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RESEARCH TOPICS

GLOBAL CHANGE AND HUMAN VULNERABILITY TO VECTOR-BORNE DISEASES

Topic Editors
Rubén Bueno-Marí and
Ricardo Jiménez-Peydró

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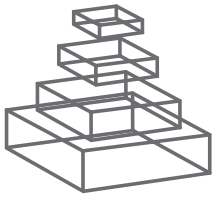

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GLOBAL CHANGE AND HUMAN VULNERABILITY TO VECTOR-BORNE DISEASES

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It is well known that several climatic, environmental and socio-demographic changes that have occurred in the last years are some of the most important causes for the emergence/resurgence of vector-borne diseases worldwide. Global change can be defined as the impact of human activity on the fundamental mechanisms of biosphere functioning. Therefore, global change includes not only climate change, but also habitat transformation, water cycle modification, biodiversity loss, synanthropic incursion of alien species into new territories, or introduction of new chemicals in nature.

On this respect, some of the effects of global change on vector-borne diseases can be currently evaluated. Globalization has enabled the movement of parasites, viruses and vectors among different countries, or even at intercontinental level. On this regard, it is important to note that the increase of imported malaria cases in different Southern European countries has led to the re-appearance of autochthonous cases of disease transmission. Moreover, the used tire trade, together with global warming, have facilitated the introduction, spread and establishment of potential Dengue tropical vectors, such as Aedes aegypti or Aedes albopictus in temperate areas. Consequently, recently the first Dengue indigenous cases in the last decades have been reported in different Southern areas of North America and Europe. Furthermore, habitat modification, mainly deforestation and transformation of aquatic environments, together with the changes in thermal and rainfall patterns, are two of the key factors to explain the increasing incidence of Leishmaniasis and several tick-borne diseases.

The aim of this Research Topic is to cover all related fields with the binomial vector-borne diseases / global change, including basic and applied research, approaches to control measures, explanations of new theories, opinion articles, reviews, etc. To discuss these issues, a holistic and integrative point of view is necessary, which only would be achieved by the close and active participation of specialists on entomology, parasitology, virology and epidemiology. Our objective is to use a systems approach to the problem of global change and vector-borne diseases. To achieve this ambitious goal and to comply with a demand of first-rate scientific and medical interest, we are very keen on asking for the participation of multiple contributors.

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PHYSIOLOGY

EDITORIAL
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Global change and human vulnerability to vector-borne diseases

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This e-book presents a collection of research and review articles related to the spread, control and basic understanding of vector borne diseases all over the world. It is well known that a multidisciplinary point of view is necessary in order to develop a global vision of this emergent problem. Therefore, in order to promote this holistic approach to the knowledge of vector borne diseases, this e-book contains a total of 19 collaborations of entomologists, epidemiologists, virologists, parasitologists, bacteriologists, zoologists and veterinarians of Europe, Africa, Asia, and America. The title perfectly reflects some of the global factors that are behind the emergence and/or reemergence of vector borne diseases.

It is now well known that several climatic, environmental and sociodemographic changes that have occurred over the past years are some of the most important causes for the resurgence of many diseases worldwide. However, global change, defined as the impact of human activity on the fundamental mechanisms of biosphere functioning, includes not only climate change, but also habitat transformation, water cycle modification, biodiversity loss, synanthropic incursion of alien species into new territories, or the introduction of new chemicals in nature.

Although there is a large and varied group of vectors worldwide, in this e-book we have examined the two most important disease vectors in our opinion: mosquitoes and ticks. Studies about the presence and transmission rates of viruses like West Nile, assays about mosquito control with new and encouraging methods, studies related to the importance of vector control strategies, research results about the role of asymptomatic cases of anthroponosis like Dengue, and investigations about the impact of climate trends on diseases transmitted by ticks and mosquitoes, are some of the issues that can be found in this Research Topic.

As editors of this Research Topic, we would like to acknowledge sincerely all coauthors for their valuable and interesting contributions and we wish the readers of this e-book a productive and enjoyable reading of some of the most innovative work related to vector borne diseases.

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ORIGINAL RESEARCH ARTICLE
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Species composition and WNV screening of mosquitoes from lagoons in a wetland area of the Algarve, Portugal

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The aim of this study was to evaluate mosquito abundance, species diversity, larval and adult population dynamics in seven lagoons integrated in the wetland coastal system of the Algarve, Portugal, in the summer of 2007, as well as the screening of these for West Nile virus (WNV). WNV has been isolated from mosquitoes in this region, in the summer of 2004, next to the putative area of infection of two linked human WN cases. Adult mosquitoes were collected with CDC traps baited with CO_2, and potential breeding sites were surveyed for immature stages. Morphological identification of 1,432 adult mosquitoes and 85 larvae revealed the presence of 10 species: *Anopheles atroparvus*, *Anopheles algeriensis*, *Coquillettidia richiardii*, *Culex modestus*, *Culex pipiens*, *Culex theileri*, *Culex univittatus*, *Culiseta longiareolata*, *Aedes caspius*, and *Aedes detritus*. Adult mosquito peak densities were recorded in July, contrasting with null larval breeding in the same month in the surveyed biotopes. Most abundant species were *C. pipiens* (52%), *C. theileri* (29%), and *A. caspius* (11%). Lagoon Salgados and Quinta das Salinas, exhibited the highest similarity of culicid fauna, despite being most distant from each other, Female mosquitoes (1,249 specimens) screened by RT-PCR, did not reveal WNV products. However, previous detection of WNV activity in this area, susceptible to re-introductions, demands for continued vigilance.

Keywords: mosquitoes, wetlands, ecology, surveillance, WNV, Portugal

INTRODUCTION

Mosquitoes act as vectors in the transmission of human diseases, such as several arboviruses belonging to the genera *Alphavirus*, *Flavivirus*, and *Bunyavirus* (Lundström, 1999). West Nile virus (WNV) currently the most widely distributed arbovirus in the world, occurring on all continents except Antarctica, has been reported to occur in mosquitoes and to infect human and other vertebrates (Kramer et al., 2008). This virus was first isolated from the blood of a sick woman in the West Nile District of Uganda in 1937 (Smithburn et al., 1940).

In recent years human or animal, mainly enzootics involving horses, WNV disease in the Eastern Hemisphere have been reported mostly from areas in the Mediterranean Basin: in Algeria in 1994, Morocco in 1996 and 2003, Tunisia in 1997 and 2003, Romania in 1996 through 2000, Czech Republic in 1997, Israel in 1999 and 2000, Russia in 1999–2001 and 2003–2007, Hungary 2003–2008, southern France in 2000, 2003–2004, Italy in 1998, 2008–2009, Spain in 2008–2009, and Greece 2010–2011 (Murgue et al., 2001; Zeller and Schuffenecker, 2004; Hayes et al., 2005; Krisztalovics et al., 2008; Rossini et al., 2008; Barzon et al., 2009; Rizzo et al., 2009; Chaskopoulou et al., 2011; Danis et al., 2011a,b; Vázquez et al., 2011).

In Portugal epidemiologic surveys for West Nile detection in human and animal populations have been carried out since the 1970s revealing seroreactivity for this virus. However, the first WNV isolation from mosquitoes was reported in 1971 (Filipe, 1972). In the summer 2004 two linked WNV cases were reported in Irish tourists, acquired in the Algarve (Connell et al., 2004) after which WNV was isolated from mosquitoes in the same region (Esteves et al., 2005).

Throughout its worldwide distribution, WNV is maintained in nature by enzootic cycles between ornithophilic mosquitoes, predominantly *Culex* species, although other genera may also be vectors, and birds (Hayes et al., 2005). Mosquitoes can also act as bridge vectors transmitting WNV out of its natural cycle to accidental hosts, such as domestic animals, particularly horses, and humans (Rappole and Hubálek, 2003; Linke et al., 2007; Kramer et al., 2008).

There are two important periods for pathogen introduction that correspond to the biannual bird migration. The first, during spring, when birds fly from Africa and can introduce pathogens in the North hemisphere especially in Mediterranean wetlands as well as in other countries from Europe. The second period, in autumn, when birds return to Africa, may carry pathogens from Northern to Southern Europe (Jourdain et al., 2007). WNV has been isolated from actively migrating birds (Malkinson et al., 2002). In European regions WNV cases occur in late summer and early fall months, when mosquito populations are at their highest densities. WNV outbreaks have occurred at urban sites near wetlands where migratory birds, mosquitoes, and humans are concentrated (Rappole et al., 2000).

Climate changes can influence significantly vector-borne disease transmission, as insects are very sensitive to meteorological conditions, namely temperature and humidity. Warmer weather and high precipitation may lead to sudden increases of mosquito populations, due to abundant larval habitats and climatic favorable conditions. Warmer weather would also influence people's behavior, leading them to spend more time outdoors. Outdoor activities, associated or not with tourism, can lead to higher exposure of humans to mosquito bites, and consequently to the transmission of mosquito borne diseases (Morgan, 2006). The ecological understanding of mosquito vectors of arboviruses is an important contribution of the surveillance regarding the risks for public health and mosquito control programs (White, 2001; Rydzanicz and Lonc, 2003; Medlock et al., 2006).

Due to its geographic localization and favorable climate, the Algarve province combines several conditions that can lead to the emergence and re-emergence of mosquito borne diseases. The presence of wetlands and lagoons, used by migratory birds can favor new viral introductions and the presence and abundance of mosquito populations can maintain viral enzootic cycles. This kind of water collections provide suitable breeding sites to some mosquito species reported as vectors of WNV (Medlock et al., 2006). The Algarve is a well known touristic place, with thousands of visitors every year.

The goals of our study included the study of the abundance and diversity of mosquitoes in the vicinity of several water collections, lagoons, of the Algarve used by migratory birds, as well as screening those mosquitoes for the presence of WNV.

MATERIALS AND METHODS

STUDY AREA

The study took place during the summer months of 2007, from June to September, in seven lagoons near the coastal line, in the province of the Algarve, southern Portugal, next to four localities, within a maximum distance of 30 km (**Figure 1**): (1, a) Lagoon Salgados (N37°5′53.33″; W8°20′3.61″), Pêra; (2, b) Lagoon Nova (N37°5′29,78″; W8°8′33,28″) and (2, c) Lagoon Antiga (N37°5′48.03″; W8°8′57.98″W), Vilamoura Natural Park, Vilamoura; (3, d) Lagoon Almargem (N37°6′8,07″; W8°21′15.97″), Quarteira;(4, e) Lagoon Dunas Douradas (N37°2′42,6″; W8°3′8,4″; (3, f) Lagoon Garrão (N37°2′42,6″; W8°2′32,6″) and Quinta das Salinas (N37°2′15,1″; W8°2′1,3″; (3, g) Garrão.

Some of these lagoons are referenced as regions of high diversity of wildlife including the presence of more than a hundred bird species. Due to this, and in particularly the Salgados lagoon was included in the national IBA's (PT035 Important Bird Area – 149 ha) being one of the more important wetlands in the Algarve, gaining national, and even international, interest, for some bird species, such as the *Porphyrio porphyrio* (Purple Swamphen) and *Aythya nyroca* (Ferruginous Duck; CCDR, 2007).

Algarve provides a wide range of outdoor activities, namely bird watching, fishery, aquaculture, and salt-works. Climate in the Algarve may be considered Mediterranean Csa type according to the Köppen–Geiger classification. For the interval 1971–2000, monthly average temperature varies from 16°C in January–December to 28°C in July–August (mean average 17°C, maximum

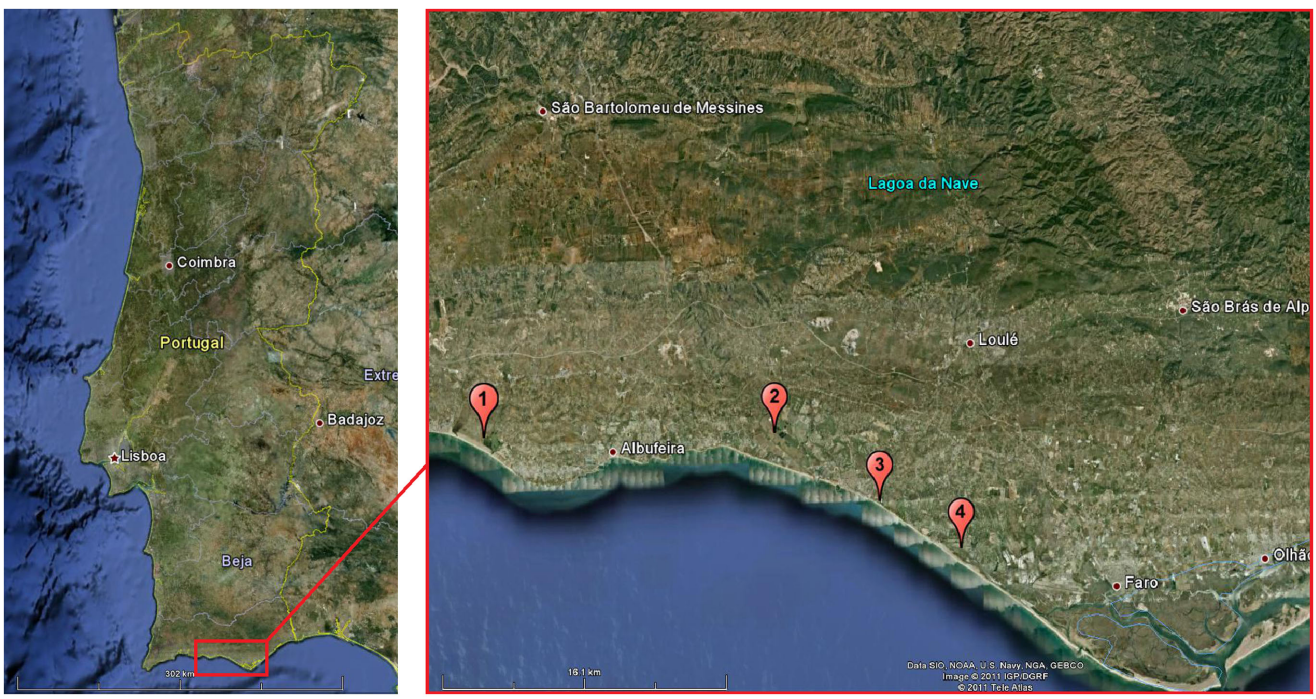

FIGURE 1 | Map showing the localization of the study area and the various surveyed lagoons: (1, a) Lagoon Salgados (Pêra); (2, b) Lagoon Nova, (2, c) Lagoon Antiga (Parque ambiental de Vilamoura); (3, d) Lagoon Almargem (Quarteira); (4, e) Lagoon Dunas Douradas, (4, f) Lagoon Garrão, and (4, g) Lagoon Quinta das Salinas (Garrão). Distance between 1 and 4 is ca. 30 km.

average 21°C, minimum average 12°C), and mean precipitation varies from 2.1 mm in August to 96 mm in December (IM, 2010).

MOSQUITO SAMPLING AND PROCESSING

Adult mosquitoes were collected monthly in the seven lagoons above, in fixed sampling sites with CDC (Centers for Disease Control; Sudia and Chamberlain, 1962) baited with carbon dioxide (CO_2; ca. 1 kg/trap; Newhouse et al., 1966), operating overnight from before dusk to after the sunrise (minimum 12 h). All traps were suspended at a height of 1.5–2 m from the ground, either in trees or human-made structures by the edge of the lagoons. Collected mosquitoes were transported live in refrigerated boxes to laboratory where they were frozen till identification.

Fixed potential breeding sites were surveyed for mosquito larvae and pupae using a standard dipper (ca. 700 ml), performing a minimum of five dips per site (3500 ml). These included margins of the lagoons, or irrigation ditches and effluents from urban wastewater treatment plants (UWTP) that discharged to the lagoons. Physical and chemical parameters (temperature, oxygen, pH, ion concentration, conductivity, total dissolved solids, salinity, and water depth) of the water were recorded. Whenever it was not possible to prospect the fixed breeding site (for example, when it was dry), an attempt was made to survey the vicinity. Immature forms were transported to the laboratory in glycerinated ethanol (4%, V/V). Physical and chemical parameters of the water collections are presented as their mean values, during the observation period, for each of these lagoons.

Adult mosquitoes were sorted by site, species, sex, and females by the gonotrophic stage, pooled accordingly to a maximum of 50 specimens per pool, and kept at −80°C until viral screening. Immatures were mounted in *Ribeiro* medium (Ribeiro, 1967). Adults and immatures were identified according to identification keys by Ribeiro and Ramos (1999).

Adult mosquito densities were calculated as mean mosquitoes per trap-night. The modified Sorensen coefficient (Southwood, 1978), grouped the surveyed lagoons according to the similarity of their adult culicid fauna, generating a respective dendrogram. Breeding site index (R) was calculated as the number of biotopes they were positive for culicids (Ribeiro et al., 1980), and larval density was calculated as the mean number of larvae per dipper.

VIRAL SCREENING

Adult mosquitoes were triturated on a vibrator for 30 s with glass beads and alundum in a 15-ml conical, screw cap plastic tube with 1.5 ml of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) at 4% (W/V). All the tubes were kept on ice. After a centrifugation (896 × G) at 4°C for 10 min three aliquots were made, using sterile pipettes, to be used on Reverse transcriptase – polymerase chain reaction (RT-PCR). RT-PCR reactions were performed using 6 µl of RT buffer, 2 µl of 0.1 M dithiothreitol, 1 µl RNA guard, 5 µ dNTP's (2.5 mM), 2.5 µl random primer (0.3 µg/µl), 8 µl ddH_2O, and 5 µl of the titration product. Samples were boiled for 5 min and cooled on ice. To this mixture it was added 1 µl of Moloney murine leukemia virus (MMLV) reverse transcriptase. The reaction was performed at 37°C for 60 min, with a final step of 95°C for 10 min.

Following reverse transcriptase, 5 µl of cDNA was transferred to 45 µl of a PCR mix containing 5 µl Taq buffer, 1.75 µl MgCl2, 5 µl dNTP's, 30 µl ddH_2O, 0.5 µl Taq, and 1.25 µl of each primer, cFD2 (5′-GTGTCCCAGCCGGCGGTGTCATCAGC-3′) and MAMD (5′-AACATGATGGGRAARAGRGARAA-3′) in the first round (Scaramozzino et al., 2001) and FS 788M (Esteves et al., 2005) for the second. The thermal cycling profile consisted on 15 min at 94°C, three initial cycles of 60 s at 94°C, 60 s at 52°C, and 60 s at 72°C, followed by 40 cycles of 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C. The amplifications products were analyzed on agarose gels (1.5 or 2% for a better resolution) containing ethidium bromide.

RESULTS

ADULTS

A total of 1,432 adult mosquitoes were caught in the seven surveyed lagoons, between June and September 2007. Mosquito abundance, mean mosquito density per CDC trap, was lowest in June and maximum in July, coinciding with the highest mean temperatures and lowest precipitation recorded for the area (**Figure 2**).

Eight species were collected as adult mosquitoes: *Anopheles* (*Anopheles*) *algeriensis* ($n = 14$), *Aedes* (*Ochlerotatus*) *caspius* ($n = 152$), *Aedes* (*Ochlerotatus*) *detritus* s.l. ($n = 7$), *Coquillettidia richiardii* ($n = 20$), *Culex* (*Culex*) *pipiens* ($n = 749$), *Culex* (*Culex*) *theileri* ($n = 415$), *Culex* (*Culex*) *univittatus* ($n = 51$), and *Culiseta* (*Allotheobaldia*) *longiareolata* ($n = 24$), The most abundant was *C. pipiens* (52%), which peaked in July, as did *A. caspius*, the third most common species (11%). However, *C. theileri* (29%), the second most abundant mosquito, exhibited a slow increase, peaking in August, but maintaining similar levels from July to September. Relative mosquito species densities varied among the seven surveyed lagoons (**Figure 3**). The dendrogram based on the modified Sorensen coefficient (Southwood, 1978), grouped the surveyed lagoons according to the similarity of their culicid fauna (**Figure 4**).

