areas or reports of the parasite found in intermediate or definitive hosts were used (Table S1). A total of 66 locations were included (Figure 1). Elevation and 19 climate variables were downloaded from WorldClim [53] with a resolution of 5 arc-minutes (100 km²; Table 1). All variables were included in the model initially. However, only the variables with the highest gain independent of others (Fig. 2) were retained, as these variables accounted for the greatest amount of the observed variation. In addition, the environmental variables that lowered the training gain the greatest when omitted were retained (Fig. 3), as these variables contained the most unique information. These variables were then retained for high multicollinearity ($|r|>0.8$) [54]. Additionally, AICc was used to evaluate the regularization of the models and to

avoid overfitting [55]. All possible combinations of the variables that did not exhibit high multicollinearity were examined. Ten-fold cross-validation was used and receiver operating characteristic (ROC) curves were created by plotting sensitivity vs. 1–specificity to evaluate the accuracy of the resulting model. The AUC were used to evaluate models. Models with an AUC score of 0.5 indicated a model preforming no better than random, while models with AUC score of 1 indicated a perfect model [11],[56]. However, AUC scores are not without limitations [57],[58] and should be used in conjunction with other model evaluation methods [59]. Consequently, I used AICc scores and model weights along with AUC scores to determine the model that best describes the current distribution of *A. cantonensis*.

IPCC 4 data for future climate conditions for the 2050s and 2080s were obtained from the International Centre for Tropical Agriculture (CIAT) [60] in order to project the potential future distribution of *A. cantonensis* at 5 arc-minutes (100 km^2). Three IPCC scenarios were evaluated; A1b (described as rapid economic growth, but with a balanced emphasis on all energy resources), A2A (characterized by slower economic growth), and B2A (which has a greater emphasis on environmental stability) using the Canadian Center for Climate Modeling Analysis Coupled Global Climate Models [60]. Results are presented as mean ± standard deviation.

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REFERENCES

1. Hughes L (2000) Biological consequences of global warming: is the signal already apparent?. Trends Ecol Evol 15: 56-61.

2. Butler CJ (2003) The disproportionate effect of climate change on the arrival dates of short distance migrant birds. Ibis 145: 484-495.

3. Parmesan C, Yohe, G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42. doi:10.1038/nature01286

4. Lafferty KD (2009) The ecology of climate change and infectious diseases. Ecology 90: 888-900.

5. Intergovernmental Panel on Climate Change (2007). Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.

6. Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, et al. (2004) Extinction risk from climate change. Nature 427: 145–148. doi:10.1038/nature02121

7. Epstein PR (2000) Is global warming harmful to health? Sci Am 283: 50-57.

8. Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife--threats to biodiversity and human health. Science 287: 443-499.

9. Peterson AT, Kluza DA (2005) Ecological niche modeling as a new paradigm for large-scale investigations of diversity and distribution of birds. USDA Forest Service General Technical Reports PSW-GTR-191: 1201-1204.

10. Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Glob Ecol Biogeogr 12: 361–371.

11. Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecol Modell 190: 231–259.

12. Peterson AT, Shaw J (2003) *Lutzomyia* vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. Int J Parasitol 33: 919-931.

13. Levine RS, Peterson AT, Yorita KL, Carroll D, Damon IK, Reynolds MG (2007) Ecological niche and geographic distribution of human monkeypox in Africa. PLoS One 2: e176.

14. Costa J, Peterson AT, Beard CB (2002) Ecological niche modeling and differentiation of populations of* *Triatoma brasiliensis** Neiva, 1911, the most important Chagas disease vector in northeastern Brazil (Hemiptera, Reduviidae, Triatominae). Am J Trop Med Hyg 67: 516-520.

15. Tanser FC, Sharp B, Le Sueur D (2003) Potential effect of climate change on malaria transmission in Africa. The Lancet 362: 1792-1798.

16. Martens WJ, Niessen LW, Rotmans J, Jetten TH, McMichael AJ (1995) Potential impact of global climate change on malaria risk. Environ Health Perspect 103: 458-464.

17. Reed, KD, Meece JK, Archer JR, Peterson AT (2008) Ecological niche modeling of *Blastomyces dermatitidis* in Wisconsin. PLoS ONE 3: e2034. Doi: 10.1371/journal.pone.0002034

18. González C, Wang O, Strutz SE, González-Salazar C, Sánchez-Cordero V, et al. (2010) Climate change and risk of Leishmaniasis in North America: predictions from ecological niche models of vector and reservoir species. PLoS Negl Trop Dis 4: e585. doi:10.1371/journal.pntd.0000585

19. Kim DY, Stewart TB, Bauer RW, Mitchell M (2002) *Parastrongylus* (=*Angiostrongylus*) *cantonensis* now endemic in Louisiana. J Parasitol 88: 1024-1026.

20. Qvarnstrom Y, et al. (2007) PCR-based detection of *Angiostrongylus cantonensis* in tissue and mucus secretions from molluscan hosts. Ap Appl Environ Microbiol 73: 1415-1419.

21. Chikweto A, Bhaiyai M, Maepherson CNL, DeAllie C, Pickney RD et al. (2009) Existence of *Angiostrongylus cantonensis* in rats (*Rattus norvegicus*) in Grenada, West Indies. Vet Parasitol 162: 160-162.

22. Lv S, Zhang Y, Liu H-X, Hu L, Yang K et al. (2009) Invasive snails and an emerging infectious disease: results from the first national survey on *Angiostrongylus cantonensis* in China. PLoS Negl Trop Dis 3: e368. doi:10.1371/journal.pntd.0000368

23. Lai C-H, Yen C-M, Chin C, Chung H-C, Kuo H-C et al. (2007) Eosinophilic meningitis caused by *Angiostrongylus cantonensis* after ingestion of raw frogs. Am J Trop Med Hyg 76: 399-402.

24. Radomyos P, Tungtrongchitr A, Praewanich R (1992) Experimental infection of yellow tree monitor (*Varanus bengalensis*) and related species with *Angiostrongylus cantonensis*. Southeast Asian J Trop Med Public Health 23: 167-168.

25. Chen HT (1935) Un nouveau nematode pulmonaire, *Pulmonema cantonensis* n. g., n. sp., des rats de Canton. Ann Parasitol Hum Comp 13: 312-317.

26. Kliks MM, Palumbo NE (1992) Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis by *Angiostrongylus cantonensis,* the nematode lungworm of rats. Soc Sci Med 34: 199-212.

27. Wang QP, Lai D-H, Zhu X-Q, Chen X-G, Lun Z-R (2008) Human angiostrongyliasis. Lancet Infect Dis 8: 621-630.

28. Patz JA, Martens WJ, Focks DA, Jetten TH (1998) Dengue fever epidemic potential as projected by general circulation models of global climate change. Environ Health Perspect 106: 147-153.

29. Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, et al. (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296: 2158-2162.

30. Hales S, de Wet N, Maindonald J, Woodward (2002) Potential effect of population and climate changes on global distribution of dengue fever: an empirical model. Lancet 360: 830-834.

31. Kutz SJ, Jenkins EJ, Veitch AM, Ducrocq J, Polley L, et al. (2009) The Arctic as a model for anticipating, preventing, and mitigating climate change impacts on host–parasite interactions. Vet Parasitol 163: 217-228.

32. Kutz SJ, Hogberg EP, Polley L, Jenkins EJ (2005) Global warming is changing the dynamics of Arctic host–parasite systems. Proc R Soc B 272: 2571-2576.

33. Lal A, Baker MG, Hales S, French NP (2012) Potential effects of global environmental changes on cryptosporidiosis and giardiasis transmission. Trends Parasitol 29: 83-90.

34. Poulin R (2006) Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132: 143-151.

35. McArthur RH (1972) Geographical Ecology: Patterns in the distribution of a species. New Jersey: Princeton University Press.

36. Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, et al. (2001) Climate Change 2001: The Scientific Basis. Cambridge: Cambridge University Press.

37. Smith G (1990) The population biology of the free living phase of *Haemonchus contortus*. Parasitology 101: 309-316.

38. Bouma MJ, Hugo JK (1996) The EI Niño Southern Oscillation and the historic malaria epidemics on the Indian subcontinent and Sri Lanka: an early warning system for future epidemics?. Trop Med Int Health 1: 86-96.

39. Bouma M J, Poveda G, Rojas W, Chavasse D, Quinones M, et al. (1997) Predicting high-risk years for malaria in Colombia using parameters of El Niño Southern Oscillation. Trop Med Int Health 2: 1122-1127.

40. Woodruff RE, Guest CS, Garner MG, Becker N, Lindesay J, et al. (2002) Predicting Ross River virus epidemics from regional weather data. Epidemiology 13: 384-393.

41. Curriero FC, Patz JA, Rose JB, Lele S (2001) The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948–1994. Am J Public Health 91:8 1194-1199.

42. Duffy MS, Miller CL, Kinsella JM, de Lahunta A (2004) *Parastrongylus cantonensis* in a nonhuman primate, Florida. Am J Trop Med Hyg 10: 2207-2210.

43. New D, Little MD, Cross J (1995) *Angiostrongylus cantonensis* infection from eating raw snails. N Engl J Med 332:1105-1106.

44. Panackel C, Cherian G, Vijayakumar K, Sharma RN (2006) Eosinophilic meningitis due to *Angiostrongylus cantonensis*. Indian J Med Microbiol 24:220-221.

45. Morgan U (2000) Detection and characterization of parasites causing emerging zoonoses. Int J Parasitol 30: 1407-1421.

46. Higgins D, Carlisle-Novack M, & Mackie J (1997) Neural angiostrongylosis in three captive rufous bettongs (*Aepyprymnus rufescens*). Aust Vet J 75: 564-566.

47. Mason, K (1987) Canine neural angiostrongylosis: The clinical and therapeutic features of 55 natural cases. Aust Vet J 64: 201-203.

48. McKenzie R, Green P, Wood A (1978) *Angiostrongylus cantonensis* infection of the brain of a captive Bennett's wallaby (*Macropus rufogrieseus*). Aust Vet J 54: 86-88.

49. Wright J, Kelly W, Wadell A, Hamilton J (1991) Equine neural angiostrongylosis. Aust Vet J 68: 58-60.

50. Monks DJ, Carlisle MS, Carrigan M, Rose K, Spratt D, Gallagher A, Prociv P (2005) *Angiostrongylus cantonensis* as a cause of cerebrospinal disease in a yellow-tailed black cockatoo (*Calyptorhynchus funereus*) and two tawny frogmouths (*Podargus strigoides*). J Avian Med Surg 19: 289-293.

51. Peterson AT, Bauer JT, Mills JN (2004) Ecologic and geographic distribution of filovirus disease. Emerg Infect Dis 10: 40-47.

52. Levine RS, Peterson AT, Benedict MQ (2004) Geographic and ecologic distributions of the *Anopheles gambiae* complex predicted using a genetic algorithm. Am J Trop Med Hyg 70: 105-109.

53. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25: 1965-1978.

54. Jones CC, Acker SA, Halperin CB (2010) Combining local- and large-scale models to predict the distributions of invasive plant species. Ecol Appl 20: 311-326.

55. Warren DL, Seifert SM (2011) Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecol Appl 21: 335–342.

56. Phillips SJ, Dudik M, Schapire RE (2004) A maximum entropy approach to species distribution modeling. Pgs. 655-662 In Proceedings of the Twenty-First International Conference on Machine Learning.

57. Lobo JM, Jiménez-Valverde A, Real R (2008) AUC: a misleading measure of the performance of predictive distribution models. Glob Ecol Biogeogr 17: 145-151. doi: 10.1111/j.1466-8238.2007.00358.x

58. Elith J, Phillips SJ, Hastie T, Miroslav D, Chee YE et al. (2011) A statistical explanation of Maxent for ecologists. Divers Distrib 17: 43-57.