IMMATURES

Collection of immature forms in the margins of the seven surveyed lagoons, irrigation ditches and UWTP effluents draining

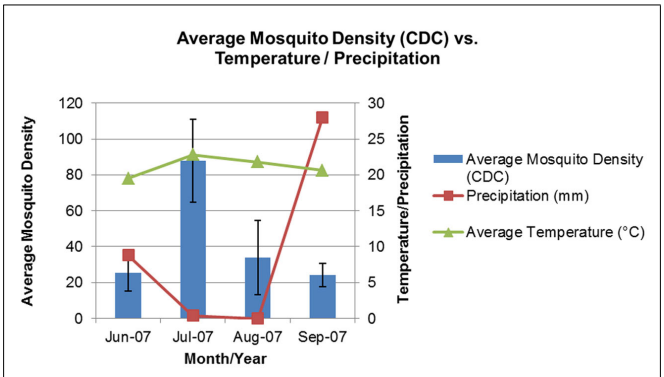

FIGURE 2 | Mean adult mosquito density collected by CDC traps, from June to September, 2007, in the seven surveyed lagoons, in coastal wetlands of the Algarve.

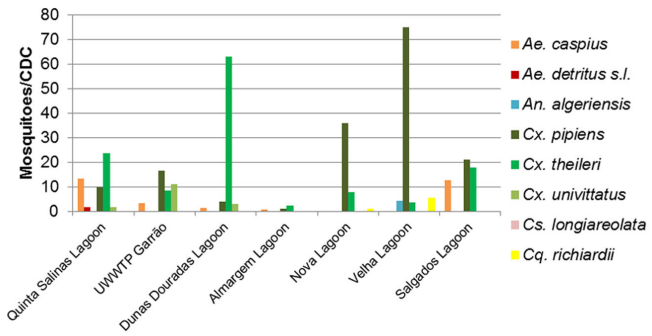

FIGURE 3 | Mean adult mosquito species composition of the seven surveyed lagoons in the wetlands of the Algarve collected by CDC traps, from June to September, 2007.

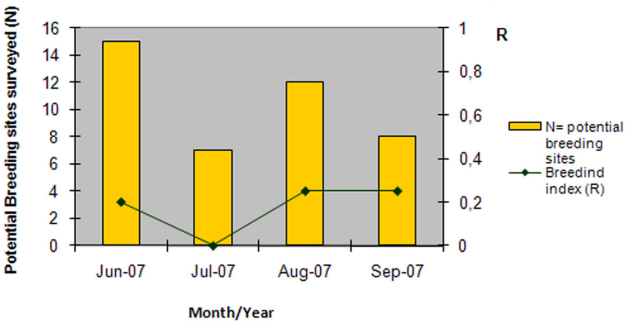

FIGURE 5 | Potential breeding sites surveyed for immature culicids along the seven lagoons surveyed, and respective breeding index, from June to September 2007.

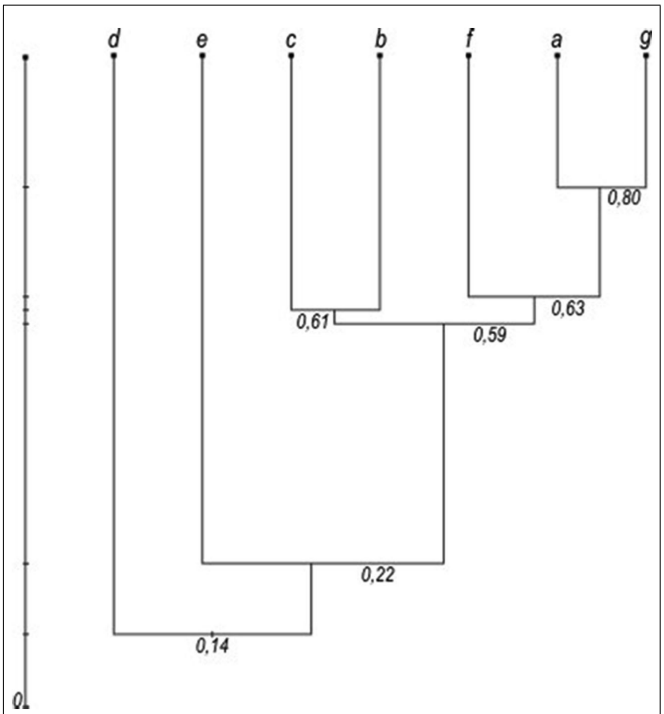

FIGURE 4 | Dendrogram based on the modified Sorensen coefficient of similarity between the surveyed lagoons: (a) Salgados (1), (b) Nova (2), (c) Antiga (2), (d) Almargem (3), (e) Dunas Douradas (4), (f) Garrão (4), and (g) Quinta das Salinas (4), (numbers in brackets refer to Figure 1).

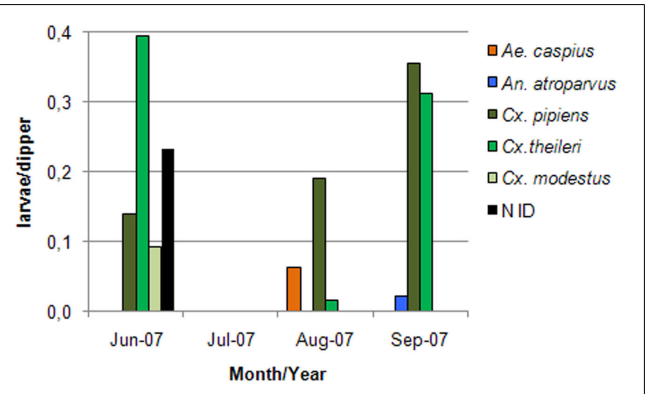

FIGURE 6 | Species composition and density (mean larvae/dipper) of the culicid immature forms collected along the lagoons surveyed. A total of 85 larvae were collected. No larvae were collected in the margins of natural lagoons.

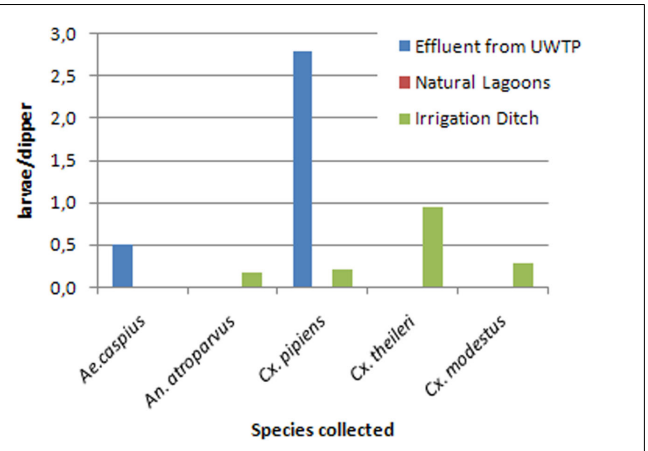

FIGURE 7 | Culicid immature species, and relative density, according to the nature of the biotope, in the surveyed lagoons.

to these, in a minimum of 7 and a maximum of 15 biotopes, yielded a total of 85 larvae, with negative collections in the month of July, and a breeding index (R) of 0.2–0.25 in the remaining months (**Figure 5**). Mean larval density was 0.18 larvae/dipper in June, 0.25 in August, and 0.7 in September. Six species were collected as immatures, *Anopheles* (*Anopheles*) *atroparvus*, *A. caspius*, *Culex* (*Barraudius*) *modestus*, *C. Pipiens*, and *C. theileri*, which varied according to the month (**Figure 6**), but also according to the nature of the biotope of collection (**Figure 7**). Physical and chemical parameters of these water collections are presented as their mean values, during the observation period, for each of these lagoons (**Table 1**).

Table 1 | Physical and chemical parameters* of the seven lagoons surveyed, communication with sea water and effluents of urban wastewater treatment plants, represented as the mean value for the observational period.

Lagoon	Sea water	UWTP	pH	Redox potential (mV)	Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	O_2 mg/l	T °C
Salgados (1, a)	Yes	Yes	9.25	−149.25	4,972.50	2,617.50	**4.43**	8.36	30.98
Nova (2, b)	–	Yes	7.98	−66.33	2,645.67	1,590.67	1.62	9.99	27.08
Antiga (2, c)	–	Yes	8.14	−77.75	2,165.00	1,212.50	1.30	9.25	26.73
Almargem (3, d)	Yes	Yes	8.05	−73.00	5,015.00	3,495.00	**11.15**	9.39	26.03
D. Douradas (4, e)	–	Yes	8.38	−96.67	1,396.00	832.67	0.87	9.38	28.00
Garrão (4, f)	–	Yes	7.62	−50.00	1,852.00	1,109.00	1.17	9.02	23.70
Salinas (4, g)	–	Yes	7.67	−54.67	16,746.67	11,623.33	**26.23**	10.32	26.57

The higher salinity values recorded are represented in bold.

**Water physical and chemical parameters were measured at about 12:00 a.m.*

*In brackets are the numbers referring to **Figure 1** and the letters referring to **Figure 4**.*

VIRAL SCREENING

A total of 1,249 female mosquitoes were screened for WNV (89% of the total collection), of which 55% ($n = 687$) were *C. pipiens*, 31.8% ($n = 397$) *C. theileri*, 6.7% ($n = 84$) *A. caspius*, 3.8% ($n = 47$) *C. univittatus*, 1.6% ($n = 20$) *C. Richiardii*, and 1.1% ($n = 14$) *A. algeriensis*. However, there was no detection of WNV nucleic acid sequences.

DISCUSSION

This survey for mosquitoes along seven lagoons located along the coastal line of the Algarve evidenced a seasonal dynamic of adults, following a typical pattern accompanying the mean temperature, with highest densities in the hottest summer months for temperate climates (Lysyk, 2010). This is not always the case as sometimes, due to low atmospheric humidity related with continued lack of precipitation, density decreases can be observed. *C. pipiens* was the most abundant species, accounting for nearly half of the collections, peaking in July, as did *A. caspius*, the third most common species. This seasonal dynamics contrasts with that registered in this area the previous year (2006), when *A. caspius* had been the dominant species, peaking in July–August, and *C. pipiens* exhibited a decrease in these months (Osório et al., 2008), despite the sampling method being also CDC traps, thus comparable to that used in this study. Another contrasting difference in our study, in 2007, was that *C. theileri* was the second most abundant species, exhibiting a slow increase and peaking in August. This also contrasts with previous findings by us, where *C. theileri* came only to fifth most common species in this same area (Almeida et al., 2008).

Diversity, similarity and relative abundance of mosquito species varied among the surveyed lagoons. Salgados (1, a) and Quinta das Salinas (4, g) exhibited similarly medium densities of *C. pipiens*, *C. Theileri*, and *A. caspius*, thus having higher Sorensen coefficients of similarity and coming closer in the dendrogram. These lagoons exhibited the highest salinity values (bold values in **Table 1**), together with Almargem lagoon (3, d), thus it is not surprising that these were where *A. caspius*, a halophile species, had higher densities, and was also found as immature forms. Almargem lagoon was dissimilar to these due to its very low mosquito density. The lagoons at Vilamoura natural park, Nova (2, b), and Antiga (2, c), were very identical between each other but different from the previous, in that *C. pipiens* was the predominating species, followed closely by Garrão (4, f), though with much lesser abundances. These three lagoons are those closer to UWTPs which can account for that predominance of *C. pipiens* and relative similarity. Dunas Douradas (4, e) exhibited a quite different culicid fauna, with *C. theileri* as the overly dominant species, thus placing it far apart from the others. However, the total small sample size may have affected the outcome of this diversity analysis.

Comparing the species collected in different life stages, two were not caught as adults but only as larvae, *A. atroparvus* and *C. modestus*, whereas five were not caught as immatures, namely *A. algeriensis*, *A. detritus* s.l., *C. univittatus*, *C. Richiardii*, and *C. longiareolata*. Furthermore, no relation could be established between species collected in both life stages in each lagoon site. *A. atroparvus* was not caught as adult, likely due to the collection method used, as this species rarely is attracted to CDC traps, and is mainly found in indoor resting collections (Almeida et al., 2008). *C. modestus* was not caught as adult probably due to its low abundance, as shown in previous surveys of this very same region, where only two, also immature forms, were caught in 1975 (Ramos et al., 1977/1978), in similarly brackish waters as in this study, or not caught at all as adults in later surveys (Ramos et al., 1992; Almeida et al., 2008). Still, this species is a known vector of WNV in the Mediterranean area (Mouchet et al., 1970).

Immature surveys were mostly negative, with breeding indexes of 0.2–0.25, and, when positive, yielded very few specimens, which may account for the smaller number of species collected in relation to adults. The type of biotopes surveyed, which might not be the preferred ones, may also justify the lesser representation of species as immature stages. The particular bioecology of *C. richiardii*, whose immature stages live attached to stems of aquatic vegetation, requiring special techniques for their collection, is also a likely justification for their absence in immature collections, despite their continued presence as adults in these lagoonal ecosystems (Almeida et al., 2005, 2008). The low level of precipitation, along with increased evaporation, during the summer months, with the consequent decrease in water level and some degree of eutrophication, can be a possible explanation for the low breeding indexes registered. As to the physic-chemical parameters of the water, a pH higher than expected (>8.2) was detected in Salgados and Dunas Douradas lagoons, although this did not reveal to be exclusive of mosquito larval presence, as these were detected

in Salgados, which had the highest pH registered. The levels of dissolved oxygen measured were always above 5 mg/l, at around noon time, which could indicate much lower levels in the night and dawn as observed in this coastal system (Newton et al., 2010). Furthermore, in the lagoons Garrão and Dunas Douradas, the ichthyofauna was inexistent in 2007 probably due to anoxia in the water and lack of communication with the sea, and high mortality was observed in the Salgados lagoon (CCDR, 2007). Thus, if on one hand, conditions for the presence of mosquito larvae in the waters of these lagoons, do not seem to be very favorable, on the other hand, if colonization by mosquito immatures happened, it could be difficult to be naturally controlled due to the absence of fish, and other, natural predators, such as coleoptera and ephemeroptera, which have also very low densities, or are totally absent, in these lagoons (CCDR, 2007).

It is also noteworthy the presence of immature culicids and their species in relation to the nature and site of the biotope. First and foremost, no larvae were found in the margins of any of these lagoons, but only in the ditches discharging to them. In irrigation ditches *C. theileri*, *C. modestus*, *C. Pipiens*, and *A. atroparvus* could be found in progressively lower densities, whereas *A. caspius* was found just in UWTP effluents. *C. pipiens* was the only species that was found in both biotopes, however, with much higher densities in the effluents from UWTP, clearly illustrating the capacity of this species to breed in somewhat polluted waters.

Algarve is known to be one of the most touristic places of Portugal, with activities that include bird watching. Some of these lagoons are referenced as bird sanctuaries, with more than a hundred species registered. On the other hand, some of the birds regularly seen in these lagoons, such as *Anas platyrhynchos* (Mallard) in Salgados, *Hirundo rustica* (Barn Swallow), and *Pandion haliaetus* (Osprey) in Garrão, have been found infected with arboviruses such as WNV (CDC, 2011). Some studies also reported the presence of neutralizing antibodies against WNV in birds from Portugal like *Buteo buteo* (Common Buzzard), *Falco tinnunculus* (Common Kestrel), and *Ciconia ciconia* (White Stork) among others (Formosinho et al., 2006), and in other Mediterranean countries like the *Turdus merula* (blackbirds) and *Passer domesticus* (house sparrows; Figuerola et al., 2008). Furthermore, some of the bird species found in Portugal like *A. platyrhynchos* (Mallard), *Anas querquedula* (Garganey), *Ardea purpurea* (Purple Heron) are referenced as migratory, thus presenting the risk of pathogen introduction. (Feith, 2007, 2010; Jourdain et al., 2007).

Mosquito species found in this survey, such as *A. atroparvus*, *A. caspius*, *A. detritus* s.l., *C. richiardii*, *C. pipiens*, *C. theileri*, *C. modestus*, and *C. univittatus*, are known vectors of several arboviruses. Previous human cases of WNV have been putatively acquired in this region (Connell et al., 2004), followed by detection of WNV infected *C. pipiens* and *C. univittatus* in the same area (Esteves et al., 2005). A phylogenetic analysis strongly suggested a re-introduction of the virus, since it had first been detected in 1971 (Parreira et al., 2007). In fact, during the last decade WNV has been active in countries lining the Mediterranean (Morocco, Italy, France, Spain, and Greece; Zeller and Schuffenecker, 2004; Rossini et al., 2008; Barzon et al., 2009; Rizzo et al., 2009; Danis et al., 2011a,b). Despite the fact that no mosquito infections were detected in the present study, which may also be due to the small sample size, surveillance should be continued as all previous evidence support the idea that the Algarve, has the conditions for WNV re-mergence, and one of the species previously found infected, *C. pipiens*, is still the most abundant in several locations and is a known bridge vector, feeding both in birds and humans (Gomes et al., 2009).

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Larval habitat associations with human land uses, roads, rivers, and land cover for *Anopheles albimanus*, *A. pseudopunctipennis*, and *A. punctimacula* (Diptera: Culicidae) in coastal and highland Ecuador

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Larval habitat for three highland *Anopheles* species: *Anopheles albimanus* Wiedemann, *Anopheles pseudopunctipennis* Theobald, *and Anopheles punctimacula* Dyar and Knab was related to human land uses, rivers, roads, and remotely sensed land cover classifications in the western Ecuadorian Andes. Of the five commonly observed human land uses, cattle pasture ($n = 30$) provided potentially suitable habitat for *A. punctimacula* and *A. albimanus* in less than 14% of sites, and was related in a principal components analysis (PCA) to the presence of macrophyte vegetation, greater surface area, clarity, and algae cover. Empty lots ($n = 30$) were related in the PCA to incident sunlight and provided potential habitat for *A. pseudopunctipennis* and *A. albimanus* in less than 14% of sites. The other land uses surveyed (banana, sugarcane, and mixed tree plantations; $n = 28$, 21, 25, respectively) provided very little standing water that could potentially be used for larval habitat. River edges and eddies ($n = 41$) were associated with greater clarity, depth, temperature, and algae cover, which provide potentially suitable habitat for *A. albimanus* in 58% of sites and *A. pseudopunctipennis* in 29% of sites. Road-associated water bodies ($n = 38$) provided potential habitat for *A. punctimacula* in 44% of sites and *A. albimanus* in 26% of sites surveyed. Species collection localities were compared to land cover classifications using Geographic Information Systems software. All three mosquito species were associated more often with the category "closed/open broadleaved evergreen and/or semi-deciduous forests" than expected ($P \leq 0.01$ in all cases), given such a habitat's abundance. This study provides evidence that specific human land uses create habitat for potential malaria vectors in highland regions of the Andes.

Keywords: Ecuador, *Anopheles*, land use, land cover, highland malaria

INTRODUCTION

Recent studies have identified the presence of multiple *Anopheles* species as well as the occasional small-scale epidemic of malaria in the highlands of the northern and central Andes (Rutar et al., 2004; Pinault and Hunter, 2011a). Although land use and land cover are strongly associated with *Anopheles* larval habitat, increasing populations of the vectors, and malaria incidence in the highlands of Africa (e.g., Brinkmann, 1994; Shanks et al., 2000; Hay et al., 2002; Afrane et al., 2005), the associations of the highland-occurring *Anopheles* species in the Andes with land uses have been hitherto unknown.

Conversion of a property to a specific land use may alter the presence and abundance of local *Anopheles* species by creating potentially suitable larval habitats (reviewed in Reiter, 2001; Chhabra et al., 2006). Certain land uses are frequently associated with anopheline larval habitat on different continents, including rice farms (Brinkmann, 1994; Reiter, 2001), fish farms, particularly abandoned fish ponds (Reiter, 2001), as well as cattle pastures where cattle footprints create permanent depressions that fill with rainwater (Reiter, 2001).

In addition to creating potential larval habitat, reduced canopy cover in the highlands (in disturbed or deforested habitats) may influence anopheline habitat suitability by altering the local microclimate (Patz et al., 2004; Minakawa et al., 2005; Chhabra et al., 2006; Guerra et al., 2006; Pattanayak et al., 2006; Yasuoka and Levins, 2007). Deforested areas are approximately 0.5°C warmer than adjacent forested regions in highland Kenya (Afrane et al., 2005, 2006). *Anopheles gambiae* Giles in highland Kenya have 64.8–79.5% higher fecundity rates, a 40% higher net reproductive rate and a 29% higher vector capacity (Afrane et al., 2006), as well as a shortened gonotrophic cycle in deforested rather than forested regions (Afrane et al., 2005). Increasing temperatures have been shown to have non-linear, positive effects on anopheline larval development rates (Paaijmans et al., 2010). In South America, deforestation has also been associated with malaria incidences, particularly for road building, gold mining, or permanent colonization or urbanization, most prominently in parts of the Amazon (Pinheiro et al., 1977; Walsh et al., 1993; Tadei et al., 1998; Póvoa et al., 2003; Caldas de Castro et al., 2006; Guerra et al., 2006; Yomiko Vittor et al., 2006; Pan et al., 2010; de Oliveira et al., 2011).

In malaria-endemic highland regions of Africa, anopheline larval habitat is limited by steep topography to areas where water accumulates (e.g., Balls et al., 2004), often in valley bottoms (Munga et al., 2009). In the Usambara mountains of Tanzania, *Anopheles* larvae live in sunlit pools that offer a warmer microclimate, and may be associated with the irrigated vegetable-growing terraces (Bødker et al., 2000). *A. gambiae* are associated with open habitat in farmlands and pastures in highland Kenya, particularly in water-collecting valley bottoms (Munga et al., 2009).

In South America, where highland malaria is less well-studied, Andean highland valleys were afflicted with cases of highland malaria during the 1940s, vectored by *A. pseudopunctipennis* (Levi Castillo, 1945). However, Levi Castillo (1945) documented the removal of this vector from highland valleys near Quito, Ecuador through the elimination of larval habitat and the use of chemical insecticides. Since that time, highland areas have been generally considered malaria-free (Pinault and Hunter, 2011a).

Land use changes in the Ecuadorian Inter-Andean valleys, particularly the conversion of highland páramo and forests to crops and pasture, occurred over vast areas during pre-Colombian times (Sarmiento, 2002). However, widespread land use changes have occurred in Ecuador during the last century in the coastal and Amazonian regions. The humid tropical forests of the coast were colonized during the cocoa boom in the 1920s and the banana boom in the 1950s, during which an extensive road network was built linking ports to inland coastal cities and highland Andean cities (Wood, 1972; Bromley, 1981). This road building led to informal colonization of foothill regions (ca. 500–1500 m) to grow sugarcane and oranges (Wood, 1972; Bromley, 1981). The northern Amazon was heavily colonized during the oil boom in the 1970s, when large-scale road networks were built to accommodate the oil industry (Wood, 1972; Bromley, 1981). Settler colonies built slash and mulch polycultures to farm rice, yams, corn, plantain, and yucca, that were later converted to pasture (Hiraoka and Yamamoto, 1980).

More recent studies of land use in Ecuador indicate the presence of permanent crops at lower elevations of the Andes, with denser human populations associated with temporary crops. Grasslands for pasture are associated with fertile soil, whereas economically poor areas are often associated with natural vegetation (no human land use; de Koning et al., 1998). On the coast, permanent export crops with widespread irrigation are favored, whereas in the Amazon, there is a trend for agricultural colonists to convert forest to grassland to be used as pasture (de Koning et al., 1998).

In this study, we evaluate the hypothesis that land use change is responsible for the proliferation of multiple vector species in the western highlands of Ecuador by creating suitable larval habitat. We predict that current land uses would provide potentially suitable habitat for larvae of three species of western-occurring malaria vectors. In this study, we relate the most common land uses, as well as the presence of rivers and roads on the western cordillera of the Andes, to potentially suitable larval habitat for *Anopheles albimanus*, *Anopheles pseudopunctipennis*, and *Anopheles punctimacula*. We also compare the distribution of these species based on recent collections to published land cover classification maps. The purpose of this study was to identify human land uses and land covers that provide potentially suitable larval habitat for each of the three common *Anopheles* species, as well as to relate any of these species to the presence of roads and rivers that may serve as lowland-highland corridors, and thereby identify priority larval habitat types. Although the incidence of malaria has declined substantially in Ecuador in recent years due to government intervention, malaria persists at low levels in certain coastal and Amazonian regions (SNEM, 1995–2009), therefore, studies of vector ecology are still pertinent.

MATERIALS AND METHODS

LAND USE

In the present study, we attempt to characterize anopheline larval habitat availability in the highlands, i.e., steep topography areas of the western Andes of Ecuador. These lie conservatively from 500 to 2000 m in elevation, the latter being the current maximum altitudinal limit for collections of the three more common species (Pinault and Hunter, 2011a). To determine potential habitats made available by highland land uses, it was first necessary to characterize the most common land uses present between 500 and 2000 m. To that end, researchers surveyed land uses present along six altitudinal transects in the western Andes in 2009 and 2010, along the east-west roads from: Quito to La Independencia (ca. N01°02′), Pilaló to Quevedo (ca. S00°53′), Guaranda to Pueblo viejo (ca. S01°35′), Chimbo to Babahoyo (ca. S01°45′), Alausí to El Triunfo (ca. S02°18′), and Loja to Machala (ca. S03°52′). Researchers progressed very slowly along the roadway in a motor vehicle and stopped at 100 m elevation intervals to record the dominant land use, vegetation type, presence of water bodies, and geographical coordinates and altitude (using a Garmin® GPS eTrex Summit) observed on the right-hand side of the roadway (along the downhill trip). The six most commonly observed land uses were ascertained graphically and used in subsequent analyses.

To determine the potential anopheline larval habitat made available on the most common land uses within the highlands (determined above), researchers traveled along accessible roads in the western Ecuadorian Andes in summer, fall, and winter during 2009 and 2010 and searched for the following land uses: cattle farms, banana plantations, mixed tree plantations (including citrus fruits), sugarcane plantations, and empty lots/cleared land. To randomly select half of the available sites, at each potential site researchers flipped a coin to determine if the property would be sampled, then, permission was sought from the landowner or manager to enter the property for the purpose of the survey (except empty lots/cleared land, which were most often public domain). Between 21 and 30 sites were sampled for each land use type.

At each site, researchers recorded the geographic coordinates and altitude using a GPS as above, and the air temperature. Researchers then moved about the property and observed every potential anopheline habitat (i.e., standing water or slow-moving water bodies), recording the length, width, and depth, water temperature (Hanna Instruments HI98129 combination water tester), percentage cover of algae and macrophyte vegetation (10% classes), incident sunlight (10% classes), and water clarity (10% classes). In every water body, water was dip-sampled using a white plastic dipper (BioQuip) for *Anopheles* larvae a standard of 30 times (although five dips were used for very small water

bodies). When larvae were collected, these were placed in vials containing 95% ethanol, transported to the laboratory, and identified to species using the morphological key of Gorham et al. (1973) and molecular techniques as in Pinault and Hunter (2011a). Specimens not destroyed for molecular sequencing were deposited in the Ecuadorian National Collection in Quito (PUCE) and nucleotide sequences were deposited in GenBank (accession numbers JN412826–JN412843) When property sizes were greater than five hectares, researchers sub-sampled a portion of the property (approximately two hectares in size).

For sites with water bodies, an average value of the parameters above was obtained for each site and entered into a principal components analysis (PCA) to determine its relation to land use type (entered individually with dummy variables; CANOCO, 2002). Data were standardized by conversion of values to Z-scores using the mean and SD of the data set to ensure that factors were similarly weighted, and centered around zero prior to analysis (Whitlock and Schluter, 2009). Water bodies were then judged for potential suitability for *A. albimanus*, *A. pseudopunctipennis*, and *A. punctimacula* larvae using the following criteria, derived from field-based observations made during work conducted in Pinault and Hunter (2012). We therefore chose limiting temperatures and other criteria that would include all observed values from field sites. *A. albimanus*-suitable habitats were permanent water bodies with some floating algae (at least 10% cover) and within a temperature range of 22–33°C. *A. pseudopunctipennis*-suitable habitat contained some floating algae (at least 10% cover), 40% clarity or greater, less than 10% cover of emergent vegetation, and temperatures within the range of 20.3–37.0°C. *A. punctimacula*-suitable habitats were water bodies with less than 70% incident sunlight and less than 50% algae cover, with temperatures in the range of 19.5–28.6°C. All criteria represent values that are conservative, i.e., are more likely to include a site as potentially suitable for larvae than not, since there is some natural variation in larval habitat site suitability.

RIVER AND ROAD EDGES

Water bodies associated with rivers and roads were assessed as potential anopheline larval habitats in highland regions. Since roads and rivers form continuous rather than discrete sample areas, the site-selection process for sites was different than for land uses. Researchers searched in the western Andes for river and road sites that had at least one stagnant or slow-moving body of water that may have been potentially suitable for anopheline larvae, and flipped a coin to determine if the site would be used or not. At each site, all water bodies were scrutinized using the same criteria and sampled for anopheline larvae as above (for land uses) within a 10 m circular study radius. A total of 41 river sites and 38 road edge sites were surveyed. Data were analyzed using a PCA and assessed for potential species suitability as described above.

To determine the spatial distribution of road edge habitats, researchers undertook three altitudinal transects along the following roads: Ibarra to Lita (ca. N00°50′), Quito to Puerto Quito (ca. N00°06′), and Alausí to El Triunfo (ca. S02°18′). We were restricted to these roads due to safety issues regarding conducting research in traffic-heavy sections of other roads. Researchers traveled slowly along the road and stopped at every stagnant or slow-moving water body observed on the right side of the road. Water type, size, coordinates, and altitude were recorded as above. To determine the degree of clustering of water bodies along each road, water bodies were plotted in ArcGIS v. 9.2 (ESRI, 2008) and analyzed using the Spatial Autocorrelation (Moran's I index) tool, weighted by total water surface area. Moran's I (MI) determines whether spatial data is clustered, dispersed, or randomly distributed (Moran, 1950).

LAND COVER

Land use can affect larval habitat availability through changes to the vegetation architecture and degree of canopy openness. For this reason, we determined the land cover for each of the positive collection localities from Pinault and Hunter (2011a) for each of the three *Anopheles* species above. Collection sites were plotted in Arc GIS v. 9.2 with the GlobCover© 2009 land cover map, published by the European Space Agency and the Université Catholique de Louvain. The GlobCover© data presents mosaics of the 22 land cover classes of the United Nations Land Cover Classification System at 300 m resolution, derived from a time series of global MERIS (MEdium Resolution Imaging Spectrometer; Bontemps et al., 2011; available at ArcGIS online, 2011). Land use values on raster tiles corresponding to distribution (collection) points were enumerated and compared to expected values derived from the availability in the overall landscape, using a Chi-square test.

RESULTS

LAND USE

Land uses on total of 263 sites were recorded along five altitudinal transects. Of these, 117 sites were observed to be "natural," i.e., were not used for any discernable purpose and were not recently disturbed in any way, with an average altitude of 1705 ± 654 m (mean + SD). Of natural sites, 47 of these were too steep to be used by humans (i.e., cliffs), with an average altitude 1617 ± 313 m. Human-use sites observed along the transects are plotted in **Figure 1**. Within the elevations of interest (500–2000 m), the most common land uses observed are: cattle pastures (51 sites), human habitations (13 sites), banana plantations (9 sites), sugarcane plantations (8 sites), mixed tree plantations, including citrus fruit species (6 sites), and cleared land/empty lot/construction sites (4 sites; **Figure 1**). In a concurrent study, human habitations provided almost no standing water other than cement laundry tanks, rain barrels, and septic tanks, all of which are unsuitable for anopheline larvae (Pinault and Hunter, 2011b). Therefore, the remaining five land uses were used in the subsequent land use survey.

In the survey of properties of pre-established land uses, the largest of the five property types were cattle pastures ($n = 30$; area 36117 ± 77351 m^2), with an elevation of 1435 ± 517 m, then banana plantations ($n = 28$; area 13215 ± 32835 m^2), with an elevation of 887 ± 534 m, sugarcane plantations ($n = 21$; area 6870 ± 8682 m^2), with an elevation of 1249 ± 437 m, mixed tree plantations ($n = 25$; area 6012 ± 6198 m^2), with an elevation of 795 ± 502 m and finally empty lots ($n = 30$; area 2335 ± 2504 m^2) with an elevation of 1294 ± 557 m. The five human land uses were related to water body characteristics measured at each

water-present site using a PCA (**Figure 2**). In the first bi-plot, banana plantations and mixed tree plantations are strongly related on Axis 1 to depth and negatively related to incident sunlight, while empty lots are strongly associated with incident sunlight (**Figure 2A**). On Axis 2, cattle pasture is strongly related to macrophyte vegetation, increasing surface area, clarity, and algae cover (**Figure 2A**). In the second bi-plot, banana plantations and empty lots are associated with incident sunlight and to a lesser extent, depth, and higher temperature on Axis 1 (**Figure 2B**). On Axis 3, cattle farms are associated with macrophyte vegetation and algae cover, as well as with altitude, and surface area (**Figure 2B**). A total of 54.7% of the cumulative variance was explained by the first three Axes, with the following Eigenvalues: Axis 1: 0.215, Axis 2: 0.183, Axis 3: 0.149.

All sites with standing water were analyzed for potential habitat suitability for the three anopheline species, and plotted in **Figure 3A**. The human land use type with the most sites with available standing water were empty lots (40% of sites with some type of standing water), 13.3% of which were potentially suitable for *A. pseudopunctipennis* and 10% potentially suitable for *A. albimanus* (**Figure 3A**). In second place, 33.3% of cattle pastures had some type of standing water, 13.3% potentially suitable for *A. punctimacula*, and 10% potentially suitable for *A. albimanus* (**Figure 3A**). *A. albimanus* larvae were collected from one empty lot site (9 larvae) and one cattle pasture site (93 larvae). The other land uses in highland regions were quite dry, with 16% of mixed tree plantations, 14.3% of sugarcane, and 7.1% of banana farms containing any standing water, most of which was unsuitable for anopheline larvae (**Figure 3A**). *Anopheles* larvae were not collected at any other sites.

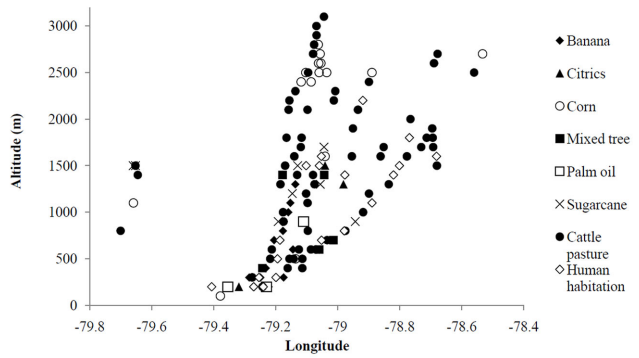

FIGURE 1 | Generalized land uses observed along six altitudinal transect in the western Ecuadorian Andes, plotted by longitude. Elevations of biological interest are indicated within a black rectangle and land use types are indicated by symbols (see legend).

RIVER AND ROAD EDGES

Road and river sites were sampled at mean elevations of 1411 ± 407 and 1034 ± 537 m, respectively. **Figure 4** presents the bi-plot results of a PCA relating roads and rivers to water body characteristics. In the first bi-plot, rivers are associated with clarity and to a lesser degree, depth, whereas roads are weakly associated with

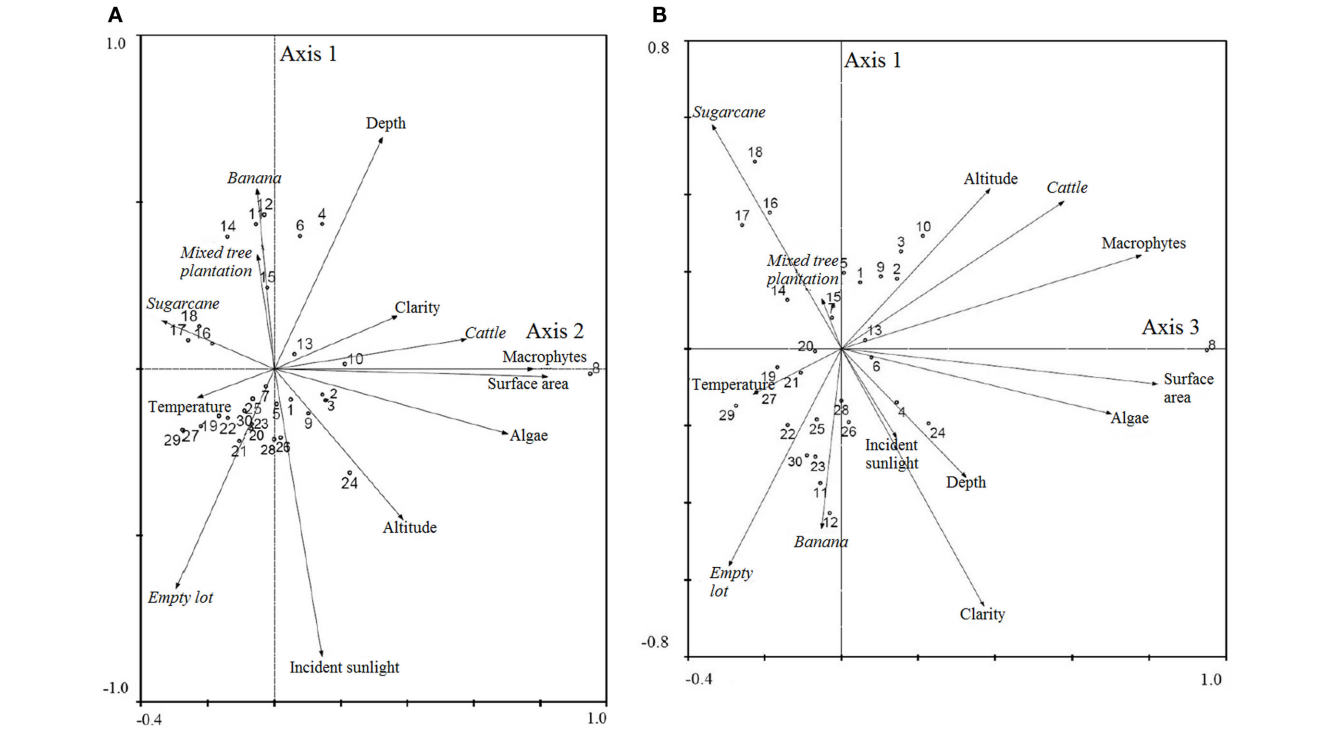

FIGURE 2 | Bi-plot results of principal components analysis of land uses and habitat characteristics for (A) Axes 1 and 2 and (B) Axes 1 and 3. Output from CANOCO (2002).

macrophyte vegetation cover along Axis 2 (**Figure 4A**). The water body characteristics of temperature, incident sunlight, and algae were inversely related to altitude (**Figure 4A**). In the second bi-plot, rivers and roads are separated along Axis 3 (**Figure 4B**). Rivers are weakly associated with temperature, depth, algae cover, clarity, and incident sunlight, whereas roads are related to increasing altitude (**Figure 4B**). The number of active samples in the PCA were 79, and a total of 57.2% of the cumulative variance was explained by the first three Axes, with the following Eigenvalues: Axis 1: 0.296, Axis 2: 0.160, Axis 3: 0.116.

Roads and rivers were judged to have a high proportion of sites that would be potentially suitable for anopheline larvae. Roads were judged to provide the most potential habitat for *A. punctimacula* (44.7%), followed by *A. albimanus* (26.3%; **Figure 3B**). Rivers, on other hand, provided the most potentially suitable habitat for *A. albimanus* (58%), followed by *A. pseudopunctipennis* (29.3%; **Figure 3B**). In terms of actual collections of anopheline larvae, one road site was positive for *A. albimanus* larvae (three larvae), three sites were positive for *A. punctimacula* larvae (4.3 ± 1.2 larvae; mean $\pm$ SD), and one site was positive for *A. pseudopunctipennis* larvae (6 larvae). Rivers provided the most current *Anopheles* habitat, with a total of 19 larval-present sites: 12 *A. pseudopunctipennis* sites (41.2 ± 56.8 larvae), five *A. punctimacula* sites (29.0 ± 34.4 larvae), and two *A. albimanus* sites (34.5 ± 38.9 larvae).

Of the three roadside water transects, Ibarra to Lita provided 21 water-present sites, followed by 15 sites for Quito to Puerto Quito and 6 sites for Alausi to El Triunfo. Most water bodies were roadside ditches, but permanent tire tracks, inland ditches, and pools formed by construction activities were also associated with roads. Spatial Autocorrelation analysis indicated that all three transects were significantly randomly distributed, rather than clustered at specific elevations (Ibarra-Lita transect: MI = 0.21, $Z = -0.57$; Quito-Santo Domingo transect: MI = −0.02, $Z = -0.08$; Alausi-El Triunfo transect: MI = −0.22, $Z = -0.04$). *Anopheles* larvae were only collected at four sites, with *A. pseudopunctipennis* larvae collected at two sites of ca. 1500 m, and *A. albimanus* larvae collected at two sites of ca. 600 m in elevation.