59. So H-C, Sham PC (2010) A unifying framework for evaluating the predictive power of genetic variants based on the level of heritability explained. PLoS Genet 6: e1001230. doi:10.1371/journal.pgen.1001230

60. Ramirez J, Jarvis A (2008) High resolution statistically downscaled future climate surfaces. Available: http://www.ccafs-climate.org/. Accessed 5 September 2012.

61. Lindo JF, Waugh C, Hall J, Cunningham-Myrie C, Ashley D, et al. (2002) Ezoonotic *Angiostrongylus cantonensis* in rats and snails after an outbreak of human eosinophilic meningitis, Jamaica. Emerg Infect Dis 8: 324-326.

62. Stokes VL, Spratt DM, Banks PB, Pech RP, Williams RL (2007) Occurrence of *Angiostrongylus species* (Nematoda) in populations of *Rattus rattus* and *Rattus fuscipes* in coastal forests of south-eastern Australia. Aust J Zool 55: 177-184.

63. Lv S, Zhang Y, Steinmann P, Zhou X-N (2008) Emerging angiostrongyliasis in mainland China. Emerg Infect Dis 14: 161-164.

64. Lin JX, Li YS, Zhu K, Chen BJ, Cheng YZ, et al. (2003) Epidemiological study on group infection of *Angiostrongylus cantonensis* in Changle City. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 21: 110-112.

65. Luo B, Chen JY, Ye DG, Liu BR, Zheng P (2005). A survey on epidemic focus of *Angiostrongylus cantonensis* in Fuzhou. Zhongguo Ren Shou Gong Huan Bing Za Zhi 21: 829-830.

66. Wei LP, Zheng KW, Wei Y (2005) A report on clinically nursing 9 angiostrongyliasis patients. J Clin Nurs 4: 21–22.

67. Foronda P, Lopez-Gonzalez M, Miquel J, Torres J, Segovia M, et al. (2010) Finding of *Parastrongylus cantonensis* (Chen, 1935) in *Rattus rattus* in Tenerife, Canary Islands (Spain). Acta Trop 114: 123-127.

68. Aguiar PH, Morera P, Pascual J (1981) First record of *Angiostrongylus cantonensis* in Cuba. Am J Trop Med Hyg 30: 963-965.

69. Ash LR (1968) The occurrence of *Angiostrongylus cantonensis* in frogs of New Caledonia with observations on paratenic hosts of metastrongyles. J Parasitol 54: 432-436.

70. Andersen E, Gubler DJ, Sorensen K, Beddard J, Ash LR (1986) First report of *Angiostrongylus cantonensis* in Puerto Rico. Am J Trop Med Hyg 35: 319-322.

71. Bowden DK (1981) Eosinophilic meningitis in the New Hebrides two outbreaks and two deaths. Am J Trop Med Hyg 30: 1141-1143.

72. Yii C-Y, Chen C-Y, Chen E-R, Hsieh H-C, Shih C-C, et al. (1975) Epidemiological studies of eosinophilic meningitis in Southern Taiwan. Am J Trop Med Hyg24: 447-454.

73. Punyagupta S, Bunnag T, Juttijudata P, Rosen L (1970) Eosinophilic meningitis in Thailand. Am J Trop Med Hyg 19: 950-958.

74. Yong WK, Welch JS, Dobson C (1981) Localized distribution of *Angiostrongylus cantonensis* among wild rat populations in Brisbane, Australia. Southeast Asian J Trop Med Public Health 12: 608-609.

75. Hochberg NS, Park SY, Blackburn BG, Sejvar JJ, Gaynor K, et al. (2007) Distribution of esosinophilic meningitis cases attributable to *Angiostrongylus cantonensis*, Hawaii. Emerg Infect Dis 13: 1675-1680.

76. Lin W, Wang XT (2004) Epidemiology of *Angiostrongylus cantonensis* in mainland. Zhongguo Ren Shou Gong Huan Bing Za Zhi 20: 1004-1007.

77. Carney WP, Stafford EE, Purnomo, Tanudjaja S (1978) Angiostrongyliasis in Indonesia: additional geographic and host occurrence records. Southeast Asian J Trop Med Public Health 9: 516-519.

78. Uchikawa R, Takagi M, Matayoshi S, Sato A (1984) The presence of *Angiostrongylus cantonensis* in Viti Levu, Fiji. J Helminthol 58: 231-234.

79. Raccurt CP, Blaise J, Durette-Desset MC (2003) Presence of *Angiostrongylus cantonensis* in Haiti. Trop Med Int Health 8: 423-426.

80. Scrimgeour EM, Welch JS (1984) *Angiostrongylus cantonensis* in east New Britain, Papua New Guinea. Trans R Soc Trop Med Hyg 78: 774-775.

81. Vargas, M, Gomez Perez JD, Malek EA (1992) First record of *Angiostrongylus cantonensis* (Chen, 1935) (Nematoda: Metastrongylidae) in the Dominican Republic. Trop Med Parasitol 43: 253-255.

82. Nishimura K, Moqi M, Okazawa T, Sato Y, Toma H, Wakibe H (1986) *Angiostrongylus cantonensis* infection in *Ampullarius canaliculatus* (Lamarck) in Kyushu, Japan. Southeast Asian J Trop Med Public Health 17: 595-600.

Figure 1. Current distribution of *A. cantonensis*. The locations taken from the literature where *A. cantonensis* (n=66) has been reported.

Figure 2. Jackknife of regularized training gain for individual variables. Withholds all variables but one. The higher the gain, the more important the variable.

Figure 3. Jackknife of regularized training gain omitting each variable is shown below. Withholds one variable. The lower the gain, the more unique information is embedded in the variable.

Figure 4. Probability of *A. cantonensis* presence in response to ecogeographical variables in the best fit models.

Figure 5. The Maxent model of the projected current distribution for *A. cantonensis.*

Figure 6. Comparison of the model runs for *A. cantonensis*. The probability of *A. cantonensis* occurrence is color coded in the legend; the brick red shade shows an area with >0.5 probability of occurrence.

Figure 7. The Northern and Southern Hemisphere centroids. Indicated by stars. Shows the geometric center of the distribution for *A. cantonensis* under the A1B, A2A and B2A scenarios.

Figure 1:

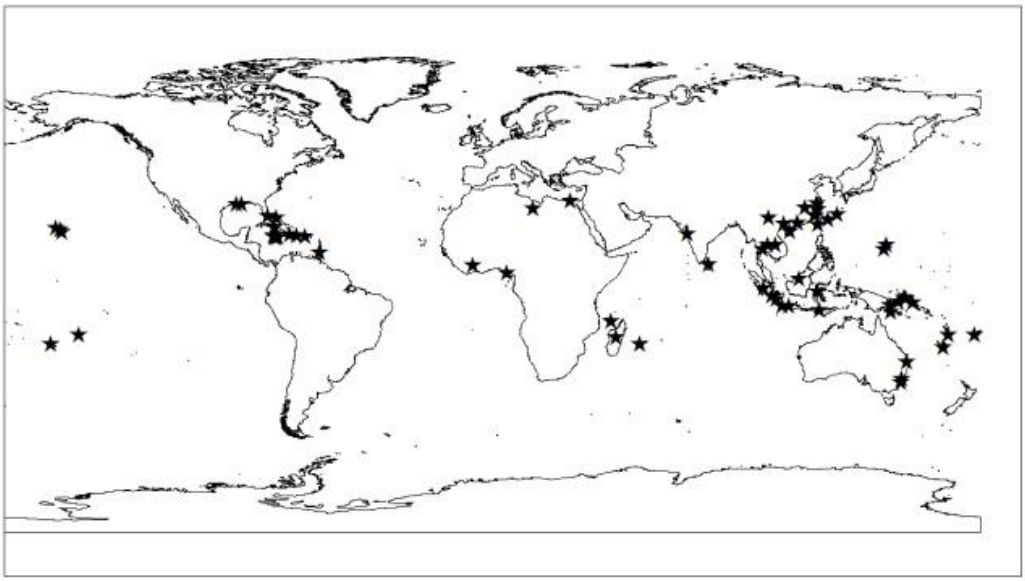

Figure 2:

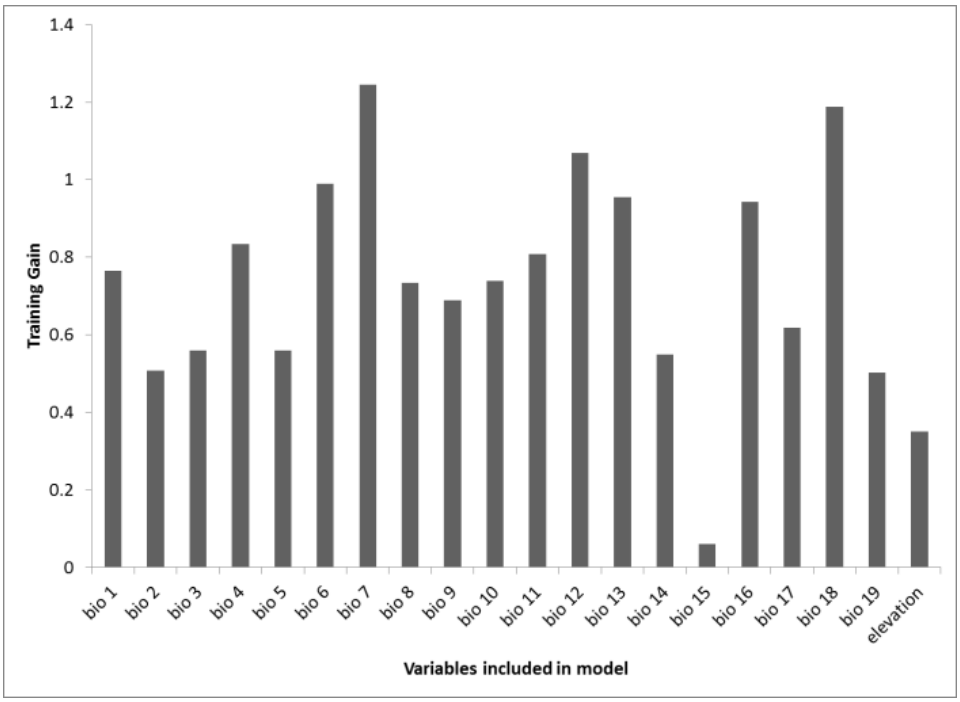
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Training Gain
bio 1
bio 2
bio 3
bio 4
bio 5
bio 6
bio 7
bio 8
bio 9
bio 10
bio 11
bio 12
bio 13
bio 14
bio 15
bio 16
bio 17
bio 18
bio 19
elevation
Variables included in model

Figure 3:

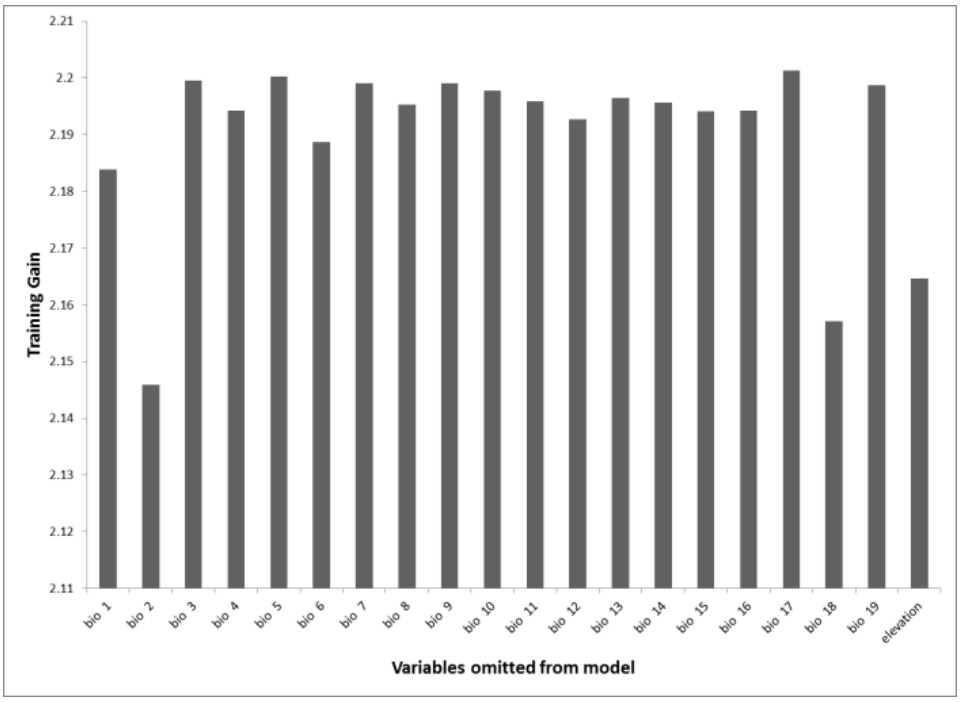
Training Gain
2.21
2.2
2.19
2.18
2.17
2.16
2.15
2.14
2.13
2.12
2.11
bio 1
bio 2
bio 3
bio 4
bio 5
bio 6
bio 7
bio 8
bio 9
bio 10
bio 11
bio 12
bio 13
bio 14
bio 15
bio 16
bio 17
bio 18
bio 19
elevation
Variables omitted from model

Figure 4:

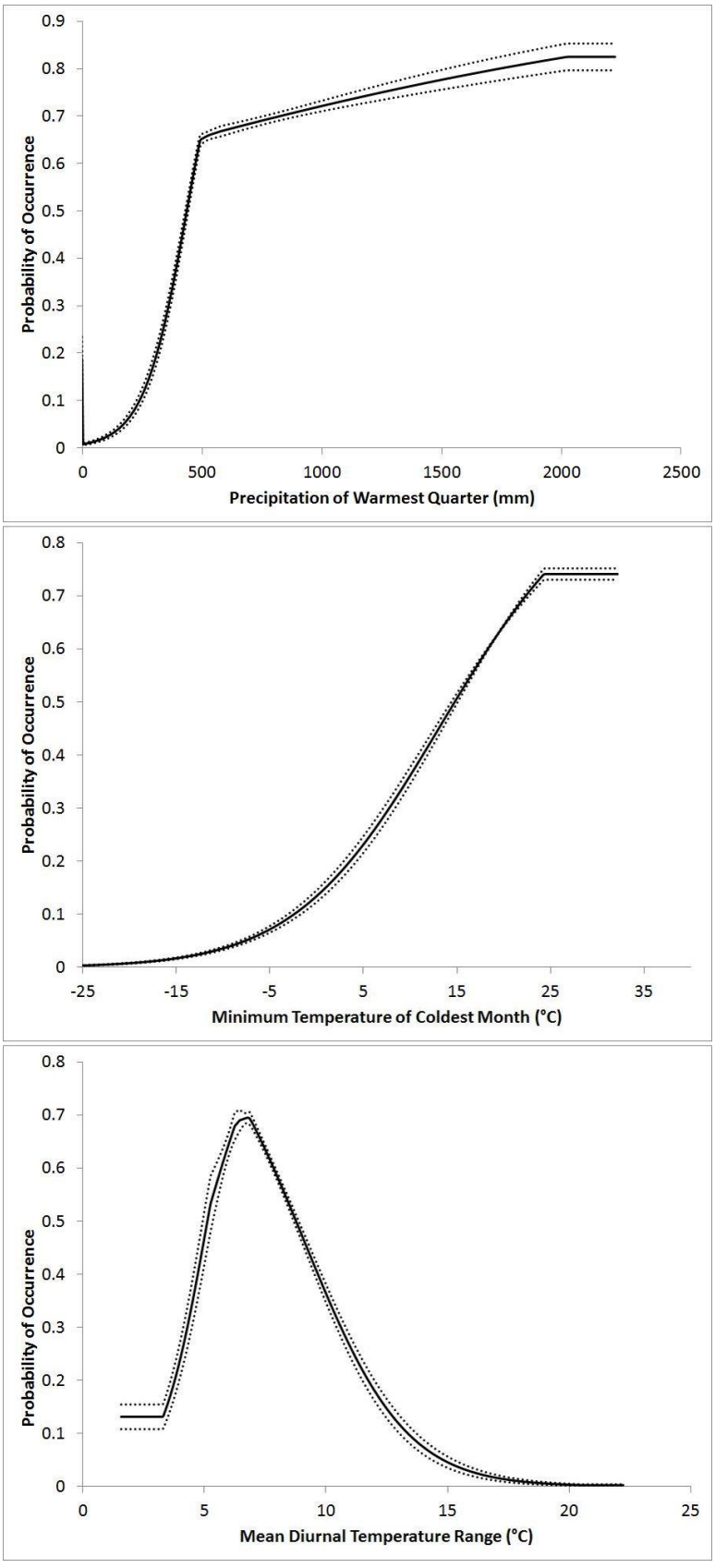
Probability of Occurrence
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
2500
Precipitation of Warmest Quarter (mm)
Probability of Occurrence
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-25
-15
-5
5
15
25
35
Minimum Temperature of Coldest Month (°C)
Probability of Occurrence
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
Mean Diurnal Temperature Range (°C)

Figure 5:

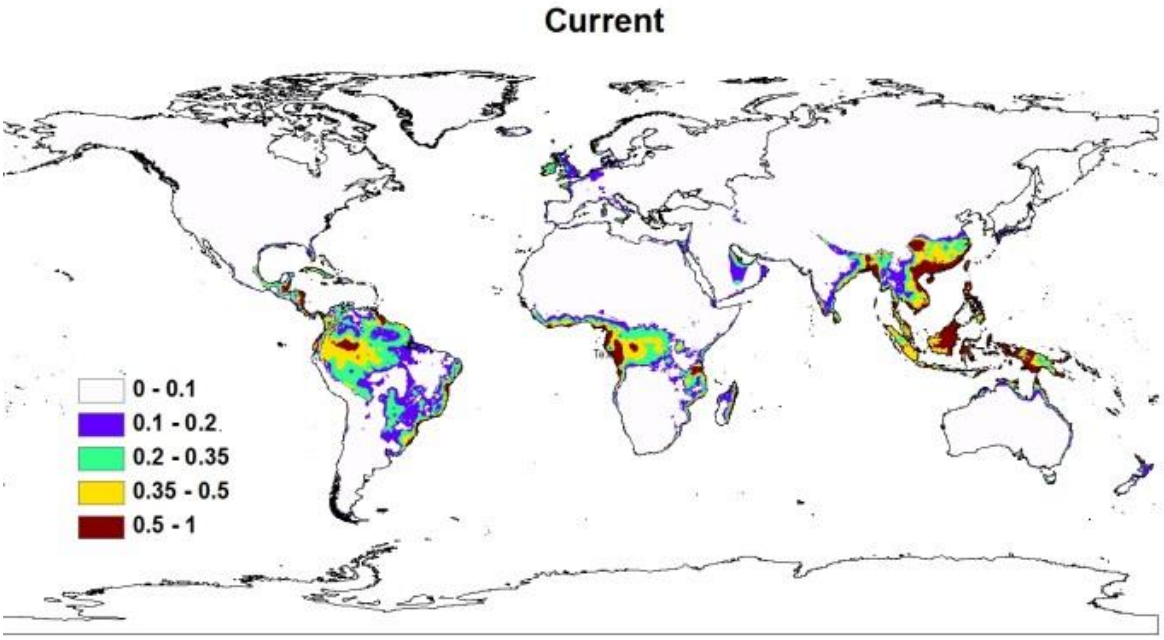

Figure 6:

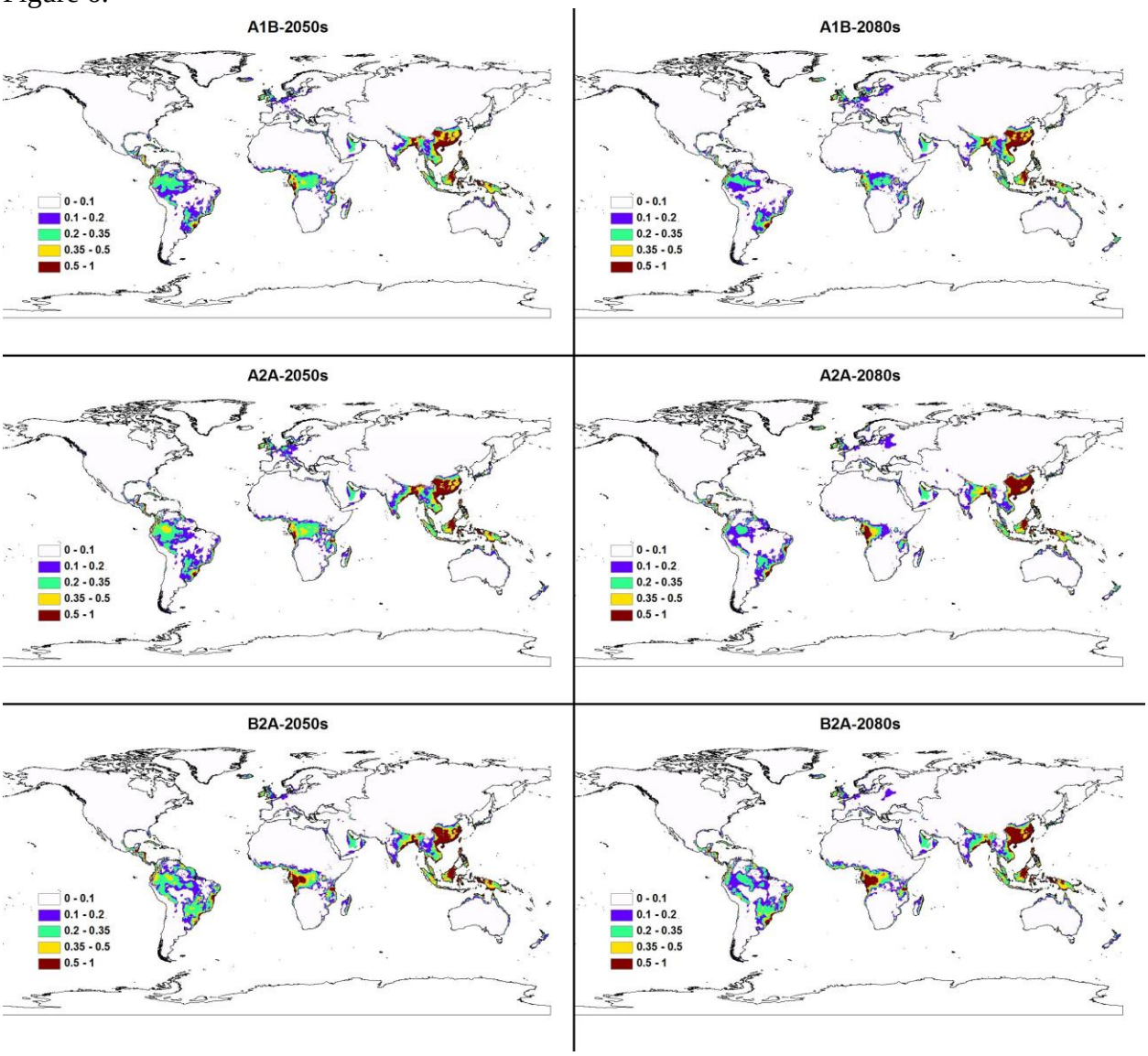
A1B-2050s
A1B-2080s
A2A-2050s
A2A-2080s
B2A-2050s
B2A-2080s
0 - 0.1
0.1 - 0.2
0.2 - 0.35
0.35 - 0.5
0.5 - 1