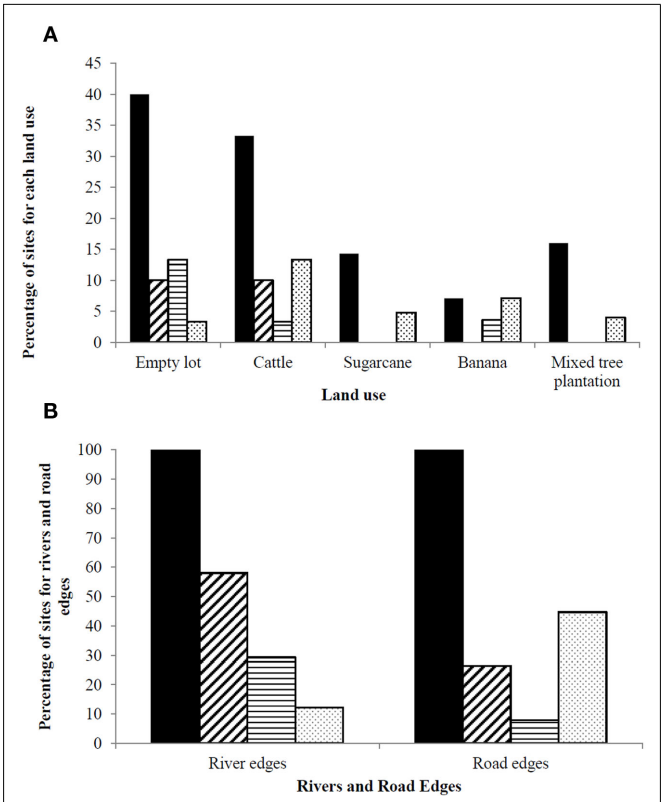

FIGURE 3 | Percentage of sites with: standing water available (black bars) and potentially suitable habitat for anopheline larvae: *A. albimanus* (diagonal bars), *A. pseudopunctipennis* (horizontal bars), and *A. punctimacula* (shaded); for (A) different land uses and (B) river and road edges. Note difference in y-axis ranges between **(A)** and **(B)**.

LAND COVER

Collection localities for the three species were related in ArcGIS to the U.N. land cover classification system. All three species were the mostly strongly related to the classification "closed/open broadleaved evergreen and/or semi-deciduous forest" (**Figure 5**), significantly more often than expected given its availability in the landscape (*A. albimanus*: $\chi^2 = 18.70$, df = 2, $P < 0.01$; *A. pseudopunctipennis*: $\chi^2 = 8.88$, df = 2, $P = 0.01$; *A. punctimacula*: $\chi^2 = 19.33$, df = 2, $P < 0.01$). *A. albimanus* were also often associated with "mosaic cropland (50–70%)/vegetation," while *A. pseudopunctipennis* was less specifically associated with one land cover type; rather, it was broadly associated with several land cover classifications (**Figure 5**). *A. punctimacula* was more specific and collected from fewer land cover types (**Figure 5**).

DISCUSSION

Our study has identified land uses (including rivers) that may be more amenable to the development of anopheline larvae in the western highlands of Ecuador. Cattle pasture can be considered the managed traditional land use most of concern. Cattle pasture was the land use most commonly observed during the transects (i.e., it is abundant), as well as having the largest property size of all the land uses examined, with an average area of 36617 m^2. Thirty-three percent of cattle pastures surveyed contained standing water, with less than 13.3% potentially suitable for each of the anopheline species. Although the water bodies on cattle pasture were associated in the PCA with higher water clarity, which is favorable for all three species, and algal cover, which is very favorable for *A. albimanus* and *A. pseudopunctipennis*, water bodies were also associated with macrophyte vegetation cover, which is unsuitable for all three species (Pinault and Hunter, 2012). Given the extent of cattle pasture in the highlands of Ecuador, priority ought to be given to ponds and other "natural" water bodies used to provide cattle with drinking water. The use of drainable troughs, including the frequently used half-tire troughs, may be recommended to cattle farmers to eliminate potentially suitable pooling of water. Similarly, flooded parts of pasture are of concern as well; in Mexico, *A. albimanus* are associated with regions of flooded cattle pasture (Rejmankova et al., 1991).

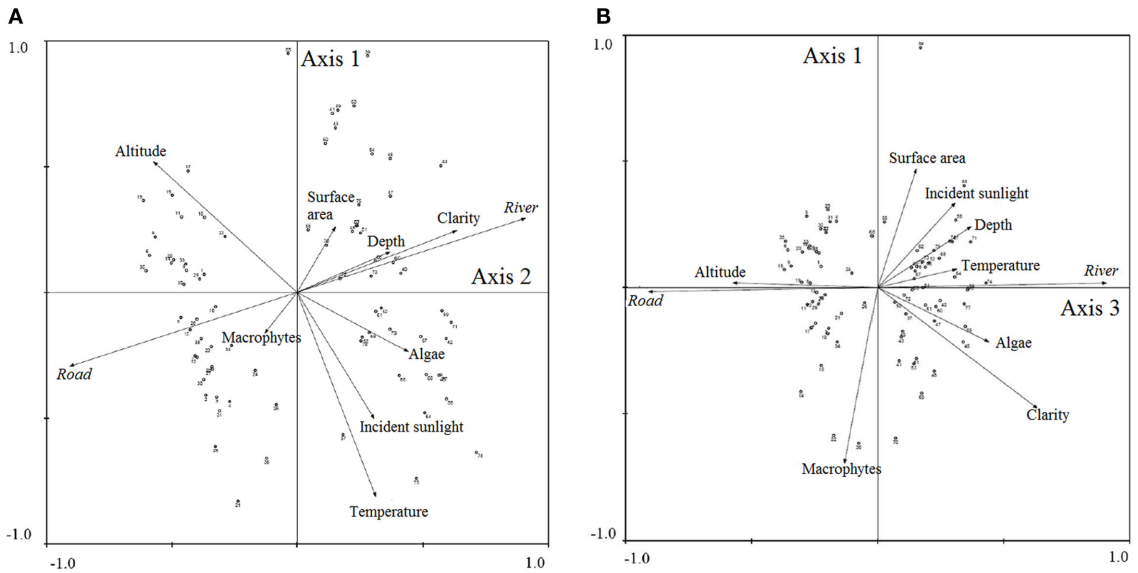

FIGURE 4 | Bi-plot results of principal components analysis of rivers/roads and habitat characteristics for (A) Axes 1 and 2 and (B) Axes 1 and 3. Output from CANOCO (2002).

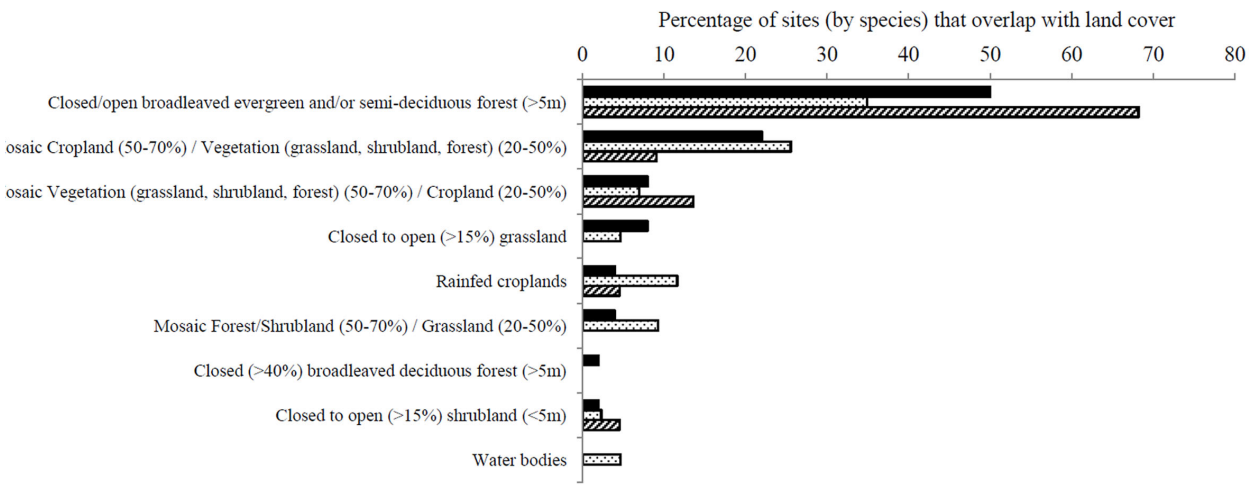

FIGURE 5 | Percentage of collection sites of *A. albimanus* (black bars), *A. pseudopunctipennis* (spotted bars), and *A. punctimacula* (diagonal bars) larvae in Ecuador, categorized by geographic overlap of land cover categories from GlobCover© 2009 MERIS (MEdium Resolution Imaging Spectrometer FR mosaics for 2009), which uses 22 land cover classes from the UN Land Cover Classification System at 300 m raster tile resolution (Data from ArcGIS online, ESRI). Analysis conducted in ArcGIS v.10 (ESRI, 2008).

Although more standing water was observed on empty lots, these were the least-observed land use in the altitudinal transects and the smallest in size. Incident sunlight is high, favoring *A. albimanus* and *A. pseudopunctipennis* larvae, where water bodies exist. Most often, construction activities and heavy machinery tear apart the surface of the earth, creating permanent depressions, including permanent tire tracks, that fill with water. Better management of these sites for mosquito habitat elimination would involve ensuring that the ground is carefully leveled prior to its temporary or permanent abandonment by a construction company.

Unlike on the coast, banana, sugarcane, and mixed tree plantations are generally dry in highland (steep) regions and do not provide much, if any, potentially suitable standing water for *Anopheles* larvae. Therefore, none of these common land uses ought to be considered priority areas for standing water elimination or malaria prevention in the highlands of Ecuador. In Belize, the runoff from sugarcane plantations can lead to a bloom of *Typha domingensis* Personnel., which is negatively correlated to the presence of *A. albimanus* larvae (Grieco et al., 2006), and may exclude larvae from sugarcane-adjacent water bodies in Ecuador as well.

Roads were associated in the PCA with macrophyte vegetation cover, which is a less suitable characteristic for *Anopheles* larval habitat. Although, roads often provide sunlit, open habitat, which were judged in 44% of cases to be potentially suitable for *A. punctimacula* and 26% of cases for *A. albimanus*, *A. albimanus*, and *A. pseudopunctipennis* were actually collected in two clusters of sites during roadside transects. Five out of the 38 sites sampled provided larvae during sampling, and densities of larvae collected in roadsides were similar to those observed during other collection efforts (Pinault and Hunter, 2011a). Despite the apparent clustering pattern, water bodies were not clustered at specific altitudes with less severe slopes, but rather, randomly distributed along roadways. Cement ditches placed along the roadways often became clogged with debris from adjacent trees and rocks, and subsequently filled with water permanently. Although road workers do periodically remove this debris, this study documents the use of these water bodies by anopheline larvae, necessitating more vigilance, and ensuring that roadside ditches along highways are frequently drained, especially at lower elevations.

River-associated habitat appeared to be important to anopheline larvae in the western highlands of Ecuador. Rivers were associated with greater water clarity, higher temperatures, the presence of algae, and incident sunlight, all of which are very suitable characteristics for *A. albimanus* and *A. pseudopunctipennis*. Although 58% of sites were judged suitable for *A. albimanus* and 29% for *A. pseudopunctipennis*, *A. albimanus* were collected more often in low-altitude river systems, while *A. pseudopunctipennis* were abundant in highland rivers that feed from most higher-altitude watersheds. *A. punctimacula* frequently co-occurs in the same river alongside *A. pseudopunctipennis*, although the two were never found to cohabit the same water bodies. Nineteen of the 41 sites sampled had larvae present, and larval abundance in these water bodies was similar in density to those from previous sampling efforts (Pinault and Hunter, 2011a). The presence of *Anopheles* larvae in highland rivers was observed historically in Balzapamba (650 m), where *A. pseudopunctipennis* larvae were collected in river-associated pools formed by an earthquake and subsequent landslide (Hanson and Montalvan, 1938). Similarly, Levi Castillo (1947) documents *A. pseudopunctipennis* in highland river edges in the late 1940s.

If rivers produce a large proportion of suitable habitat, they may provide corridors for the passage of anopheline mosquitoes to move into higher-altitude regions with favorable meteorological conditions. Although rivers are not technically a human land use, rivers in Ecuador are modified by adjacent land uses that sometimes includes the dumping of rocks and boulders into the stream, the construction of off-shoot canals for irrigation and drainage (that are not well-maintained) and the presence of water-collecting tubes that force water to collect in pools and slow-moving streams on the edges of rivers. Another possible source of larval habitat is the reduction of river flow due to either the diminished glacier-fed streams from glacier disappearance due to climate change (Bradley et al., 2006; Vergara et al., 2007) or to the modification of the river flow due to the construction of hydroelectrical dams (Vergara et al., 2007; L. Pinault, personal observation). When the flow is sufficiently reduced, a greater surface area is available on the river edge within the original streambed for the formation of rock pools and slow-moving water streams. It is therefore the author's recommendation that highland rivers and streams (ca. 1500–2500 m) be monitored for the presence of *Anopheles* larvae in the western Andes to forecast and prevent future highland malaria incidences. As well, construction and hydrological projects that reduce river flow ought to ensure that the river edge walls are sufficiently steep, or that boulders and other impediments are removed from the river, to prevent the formation of water pools and slow-moving offshoots on the edges of rivers.

Since land use including roads and rivers is a strong driver of highland malaria, several authors have stressed the importance of relating specific land use changes to the distribution of infectious disease to predict and control future incidences and epidemics (e.g., Patz et al., 2004; Munga et al., 2009). For example, in Nigeria, water bodies related to specific types of farming such as trenches, dams, and irrigation have been identified as potential anopheline larval habitat (Oladepo et al., 2010). Farmers in these regions have been provided with these data, resulting in many farmers now managing their property to prevent the formation of standing water (Oladepo et al., 2010). The identification of cattle farms, river edges, and road edges as potential sources of anopheline larval habitat in highland regions of Ecuador implies that managers of these land use types ought to be targeted in source reduction educational programs.

In the management of highland anopheline species, insecticide spray programs used with larval habitat management have been demonstrated to be particularly effective due to the limited number of habitats available (e.g., de Zulueta et al., 1964). The identification of specific highland habitats associated with land uses, such as permanent cattle watering holes/ponds and modified rivers, allows malaria prevention programs to target these habitats in highlands and thereby reduce populations of potential malaria-vectoring mosquitoes. Finally, regional mapping of highland larval habitats based on our findings may allow community members and malaria control personnel to either eliminate habitat or initiate spray programs when required.

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Impact of climate trends on tick-borne pathogen transmission

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Recent advances in climate research together with a better understanding of tick–pathogen interactions, the distribution of ticks and the diagnosis of tick-borne pathogens raise questions about the impact of environmental factors on tick abundance and spread and the prevalence and transmission of tick-borne pathogens. While undoubtedly climate plays a role in the changes in distribution and seasonal abundance of ticks, it is always difficult to disentangle factors impacting on the abundance of tick hosts from those exerted by human habits. All together, climate, host abundance, and social factors may explain the upsurge of epidemics transmitted by ticks to humans. Herein we focused on tick-borne pathogens that affect humans with epidemic potential. *Borrelia burgdorferi* s.l. (Lyme disease), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), and tick-borne encephalitis virus (tick-borne encephalitis) are transmitted by *Ixodes* spp. Crimean–Congo hemorrhagic fever virus (Crimean–Congo hemorrhagic fever) is transmitted by *Hyalomma* spp. In this review, we discussed how vector tick species occupy the habitat as a function of different climatic factors, and how these factors impact on tick survival and seasonality. How molecular events at the tick–pathogen interface impact on pathogen transmission is also discussed. Results from statistically and biologically derived models are compared to show that while statistical models are able to outline basic information about tick distributions, biologically derived models are necessary to evaluate pathogen transmission rates and understand the effect of climatic variables and host abundance patterns on pathogen transmission. The results of these studies could be used to build early alert systems able to identify the main factors driving the subtle changes in tick distribution and seasonality and the prevalence of tick-borne pathogens.

Keywords: tick, model, genetics, climate, *Borrelia*, *Anaplasma*, virus

INTRODUCTION

Ticks are obligate hematophagous ectoparasites of wild and domestic animals and humans that are classified in the subclass Acari, order Parasitiformes, suborder Ixodida, and distributed from Arctic to tropical regions of the world. Despite efforts to control tick infestations, ticks and the pathogens they transmit continue to be a serious constraint to human and animal health worldwide (de la Fuente and Kocan, 2006).

Several events that occurred during the final decades of the twentieth century and the beginning of the twenty-first century suggest a rise of tick-borne infections worldwide. These events include recent national and regional epidemics of known diseases such as tick-borne encephalitis (TBE) in Central and Eastern Europe, Kyasanur forest disease (KFD) in Karnataka state in India, Crimean–Congo hemorrhagic fever (CCHF) in northern Turkey and the southwestern regions of the Russian Federation, and Rocky Mountain spotted fever (RMSF) in Arizona, United States, and Baja California, Mexico (Pattnaik, 2006; Randolph, 2008; Maltezou et al., 2010; McQuiston et al., 2010). Globally, the recognized number of distinct and epidemiologically important diseases transmitted by ticks has increased considerably during the last 30 years. For example, more than 10 newly recognized spotted fever rickettsioses have been identified since 1984 (Parola et al., 2005; Paddock et al., 2008). In the United States, the list of national notifiable diseases include four tick-borne diseases, Lyme disease (*Borrelia burgdorferi* s.l.), human granulocytic anaplasmosis (HGA; *Anaplasma phagocytophilum*), human babesiosis (*Babesia* spp.), and human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), each of which has increased steadily in average annual incidence. From 2000 to 2007, the incidence of infections caused by *B. burgdorferi*, *A. phagocytophilum*, and *E. chaffeensis* increased linearly (Bacon et al., 2008). Although advances in molecular technology have contributed to the identification of these pathogens, rapidly expanding pathogen diagnosis and increasing incidence have raised concerns about the accuracy of case counts and epidemiology reports (Mantke et al., 2008). The problem of analyzing the incidence of tick-borne pathogens in humans is the concurrency of factors affecting the whole system such as climate, driving the life cycle of the ticks, the availability, occurrence and seasonal patterns of competent reservoirs, and social habits, leading the

contact with tick-infested areas need to be considered. All these factors should be analyzed with increasing levels of complexity that obscure the relationships between climate and the final impact of tick-borne pathogens.

Ticks and tick-borne pathogens have co-evolved molecular interactions involving genetic traits of both the tick and the pathogen that mediate their development and survival (Narasimhan et al., 2002, 2007; Kocan et al., 2004; de la Fuente et al., 2007a; Zivkovic et al., 2009; Rikihisa, 2010). These mechanisms are not well defined and the impact of environmental factors such as climate adds additional complexity to their study. However, the complexity of tick–pathogen relationships are emerging to show that it is difficult to describe a simple effect on the cascade of social and biological events affecting the transmission of tick-borne pathogens.

In these review, we evaluated pathogens that infect humans with epidemic potential such as *B. burgdorferi* s.l. (Lyme disease), *A. phagocytophilum* (human granulocytic anaplasmosis; HGA), and tick-borne encephalitis virus (TBEV; TBE) and Crimean–Congo hemorrhagic fever virus (CCHFV; CCHF). These pathogens are transmitted by *Ixodes* spp. and *Hyalomma* spp. ticks (de la Fuente et al., 2008) and were used to discuss how these tick species occupy the habitat as a function of different environmental factors, and how these factors together with molecular events at the tick–pathogen interface impact on tick survival and seasonality and pathogen transmission. The results of existing models could be used to build early alert systems able to identify the main factors driving changes in tick distribution and seasonality and the prevalence of tick-borne pathogens.

EFFECT OF CLIMATE AND OTHER ABIOTIC AND BIOTIC FACTORS ON TICK DEVELOPMENT, SURVIVAL, AND QUESTING

As many other arthropods, ticks are very sensitive to climate. Ticks spend most of their life cycle in the environment, and all tick life cycle stages are dependent on a complex combination of climate variables for development and survival. Host availability and vegetation significantly modulate the dynamics of tick populations. They probably have smaller contributions than climate in delimiting tick distribution as climate is probably the major driver to the presence or absence of a tick species in a given territory (Cumming, 2002). However, vegetation is a great modifier of the local weather or the so-called microclimatic conditions, the one that the ticks must adapt to for development and survival. Though many animal species can serve as tick hosts, there are several determinants of host suitability and the specificity of tick-reservoir host–pathogen relationships is key to our understanding of the complex processes conditioning the transmission of pathogens by ticks (Randolph, 2009). For example, host availability in time and space is an important determinant of tick bionomics. Shelter and protection from environmental extreme conditions are critical to tick survival, because questing and diapausing ticks are vulnerable to extremes temperatures and humidity.

Concerns exist about how predicted climate changes may alter tick–host–pathogen relationships and particularly tick potential for invasion of new areas and pathogen transmission. Although surveillance and reporting of changes in the distribution of tick populations is generally inadequate, some well-documented reports support the slow but apparently continuous expansion of the historical frontiers of some tick species into areas where they were previously absent (Gray et al., 2009). Warmer temperatures have been suggested as the main driver of some tick geographic range changes (Lindgren et al., 2000; Danielova et al., 2006). However, the potential influence of changing rainfall patterns has largely been ignored although this may have a greater effect than temperature on the ability of tick populations to establish in new areas. Invasive events (the transportation of an exotic tick species into an area far from its native range) are also well-documented and seem to be related to unrestricted domestic animal movements or over-abundance of certain wild hosts. Finally, there is little doubt that human-induced changes in abiotic (climate, land cover, habitat structure) and biotic (distribution and abundance of tick hosts) conditions have occurred over the past few decades, and there is equally indisputable evidence for the increase in recorded human cases of some tick-borne diseases (Randolph, 2009).

Many tick species are exophilous, meaning that they develop and quest in open habitats, not in the protected environment of a shelter as do the endophiluous ticks. The ticks have three developmental instars, larvae, nymphs, and adults that feed on one (one-host ticks) or multiple (two- and three-host ticks) animal hosts. Ticks take a continuous blood meal on the same host for 3–10 days, depending on the instar. When fully engorged, ticks fall off their host and undergo development to the next instar. The mortality observed during tick development is regulated by water losses, which in turn are greatly influenced by the temperature and the physical properties of the arthropods cuticle. Climate thus affects tick survival mostly during non-parasitic periods of their life cycle because ticks survival and host-seeking activity is inhibited outside certain ranges of temperature and rainfall (Randolph, 1997; Ogden et al., 2004). If developing and host-seeking ticks suffer mortality at an approximately constant rate in nature (Vail and Smith, 1998), then the lower the temperature, the longer is the developmental cycle and the higher is tick mortality. Therefore, warmer than current climate conditions would favor the presence of permanent populations of the tick species living across the northern temperate zone, where water stress is not an important factor. Therefore, climate may contribute to increased tick abundance (because faster development and lower mortality) and the probability to survive in a newly colonized territory (Ogden et al., 2004). However, this relationship is not lineal. It is important to stress that relative humidity is not an adequate concept to understand water stress in ticks. Low air water contents are responsible of water losses in ticks (like in any other arthropod). An increased in temperature contributes to desiccation by water losses (Perret et al., 2000, 2003). The temperature, together with the deficit in water content of the air are thus the drivers of tick desiccation.

Climate can affect not only tick development and mortality, but also their activity rates, a feature that deeply affects infestation risks for humans and the intensity of tick infestations in reservoir hosts. Questing is the mechanism by which ticks find a host, climb on it, and feed. Ticks quest at variable heights in the vegetation, driven by factors such as temperature and relative humidity (Vail and Smith, 2002; Busby et al., 2012). It is known that different tick species, and even different instars of the same species, may quest at

different heights, therefore experiencing different mortality rates at various temperatures.

IMPACT OF WEATHER-INDUCED TICK STRESS RESPONSE ON TICK SURVIVAL, QUESTING, AND PATHOGEN TRANSMISSION

The heat shock and other stress responses are a conserved reaction of cells and organisms to elevated temperatures and other stress conditions such as toxicity and pathogen infection (Tutar and Tutar, 2010). Crucial to cell survival is the sensitivity of proteins and enzymes to heat inactivation and denaturation. Therefore, adaptive mechanisms exist that protect cells from the proteotoxic effects of heat stress. The heat shock proteins (HSPs) and other stress response proteins (SRPs) protect cells and organisms from damage, providing higher levels of tolerance to environmental stress.

Tick HSPs and other SRPs such as glutathione-*S*-transferase, selenoproteins, metallothioneins, and ferritin have been shown to be involved in the cellular response to different stress conditions such as heat shock, oxidative stress, tick attachment, blood feeding, and pathogen infection (Macaluso et al., 2003; Mulenga et al., 2003, 2007; Rudenko et al., 2005; Ribeiro et al., 2006; de la Fuente et al., 2007b; Rachinsky et al., 2007, 2008; Hajdusek et al., 2009; Zivkovic et al., 2009; Kongsuwan et al., 2010; Lew-Tabor et al., 2010; Villar et al., 2010; Busby et al., 2012). For example, recent studies demonstrated that the stress response is activated in ticks and cultured tick cells after *Anaplasma* spp. infection and heat shock (Villar et al., 2010; Busby et al., 2012). However, under natural vector–pathogen relationships such as those occurring in *I. scapularis* infected with *A. phagocytophilum*, HSPs, and other SRPs are not strongly activated, probably reflecting tick–pathogen co-evolution (Villar et al., 2010). Nevertheless, at least as shown by proteomics analysis of *I. scapularis* ISE6 tick cells in response to *A. phagocytophilum* infection, some HSPs such as the HSP70 family are over-expressed while other putative HSPs such as HSP20 are under-expressed in infected cells (Villar et al., 2010).

Tick questing behavior affects host-seeking activity and pathogen transmission through the interaction of several factors including tick stress response (Belozerov, 1982; Daniel and Dusbabek, 1994; Randolph, 2004). The effect of temperature and relative humidity on *I. scapularis* questing behavior and abundance has been demonstrated in field and laboratory studies (Schulze et al., 2001; Vail and Smith, 2002; Busby et al., 2012). Lefcort and Durden (1996) demonstrated the effect of pathogen infection on ticks questing behavior by showing that infection with *B. burgdorferi* negatively affects adult *I. scapularis* questing behavior. Although still controversial, Herrmann and Gern (2010) provided evidence that that infection with *B. burgdorferi* and *B. afzelii* confers survival advantages to *I. ricinus* under challenging thermohygrometric conditions.

Recent studies demonstrated that subolesin and HSPs are involved in the control of *I. scapularis* response to the stress produced by heat shock, blood feeding, and *A. phagocytophilum* infection (Busby et al., 2012). These results showed that at high temperatures and during blood feeding, when *hsp20*, *hsp70*, and subolesin are over-expressed, *I. scapularis* ticks are protected from stress and pathogen infection and have a higher questing speed. These responses help *I. scapularis* to increase survival by inducing stress responses and preventing desiccation because higher water losses at high temperatures with higher questing speed to increase chances to attach to a host. Because pathogen infection occurs during blood feeding, ticks also have developed a protective response to limit pathogen infection levels, also contributing to their survival.

Taken together, these results suggest a connection between tick stress response, questing behavior, and pathogen transmission. Tick stress response is activated as a consequence of different stress conditions such as those caused by temperature changes, blood feeding, and pathogen infection. The stress response counteracts the negative effect of heat shock and pathogen infection on tick questing behavior and increase tick survival, those playing an important role in pathogen transmission and the adaptation of tick populations to challenging environmental conditions.

IMPACT OF CLIMATE TRENDS ON TICK–PATHOGEN TRANSMISSION

BORRELIA BURGDORFERI s.l., THE CAUSATIVE AGENT OF LYME DISEASE

Lyme disease is caused by *B. burgdorferi* s.l. infection and is transmitted by *Ixodes* spp. ticks. Lyme disease is one of the most prevalent human arthropod-borne diseases in United States and Europe (Bacon et al., 2008; Gray et al., 2009). However, Lyme disease records in Europe are commonly produced by large administrative divisions, which are extremely heterogeneous in climate, abundance of reservoir hosts and landscape composition, all factors affecting pathogen prevalence in the tick vector (Estrada-Peña et al., 2011a). The association between *B. burgdorferi* and its vector is quite specific and only a small group of tick species within the genus *Ixodes* are known to be vector competent. These ticks have infections that spread from the gut to the salivary glands for transmission to susceptible hosts. *Ixodes* spp. ticks feed upon small to medium-sized reservoir hosts (usually mice, birds, and lizards) as immatures, and medium to large hosts (ungulates) as adults. Host range for all stages of *Ixodes* spp. is much broader than for most other ticks (Keirans et al., 1999). Microclimatic and biologic requirements for *Ixodes* ticks that transmit the spirochete are: (1) suitable host availability, (2) temperature fluctuations between −10 and +35°C, with tolerance to the extremes for only brief periods, and (3) a constant relative humidity not lower than 80% in the air and near saturation in the soil. Therefore, the ticks have well quantified requirements for its development and survival.

A recent study by Ogden et al. (2004) demonstrated that, under the conditions in the northern United States, local populations of *I. scapularis* are not affected by water stress, therefore temperature and photoperiod would be the only regulatory variables of tick activity and development. Ticks acquire *B. burgdorferi* infection while feeding on an infected competent reservoir host, a species capable of transmitting infection. After molting, ticks quest for another host among the herbage of the woodland floor, and infected ticks will transmit the pathogen and infect any susceptible host they feed on. Two factors have facilitated the spread of *B. burgdorferi* in northern United States. First, the increasing population size and geographical range of *I. scapularis* ticks, which is believed to be driven by restored woodlands and growing

white-tailed deer populations, an important tick host (Kurtenbach et al., 2006). Second, the role of migratory birds in the spread of feeding *I. scapularis* nymphs when flying from their winter quarters to northern territories in Canada (Ogden et al., 2005a,b). Not only these migratory birds can introduce ticks into new territories, but also move further north established tick populations, likely because warmer weather periods in autumn and winter. These studies complemented previous reports (e.g., Estrada-Peña, 2002) derived from models about the increasing suitability of the weather in United States to support permanent *I. scapularis* populations. Each of these weather-derived factors affect tick survival rates, influencing the densities of endemic tick populations and the threshold number of immigrating ticks needed to establish a tick population in a new focus.

The situation in Western Palearctic is very different (Kurtenbach et al., 2006). The temporal pattern of the incidence of Lyme disease in Europe seems to be more stable than in the northeastern United States, although local temporal fluctuations in tick infection prevalence have been recorded (Kurtenbach et al., 2006). Unlike in the northeastern United States, in Europe, most species or even subtypes of *B. burgdorferi* s.l. are specialized to infect different groups of vertebrates. *I. ricinus*, the main tick vector of *B. burgdorferi* in Europe, needs areas with a good cover of vegetation and a mat of decaying vegetation with a relative humidity of at least 80% during the driest times of the year (Gray, 2008). When it is too dry or too cold, ticks will withdraw to the litter area to prevent desiccation and freezing (Gray, 2008). Geographical range of *I. ricinus* ticks is limited in its northern, or high-altitude, range by temperature (Lindgren et al., 2000; Jouda et al., 2004) and in its southern range by humidity (Estrada-Peña et al., 2004). The ecology of the tick and its habitat in northern Africa, where the pathogen also circulates in a dry, Mediterranean type environment, has not been characterized. Due to the effect of temperature and humidity on *I. ricinus*, its activity varies in different regions. In central Europe, occurrence of ticks shows two peaks for all developmental stages, with maximum in May–June and September–October (Estrada-Peña et al., 2004). In Northern Europe, these two peaks converge into a single maximum in the summer, although this pattern is not constant in all regions (Lindgren et al., 2000). Jouda et al. (2004) demonstrated that tick seasonality changes with variations in altitude. However, these bimodal or unimodal activity patterns may change from year to year in the same area (Jouda et al., 2004). In the Mediterranean, an adult tick maximum occurs between November and January, but nymphs are active in spring (Dsouli et al., 2006).

The association between the prevalence of *B. burgdorferi* in nymphal *I. ricinus* ticks exists across a large geographical range in the western Palearctic and this association is partially correlated with some continuous traits of the regional weather (Estrada-Peña et al., 2011a). Some climate gradients and phenological features together with habitat fragmentation provide better conditions for *B. burgdorferi* infection of *I. ricinus* and for the maintenance of highly tick-infected foci (Estrada-Peña et al., 2011a). Some studies have indicated the influence of tick life cycle traits on the distribution of *B. burgdorferi* genotypes in the United States as a consequence of different climate patterns affecting tick phenology. Kurtenbach et al. (2006) predicted and it was later confirmed by empirical data (Gatewood et al., 2009) that the asynchrony of infected nymphs and uninfected larvae favors pathogen persistence strategies, whereas synchrony of these tick stages combined with a short annual period of activity should favor short-lived strategies and the capacity for co-feeding transmission. Because the climate modulates such a tick phenology in keeping both larval and nymphal stages feeding on host at the same time or at different moments of the year, climate can be considered responsible for the persistence of different strains of *B. burgdorferi* s.l. in the United States and Europe. The abundance of infected ticks is determined not only by climate trends but also by the extrinsic incubation period of spirochetes in ixodid ticks, which equals the duration of development from larvae to nymphs that is climate sensitive (Randolph and Rogers, 2000). Therefore, patterns of abundance and genotype distribution of the populations of *B. burgdorferi* are shaped substantially by the environmental cues that act on the tick populations, whereas additional layers of complexity are introduced into the system by host population dynamics and the host immune response to both bacteria and the ticks.

ANAPLASMA PHAGOCYTOPHILUM, THE CAUSATIVE AGENT OF HUMAN GRANULOCYTIC ANAPLASMOSIS

Similar processes to those described for *B. burgdorferi* could be considered for *A. phagocytophilum* (formerly *E. phagocytophila*, *E. equi*, and *A. phagocytophila*), the causative agent of HGA and also transmitted by ticks of the *I. ricinus* complex. *A. phagocytophilum* was first identified and described in humans in 1994 (Dumler et al., 2001). HGA is an emerging zoonotic disease in Asia and Europe (Parola and Raoult, 2001) and is reported in the United States at a rate of 4.2 cases per million persons in 2008 (http://www.cdc.gov/anaplasmosis/stats/). *A. phagocytophilum* is widespread in many species of wild and domestic animals, including rodents, carnivores, equids, ruminants, and birds (Sréter et al., 2004). Although, the same tick species involved in the transmission of *B. burgdorferi* are also involved in the transmission of *A. phagocytophilum*, the range of competent reservoir hosts and tick vectors is different and involves a larger number of species, thus affecting the epidemiology of the disease. *A. phagocytophilum* has been detected in animals and ticks throughout the United States and in nearly all European countries, but the strains or variants and the resulting diseases vary with the geographic location (de la Fuente et al., 2005; Massung et al., 2006; Stuen, 2007; Reichard et al., 2009; Woldehiwet, 2010; Gaowa et al., 2012; Jin et al., 2012). Additionally, recent evidence suggested that pathogen strains infecting humans differ genetically from ruminant straits and may be maintained in nature in different reservoir hosts (de la Fuente et al., 2005).

Transmission of *A. phagocytophilum* was shown to be transstadial, in which infection is acquired by the feeding of larvae or nymphs on infected hosts and transmission occurring by the next tick stage, nymphs or adults (Hodzic et al., 1998). However, the low abundance of *I. ricinus* in some areas in which *A. phagocytophilum* has been identified suggests that other tick species may be involved in pathogen transmission (MacLeod, 1932; Holden et al., 2003; de la Fuente et al., 2004; Alberti et al., 2005; Cao et al., 2006; Naranjo et al., 2006; Barandika et al., 2008). Tick transmission of *A. phagocytophilum* variants by these tick species may have different transmission patterns and target hosts which have not

been reported so far. Notably, Baldridge et al. (2009) demonstrated transovarial transmission of *A. phagocytophilum* variants in *D. albopictus*, which is of interest because this mode of transmission is not considered to occur with other *Anaplasma* spp. Transovarial transmission of *A. phagocytophilum* variants in nature would reduce their dependence on mammalian reservoirs. However, further studies on *A. phagocytophilum* transmission by different tick species and pathogen strains and variants are needed to fully define the role of ticks in the transmission of this pathogen.

Anaplasma phagocytophilum host infection levels at the time of tick feeding influence tick infection rates. The infection of *A. phagocytophilum* in *I. scapularis* nymphs correlated with the bacteremia level in the mouse blood (Hodzic et al., 1998). However, once ticks become infected, even with a low number of bacteria, pathogen replication in ticks appears to compensate for the low infection rates and enhanced transmission (Hodzic et al., 1998). Transmission of *A. phagocytophilum* by ticks was shown to occur between 24 and 48 h after tick attachment (Sukumaran et al., 2006).

Despite these advances in the study of tick–host–pathogen interactions, available information is not enough to model infection risks associated with *A. phagocytophilum* distribution and the effect of abiotic factors on pathogen transmission.

TICK-BORNE ENCEPHALITIS VIRUS, THE CAUSATIVE AGENT OF TBE

Tick-borne encephalitis is caused by an important arbovirus of the genus *Flavivirus*. The disease is reported from many areas in central and northern Europe. The geographical range of TBE clinical cases do not overlap with the known distribution of the vector, *I. ricinus*, which is recognized as the only vector in Western Palearctic. Therefore, it is inferred that factors other than the simple presence of the vector, are driving the range of the virus.

Recent results show that tick saliva contains factors that modulate host inflammatory, coagulation and immune response to improve tick blood feeding and pathogen transmission (Jones et al., 1989; Alekseev et al., 1991; Labuda et al., 1993a; Randolph, 2009). This so-called "saliva-assisted transmission" (SAT) was reviewed by Nuttall and Labuda (2008). Inoculation of salivary glands extracts and TBEV into laboratory animal hosts resulted in enhanced transmission from hosts to nymphal ticks when compared with pathogen inoculation alone (Alekseev et al., 1991; Labuda et al., 1993b). SAT helped to explain the mechanism behind the equally novel observation of TBEV transmission between co-feeding ticks in the absence of a systemic infection (Labuda et al., 1993a,b; Randolph, 2009).

Co-feeding transmission imposes constraints because it requires co-feeding by at least two tick stages in synchrony in their seasonal activity (Randolph et al., 2000). The long and slow life cycle typical of temperate tick species, caused by low temperature-dependent developmental rates and overwinter diapause, slows the pace of pathogen transmission. As tick phenology is reset each year by winter conditions (Randolph et al., 2002), the critical stages (larvae and nymphs for TBEV) may emerge from diapause in more or less synchrony in the spring, depending on whether temperatures rise sufficiently rapidly to cross the threshold for larval activity (ca. 10°C mean daily maximum) soon after the threshold for nymphal activity (ca. 7°C mean daily maximum; Randolph and Sumilo, 2007). The variability of thermal conditions associated with seasonal synchrony between tick stages has been identified as the key determinant of the focal distribution of TBEV across Europe (Randolph et al., 2000), allowing the predicted risk of TBE to be mapped using climatic surrogates sensed from space (Randolph et al., 2000).

Altogether, this information suggests that climate exerts an extreme control of the natural cycles of TBEV and delineates both their intensity (in terms of field tick prevalence rates) and their geographical distribution. According to the prevalent hypothesis outlined before, the climate at the beginning of the spring exerts a regulatory action on the synchrony of the active immature ticks, conditioning the necessary coexistence of nymphs and larvae on the same hosts. Because the short time of feeding for both larvae and nymphs, small changes in the temperature in that period may promote a lack of synchronicity of a few days, enough to prevent the "backward" transmission of the virus. These events have not yet been captured by a process-driven model, which could be a welcomed addition to our array of epidemiological tools, necessary to understand the TBEV epidemiology and design intervention for its prevention.