Figure 7:

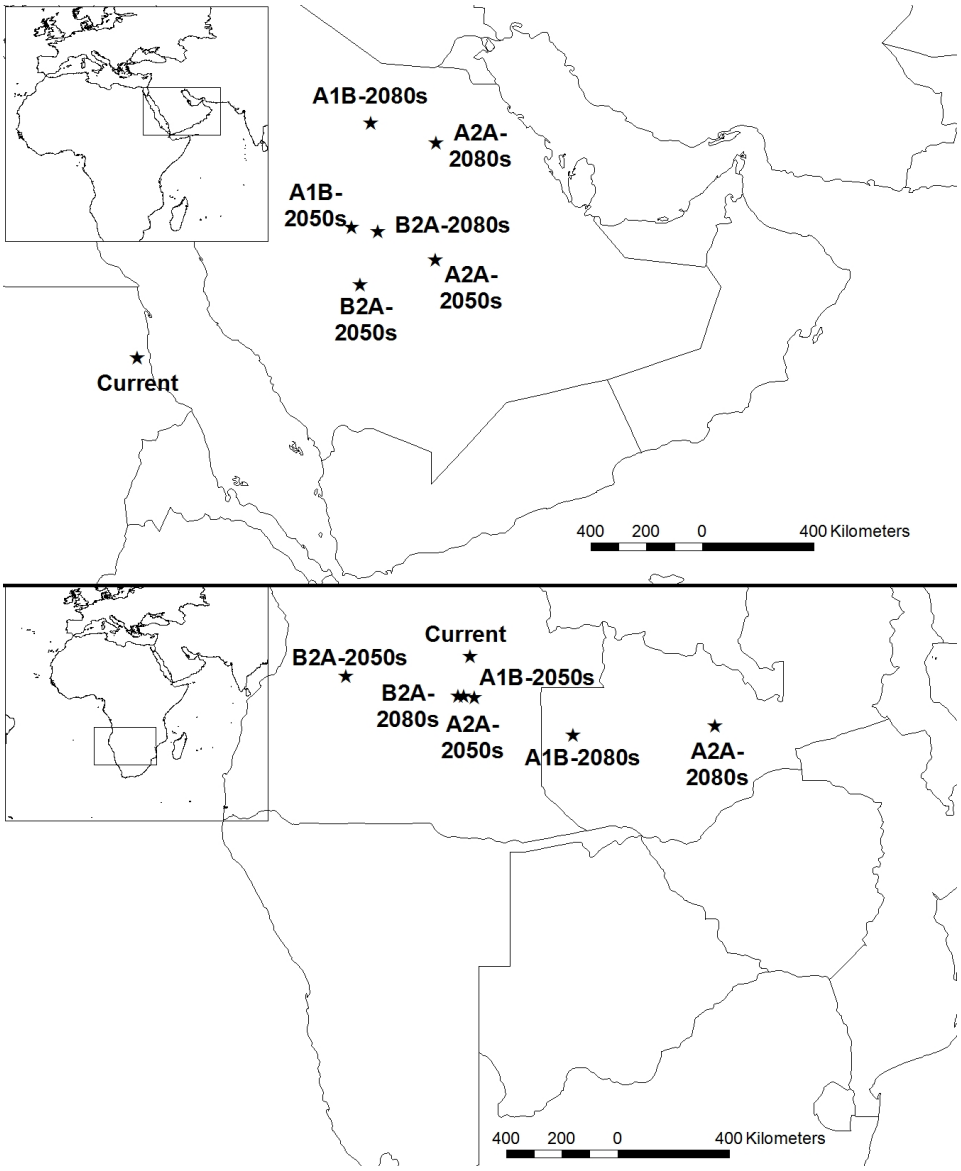
A1B-2080s
A2A-2080s
A1B-2050s
B2A-2080s
A2A-2050s
B2A-2050s
Current
400 200 0 400 Kilometers
Current
B2A-2050s
A1B-2050s
B2A-2080s
A2A-2050s
A1B-2080s
A2A-2080s
400 200 0 400 Kilometers

Table 1. Bioclimatic variables used in the construction of the niche models.

Variable	Definition
BIO 1	Annual mean temperature
BIO 2	Mean diurnal range (Mean of monthly [max temp - min temp])
BIO 3	Isothermality (BIO 2 / BIO 7) * 100
BIO 4	Temperature seasonality (standard deviation * 100)
BIO 5	Max temperature of warmest month
BIO 6	Min temperature of coldest month
BIO 7	Temperature annual range (BIO 5 - BIO 6)
BIO 8	Mean temperature of wettest quarter
BIO 9	Mean temperature of driest quarter
BIO 10	Mean temperature of warmest quarter
BIO 11	Mean temperature of coldest quarter
BIO 12	Annual precipitation
BIO 13	Precipitation of wettest month
BIO 14	Precipitation of driest month
BIO 15	Precipitation seasonality (coefficient of variation)
BIO 16	Precipitation of wettest quarter
BIO 17	Precipitation of driest quarter
BIO 18	Precipitation of warmest quarter
BIO 19	Precipitation of coldest quarter
Elevation	Elevation above sea level

Table 2. Comparison of the top three possible models.

Variables in Model	Log Likelihood	AICc scores	ΔAICc	wAICc	Mean AUC
BIO 2, BIO 6, BIO 18	-815.871	1664.742703	0	0.904935182	0.947
BIO 2, BIO 6	-827.454	1670.838066	6.095362635	0.042956243	0.941
BIO 2, BIO 6, BIO 12, BIO 18	-812.654	1672.058673	7.31596974	0.023333228	0.946

*Log-likelihood is the natural log of the probability of the data given in the model. AICc is a corrected AIC score, used for a small sample size by increasing the cost for each parameter. Delta AICc is the difference between the model with the lowest score (the "best" model) and the AICc score for each model. The model weight (wAICc) is the relative likelihood for each model, divided by the total relative likelihood for all models that were considered. AUC (area under the curve) is a measure of the accuracy of the model.

Table 3. The total area predicted to have >50% probability of suitable habitat conditions for *A. cantonensis* under each climate change scenario.

Scenario	Area (km^2)	% change in area	Area common to current (km^2)	% of current distribution retained
Current	6023577.53			
2050s – A1B	3819401.98	-36.59 %	3019561.24	50.13 %
2050s – A2A	4492528.57	-25.42%	3247301.62	53.91 %
2050s – B2A	5397195.02	-10.40%	3908147.80	64.88 %
2080s – A1B	3653893.62	-39.34%	2457742.65	40.80 %
2080s – A2A	4524055.46	-24.89%	2795431.91	46.41 %
2080s – B2A	5122298.26	-14.96%	3402096.64	56.48 %

Table 4. A summary of the distance from each projected centroid for each scenario (A1B, A2A, B2A) to the current centroid as well as the rate per decade.

Scenario	Distance (km) to current centroid	Rate of km per decade
Current North		
2050s – A1B	852.66 (NE)	170.53 km / decade
2050s – A2A	984.38 (ENE)	196.88 km / decade
2050s – B2A	745.35 (ENE)	149.07 km / decade
2080s – A1B	2281.37 (NE)	285.17 km / decade
2080s – A2A	1060.91 (NE)	132.61 km / decade
2080s – B2A	2531.59 (NE)	316.45 km / decade
Current South		
2050s – A1B	148.26 (S)	29.65 km / decade
2050s – A2A	145.14 (S)	29.03 km / decade
2050s – B2A	442.23 (WSW)	88.45 km / decade
2080s – A1B	457.45 (SE)	57.18 km / decade
2080s – A2A	889.55 (ESE)	111.19 km / decade
2080s – B2A	146.94 (S)	18.37 km / decade

Table S1: Locality Data

Geographic Region:	Location:	Latitude	Longitude	Literature Source:
Africa – Eastern	Madagascar	-18.766947	46.869108	26
Africa - Eastern	Mayotte	-12.827436	45.166281	27
Africa – Eastern	Réunion Island	-21.115142	55.536383	26
Africa – Northern	Egypt (Cairo)	26.820553	30.802497	26
Africa – Western	Cote d'Ivoire	7.539989	-5.547081	26
Africa – Western	Port Harcourt, Nigeria	4.8	7	26
Asia - Eastern	Changle, Fujian, China	25.963119	119.523383	64
Asia - Eastern	Fuzhou, Fujian, China	26.074508	119.296494	65
Asia - Eastern	Guangdong, China	23.132192	113.266531	63
Asia - Eastern	Guangxi, China	22.815478	108.327544	63
Asia - Eastern	Canton/Guangzhou, China	23.129164	113.264436	25
Asia - Eastern	Hainan, China	20.017378	110.349228	63
Asia - Eastern	Jiangxi, China	28.674425	115.909175	63
Asia - Eastern	Kunming, Yunnan, China	25.037722	102.722203	66
Asia - Eastern	Wenzhou, Zhejiang, China	27.994267	120.699367	63
Asia – Eastern	Zhejiang, China	30.266292	120.153822	76
Asia – Eastern	Ishigaki Island, Okinawa	24.4	124.4	82
Asia - Eastern	Okinawa, Japan	26.2124	127.680933	26
Asia - Eastern	Pingtung Hsien, Taiwan	22.655789	120.470289	72
Asia – Southern	Bombay, India	19.017614	72.856164	26
Asia – Southern	Sri Lanka	7.873053	80.771797	44
Asia - South-Eastern	Sarawak, Borneo	2.607342	113.648944	26
Asia –South-Eastern	Central Java	-7.150975	110.140258	77
Asia –South-Eastern	West Java	-7.090883	107.668861	77
Asia –South-Eastern	Lampung	-4.558586	105.406808	77
Asia –South-Eastern	East Nusa Tenggara	-8.657383	121.079369	77
Asia –South-Eastern	South Sumatra	-3.319436	103.9144	77
Asia –South-Eastern	West Sumatra	-0.739939	100.800006	77
Asia - South-Eastern	Bangkok, Thailand	13.716731	100.54064	73
Asia - South-Eastern	Korat, Thailand	15.017228	102.316944	73
Asia - South-Eastern	Ubon, Thailand	15.072708	105.219481	73
Asia –South-Eastern	North Sulawesi	-1.847908	120.527911	77
Australia	Beecroft Peninsula, Australia	-35.082594	150.813367	62
Australia	Sydney, Australia	-33.89175	151.199522	50
Caribbean	Nassau, Bahamas	25.06	-77.345	26
Caribbean	Santo Domingo, Dominican Republic	18.499997	-69.983331	81
Caribbean	Cuba	21.521758	-77.781167	68
Caribbean	Haiti	18.971186	-72.285214	79
Caribbean	Black River, Jamaica	18.03085	-77.852158	61

Caribbean	Kingston, Jamaica	17.992731	-76.792008	61
Caribbean	Lucea, Jamaica	18.44275	-78.178628	61
Caribbean	Mandeville, Jamaica	18.039661	-77.513283	61
Caribbean	Montego Bay, Jamaica	18.466667	-77.916667	61
Caribbean	Puerto Rico	18.220833	-66.59015	70
Caribbean	Grenada, West Indies	12.262775	-61.604172	21
Europe –Southern	Tenerife, Canary Islands	28.291564	16.629131	67
Melanesia	Viti Levu, Fiji	-17.848319	178.011847	78
Melanesia	Noumea, New Caledonia	-22.2758	166.458	69
Melanesia	Efate, New Hebride	-17.735261	168.321731	71
Melanesia	Bougainville, Papua New Guinea	-6.0536	155.190681	80
Melanesia	Kimbe, West New Britain, Papua New Guinea	-5.550433	150.142808	80
Melanesia	Lae, Papua New Guinea	-6.723669	146.990906	80
Melanesia	New Ireland, Papua New Guinea	-4.285325	152.920592	80
Melanesia	Port Moresby, Papua New Guinea	-9.481553	147.190242	80
Melanesia	Rabaul, Papua New Guinea	-4.196161	152.172961	80
Micronesia	Guam	13.444275	144.793731	26
Micronesia	Saipan	15.1778	145.750967	26
Northern America	Miami, Florida	25.611517	-80.397781	42
Northern America	Big Island, Hawaii	19.693236	-155.537814	75
Northern America	Honolulu, Hawaii	21.301281	-157.860656	75
Northern America	Maui, Hawaii	20.808581	-156.319975	75
Northern America	New Orleans, Louisiana	29.952858	-90.071242	43
Northern America	New Iberia, Louisiana	30.003536	-91.815367	19
Polynesia	Rarotonga, Cook Islands	-21.229236	-159.77635	26
Polynesia	Tahiti	-17.650919	-149.426042	26

Ecological Niche Model based Survey and Molecular Identification of *Angiostrongylus cantonensis* in Rodents from Southeast Oklahoma and Louisiana.

ABSTRACT

The majority of emerging infectious diseases (EIDs) are zoonotic with the potential to incur substantial costs to wildlife populations. The zoonotic parasite, *Angiostrongylus cantonensis*, causes eosinophilic meningitis in humans and neurological disorders in animals. *A. cantonensis* has been documented in Louisiana and provides an excellent opportunity to evaluate tools like ecological niche modeling (ENM) and real-time PCR. I sampled a total of 146 rodents and two insectivores in areas of predicted suitable (Louisisana) and non-suitable (Oklahoma) habitat within the SE United States for *A. cantonensis.* All rodent lungs were negative for adult parasites following lung floatation. Real-time PCR analysis (Qvarnstrom, 2010) identified 34 tissue samples as potentially positive for *A. cantonensis*. To definitively identify *A. cantonensis*, a 105 base pair fragment of the internal transcribed spacer 1 was sequenced. Only three brain samples (two from Louisiana and one from Oklahoma) produced sequences having a 92-99% match with those found on GenBank for *A. cantonensis*. The remaining 31 samples were then classified as false positives. The program MEGA was used to generate a maximum likelihood tree to show the relationship between the three samples, *A. cantonensis* and two closely related nematode species. Although predicted unlikely to be found within Oklahoma, my sequencing results indicate the presence of *A. cantonensis* within the state. My results demonstrate the necessity for constant surveillance of pathogens to prevent their spread. Through continuous re-evaluation of methodology, more efficient and accurate sampling techniques may be developed, in turn influencing predictive models of pathogen occurrence.

INTRODUCTION

Emerging infectious diseases (EIDs) can have devastating effects on wildlife, including mass mortalities, local population extinctions, and global extinctions (Cunningham and Daszak, 1998; Daszak and Cunningham, 1999). Sixty percent of EIDs are caused by zoonotic pathogens, and of these, over 70% originate in wildlife (Jones, 2008). As such, the potential for disease transmission among domestic and wild populations of protected, endangered and/or susceptible species is likely (Artoi et al., 2001; Bengis et al., 2004). Disease emergence or re-emergence might be due to numerous factors (e.g. globalization of trade, increase interaction of humans and domestic animals with wildlife, anthropogenic climate change) that function independently or synergistically (Patz et al., 2000; Bengis, 2004). Consequently, the means by which pathogens are studied are changing in order to better identify, control and prevent outbreaks.

The rat-lung worm, *Angiostrongylus cantonensis,* causes eosinophilic meningitis in humans (Prociv et al., 2000). *Angiostrongylus cantonensis* was first linked to human disease in 1944 (Nomura and Lin, 1945) but was not recognized as a significant health risk until 1964 (Beaver and Rosen, 1964). The parasite also causes various disease symptoms (meningoencephalitis, neurological disroders) in atypical host species, including wildlife and captive animals (Gardiner et al., 1990; Duffy et al., 2004). The prevalence of *A. cantonensis* varies among geographic regions and within host species but the number of infected hosts can be high in certain areas (Lindo et al., 2002; Lv et al., 2009; Vitta et al., 2011). *Angiostrongylus cantonensis* potentially poses a significant threat to the conservation of endangered wildlife, especially in those endemic to geographic regions and that have not evolved host-parasite associations.

The occurrence of *A. cantonensis* has been documented world-wide including, Southeast Asia, Australia, India, south-eastern USA and Africa (Kliks and Palumbo, 1992). The wide-spread geographical distribution of *A. cantonensis* has been attributed largely to the spread of an intermediate host, the African giant land snail (*Archachatina marginata*), and its definitive host, *Rattus* spp. (Kilks and Palubo, 1992). Moreover, the host specificity of *A. cantonensis* is not restricted, which contributes to its continuous geographic expansion (Prociv et al., 2000). Recently, *A. cantonensis* has been documented in additional snail species, *Pomacea maculata* and *Achatina fulica*, in southeastern United States. These intermediate hosts may facilitate a range expansion of the parasite within the United States, as demonstrated in China (Teem et al., 2013). These factors suggest that *A. cantonensis* is an emerging zoonotic pathogen of concern to both humans and wildlife. *Angiostrongylus cantonensis* provides an excellent template to evaluate the sensitivity and effectiveness of novel epidemiological techniques.

Ecological niche modeling (ENM) is being increasingly implemented as a tool for studying the geographic distributions of zoonotic pathogens (Costa et al. 2002; Peterson and Shaw, 2003; Levine et al. 2007). ENM uses point-occurrence data to describe and predict the potential geographic range of a species, providing wildlife epidemiologists with areas of interest to survey and hence document, the spread of pathogens. Modern molecular genetic techniques are an efficient means to survey and accurately identify zoonotic pathogens, thereby providing a better understanding of the epidemiology of zoonotic pathogens and the means to control them (Morgan, 2000). Real-time PCR (qPCR) in particular, is being used more frequently in forensic, veterinary, medical and biological investigations (Bustin, 2005). Combining qPCR techniques with field epidemiology allows for a rigorous test of the effectiveness and accuracy of modeling tools such as ENM. A recent ENM of *A. cantonensis* suggests that the potential range for the

parasite within the United States lies along the coast of Louisiana and parts of Texas and Florida (York, 2013). I tested the predictions of this model and the possible use of a species-specific Taq-man assay (Qvarnstrom et al., 2010) as a surveying technique for *A. cantonensis* by sampling rodent populations in areas predicted to have suitable and non-suitable habitat.

MATERIALS AND METHODS

Animal Collection

I sampled rodent populations in southeast Oklahoma and Louisiana based on predictions from my ENM, which included both suitable and non-suitable habitat patches. Field collection in Oklahoma occurred in rural to semi-urbanized areas, whereas in Louisiana most took place in urbanized to semi-rural areas. Sample sites from Oklahoma (non-suitable habitat) were confined to the South Central Plains ecoregion (United States Environmental Protection Agency, 2011). In Louisiana, I sampled in New Orleans, East Baton Rouge, and Lake Charles, where the parasite has been previously documented and predicted to have suitable habitat (Kim et al., 2002).

Trapping occurred during the spring, summer and fall months of the years 2010—2012 using Sherman live traps (3x3.5x9”) and tomahawk traps (5x5x16”) baited with various food types (e.g. rolled oats, apples, peanut butter, chocolate). Sherman live traps were set in transects with trap stations approximately 10 m apart. A pair of traps constituted a trap station, and 50 to 100 traps were set per transect while trapping in the field. At least 3 transects (~200 traps) were set per area of suitable habitat (Resource Inventory Committee, 1997). In urbanized areas trap lines were not practical and instead, traps were placed in areas with signs of high rodent activity (e.g. scat, chewing). One trap session consisted of setting traps in the late afternoon and examining them the following morning. All specimens captured were identified to species, with

sex, reproductive condition, and relative age recorded along with the date of collection, specific location, and weight.

Collection of Blood and Tissue Samples

Blood samples from rodents were collected from the suborbital sinus using sterile capillary tubes (Hoff, 2000) or through cardiac puncture with prior sedation using chloroform. Following blood collection, all rodents were euthanized with chloroform. Lungs were removed for floatation in 10% saline solution and brain tissue samples were taken. If adult worms are present in the lungs, they will leave the tissue. All tissue samples were labeled and transported in 70% ethanol to UCO. Dissection equipment was rinsed in 10% bleach solution or cleaned with bleach wipes between tissue collections. Voucher specimens were either skinned or placed in formalin and deposited at UCO Natural History Museum.

The handling of rodents, tissue sample collections, and subsequent analyses adhered to standard operating procedures of the American Society of Mammalogists (2011) and the Institutional Animal Care and Use Committee (IACUC) at the University of Central Oklahoma (UCO). Approvals and permits for rodent sampling were obtained from UCO IACUC, the Oklahoma Department of Wildlife Conservation, and the Louisiana Department of Wildlife and Fisheries.

Nucleic Acid Extraction and Real-time PCR (qPCR)

Adult *A. cantonensis* were kindly provided by Dr. Mark Eberhard at the Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention (CDC). A 105 base-pair (bp) fragment of ribosomal DNA first internal transcribed spacer (ITS1) was cloned (Invitrogen, cat. no. 450030) using species-specific primers, (AcanITS1F1- 5’-TTCATGGATGG CGAACTGATAG-3’) and (AcanITS1R1- 5’-GCGCCCATTGAAACATTATACTT-3’)

(Qvarnstrom et al, 2010). The thermal cycling profile was characterized by 94˚C for 5 min, followed by 40 cycles of 94˚C for 30 sec, 60˚C for 30 sec, 72˚C for 1 min, and 72˚C for 7 min. Products were sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Qiagen, cat. no. 69506) (Table 1) and compared to sequences on GenBank via BLAST with a perfect sequence match, 99% coverage, and an E value of 2e-47. The cloned fragment was used as a positive control in qPCR.

Total cellular DNA was extracted from blood and brain samples using a DNeasy blood and tissue kit (Qiagen, product number 69506) following manufactures recommendations. Prior to extraction, brain samples were homogenized with a sterilized glass rod to ensure random sampling of tissue. I tested for the presence of *A. cantonensis* using a TaqMan assay via qPCR on an AB 7500 system (Qvarnstrom et al., 2010), with the following probe and primers: AcanITS1P1 (5'-FAM-ATCGCATATCTACTATACGCAT GTGACACCTG-MGBNFQ-3'), AcanITS1F1 and AcanITS1R1. The qPCR assay was conducted in a 20 µl total volume containing Platinum qPCR Supermix-UDG (Invitrogen, cat. no. 11730-017), 0.4 µl (10 µM) each of AcanITS1F1 and AcanITS1R1, and 0.2 µl (1 µM) of AcanITS1P1. Positive (i.e. standards), non-template and blank controls were used when analyzing samples to ensure that reactions amplified.

During relative abundance qPCR, increased florescence of the blank and non-template control produced false positives near or after amplification cycle 30. Consequently, samples with increased fluorescence relative to the blank or non-template control were separated into two groups, "positive" and "potential positive". "Positive" samples crossed a threshold value between cycle 10—25. The samples that crossed the threshold value between cycles 26—30 were categorized as "potential positives." However these are generalized ranges, and each plate

amplified differently, causing additional factors which had to be taken into account (e.g. shape of the amplification curve, proximity to non-template control and blank). Because of variation between plates and the amplification of the blank and non-template control, I sequenced samples that were categorized as "positive" and "potential positive", to discriminate between true positives and potential false positives.