The situation is drastically different when series of human TBE cases are compared against a background of oscillating climatic conditions. It has been speculated that changes in climate, host abundance, social habitats, economic fluctuations, environmental changes, and to a lesser extent climate changes have increased the incidence of TBE (Lindgren and Gustafson, 2001; Zeman and Benes, 2004; Sumilo et al., 2006, 2007, 2008). However, it is very difficult to correlate series of human clinical cases against basic climatic features because climate has several collateral effects, not only affecting tick life cycle but also hosts and, most important, social habits. This has been demonstrated in a series of data for TBE cases in the countries of the Baltic Sea (Sumilo et al., 2007) and the Czech Republic (Zeman and Benes, 2004). It is thus hard to find a long, unbiased series of data on human TBE incidence, covering an adequate time, and then find simple correlations with raw climate features.

CRIMEAN–CONGO HEMORRHAGIC FEVER VIRUS, THE CAUSATIVE AGENT OF CCHF

A different situation exists regarding CCHFV, the causative agent of CCHF. One of Hoogstraal's conclusions in his review on the epidemiology of CCHF (Hoogstraal, 1979) stated that "Not a single substantial study has been made of interrelationships between the virus, [. . .] wild and domestic mammals, [. . .] and ticks during the 'silent' coursing of the virus in nature. It is disappointing to have to write [. . .] that there are still no detailed investigations on CCHF virus localization, multiplication, and dynamics in ticks." More than 30 years later it is disappointing to report that little has progressed in this area. The complex enzootic cycles of CCHFV are made up of a combination of interactions resulting in a seemingly focal geographic distribution range that does not appear to currently match that of competent vector species. These interactions include: (i) several tick species implicated as vectors, (ii) a wide array of (suspected) reservoir hosts for immature and adult stages of the ticks, (iii) trends in climate and (iv) social changes, and consequently thereafter (v) alterations in landscape and vegetation. A

recent review (Estrada-Peña et al., 2012a) discussed the possibility of missing basic factors in our understanding of the epidemiology of CCHFV and some arguments that have been already rejected as drivers of virus reemergence. It is of interest to note that epidemics or active "silent" (inter-epidemics) CCHFV transmission occurs in areas where ticks of the genus *Hyalomma* are common. It has been proposed that other tick species are involved in the transmission of the virus. Some genera other than *Hyalomma* have been tested under strict laboratory conditions, demonstrating the presence of the virus in the tick after feeding on infected hosts (Swanepoel et al., 1983). The only constant feature of CCHFV foci is the presence, and in some cases increased abundance, of *Hyalomma* ticks. These results suggest that although other tick species may be competent vectors for the virus, *Hyalomma* ticks play the major role in virus transmission.

The tick genus *Hyalomma* is widespread in different ecological areas of the Palearctic and Afrotropical regions. Some species, like *H. scupense* (one- or two-host tick) and *H. anatolicum* (two- or three-host tick) prefer to feed on the same large ungulates (mostly cattle) during all developmental stages, and adopt a ridiculous life cycle. *H. marginatum* and *H. rufipes* are two-host ticks with immatures feeding on birds or small/medium-sized mammals and adults feeding on a larger wild and domestic ungulates (Apanaskevich, 2004). The principal species implicated in transmission of CCHFV in Eurasia are *H. marginatum*, *H. turanicum*, *H. anatolicum*, and *H. scupense* (including the former *H. detritum*, now considered a synonym of *H. scupense*; Guglielmone et al., 2010). The virus has been reported to survive throughout tick's life cycle and is transmitted transstadially and transovarially (Matser et al., 2009). The long survival of the virus in ticks is important CCHFV epidemiology, but there is still a dearth of knowledge regarding host exposure rates and host immune responses particularly in populations of short-lived birds, insectivores and lagomorphs.

Similar to other tick-borne diseases, climate trends have been commonly linked to outbreaks of CCHF clinical cases. However, social factors such as abandoned arable land (and therefore secondary vegetation growth) together with an increase in the abundance of wild animal hosts for ticks, may result in the amplification of tick populations (Vatansever, personal communication). Without a framework linking the response of the tick vector to subtle climate variations, it is only possible to speculate on the effect of climate on pathogen transmission. An assessment of the effects of climate on the presence of human CCHF clinical cases in Turkey included monthly values of several climate variables and concluded that climate was not different in sites with active foci of the disease as compared with sites where *H. marginatum* is common but human cases have not been reported (Estrada-Peña et al., 2011b). Studies in the focus of the disease in Turkey demonstrated that a high landscape fragmentation, compatible with conditions of high hosts and tick population movements and turnover, is a hallmark in areas with highest case incidence of CCHF in humans (Estrada-Peña et al., 2010). These reports concluded that it is not possible to predict, based solely on climate grounds, where new CCHF cases could appear in a reasonably near future. The recent finding of CCHFV in southern Europe (Estrada-Peña et al., 2012b) opened a yet speculative door about the potential real distribution of the virus in the Western Mediterranean. One of the most striking questions in the geographical distribution of CCHFV is the lack of clinical cases in western Mediterranean, west to the main distribution area of the pathogen, which ends at western Balkans. The analysis of the viral strain recorded in southwestern Europe suggested its close phylogenetic proximity with strains commonly reported from northern Africa and suggested that migratory birds might be behind such a particular spread of the pathogen.

Results suggest that the *Hyalomma* tick vector may spread into northern Europe as a consequence of warmer winters. Migratory birds have been repeatedly implicated in dispersing immature *Hyalomma* ticks. Each spring, many thousands of ticks are introduced in Europe by migratory birds from Africa (Hoogstraal et al., 1961, 1963). The level to which these birds may be exposed to African *Hyalomma* populations and subsequently import them into Europe depends upon the habitats they frequent, their ground feeding behavior and the timing of their departure. Owing to the earlier timing of bird arrivals in western Europe compared to the period for optimal nymphal molt in Africa, the likelihood of such importation is reduced (Knudsen et al., 2007). The northern limit of *Hyalomma* potential survival will depend upon how suitable the abiotic conditions are at the arrival site to facilitate molt of engorged immatures, as well as the availability of suitable hosts. Hasle et al. (2011) reported the presence of only seven immature *H. rufipes* on 713 migratory birds collected in the southern coasts of Norway. However, this result does not guarantee their survival under local climate conditions. Further investigation is required to determine whether or not such exotic tick species can survive and establish should they arrive to a suitable habitat and with available hosts. For example, *H. rufipes* from sub-Saharan Africa and the Nile River cannot survive in the environments of the Mediterranean basin or in the northern European latitudes. Studies demonstrated that the natural distribution of *H. rufipes* is sub-Saharan Africa and adjacent regions of Africa and Arabia along the Red Sea (Apanaskevich and Horak, 2008). The records of *H. rufipes* from Europe (Macedonia, Malta, and Turkey) and North Africa (except Egypt) are an apparent consequence of the dissemination of the immature stages by migratory birds from Africa, but do not represent permanent populations (Apanaskevich and Horak, 2008). However, studies have demonstrated that the northern distribution limit of *H. marginatum*, which is a Palearctic tick species, may be moving further north owing to the trend in the autumn and winter temperatures (Estrada-Peña and Venzal, 2007). The issue is thus the evaluation of the probabilities at which a bird with infected and feeding *Hyalomma* immatures may arrive to a given site in Europe, at the precise timing for optimal tick molt and survival.

Nothing is known about the molecular interactions at the tick–host–virus interface, probably because the high biosafety level required working with CCHFV. The characterization of the tick–host–virus interactions is essential to fully understand virus infection and transmission processes.

MODELING RISKS FOR PATHOGEN TRANSMISSION AND EPIDEMIC POTENTIAL

The most common strategy to estimate the potential geographic range of a species is to characterize the environmental conditions that are suitable for the species, and then identify where suitable

environment is distributed in space. This is the fundamental strategy common to most distribution models. The environmental conditions that are suitable for a species may be characterized using either a statistical or a correlative approach. Statistical models aim to incorporate physiologically limiting mechanisms in a species tolerance to environmental conditions. The principal steps required to build and validate a correlative species distribution model are: (1) known species occurrence records and (2) a suite of environmental variables. Raw climate variables, such as daily precipitation records collected from weather stations are often processed to generate model inputs that are thought to have a direct physiological role in limiting the ability of the species to survive. The species occurrence records and environmental variables are entered into an algorithm that aims to identify environmental conditions that are associated with species occurrence. In practice, we usually seek algorithms that are able to integrate several environmental variables, since species are in reality likely to respond to multiple factors. Having run the modeling algorithm, a map can be drawn showing the predicted species distribution. The ability of the model to predict the known species distribution should be tested at this stage. A set of species occurrence records that have not previously been used in the modeling should be used as independent test data. The ability of the model to predict the independent data is assessed using a suitable statistic test. Once these steps have been completed, and if model validation is successful, the model can be used to predict species occurrence in areas where the distribution is unknown. Thus, a set of environmental variables for the area of interest is the input into the model and the suitability of conditions at a given locality is predicted. In many cases the model is used to "fill the gaps" around known occurrences.

A model to capture the range of suitable climate conditions for the tick *I. ricinus* based on occurrence records has been developed (Estrada-Peña, 1999, 2008). This model runs on a series of remotely sensed climate features, namely the average monthly temperature and the average normalized derived vegetation index (NDVI). NDVI is an indicator of plant photosynthetic activity and therefore used here as a surrogate for water stress as perceived by the tick. NDVI is the single variable that better explains the range of environmental suitability for *I. ricinus* (Estrada-Peña, 1999). **Figure 1** shows the expected range for the *I. ricinus* colonization in a wide area of western Palearctic. After training the model, a relatively long series of data can be used as information for the modeling algorithm to predict the long-term changes of the index of habitat suitability for *I. ricinus*. **Figure 1** also displays the trend in environmental (climate) suitability for the *I. ricinus* in the period 2000–2010. The model shows a clear increase in climate suitability and the possible expansion of tick populations into wide areas of southern Scandinavia, eastern Europe, and Baltic countries. These predictions agree with recent observations of increased abundance of the tick in these areas (Danielova et al., 2006; Jaenson et al., 2012). It is widely recognized that climate is not the only factor driving tick range expansion, because availability of key tick hosts may cause sharp changes in tick population abundance (Jore et al., 2011; Jaenson et al., 2012). Climate is just one of the factors involved, but the models trained only with climate as explanatory variables of tick occurrence produce a coherent result about the effects of climate on tick populations.

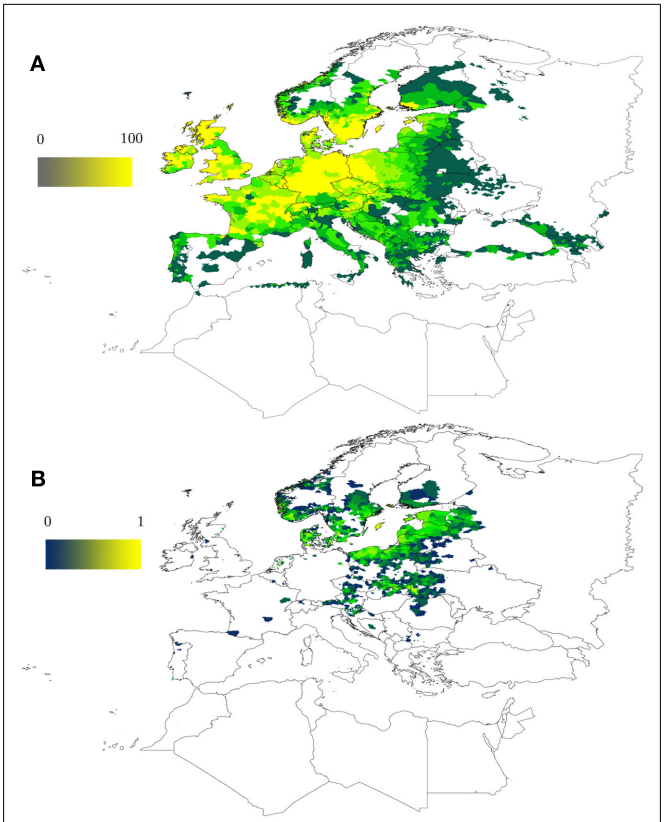

FIGURE 1 | Predicted climate suitability for the tick *I. ricinus* in the western Palearctic. (A) Predicted climate suitability (0–100) was evaluated by a model trained with more than 4,000 tick occurrence points using MaxEnt as modeling software (Phillips et al., 2006). The map is based on previous developments by Estrada-Peña et al. (2006). The ramp of colors shows the probability to find permanent tick populations as driven only by climate conditions, including a set of remotely sensed monthly average temperature and vegetation stress (NDVI, a proxy for tick water stress) from 2000 to 2010. **(B)** Changes in climate suitability for *I. ricinus* in the period 2000–2010 (from 0, the minimum, to 1, the maximum) based on the same model. Results are based on modeling climate suitability for ticks separately for each year and then evaluating the suitability index trend along years 2000–2010. Both maps **(A,B)** do not represent tick abundance but the appropriateness of the climate for the development of the tick **(A)** and how this factor evolved in time **(B)**.

However, the many factors behind the complex tick–host–pathogen interface make this approach simplistic for the purpose of understanding pathogen transmission. If we aim to decipher how a pathogen can be sustained and spread, we need a process-driven model capturing the seasonal activity of the tick. A series of studies on the life cycle of *I. scapularis* and the transmission of *B. burgdorferi* were conducted by Ogden et al. (2005a,b). These models are built over the concept of the accumulated development, i.e., the different stages of the tick have a temperature over which the tick is activated and perform developmental processes. There is a species-specific threshold of temperature (e.g., a value of accumulated temperatures) over which tick development is completed. Therefore, the temperatures recorded at a given period indicate the fraction of tick development completed. The models by Ogden et al. (2005a,b) for the *I. scapularis* life cycle also incorporate

adequate descriptions of tick questing periods and the infestation rates on hosts. These models were aimed to describe the spreading patterns of *B. burgdorferi* in southern Canada and northern United States, and to recognize the effect of key hosts in the prevalence rates by some pathogens in ticks.

While not explicitly aimed to address the concept, these deterministic models are focused on the understanding of the basic reproduction number, R0. The spread of pathogens is typically characterized by this index, which is defined as the expected number of secondary cases produced by a single primary case in a wholly susceptible population when there is a homogeneous and well-mixed population of hosts. In the case of the pathogens surviving in an *Ixodes*-reservoir rodent cycle, the duration of rodent infectivity for ticks must span any gap between the seasons of nymphal and larval activity (Randolph, 2001). The duration of infectivity is a crucial measure of fitness in different host species for vector-borne pathogens and particularly for *B. burgdorferi* in northeastern Unites States (Tsao et al., 2004). However, for *I. scapularis*-borne zoonoses investigated so far, recovery of rodents from acute, highly transmissible infections is not complete and the rodents remain persistently infective carriers that transmit infection to ticks with low efficiency (Derdáková et al., 2004; Ogden et al., 2005b). The potential capacity for *B. burgdorferi* or *A. phagocytophilum* to be transmitted between ticks co-feeding on the same host irrespective of systemic host infection adds further complexity to the transmission dynamics. Furthermore, relative rodent birth and death rates between nymphal and larval appearance each year very likely also affect pathogen transmission cycles (Schauber and Ostfeld, 2002).

These examples show that, for a complete understanding of the complex cycles among ticks, reservoir hosts, and pathogens, it is necessary to develop a system able to reliably predict, at least, the periods of activity of the tick vectors. It must also coherently compute the tick density-dependent mortality rates, which are a feature derived from the abundance of both ticks and hosts. A model displaying the seasonal patterns of the tick is thus necessary before R0 can be calculated for tick-transmitted pathogens. A statistical model derived from the relationship between the tick and the climate will probably provide a reliable picture of the geographical range of the tick, but not the environment necessary for the potential evaluation of R0.

A process-driven model for *H. marginatum* (Estrada-Peña et al., 2011b, 2012c) examined the potential effects of a changing environment on the colonization potential of the tick in areas outside its current range. This study specifically described areas where climatic features and a critical habitat configuration for host dispersal results in major changes in tick turnover. The study used a model based on host movement rules over the interface of the process-driven tick model, regulated by the daily climate obtained for a spatially interpolated 10 min grid over the target region. The results of this model, based on a dynamic evaluation of tick developmental and mortality rates, predicted different tick suitability areas when compared to a previously developed model based on tick occurrence patterns (Estrada-Peña and Venzal, 2007; **Figure 2**). Process-driven models tend to map a larger area of potential tick range, even if plotted at the same resolution of raw explanatory climate variables. This is probably because these models use a higher number of features, therefore providing a better environment to develop a reliable background to evaluate R0 rates.

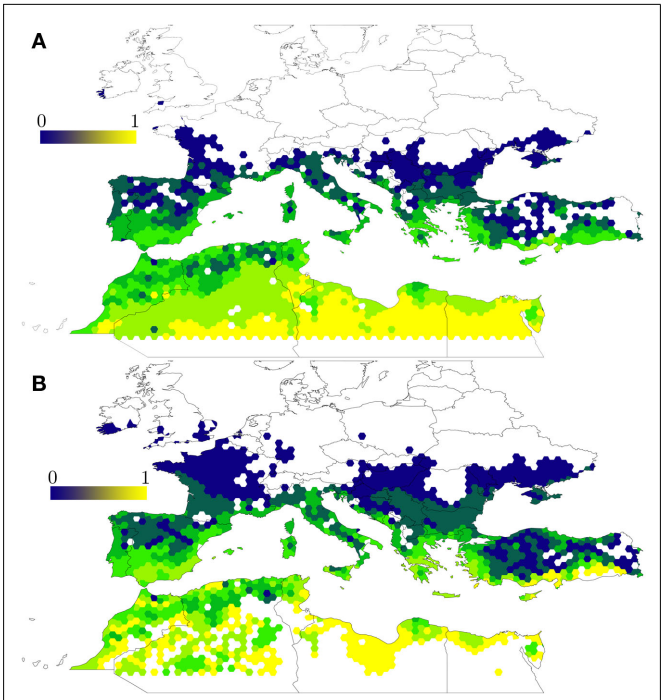

FIGURE 2 | Compared output between a statistical and a process-driven model of *H. marginatum* in the Mediterranean basin. **(A)** The statistical model was trained with records of tick occurrence in the region and displays the probability to find permanent tick populations (in the range 0–1) as reported by Estrada-Peña and Venzal (2007). The model is based only on climate features found at the sites where the tick has been recorded. **(B)** The process-driven model uses the same set of climate explanatory variables (average monthly temperature and water deficit) and represents the same probability based on tick development and mortality rates over a period of 1 year (Estrada-Peña et al., 2011b). The process-driven model predicted a larger range northern to the Mediterranean area, sites which were regarded as unsuitable by the statistical model, and reported no suitability in large areas of the Sahara desert.

Climate scenarios for the years 2020, 2050, and 2080 on the life cycle of *H. marginatum* ticks in the western Palearctic showed that the net growth rate of tick populations increase in every scenario tested compared to current climate baseline. These results support the expectations of increased tick survival and increased population turnover in future climate scenarios. Such a model of host dispersal linked to the process-driven life cycle model demonstrated that current eastern (Turkey, Russia, Balkans) populations of *H. marginatum* are well separated and have little mixing with western (Italy, Spain, northern Africa) populations. The cold areas in the Balkans, Alps, and Pyrenees mark the northern limit for tick survival. Under the warmer conditions predicted by future climate scenarios, tick expansion to new areas previously free of the vector is expected to increase, mainly in the Balkans and southern Russia, thus increasing the northern limit of the tick range.

Another area in which models can help understating the risks associated with tick-borne pathogen transmission is modeling

vaccination strategies. It has been shown that immunization with tick antigens can reduce pathogen transmission by reducing tick populations and vector capacity (de la Fuente et al., 1998, 2006, 2007a,c; de la Fuente and Kocan, 2003, 2006; Labuda et al., 2006; Pedra et al., 2006; Narasimhan et al., 2007; Tsuji et al., 2007; Merino et al., 2011; Carreón et al., 2012). Gomes-Solecki et al. (2006) and Tsao et al. (2004) recently demonstrated the feasibility of vaccinating animal host populations to decrease the prevalence of tick-borne pathogens in ticks, thus reducing the risk for pathogen transmission to humans and animals. In this research, *B. burgdorferi* infections in *I. scapularis* were reduced when ticks fed on mice immunized with the bacterial protein OspA, possibly by blocking bacterial adhesion to the tick receptor. A dynamic model of *B. burgdorferi* transmission in mice was then developed by Tsao et al. (2012) to evaluate the effect of vaccinating mice for the control of Lyme disease. They showed that a mouse-targeted vaccine would reduce *B. burgdorferi* infection prevalence in ticks but to further reduce risks of human infection, vaccination should also target other host species and measures should be implemented to reduce tick populations by vaccination and/or acaricide application. However, these models need to incorporate other variables related to climate and factors described herein to fully explore the effect of vaccines on the control of pathogen transmission.