Sequencing and Phylogenetic Analyses

"Positive" and some "potential positive" results produced by qPCR were reamplified using AcanITS1F1 and primer AngioR58sR4 (5'-TACCTGCGTTTTTCATCGATA-3') (Qvarnstrom, 2010), and Amplitaq Gold master mix (Applied Biosystems, cat. no. 4398876) to generate a larger ITS1 fragment for better species discrimination. The thermal cycling profile consisted of an initial denaturation step of 95˚C for 5 min, followed by 45 cycles of 95˚C for 30 sec, 56˚C for 30 sec, 72˚C for 1 min, and 72˚C for 7 min. Following PCR, samples were purified with a Mini Elute kit, sequenced with BigDye Terminator v3.1 cycle sequencing kit, and purified once more with an Edge Bio kit. I compared my samples against a 267 bp fragment generated from the known sample of *A. cantonensis* (Table 1). All positive and selected "potential positive" samples were analyzed on an ABI 3130 Genetic Analyzer (Applied Biosystems) three times to detect possible errors incurred by the misincorporation of dNTPs by Taq polymerase (Saiki et al., 1988). Generated sequences were aligned using Muscle in MEGA 5.2 (Tamura et al., 2011) and manually inspected for consensus. The generated consensus sequences were then compared to those on GenBank via BLAST, to identify the most likely origin (i.e. organism) of the sequence.

For phylogenetic analysis, consensus sequences for the ITS1 region of *A. cantonensis* (GU733321.1, GU733323.1. GU587762.1) and two closely related species, *Angiostrongylus vasorum* (GU733324.1, GU733325.1, GU045370.1) and *Angiostrongylus costaricensis*

(GU587745.1, GU587746.1, GU587747.1), were generated from GenBank using BioEdit 7.2.0 (Hall, 1999). All consensus sequences were manually pruned to maximize homology.

Muscle in MEGA 5.2 (Tamura et al., 2011) was used to realign all consensus sequences and the pairwise distances between the sequences were noted. I used maximum likelihood (ML) to reconstruct a phylogeny of the generated sequences from the positive samples and the four designated taxa. Model selection in MEGA identified Tamura 3-parameter as the best-fit model for nucleotide substitution. The ML tree was constructed using the Tamura 3-parameter model and default parameters in MEGA 5.2, with 10,000 bootstrap replicates. Any branches with a bootstrap value below 50 were collapsed. To confirm tree topology, I used additional phylogenetic methods: neighbor-joining (NJ), minimum evolution (ME), and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with the Tamura 3-parameter model, and maximum parsimony (MP).

RESULTS

Field Collection

There was a total of 1950 trap nights in Oklahoma: 350 at the Oklahoma State Forest Resource Center, and 1600 in the Red Slough Wildlife Management Area (WMA). Forty-two rodents and three insectivores were collected from McCurtain County (Table 2). Three hundred sixty-three trap nights were in Louisiana: 121 in New Orleans, 118 in Baton Rouge, and 124 in Lake Charles. Forty-seven rodents were collected from Louisiana (Table 2). The collection number, species and GIS coordinates for each rodent captured is shown in Table 1 of the supplementary material. In addition, to those collected in the field, tissue samples from 56 *Rattus norvegicus* were obtained from the City of New Orleans Mosquito, Termite, and Rodent Control Board. All voucher specimens were deposited at the UCO Natural History Museum.

Blood and Tissue Processing

Lung floatation was performed on 148 rodents. All samples were negative for adult *A. cantonensis*. Due to extraction inhibitors, DNA from blood and brain samples was obtained from only 134 and 137 specimens, respectively. Following qPCR, 7 samples were classified as "positive" and 27 samples as "potential positive." Only three brain samples of the 34 "positive" and "potential positive" samples produced a sequence (Table 3). These samples 32, 70 and 76 were obtained from *a Sigmodon hispidus* and two *Rattus norvegicus*, respectively. The samples were classified as true positives and the remaining samples were classified as false positives. Upon evaluation of the sequences, brain sample 32 appeared to be a mixed sample due to slippage following a TA repeat region (Clarke et al., 2001). Comparison of the three brain samples 32, 70 and 76 to those on GenBank via BLAST showed a match with *A. cantonensis* (Table 4). The next closest species match for brain samples 70 and 76 was *A. vasorum*, with it being 81% identical to the samples and an e value of 2e-42 and 4e-44, respectively. Following species confirmation, the prevalence of *A. cantonensis* in the Red Slough WMA was determined to be 3% and 2% in New Orleans.

Phylogenetic Analysis

Aligned consensus sequences for three taxa and 3 samples consisted of 291sites with gaps. Pairwise distances between *A. cantonensis*, the three brain samples and other *Angiostrongylus* spp. are shown in Table 5. The phylogenetic trees constructed using ML, NJ, ME, UPGMA and MP methods showed some variation in tree topology. However, samples 32, 70, and 76 grouped with *A. cantonensis* with bootstrap values of 100% for all phylogenetic methods. The ML consensus tree is shown in Figure 1.

DISCUSSION

Angiostrongylus cantonensis poses a significant health risk to humans and wildlife worldwide, demonstrating a need for research that can shed light on its location and dispersal. I surveyed New Orleans, East Baton Rouge and Calcasieu parishes in Louisiana for the presence of *A. cantonensis.* Two of the parishes (New Orleans and East Baton Rouge) had previously documented *A. cantonensis* (Kim et al., 2002). I also surveyed for the parasite in McCurtain County, Oklahoma. Of the 148 specimens collected, qPCR identified 34 as positive for the parasite. However, sequencing analysis with species-specific primers revealed only 3 of the samples contained *A. cantonensis*. Phylogenetic analyses grouped the sequenced samples with *A. cantonensis*. This affirmed the presence of the parasite in SE Oklahoma and Louisiana and the validity of ITS1 as an important molecular maker for parasite detection.

Integration of ecological niche modeling (ENM) and qPCR suggests a new perspective of the distribution of *A. cantonensis* within the United States. Previous reports note that *A. cantonensis* is found in Louisiana, Mississippi, and Florida (Kim et al., 2002; Duffy et al. 2004). However, studies on the prevalence of the parasite within definitive hosts in the southeastern United States are lacking. Although sampling in Louisiana was limited, a prevalence of two percent is disconcerting, especially given that infected rodents were collected in densely populated areas. Importantly, *A. cantonensis* was documented within a novel host species (*S. hispidus*) in Oklahoma, an area predicted to lack suitable habitat for the parasite. This northward range expansion increases significantly the risk of disease spread to both host species and other wildlife that might feed on *A. cantonensis* larvae, including humans and protected species of birds and mammals.

EIDs are hypothesized to originate from either novel or endemic pathogens (Rachowicz et al. 2005). Novel pathogens are recently introduced pathogens to new geographic regions that encounter species highly susceptible to infection, resulting in an outbreak. By contrast, endemic pathogens are already present in the environment and have acquired new host species or increased in pathogenicity. Because their disease management is different, it is crucial to determine whether a pathogen is novel or endemic. Based on previous work, *A. cantonensis* would have been described as a novel pathogen within SE United States. However, it is now characterized as endemic (Kim et al., 2002). Changes in the classification of *A. cantonensis* accentuate the need for techniques that monitor the extent to which the parasite infiltrates geographic areas and the threat it poses to native wildlife.

Reat-time PCR has become an increasingly implemented technique to screen tissue samples for various pathogens and microbes, enabling species-specific identification (Cummings and Tarleton, 2003; Kriger et al., 2006). However, there is a growing need for such diagnostic techniques to detect pathogens in a wide variety of host species, particularly for those that have strong potential to spread disease worldwide. This is especially true for *A. cantonensis*, where definitive and intermediate hosts are found worldwide and have contributed greatly to the spread of the parasite (Kliks et al., 1992). I used a qPCR Taq-Man assay designed specifically for identification of *A. cantonensis* within mollusks (Qvarnstrom et al. 2010) to screen rodent tissue for the parasite. This method provided rapid screening for *A. cantonensis* by identifying samples of interest from definitive hosts, validating its potential use as an effective epidemiological screening technique and suggests the possibility of its application for other pathogens in other mammalian species.

Although my study suggests that the Taq-Man assay might be of value to epidemiologists, there is also a concern. All samples, including non-template and blank controls, showed ranges of increased florescence leading to an occurrence of false-positives. The discrepancy between qPCR and sequencing analysis might result from lowered quenching of the fluorophore by the quencher. In contrast to the present study, which used a MGB non-fluorescent quencher, Qvarnstrom et al. (2010) employed a black hole quencher (BHQ). Both MGB and black hole quenchers rely on Förster Resonance Energy Transfer (FRET) to prevent escape of fluorophore emissions (Marras et al., 2002). Similar to the non-fluorescent quencher, BHQ absorbs excitation energy from the fluorophore and releases energy as heat as long as the fluorophore remains within a certain distance from the quencher (Marras et al., 2002). However, BHQ differs from MGB by providing a more broad spectral overlap which in turn increases the efficiency of quenching and lowers background fluorescence (Marras et al., 2002). Moreover, BHQ has greater quenching efficiency when paired with FAM (Marras et al., 2002). Therefore, use of the MGB non-fluorescent quencher may not have fully masked the fluorophore, resulting in background fluorescence. However, background fluorescence does not invalidate my results. It is recommended, however, that future studies on *A. cantonensis* use the MGB probe in conjunction with BHQ probe.

Genetic evidence of *A. cantonensis* within Louisiana supports the ENM predictions for it to be present in that region. In contrast, although the ENM predicted unsuitable habitat for *A. cantonensis* within SE Oklahoma, my results from sequencing analysis confirmed the parasite presence in the state. My study highlights the difficulty in modeling the potential ranges and distributions of parasites with complex life cycles. Without considering the complexity of parasite-host interactions, environmental requirements, as well as the distribution of known host

species and potential for new host species, the current ENM might not fully capture the realized niche of the parasite (Peterson, 2006). When developing an ENM that primarily focuses on species occurrences, the generated model is known as a "black box" (Peterson, 2006) in that it provides an overall picture of disease ecology but not the chain of transmission as in this study. Regardless, this study suggests that an ENM based on basic information about the location of the parasite can provide an important starting point for disease surveillance and identifies bioclimatic variables that most likely influence the parasite's distribution.

The advent of modern molecular techniques has allowed wildlife epidemiologists to identify and characterize pathogens of medical, veterinary and wildlife significance in greater detail compared to classical procedures (Morgan, 2000). An emerging consensus is that there has been a significant global increase in the overall number and diversity of pathogens (Daszak et al. 2000; Jones et al., 2008). The apparent increase in EID incidence is the result of greater reporting effort of zoonotic pathogens worldwide in conjunction with the power of new diagnostic techniques (Bengis et al., 2004). However, this documentation basis was recently evaluated and controlled for with findings showing a significant increase in the number of EIDs originating in wildlife over time (Jones et al., 2008).

With an estimate of 86% of the world's terrestrial species not taxonomically described, there are potentially numerous reservoirs of unknown pathogens worldwide (Mora et al., 20011). Global travel, human encroachment into wildlife habitat and climate change will significantly influence the distribution and emergence of disease (Bengis et al., 2004; Cunningham, 2005) and might lead to a greater probability of species extinctions. Improvements in detection capabilities, constant surveillance for and documentation of pathogens will aid in understanding the ecological niche of pathogens. By incorporating ENM, field epidemiology and qPCR techniques

to determine the geographic distribution of a pathogen, major advances can be made in controlling and preventing the spread of outbreaks of wildlife diseases. Future work should refine each of these techniques and their application to epidemiology and wildlife disease.

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LITERATURE CITED

Artois M, Delahay R, Gubert V, Cheeseman C. 2001. Control of infectious diseases of wildlife in Europe. *Vet J* 162:141-152.

Bengis RG, Leighton FA, Fischer JR, Artois M, Morner T, Tate CM . 2004. The role of wildlife in emerging and re-emerging zoonoses. *Rev - Off Int Epizoot* 23:497-511.

Beaver PC, Rosen L. 1964. Memorandum on the first report of *Angiostrongylus* in man, by Nomura and Lin, 1945. *Am J Trop Med Hyg* 13:589-590.

Bustin SA. 2005. Real-time, fluorescence-based quantitative PCR: a snapshot of current procedures and preferences. *Expert Rev Mol Diagn* 5:493-498.

Clarke LA, Rebelo CS, Gonçalves J, Boavida MG, Jordan P. 2001. PCR amplification introduces errors into mononucleotide and dinucleotide repeat sequences. *J Clin Pathol: Mol Pathol* 54:351–353.

Costa J, Peterson AT, Beard CB. 2002. Ecological niche modeling and differentiation of populations of *Triatoma brasiliensis* Neiva, 1911, the most important Chagas disease vector in northeastern Brazil (Hemiptera, Reduviidae, Triatominae). *Am J Trop Med Hyg* 67:516-520.

Cunningham AA, Daszak, P. 1998. Extinction of a species of land snail due to infection with a microsporidian parasite. *Conserv Biol* 12:1139-1141.

Cummings KL, Tarleton RL. 2003. Rapid quantitation of *Trypanosoma cruzi* in host tissue by real-time PCR. *Mol Biochem Parasitol* 129:53-59.

Cunningham AA. 2005. A walk on the wild side-emerging wildlife diseases. *BMJ* 331:1214-1215.

Daszak P, Cunningham AA. 1999. Extinction by infection. *Trends Ecol Evol* 14: 279.

Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science* 287:443-449.

Duffy M, Miller C, Kinsella J, Lahunta A. 2004. *Parastrongylus cantonensis* in a nonhuman primate, Florida. *Emerg Infect Dis* 10:2207-2210.

United States Environmental Protection Agency: Western Ecology Division. 2011. http://www.epa.gov/wed/pages/ecoregions/ok_eco.htm Gardiner et al 1990. Accessed July 2012.

Hall TA. 1999. BioEdit: a user-friendly biologicalsequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 41:95-98.

Hoff J. 2000. Methods of blood collection in the mouse. *Lab Anim* 29:47-53.

Jones K, Patel N, Levy M, Storeygard A, Balk D, Gittleman J, Daszak P. 2008. Global trends in emerging infectious diseases. *Nature* 451:990-993.

Kim D, Stewart T, Bauer R, Mitchell M. 2002. *Parastrongylus* (=*Angiostrongylus*) *cantonensis* now endemic in Louisiana. *J Parasitol* 88:1024-1026.

Kliks M, Palumbo N. 1992. Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis by *Angiostrongylus cantonensis*, the nematode lungworm of rats. *Soc Sci Med* 34:199-212.

Kriger KM, Hero JM, Ashton KJ. 2006. Cost efficiency in the detection of chytridiomycosis using PCR assay. *Dis Aquat Organ* 71:149-154.

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase–PCR assay. *J Clin Microbiol* 38:4066–4071.

Levine RS, Peterson AT, Yorita KL, Carroll D, Damon IK, Reynolds MG. 2007 Ecological niche and geographic distribution of human monkeypox in Africa. *PLoS One* 2:e176.

Lindo J, Waugh C, Hall J, Cunningham-Myrie C, Ashley D, Eberhard M, Sullivan J, Bishop H, Robinson D, Holtz T, Robinson R. 2002. Enzootic *Angiostrongylus cantonensis* in rats and snails after an outbreak of human eosinophilic meningitis, Jamaica. *Emerg Infect Dis* 8:324-326.

Lv S, Zhang Y, Liu H-X, Hu L, Yang K, Steinmann P, Chen Z, Wang L-Y, Utzinger J, Zhou X-N. 2009. Invasive snails and an emerging infectious disease: result from the first national survey on *Angiostrongylus cantonensis* in China. *PLoS Negl Trop Dis* 3:e368.

Marras SA, Kramer FR, Tyagi S. 2002. Efficiencies of fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes. *Nucleic Acids Res* 30:e122.

Mora C, Tittensor DP, Adl S, Simpson AG, Worm B. 2011. How many species are there on Earth and in the ocean?. *PLoS Biol*, 9 e1001127.

Morgan U. 2000. Detection and characterization of parasites causing emerging zoonoses. *Int J Parasitol* 30:1407-1421.

Nomura S, Lin PH. 1945. First case of human infection with *Haemostrongylus ratti* Yokogawa. *Taiwan no Ikai*. 3:589.

Patz JA, Graczyk TK, Geller N, Vittor AY. 2000. Effects of environmental change on emerging parasitic diseases. *Int J Parasitol* 30:1395-1405.

Peterson AT, Shaw J .2003. *Lutzomyia* vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. *Int J Parasitol* 33:919-931.

Peterson AT. 2006. Ecological niche modeling and spatial patterns of disease transmission. *Emerg Infect Dis* 12:1822-1826.

Prociv P, Spratt DM, Carlisle MS. 2000. Neuro-angiostrongyliasis: unresolved issues. *Int J Parasitol* 1295-1303.

Qvarnstrom Y, Silva A, Teem J, Hollingsworth R, Bishop H, Graeff-Teixeira C, Silva A. 2010. Improved molecular detection of *Angiostrongylus cantonensis* in mollusks and other environmental sample with a species-specific ITS1-based TaqMan assay. *Appl Environ Biol* 76:5287-5289.

Rachowicz LJ, Hero JM, Alford RA, Taylor JW, Morgan JAT, Vredenburg VT, Collins JP, Briggs CJ. 2005. The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. *Conserv Biol* 19:1441-1448.

Resource Inventory Committee. 1997. Standardized inventory methodologies for components of British Columbia's biodiversity: shrews, voles, mice and rats. http://www.ilmb.gov.bc.ca/risc/pubs/tebiodiv/smallmammals/cip.htm. Accessed May 2010.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science* 239:487-491.

Sikes RS, Gannon WL, the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal* 92:235-253.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731-2739.

Teem JL, Qvarnstrom Y, Bishop H S, da Silva AJ, Carter J, White-Mclean J, Smith T. 2013. The occurrence of the rat lungworm, *Angiostrongylus cantonensis*, in nonindigenous snails in the Gulf of Mexico region of the United States. *Hawaii J Med Public Health* 72:11.

Qu ZY, Yang X, Cheng M, Lin YF, Liu XM, He A, Wu ZD, Zhan XM. 2011. Enzootic angiostrongyliasis, Guangdong, China, 2008–2009. *Emerg Infect Dis* 17: 1335.

York EM. 2013. Emerging infectious disease: ecological niche modeling and molecular identification of *Angiostrongylus cantonensis* in rodents form Oklahoma and Louisiana. M.S. Thesis, Forensic Science Institute, University of Central Oklahoma, Edmond.

Table 1. Sequence fragments generated from *A. cantonensis* control specimen of the ITS1 region.

Species-specific region, 105 bp (Qvarnstrom, 2010)	5'-TTTCATGGATGGCGAACTGATAGTATCATCGCATATATA CTATAC GCATGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTATAA TGTTTCAATGGGCGC-3'
267 bp fragment used in phylogenetic analyses	5'-TTCATGGATGGCGAACTGATAGTATCATCGCATATA TACTATAC GCATGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTATAA TGTTTCAATGGGCGCCAACGTAGCAACAGAACAGTTTTTCTACACGT GAAAATGTGGAACGAGATACACAGGATGTATATATATATATATACA CA TATATATATGTGTATGGAAATTGATATACTAGCTTCAGCGATGG ATCGGTCGATTCGCGTATCGATGAAAAACGC ATCTAAA-3'

Table 2. Location, species, and number of rodents caught.

Location	Species	No. Caught
Idabel, OK	*Neotoma floridana*	2
Red Slough (WMA), OK	*Blarina carolinensis*	3
Red Slough (WMA), OK	*Oryzomys palustris*	2
Red Slough (WMA), OK	*Peromyscus leucopus*	1
Red Slough (WMA), OK	*Reithrodontomys fulvescence*	7
Red Slough (WMA), OK	*Sigmodon hispidus*	30
New Orleans Parish, LA	*Rattus norvegicus*	36
East Baton Rouge Parish, LA	*Rattus rattus*	3
Calcasieu Parish, LA	*Rattus norvegicus*	8

[a] Wildlife Management Area (WMA)

Table 3. Sequences generated from the three positive samples.

Species	Location	Sample No.	Generated Sequence from Brain Samples
Sigmodon hispidus	Red Slough WMA, OK	32	5'-TTCATGGATGGCGAACTGATAGTATCATCGCATATCTACTATAC GCATGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTA TAATGTTTCAATGGGCGCCAACGTAGCAACAGAACAGTTTTTCA CACGTGAAAATGTGGAACGAGATACACAGGATGtatatataTATATA TATATATATACACATATATRTGTGTRTGGAAATAGATATACTAKCT TCAGMGAKGRWKCGSGYGATTCGCGTATCTAAGAAAAACACA-3'
Rattus norvegicus	New Orleans, LA	70	5'-TTCATGGATGGCGAACTGATAGTGTCATCGCATATCTACTATA CGCATGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTAT A ATGTTTCAATGGGCGCCAACGTAGCAACAGAACAGTTTTTCT ACACGTGAAAATGTGGAACGAGATACACAGGATGTATATATATA TATATATACACATATATATGTGTATGGAAATTGATATACTAGCTTC AGCGATGGATCGGTCGATTCGCGTATCGATGAAAAACGCATCTA-3'
Rattus norvegicus	New Orleans, LA	76	5'-TTCATGGATGGCGAACTGATAGTATCATCGCATATCTACTATA CGCATGTGACACCTGATTGACAGGAAATCTTAATGACCCAAGTA TAATGTTTCAATGGGCGCCAACGTAGCAACAGAACAGTTTTTCT ACACGTGAAAATGTGGAACGAGATACACAGGATGTATATATATAT ATATATACACATATATATGTGTATGGAAATTGATATACTAGCTTCA GCGATGGATCGGTCGATTCGCGTATCGATGAAAAACGCAGCT A-3'

Table 4. NCBI BLAST results when compared to *A. cantonensis*.

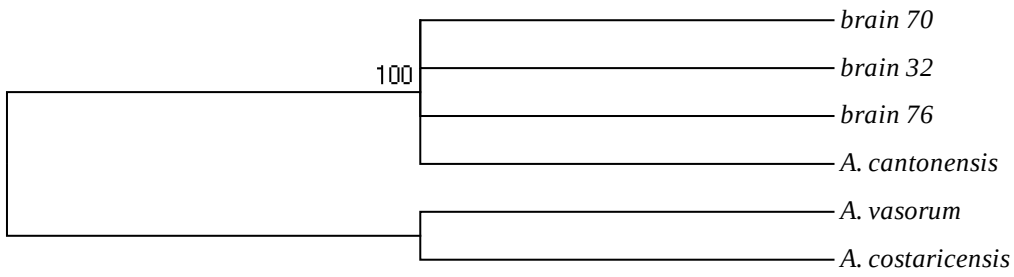
Sample No.	Host Species	Location	Match	Coverage	e value
32	*Sigmodon hispidus*	Red Slough WMA, OK	92%	98%	3e-105
70	*Rattus norvegicus*	New Orleans, LA	99%	100%	3e-130
76	*Rattus norvegicus*	New Orleans, LA	99%	100%	1e-133

Table 5. Shows the pairwise distances between *A. cantonensis* and sequences.

A. cantonensis					
brain 76	0.005				
brain 70	0.011	0.005			
brain 32	0.033	0.027	0.033		
A. vasorum	0.178	0.178	0.187	0.220	
A. costaricensis	0.213	0.203	0.213	0.247	0.141

Figure1. Maximum Likelihood Bootstrap Consensus Tree using Tamura 3-parameter model; demonstrates the relationship between the generated sequences and *Angiostrongylus* spp.