CONCLUSION AND FUTURE DIRECTIONS

Several reliable models use climate variables as drivers to predict the current and future distribution of ticks or even their particular phenological patterns. Populations of the tick vector are regulated by a series of admittedly complex factors and climate traits. However, the "risk," conceived as the probability for humans to get infected by tick-transmitted pathogens, is dependent upon a large array of genetic factors (at the pathogen, tick, and host levels), social factors, and the dynamics of competent reservoir hosts, thus adding further layers of complexity. Therefore, other than the modulation of the development and mortality rates of the tick vector, climate, and other factors may regulate the intrinsic rates of the population density of the many tick hosts available. It is necessary to understand how regional weather and pathogen infection rates affect the molecular events in the tick vector and pathogen transmission. The field of compared ecology and behavioral processes is still widely open, which needs new developments and harmonized approaches to extricate the complex processes at the host–vector–pathogen interface. Recent results support that climate is playing a pivotal role in the spread, seasonality, and abundance patterns of several tick species with economical importance and/or impact on human and animal health.

Future research directions include the development of models that could handle the complex relationships between cohorts of newly borne and adult reservoir hosts, integrating tick developmental and mortality rates into a dynamic framework. Currently, adequate methods do not exist to model the composition of tick populations, the impact of climate on the individuals in the population as related to tick questing activity. This is of special relevance to compute the infestation rates on hosts and estimate density-dependent mortality rates and thus the recruitment of individuals into the next tick activity season. This is expected to be of particular importance in the development of reliable R0 values.

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Modulation of La Crosse virus infection in *Aedes albopictus* mosquitoes following larval exposure to coffee extracts

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The mosquito-borne La Crosse virus (LACV; Family Bunyaviridae) may cause encephalitis, primarily in children, and is distributed throughout much of the eastern United States. No antivirals or vaccines are available for LACV, or most other mosquito-borne viruses, and prevention generally relies on mosquito control. We sought to determine whether coffee extracts could interfere with LACV replication and vector mosquito development. Both regular and decaffeinated coffee demonstrated significant reductions in LACV replication in direct antiviral assays. This activity was not due to the presence of caffeine, which did not inhibit the virus life cycle. *Aedes albopictus* (Skuse; Diptera: Culicidae) mosquito larvae suffered near total mortality when reared in high concentrations of regular and decaffeinated coffee and in caffeine. Following larval exposure to sublethal coffee concentrations, adult *A. albopictus* mosquitoes had significantly reduced whole-body LACV titers 5 days post-infection, compared to larvae reared in distilled water. These results suggest that it may be possible to both control mosquito populations and alter the vector competence of mosquitoes for arthropod-borne viruses by introducing antiviral compounds into the larval habitat.

Keywords: arbovirus, mosquito larvicide, antiviral, La Crosse virus, Bunyaviridae

INTRODUCTION

La Crosse virus (LACV) is an arthropod-borne virus (arbovirus) in the California serogroup of the genus *Orthobunyavirus*, of the family Bunyaviridae and is distributed throughout most of the eastern United States. The virus is maintained in nature by transovarial passage from an infected female mosquito to her progeny via the egg and through an amplification cycle involving chipmunks and squirrels, which produce a high viremia capable of infecting other mosquitoes (Borucki et al., 2002). The natural vector is the eastern tree hole mosquito, *Ochlerotatus triseriatus* (Say), but other mosquitoes are also competent, including the following two introduced species: *Aedes albopictus* (Skuse; Grimstad et al., 1989) and *Ochlerotatus japonicus* (Theobald; Sardelis et al., 2002).

Human infections with LACV are common, though disease is rare, with an average of ∼70 cases reported annually. Infection typically presents with a generic febrile illness that may progress to severe central nervous system involvement, including seizures, mental impairment, coma, and death. Sequelae may consist of persistent seizures for over 10 years and learning disabilities. Death is rare, occurring in <2% of those displaying severe symptoms (Haddow and Odoi, 2009). There are no vaccines or antivirals used to treat infection with LACV and most other arboviruses. Prevention generally relies on avoidance of mosquito bites, either through personal protective measures or anti-mosquito insecticides.

Mosquito control generally uses either chemical insecticides or toxins derived from *Bacillus* species bacteria, although resistance to most control agents develops rather quickly (e.g., Cui et al., 2006; Paris et al., 2011). Novel mechanisms to prevent transmission of LACV and other mosquito-borne pathogens are therefore needed (Lambrechts et al., 2009; Luckhart et al., 2010). Environmentally friendly products derived from plants have been proffered as one source of mosquitocidal compounds, and many different extracts of plants have demonstrated mosquito larvicidal activity, though the effective concentrations vary widely among plant species and among extraction methods (Shaalan et al., 2005; Fallatah and Khater, 2010). Both caffeine and coffee extracts have been shown to inhibit the development of *A. aegypti* (L.) larvae (Laranja et al., 2003, 2006).

Further, plant extracts offer a potential source of antiviral compounds, and many have shown such activity against a broad spectrum of viruses (Jassim and Naji, 2003; Mukhtar et al., 2008). For example, coffee extracts from various sources have been shown to inhibit the replication of herpes simplex virus type 1 and poliovirus, and this antiviral activity did not require the presence of caffeine (Utsunomiya et al., 2008). In subsequent experiments, caffeine alone was shown to possess antiviral activity (Murayama et al., 2008), as did a newly identified compound in coffee, *N*-methyl-pyridinium formate (Tsujimoto et al., 2010).

In the present study, we investigated whether coffee extracts can interfere with mosquito development and LACV replication.

MATERIALS AND METHODS

ANTIVIRAL ASSAYS

La Crosse virus was kindly provided by Sally Paulson (Virginia Polytechnic Institute and State University) and was isolated from *O. triseriatus* mosquitoes in southwest Virginia. Virus stocks were generated by inoculation of confluent monolayers of African green monkey kidney (Vero) cells, harvested from supernatants when ∼90% of the monolayer exhibited cytopathic effect, and titrated by plaque assay in Vero cells.

Regular (Maxwell House Dark Roast) or decaffeinated (Great Value Classic Decaf Medium Roast) ground coffee beans were prepared according to the package directions by extracting one teaspoon coffee grounds with six fluid ounces deionized water (∼26.7 g coffee/L water) using a Mr. Coffee drip coffee maker. Whole coffee extracts were then sterilized by passage through a 0.22-μm membrane filter and serially twofold diluted in Medium 199 (M199; Cellgro) supplemented with 5% fetal bovine serum (FBS), amphotericin B (25 μg/100 mL), and gentamycin (15 μg/100 mL). Direct virucidal assays were performed by incubating ∼10^5 plaque-forming units (PFUs) LACV in 800 μL M199 with 200 μL of each solution for 30 min at room temperature. Each dilution was then serially 10-fold diluted in M199, and 400 μL of each dilution was plated onto confluent Vero monolayers in six-well plates (BD) for 1 h at 37°C and 5% CO_2. After incubating, the supernatant was removed by aspiration and replaced with M199 containing 0.8% gum tragacanth (MP Biomedicals) and FBS and antibiotics as above. Following a 3-day incubation at 37°C and 5% CO_2, the medium was poured off and the plates were stained in 1 mg/mL crystal violet in 10% formalin. Virus mortality was determined by comparing plaque numbers in coffee compound treated virus dilutions to virus incubated only in M199, which was set to 100% viability. Three independent replicates of each experiment were performed.

We also tested whether regular coffee could interfere with virus replication by adding undiluted coffee to Vero cells 1 or 24 h before or after infections with LACV as described above. Coffee was allowed to incubate on the Vero cells for 1 h at 37°C and 5% CO_2, and was then replaced with either normal M199, with LACV diluted to ∼10^3 PFUs/mL, or with M199 with gum tragacanth as above.

LARVICIDAL ASSAYS

Mosquitoes used were from a colony established from host-seeking *A. albopictus* collected in the Radford, Virginia, area, which has been maintained for 4 years with new field-collected adults added seasonally. This species was used because of its ease of laboratory colonization and because it is an efficient vector for a number of arboviruses, including LACV (Grimstad et al., 1989). All mosquito stages were maintained in an insectary held at 27°C and 80% relative humidity. Eggs were stimulated to hatch in 1.5 L deionized water containing ∼5 mL of a slurry of bovine liver powder (9.375 g/L) and brewer's yeast (3.125 g/L), which resulted in hatch within 1–2 days. Whole coffee extracts were serially twofold diluted from 1:2 to 1:8 in deionized water. Twenty-five *A. albopictus* larvae, mostly L1 but a few L2, were transferred 1–2 days post-hatching to 250-mL glass beakers containing 100 mL of each coffee dilution (50, 25, 12.5, or H_2O). Larvae were fed the bovine liver powder slurry as needed, generally ∼0.5 mL every other day. Surviving larvae were counted daily until all had died or pupated; a mosquito was considered living if it responded to gentle prodding with a transfer pipet. Pupae were removed from the beaker and were counted as surviving on all subsequent days, though we did not monitor survival of pupae and adults after their removal. Larvae reared in deionized water served as a control. Three independent replicates of each experiment were performed.

ADULT MOSQUITO INFECTIONS

To determine whether mosquitoes exposed to coffee during the larval stages have an altered susceptibility to LACV infection, we reared ∼100 *A. albopictus* larvae in 1 L of either deionized water or 15% regular coffee, which induces only a moderate mortality in larvae. Mosquitoes were housed in 30 cm × 30 cm × 30 cm cages, allowed to freely mate, and given cotton balls soaked with 10% sucrose, which was removed 24 h prior to blood feeding. Approximately 7 days post-eclosion, adults were given a blood meal containing 5.82 $\log_{10}$ PFU/mL LACV and held for 5 days at 27°C and 80% relative humidity. We selected the 5-day time point because the virus is not expected to have fully disseminated from the midgut (Paulson and Grimstad, 1989; Chandler et al., 1998), and therefore would pose only a minimal risk of accidental transmission. Mosquitoes that did not blood feed or only took a partial meal were destroyed. Individual mosquitoes were then homogenized in 50 μL M199 using a plastic pestle, an additional 100 μL M199 was added, and the homogenate was centrifuged 1 min at 14,000 × *g* to pellet debris. The supernatant was serially 10-fold diluted in M199, and plaque assays were performed with 200 μL of each dilution on Vero monolayers in 12-well plates (Costar) as described above, except antibiotic levels were increased to 125 μg/100 mL amphotericin B and 0.75 μg/100 mL gentamycin. Whole-body LACV titers were calculated for each mosquito.

OVIPOSITION

A small cohort of adult mosquitoes reared in 15% coffee or in deionized water were allowed to take a non-infectious blood meal from a human arm. Those that fed to repletion were individually housed in 21 mm × 70 mm shell vials containing ∼1 cm water and a strip of moist seed germination paper on which to oviposit. After 4 days, the number of eggs was counted under a dissecting microscope. Individuals that did not oviposit were discarded from the analysis.

STATISTICAL ANALYSES

All analyses were performed using JMP software (SAS Institute, Cary, NC, USA). Significance of antiviral assays was determined by ANOVA with Dunnett's test performed *post hoc*, comparing coffee-exposed virus levels to the virus control which was set to 100%. Differences in larval survival following exposure to coffee dilutions were identified by Wilcoxon comparison of Kaplan–Meier survival curves. Individuals that pupated were censored at the date when all larvae had either pupated or died. When the Wilcoxon test among groups was significant, pairwise comparisons were made to the control group. Mosquito infections were compared by unpaired *t*-test of log-transformed whole-body titers. Egg numbers were compared by unpaired *t*-test.

RESULTS

ANTIVIRAL ASSAYS

When regular Maxwell House Dark Roast coffee was incubated with LACV, direct antiviral activity was evident, and this activity was lost upon dilution (**Figure 1**). The coffee extract significantly reduced virus viability at final concentrations between 1.25 and 20%, but not at 0.625%. Similarly, Great Value

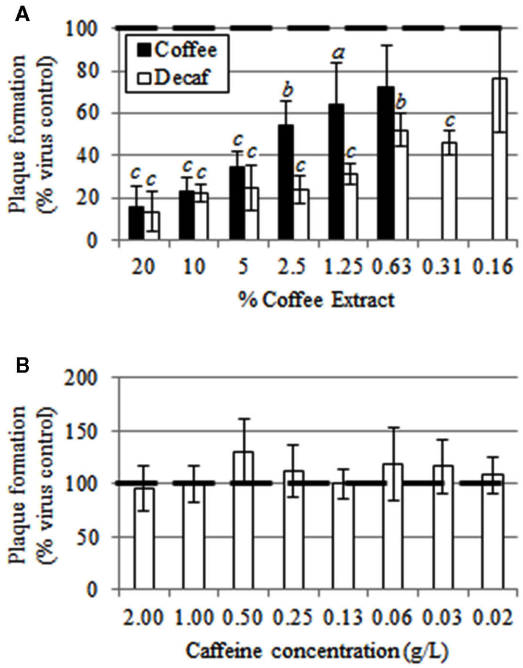

FIGURE 1 | (A) Direct anti-La Crosse virus effect of regular (black bars) and decaffeinated (white bars) coffee. **(B)** Lack of a direct anti-La Crosse virus effect upon exposure to caffeine. Error bars represent the SD of three independent replicates. *a*: $p < 0.05$; *b*: $p < 0.01$; *c*: $p < 0.0001$. The heavy, dashed, black line indicates the virus control, which was set to 100%.

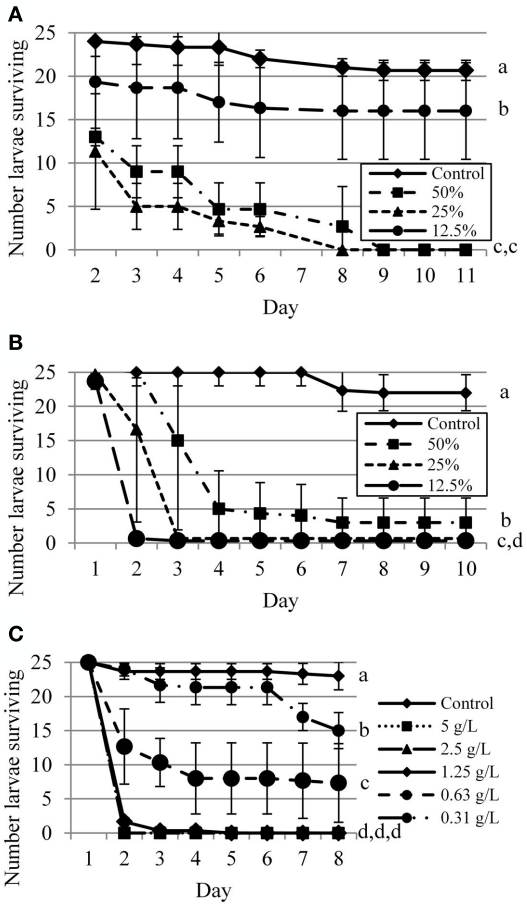

FIGURE 2 | Survival curves for *Aedes albopictus* larvae exposed to varying concentrations of regular coffee (A), decaffeinated coffee (B), or caffeine (C). Curves followed by different letters are significantly different ($p < 0.05$) by Wilcoxon analysis of Kaplan–Meier survival curves.

brand decaffeinated coffee exhibited strong antiviral effect; virus viability was significantly reduced at concentrations of 0.625–20% ($p \leq 0.0002$), while the 0.15625% level was marginally significant ($p = 0.0840$; **Figure 1A**). Thus, it is likely not the caffeine that has the antiviral activity, which was verified by the lack of virucidal activity seen with pure caffeine (**Figure 1B**). No toxic effects of the coffee on the Vero cells were noted.

Coffee extracts did not interfere with LACV replication when added to Vero cells 24 h before ($p = 0.8105$, $t = 0.2561$) or 24 h after ($p = 0.0777$, $t = 2.3590$) infection with LACV. When Vero cells were exposed to coffee extracts 1 h before infection, the production of LACV plaques was reduced by 52.5% ($p = 0.0002$, $t = 13.2500$). Similarly, a 23.8% reduction in plaques was evident when coffee was added 1 h after LACV infection ($p = 0.0200$, $t = 3.7482$; data not shown).

LARVICIDAL ACTIVITY

Mosquitoes reared in 25 or 50% regular coffee did not survive to pupation, and both concentrations exhibited similar survival curves; those reared in 12.5% coffee showed reduced mortality (**Figure 2A**). All pairwise comparisons were significantly different at $p \leq 0.0002$, with the exception of 50 vs. 25% ($p = 0.1320$). Decaffeinated coffee was largely lethal at all concentrations tested, though the 50% concentration surprisingly allowed a few mosquitoes to pupate while the lower concentrations killed all larvae (**Figure 2B**). All pairwise comparisons were significantly different at $p < 0.0001$. In contrast to the antiviral results, caffeine showed relatively strong larvicidal action, especially at concentrations $\geq$1.25 g/L (**Figure 2C**). All pairwise comparisons were significantly different at $p < 0.001$, except 1.25 vs. 2.5 and 5.0 g/L ($p = 0.1739$). We noted, but did not quantify here, that mosquitoes reared in coffee tended to pupate more quickly than water-reared controls.

MODULATION OF VIRAL TITERS IN ADULT MOSQUITOES

We reared mosquitoes in 15% coffee extract to yield sufficient surviving individuals to infect with LACV, yet be exposed to a relatively high concentration of coffee. These mosquitoes were then infected with a LACV-containing blood meal, and whole-body titers were determined after 5 days' infection. Exposure to coffee during the larval stages resulted in a significant reduction in the whole-body titer of mosquitoes infected with LACV as adults ($p = 0.0090$; $t = 2.780$). Mosquitoes reared in distilled water ($N = 17$) had an average titer of 4.65 $\log_{10}$ PFU/mosquito, while those reared in coffee ($N = 42$) had a titer of 3.07 $\log_{10}$ PFU/mosquito (**Figure 3**). Differences in body size were not obvious, though we did not measure wing lengths to verify this.

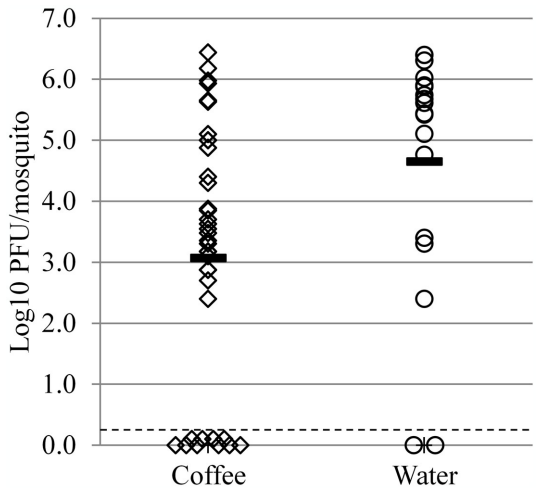

FIGURE 3 | Full-body La Crosse virus titers of *Aedes albopictus* females reared in 15% coffee extract (diamonds) or distilled water (circles). Solid horizontal lines indicate the mean for each group. Dashed line represents the limit of detection. Means are significantly different by *t*-test ($p = 0.0090$).

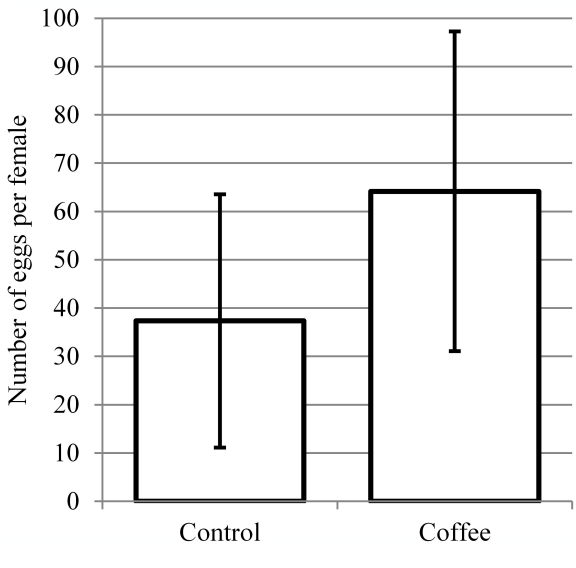

FIGURE 4 | Oviposition by females reared in water ($N = 12$) or 15% coffee ($N = 13$). Means are significantly different by *t*-test ($p = 0.0356$).