Figure 1.


100
brain 70
brain 32
brain 76
A. cantonensis
A. vasorum
A. costaricensis

SUPPLEMENTARY MATERIAL

Table 1. List of all rodents collected with collection number and coordinates.

Collection No.	Species	Latitude	Longitude
3	*Sigmodon hispidus*	33° 44.204' N	94° 40.627' W
4	*Sigmodon hispidus*	33° 43.281' N	94° 41.376' W
5	*Oryzomys palustris*	33° 43.281' N	94° 41.376' W
6	*Sigmodon hispidus*	33° 44.123' N	94° 41.601' W
7	*Oryzomys palustris*	33° 44.145' N	94° 41.573' W
11	*Sigmodon hispidus*	33° 44' 9.552" N	94° 38' 31.795" W
12	*Sigmodon hispidus*	33° 44' 9.552" N	94° 38' 31.795" W
13	*Sigmodon hispidus*	33° 42' 29.134" N	94° 38' 10.228" W
14	*Sigmodon hispidus*	33° 42' 29.134" N	94° 38' 10.228" W
15	*Sigmodon hispidus*	33° 42' 29.134" N	94° 38' 10.228" W
16	*Blarina carolinensis*	33° 42' 29.134" N	94° 38' 10.228" W
17	*Neotoma floridana*	33° 53' 40.15" N	94° 45' 14.43" W
18	*Blarina carolinensis*	33° 42' 29.134" N	94° 38' 10.228" W
19	*Reithrodontomys fulvescense*	33° 42' 29.134" N	94° 38' 10.228" W
20	*Sigmodon hispidus*	33° 42' 29.134" N	94° 38' 10.228" W
21	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
22	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
23	*Neotoma floridana*	33° 53' 40.15" N	94° 45' 14.43" W
24	*Reithrodontomys fulvescense*	33° 45' 24.76" N	94° 38' 36.96" W
25	*Sigmodon hispidus*	33° 42' 29.134" N	94° 38' 10.228" W
26	*Reithrodontomys fulvescense*	33° 42' 29.134" N	94° 38' 10.228" W
27	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
28	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
29	*Reithrodontomys fulvescense*	33° 43' 17.598" N	94° 41' 6.673" W
30	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
31	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
32	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
33	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
34	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
35	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
36	*Reithrodontomys fulvescense*	33° 42' 29.134" N	94° 38' 10.228" W
37	*Blarina carolinensis*	33° 43' 17.598" N	94° 41' 6.673" W
38	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
39	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
40	*Reithrodontomys fulvescense*	33° 43' 17.598" N	94° 41' 6.673" W
41	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
42	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
43	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
44	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W

45	*Peromyscus leucopus*	33° 43' 17.598" N	94° 41' 6.673" W
46	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
47	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
48	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
49	*Sigmodon hispidus*	33° 43' 17.598" N	94° 41' 6.673" W
50	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
51	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
52	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
53	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
54	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
55	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
56	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
57	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
58	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
59	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
60	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
61	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
62	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
63	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
64	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
65	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
66	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
67	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
68	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
69	*Rattus norvegicus*	29° 57' 32.29" N	90° 03' 37.22" W
70	*Rattus norvegicus*	29° 57' 03.58" N	90° 04' 52.57" W
71	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
72	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
73	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
74	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
75	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
76	*Rattus norvegicus*	29° 57' 33.03" N	90° 03' 36.02" W
77	*Rattus norvegicus*	29° 57' 03.58" N	90° 04' 52.57" W
78	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
79	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
80	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
81	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
82	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
83	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
84	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
85	*Rattus norvegicus*	29° 56' 36.32" N	90° 04' 37.58" W
86	*Reithrodontomys fulvescense*	33° 44' 2.54" N	94° 38' 39.37" W
87	*Rattus rattus*	30° 31' 47.27" N	91° 11' 23.82" W

88	*Rattus rattus*	30° 31' 50.46" N	91° 11' 44.35" W
89	*Rattus rattus*	30° 31' 50.46" N	91° 11' 44.35" W
90	*Rattus norvegicus*	30° 10' 22.88" N	93° 10' 26.10" W
91	*Rattus norvegicus*	30° 10' 22.88" N	93° 10' 26.10" W
92	*Rattus norvegicus*	30° 10' 22.88" N	93° 10' 26.10" W
93	*Rattus norvegicus*	30° 10' 22.88" N	93° 10' 26.10" W
94	*Rattus norvegicus*	30° 10' 22.88" N	93° 10' 26.10" W
95	*Rattus norvegicus*	30° 10' 22.88" N	93° 10' 26.10" W
96	*Rattus norvegicus*	30° 14' 14.59" N	93° 05' 03.93" W
97	*Rattus norvegicus*	30° 12' 57.05" N	93° 14' 58.87" W

Conclusion

Climate change is expected to profoundly influence ecosystems by causing numerical (amplification of parasite populations), functional (shift or range expansion of parasites and hosts) or microevolutionary (local adaption or directional shit in gene frequencies) alterations in host, parasite and pathogen interactions, and resulting ultimately in the emergence of diseases (Brooks & Hoberg, 2007). Epidemiological diagnostic techniques and modeling facilitates our understanding of the potential impacts climate change will have on host-parasite interactions.

This study utilized ecological niche modeling (ENM) to predict the present and future distribution of *A. cantonensis* and integrated field epidemiology and modern molecular techniques to test predictions of the generated ENM. I sampled rodents for *A. cantonensis* in regions predicted to have suitable and non-suitable habitats and identified inconsistencies between the ENM and where the parasite was found. Although *A. cantonensis* was not detected through visual examination of rodent tissue, both real-time PCR and sequencing methods detected the parasite, reaffirming the benefits and sensitivity of molecular techniques. This is the first documentation of the parasite within Oklahoma, and within the host, *Sigmodon hispidus*. This is surprising, because Oklahoma was predicted to be a region of non-suitable habitat, altering our understood current range of *A. cantonensis.*

In addition to the current range expansion of *A. cantonensis*, future predictions indicate that global climate change will continue to have a functional effect on *A. cantonensis*. Under all three climatic scenarios, the model predicts a decrease in suitable habitat for *A. cantonensis*, as well as a range shift north and eastward in the Northern hemisphere and southward in the Southern hemisphere. These results are not surprising considering that climate change is predicted to cause periods of extreme drought in new regions, negatively impacting intermediate

hosts (e.g. snails, slugs) that *A. cantonensis* is dependent on (Houghton et al., 2001). Such changes in the distribution of the parasite are likely to lead to disease emergence in wildlife previously unaffected.

Limitations of this study include small sample size and a need for refinement of molecular and modeling techniques. Do to the high number of false positives; further evaluation of the qPCR techniques is necessary. For now, the most likely cause of false positives is lack of a black hole quencher (BHQ), which better masks additional fluorescence. However, because this is the first time this probe was used on definitive host tissue, other complications may arise and require modification. Furthermore, ENM predictions regarding suitable habitat in Oklahoma, and my findings of *A. cantonensis* within the state, contradict. This discrepancy illustrates the difficulty in predicting the distribution of organisms without field sampling, particularly those with complex life histories such as parasites, highlighting the need to consider understated yet important complexities of parasite-host interactions and the ability of parasites to acquire novel hosts. Modeling of this parasite and others will require additional information (e.g. host life cycle requirements and distribution) to generate an accurate portrayal of the species distribution.

Execution of control and preventive measures is essential for management of zoonotic EIDs and requires expertise in ecological and epidemiological techniques from various fields (Artois et al., 2001). By integrating ENM field surveillance for disease confirmation, molecular identification advancements, development of plans for pathogen/host management and increased public awareness, disease emergence and outbreak will decline. Furthermore, notification of medical and wildlife specialist in SE Oklahoma and surrounding areas of *A. cantonensis* is crucial, especially if there is a probable history of intermediate host ingestion. Future studies on

EIDs should integrate and perfect techniques such as ENM and qPCR with classical field methodology to promote our understanding of diseases.

References

Artois, M., Delahay, R., Gubert V., & Cheeseman, C. (2001). Control of infectious diseases of wildlife in Europe. *The Veterinary Journal*, 162, 141-152.

Brooks, D.R., & Hoberg, E. P. (2007). How will global climate change affect parasite-host assemblages?. *Trends in Parasitology*, 23, 571-574.

Budowle, B., Schutzer, S. E., Einseln, A., Kelley, L. C., Walsh, A. C., Smith, J. A. L., . . .Campos, J.(2003). Building microbial forensics as a response to bioterrorism. *Science Galley*, 301, 1-3.

Chikweto, A., Bhaiyai, M., Maepherson, C. N. L., DeAllie, C., Pickney, R., D.,Richards, C., & Sharma, R. N. (2009) Existence of *Angiostrongylus cantonensis* in rats (*Rattus norvegicus*) in Grenada, West Indies. *Veterinary Parasitology*, 162, 160-162.

Cunningham, A. A. (2005). A walk on the wild side-emerging wildlife diseases. *British Medical Journal*, 331, 1214-1215.

Daszak, P., & Cunningham, A. A., (1999). Extinction by infection. *Trends in Ecology and Evolution*, 14, 279.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000) Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science*, 287, 443-499.

Dobson, A., & Foufopoulos, J. (2001). Emerging infectious pathogens of wildlife. *Ecology and Evolutionary Biology*, 356, 1001-1002.

Ecker, D. J., Sampath, R., Willett, P., Wyatt, J. R., Samant, V., Massire, C., . . .Budowle, B. (2005). The Microbial Rosetta Stone Database: A compilation of global and emerging infectious microorganisms and bioterrorist threat agents. *BMC Microbiology*, 5, 1-19.

Heidejorgensen, M. P., Harkonen, T., Dietz, R., & Thompson, P. M. (1992). Retrospective of the 1988 European seal epizootic. *Diseases of Aquatic Organisms*, 13, 37-62.

Houghton , J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P.J., Dia, X.,. . .Johnson, C. A. (2001) Climate Change 2001: The Scientific Basis. Cambridge: Cambridge University Press.

Jones, K., Patel, N., Levy, M., Storeygard, A., Balk, D., Gittleman, J., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, 451, 990-993.

Kim, D., Stewart, T., Bauer, R., Mitchell, M. (2002). *Parastrongylus* (=*Angiostrongylus*) *cantonensis* now endemic in Louisiana. *Journal of Parasitology*, 88, 1024-1026.

Kliks, M. & Palumbo, N. (1992). Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis by *Angiostrongylus cantonensis*, the nematode lungworm of rats. *Social Science & Medicine*, 34, 199-212.

Lederberg, J., Shope, R. E., & Oakes, S. C. Jr., (1992). Emerging infections: microbial threats in health in the United States. Washington, DC: Institute of Medicine, National Academy Press.

Prakesh, V. (1999). Status of vultures in Keoladeo National Park, Bharatpur, Rajasthan, with special reference to population crash in *Gyps* species. *Journal of Bombay Natural History Society*. 96, 204-230.

Qvarnstrom, Y., Aramburu da Silva, A. C., Teem, J. L., Hollingsworth, R., Bishop, H., Graeff-Teixeira, C., & da Silva A. J. (2010). Improved Molecular Detection of *Angiostrongylus cantonensis* in Mollusks and Other Environmental Samples with a Species-Specific Internal Transcribed Spacer 1-Based TaqMan Assay. *Applied and Environmental Microbiology*, 76, 5287-5289.

Roelke-Parker, M. E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., . . . Appel, M. J. (1996). A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature*, 379, 441-445.

Taylor, L. H., Latham, S. M., & Woolhouse, M.E. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society: B Biological Sciences*, 356, 983-989.