OVIPOSITION

Females reared in 15% coffee ($N = 12$) laid an average of 64.2 eggs (range 1–92), whereas those reared in water ($N = 13$) deposited a mean of 37.3 eggs (range 11–113; **Figure 4**). This difference was significant ($p = 0.0356$; $t = 2.2327$).

DISCUSSION

We present here a demonstration that it is possible to modify a mosquito's vector competence following exposure to an antiviral compound during the larval stages of its development. High concentrations of coffee were required to demonstrate antiviral and larvicidal activity individually and, thus, coffee extracts are likely not suitable as part of a wide-scale larvicidal and antiviral campaign unless the active components can be isolated. One such compound, pyridinium formate, has been shown to possess antiviral activity (Tsujimoto et al., 2010). Others may eventually be isolated, though, as >700 chemicals have been identified in extracts of roasted coffee beans (Spiller, 1984). Given the large number of compounds found in coffee, it is also improbable that the same compound is acting against both virus and larvae.

The mechanism by which coffee extracts exert their mode of action is unclear. We did not observe cytopathic effect in the Vero cells following the 1-h incubations with coffee extract. Hence, the coffee must be acting directly on the virus prior to our infection of the cell layers. In this case, the coffee could be inactivating the virus or interfering with an early step in the virus life cycle. The reduction in plaque numbers seen when coffee was added 1 h, but not 24 h, before or after virus infection suggests that an early step in the life cycle is targeted. A similar early inhibition was seen in cells infected with herpes simplex 1 virus (a virus with a DNA genome) following addition of coffee (Utsunomiya et al., 2008).

In these studies, decaffeinated coffee extract demonstrated a somewhat higher larvicidal activity and antiviral activity. This was unexpected, as previous experiments had shown similar herpesvirus inactivation rates using coffee extracts from broad geographic areas and between caffeinated and decaffeinated products, though these rates were quite variable among different brands (Utsunomiya et al., 2008). It is unlikely that the ethyl acetate method used to decaffeinate the coffee beans contributes to the increased larvicidal and antiviral activities, because the ethyl acetate is thoroughly removed during the process (Ramalakshmi and Raghavan, 1999). The variation seen between these two brands and formulations of coffee is most probably due to the different origins and degree of roasting of the coffee beans that may contain different concentrations of whatever compound(s) has the antiviral and/or larvicidal activity. Such variation in coffee compounds that have antibacterial activity has been shown among different coffee brands (Almeida et al., 2006). However, we did not attempt to identify or quantify the specific active compounds in this study.

Mosquito control using synthetic chemicals has been very successful, but resistance has developed to all insecticides used in their control (Hemingway et al., 2004). Bacterial toxins, such as those produced by *Bacillus thuringiensis israelensis* (Bti), have also proven useful. Again, resistance has begun to become evident (e.g., Paris et al., 2011), though not to the extent seen with insecticides. Plant extracts, such as from coffee, could potentially be used as control agents (Shaalan et al., 2005; Fallatah and Khater, 2010), though the likelihood of these being developed seems somewhat remote given the cost and regulatory burden associated with bringing new products to market (Isman, 2006).

We have documented several altered traits that can influence the vectorial capacity, or likelihood of transmission, of LACV. Vectorial capacity incorporates measures of vector density, feeding preference, survival, and extrinsic incubation period (EIP), which is the time it takes a mosquito to become infectious following a virus-containing blood meal (Black and Moore, 2005). Larval exposure to coffee extracts did not completely prevent LACV

infection of *A. albopictus*, but did significantly reduce the virus titer in the mosquitoes. This could result in a lengthening of the EIP, the time it takes a mosquito to become infectious. Because the EIP interacts with the probability of daily survival exponentially, a small change in the EIP can have a dramatic effect on the likelihood of transmission (Anderson and Rico-Hesse, 2006). We noted that females reared in coffee tended to lay more eggs than control females, which could theoretically lead to a higher risk of virus transmission by increasing the vector density. Other measures implicit in determining vectorial capacity, such as coffee's influence on adult survival or fertility, remain to be assessed. Additionally, variation in the size of the mosquito also influences the likelihood of transmission, with smaller mosquitoes transmitting at higher rates (Paulson and Hawley, 1991; Anderson et al., 2005). This does not mean that virus titers vary, as *A. albopictus* body size does not influence the whole-body titer of dengue virus (Alto et al., 2008).

The exact nature of this resistance to infection remains to be determined, and it is unclear whether the antiviral activity seen in cell culture is actually occurring in live mosquitoes. Two mechanisms described in the literature seem possible and warrant further study following identification of the active compound(s). First, the active antiviral component of coffee may be integrated into the adult mosquito from the aquatic medium in which the larvae and pupae develop. Such carry-over from larvae to adult has been demonstrated by feeding larvae suspensions containing radioactive rubidium (Wilkins et al., 2007) or zinc (Lang, 1963); these isotopes are then detectible in emergent adults. Further, exposure to sublethal concentrations of growth regulators during the larval stages continues to have detrimental effects on the adults that survive exposure (Vasuki, 1992; Suman et al., 2010). Thus, the antiviral compound(s) may be carried over from larvae to adult and reduce LACV infectivity in the mosquito. The fact that Vero cells exposed to coffee 24 h before LACV infection do not yield a reduction in PFUs provide evidence against this possibility, however. Second, exposure to coffee compounds may increase expression of stress-related genes, which may include those involved in the insect's innate immune system. Accordingly, *A. aegypti* larvae exposed to used coffee grounds and caffeine showed altered patterns of esterase expression compared to water-reared controls (Laranja et al., 2003). *Daphnia magna* Straus exposed to the herbicide propanil up-regulate a number of stress proteins (Pereira et al., 2010), as do *Drosophila melanogaster* (Meigen) when exposed to the pesticide endosulfan (Sharma et al., 2011). Such proteins may influence arbovirus replication in mosquitoes. For example, proteins involved in the Toll (Xi et al., 2008; Ramirez and Dimopoulos, 2010) and JAK–STAT (Souza-Neto et al., 2009) pathways influence the development of dengue virus infection in *A. aegypti* mosquitoes. However, the exact mode of action remains to be determined and refined.

In conclusion, we have demonstrated that some brands of coffee extracts have both mosquito larvicidal and anti-LACV activity, and we have further shown that replication of LACV can be modulated in adult mosquitoes by exposing the larvae to coffee extracts. Our results are at least a proof of concept, and we did not identify the actual active agent(s) responsible for our observed effects. However, this represents a potential new way to interfere with arbovirus transmission at two steps in the virus's life cycle, namely by decreasing the number of vectors and by reducing or blocking the replication of the virus in vector mosquitoes exposed to the virus. The large number of antiviral plant-derived compounds deserves further study to develop this mechanism into an effective arbovirus-control strategy.

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Linking global warming, metabolic rate of hematophagous vectors, and the transmission of infectious diseases

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Climate is under constant change. In addition to its natural variability, there is plenty of evidence suggesting persistent changes overtime produced by external forces, such as anthropogenic activities. These changes are observed on patterns of precipitation and temperature, among others (Crowley, 2000). Carbon dioxide levels in the atmosphere have been recorded since the beginning of the twentieth century. The hypothesis that changes in CO_2 concentration of the atmosphere could be responsible for climate deviations, first proposed by Arrhenius (1896), is now known as the greenhouse effect. Much research has been done finding a connection between the rise of anthropogenic carbon dioxide (and other greenhouse-effect gases) to increases in the global mean temperatures. Energy balance model studies show that temperature changes during the past 100 years cannot be explained by natural factors alone (such as solar irradiation and volcanism). Global mean temperatures have increased by 0.74 ± 0.18°C during the period 1906–2005, while average CO_2 levels increased from 280 ppm before the industrial revolution to 379 ppm in 2005 (IPCC, 2007). Moreover, it has been documented that this mean increase in temperature is produced by a rise in the minimum temperatures, resulting in a narrower diurnal temperature range (Easterling, 1997).

These changes in the abiotic parameters of the environment have a direct effect on biological systems. In the light of the current climatic variations the consequences have been analyzed across various levels of organization, seeking to establish causal relations between anthropogenic climate change and physical and biological impacts (Rosenzweig et al., 2008). At an ecosystem scale, the changes observed include for example: perturbations in seasonal events (such as migrations or time of reproduction) and shifts in biogeography and biodiversity (Rosenzweig et al., 2008). These effects can be traced to individual levels. Organisms live within a certain range of temperatures at which there is a coordinated functioning of molecular, cellular, and systemic processes (Pörtner and Farrell, 2008), and generally this is portrayed by a quadratic-shaped function. This function stands for the relation between these processes and temperature and it is represented by thermal performance curves. For each process there will be an optimal temperature, and two extreme points, i.e., an upper and a lower thermo-limit of performance. Particularly, ectothermic organisms are most vulnerable to thermal changes, given that their physiological processes are affected by temperature (Chown et al., 2010). Among ectotherms, terrestrial species are the ones most affected by environmental temperature changes due to the greater amplitude of change in their habitats compared to aquatic ecosystems. Terrestrial ectotherms comprise a large proportion of organisms and within this group there are some animals of great relevance to human health. Many of the worst epidemic diseases, such as Malaria, Chagas disease, Dengue, sleeping sickness, Leishmaniasis, Tick-borne encephalitis, among others, are transmitted by hematophagous arthropods that are ectothermic organisms. Thus, temperature will affect behavioral and physiological characteristics of diseases' vectors that are relevant to disease transmission.

Vector-borne diseases provide a fertile ground to address the general question of the causality between climate and biological change, and in order to understand fluctuations within this system (past, present, and future), information from different disciplines must be gathered. Having more accurate predictions from global change on the ecology of diseases, may enable more efficient health campaigns to be developed. Classical epidemiological parameters used to study disease transmission are based on the following factors: (i) survival and reproduction rates of vectors, (ii) intensity and temporal pattern of vector activity, and (iii) rates of development, survival, and reproduction of pathogens within vectors (Kovats et al., 2001). The vectorial capacity (VC) relates all of these factors as the number of infections resulting from the bites to one infected person per day through the following equation:

$$\mathrm{VC}=\left(ma^2p^n\right)/-\ln p$$

where m, is the vector–host (human) ratio; a is the biting frequency: the number of bites per vector per day; p stands for the daily probability of survival; and n for the development time of the parasite inside the vector. Another analogous epidemiological parameter widely used is the reproductive number R_0, which takes into account the same parameters as VC, plus the transmission coefficient from vertebrate to vector and from vector to vertebrate, as well as, the rate of host recovery from infection. Both expressions use information related to vector's biology. Variations on these parameters and their interactions will be reflected on the direction of the change of VC and R_0, i.e., a rise or decline in the disease (Rogers and Randolph, 2006). Vector survival rate may be affected in different directions, depending on the temperature and of how close of its thermal optimum the individual is. The vector–host ratio, on the other hand, depends both on vector's and human population density. The former depends upon parameters such as vector survival and reproduction rate, while the latter is driven mostly by socio-economical factors, together with environmental characteristics. Consequently, it is not clear, weather to expect an increase or decrease in the vector–host ratio in response to increasing temperatures (Rohr et al., 2011). Finally, another important parameter affecting disease dynamics is the biting rate, which is inversely proportional to the amount of time between blood meals,

and has a clear relationship with metabolic and nutrient conversion rates. Biting rate can be measured in the laboratory under controlled conditions, however, in the field only indirect estimations can be performed. For example, for triatomine bugs, vectors of Chagas disease, an indirect method is based on the distribution of bloodmeal weights from large vector samples, which would represent the distribution of times since last feeding (Rabinovich et al., 1979). Another method measures the presence of colorless urine that only occurs during the first few hours after feeding (Catalá, 1991). Nonetheless, these are indirect approaches and they have their limitations (see Rabinovich et al., 1979 and Catalá, 1991 for detailed explanation of the methods). Thus, it would be possible to directly measure the biting rate in the laboratory, without the limitations of an indirect measurement, and thereafter estimate the variation of biting frequency on the field based on the measured environmental temperature and the temperature sensitivity of the vector's metabolic rate (MR).

Q_{10} is a measurement of temperature sensitivity, originally applied to rates of chemical reactions; it is the factor by which a rate changes in response to a variation of 10°C, and it is a useful way to express the temperature dependence of a process. This factor may also be applied to whole organisms, for example, the sensitivity to temperature of MRs. Ectotherm's MRs are very sensitive to temperature, with Q_{10} values around 2, which means that a 10°C increase will double MR (Randall et al., 2001). Warmer temperatures speed up biochemical reactions, which in turn bring an increase in MR and nutrient transformation rate. As a consequence an increase on the frequency of biting would be expected. Thus, epidemiological mathematical models could use an estimated Q_{10} of 2 or a calculated Q_{10} based on measurements of MR at different temperatures from the species of interest as a simple way to account for changes in biting rate in response to thermal variations.

Thermal variations may have many effects on the physiology of vectors and consequently in vector-borne diseases transmission. Although common knowledge suggests that higher temperatures will lead to an increase in vector-borne diseases, recently this has been questioned and it has been proposed that a shift rather than an expansion in geographic range of disease, might occur. This consequence may be driven by variations in habitat suitability, which depends on climatic factors as temperature and humidity, as well as on barriers to dispersal and competition (Lafferty, 2009). Thermal changes are heterogeneous around the globe, as well as thermal tolerance among species, which is evidenced by their diversity across latitudes; at higher latitudes thermal tolerance is broader, while tropical organisms show more sensitivity to temperature changes (Chown and Nicolson, 2004). This implies that the consequences of an increase in environmental temperature will differ across latitudes (Deutsch et al., 2008) and species. Another factor that is generally left aside, are thermal daily variations. Most studies on the temperature effects on animal physiology are done under different constant temperatures, despite the fact that environmental temperature has a daily variation. However research has increasingly started to take more realistic daily variations into account. For example, in vectors of diseases, a variation on daily temperature preference has been observed in different species of kissing bugs, moreover, this preference is affected by the degree of starvation (e.g., Lazzari, 1991; Schilman and Lazzari, 2004). In addition, Paaijmans and collaborators showed that malaria transmission intensity is affected by the degree of daily temperature variations. Fluctuations around a low temperature increase rates of malaria parasite transmission, and mosquito development times and survival, while fluctuations around a high temperature slows the rates of these processes (Paaijmans et al., 2010). These results suggest that the molecular and physiological mechanisms underlying responses to temperature variations are complex and non-linear, and it is important to integrate them into current research of climate change impact.

In summary, several physiological factors affected by global warming should be considered to predict the dynamics of worldwide diseases' transmission. How these factors vary in response to thermal changes will be ultimately observed in modifications on the epidemiology of the disease. It has been observed that the underlying processes linking environmental temperature and rate of transmission diseases are not simple and direct linear relations. On one hand, the effects of daily temperature cycles on life history and survival of pathogens and vectors, as well as the differential sensitivity to thermal variations across different species and populations portray a complex system yet to be explored. On the other hand, and to construct the theoretical predictions of upcoming trends on vector-borne diseases simplified approaches might be useful. Thus, mathematical models could be developed taking into account the modulation of biting frequency by environmental temperature with the use of temperature sensitivity or coefficient Q_{10} of the MR. In other words, based on the close relationship of ectothermic organisms between environmental temperatures, MRs, biting frequencies, and diseases' transmission rates, more specific models could be developed in future analyses.

Climate change is a global issue that should be approached in a global fashion. Therefore, we hope that the field of invertebrate physiology could provide more tools to improve the formulation of mathematical models to predict, among other things, upcoming trends on vector-borne diseases in a global climate change scenario.

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Eventual role of asymptomatic cases of dengue for the introduction and spread of dengue viruses in non-endemic regions

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In dengue virus infections the asymptomatic cases are much more frequent than the symptomatic ones, but their true role in the introduction and subsequent spread of dengue viruses in non-endemic regions remains to de clarified. We analyzed data from English and French literatures to assess if viremia in asymptomatic dengue infections might be sufficient to represent a true risk. During outbreaks of dengue a large number of individuals are infected and since viremia levels in symptomatic patients are known to vary by many orders of magnitude, it is reasonable to augur that a proportion of asymptomatic cases might reach levels of viremia sufficient to infect competent mosquitoes. In addition, a number of new ways of contamination in man by dengue viruses were recently described such as blood transfusion, bone marrow transplantation, and nosocomial infections that may be worth considering.

Keywords: dengue virus infection, infected travelers, asymptomatic infections, viremia, disease severity

INTRODUCTION

During the three past decades dengue fever (DF) has shown an unprecedented geographic expansion whilst the annual number of cases and the severity of the disease increased dramatically. This mosquito-borne disease is at present the first arboviral disease infecting mankind worldwide. An estimated 2.5 billion persons live in virus endemic areas and 50–100 million cases occur each year, inducing 500,000 hospitalizations (Halsitead, 2007) and about 20,000 deaths (Strait et al., 2011).

As a direct consequence of the recent establishment of the *Aedes albopictus* mosquito in many European countries where imported cases of dengue occur among travelers returning from endemic areas, European Union Member States are at risk of local outbreaks. This is particularly evident for Northern Italy (Rovida et al., 2011) and above all for Southern France (Laruche et al., 2010) and Croatia (Gjenero-Margan et al., 2011). In each of these last two countries two autochthonous cases of dengue occurred in September 2010. These few cases were efficiently detected as a result of the recent implementation of European surveillance programs in France, Croatia, and Germany. It is the first time that autochthonous transmission of dengue viruses was recorded in Europe since the historical Greek outbreak of 1927–1928 (Chastel, 2009).

Increased global movement of humans, plants, and hematophagous arthropods via shipping and air traffic has multiplied the opportunities for infected travelers and other items to export dengue viruses from tropical to temperate regions (Chastel, 2007). Whether or not global climate change has worsened the situation is debatable (Reiter, 2001; Chastel, 2002; Barklay, 2008; Gould and Higgs, 2009) but it can no longer be ignored.

Moreover, in dengue as in other mosquito-borne arbovirus infections (Chastel, 2011) the asymptomatic cases are much more frequent than the symptomatic ones although their true role in introduction and subsequent spread of dengue viruses in non-endemic regions remains to be clarified.

This short review therefore examines the concept that asymptomatic disease is an increasingly contributor to the dispersal of dengue viruses than it may have been previously thought.

METHODS

We have collected pertinent data from English and French literatures from the 1980s to 2011 through PubMed and other bibliographic sources including Dengue Net. Data were analyzed to assess if viremia in asymptomatic dengue infections (dengue 1–4 viruses) might be sufficient to represent a true risk for the introduction of these viruses in non-endemic regions. The key-words we used in searching in databases were: "dengue virus infections," "introduction by infected travelers," "asymptomatic infections," "viremia," and "disease severity." We thus selected five articles dealing with the frequency of asymptomatic cases and seven articles related to viremia in symptomatic or asymptomatic cases. In addition we have taken in account both the classical way of transmission, i.e., by mosquito bite, and the new ones recently documented such as blood transfusion, organ transplantation, needle stick injury, or *intrapartum*.

Moreover, some studies concerning the introduction of dengue viruses in new areas and the Public Health measures implemented to fight against the consequences of such events were analyzed (see Beckett et al., 2005; Shu et al., 2005; Shang et al., 2010, for instance).

RESULTS

ATTEMPTS TO MINIMIZE THE INTRODUCTION AND SPREAD OF DENGUE VIRUSES IN NEW AREAS

It is almost impossible to intercept asymptomatic or mildly symptomatic travelers arriving from tropical regions and entering a

non-endemic one. However, it is important to emphasize that mild symptoms are a very common feature of arboviruses including the four dengue viruses (DENV 1–4).

In order to detect potential imported cases of DF and thereby to reduce the local spread of the introduced viruses, the Taiwanese Disease Control authorities established an integrated control program that included a variety of surveillance systems, capable of screening for fever in arriving passengers, a network of rapid diagnostic laboratories, and other integrated control measures. This strategy applied between July 2003 and June 2004 identified 40 confirmed cases of DF, all of which were *viremic* when tested by RT-PCR (Shu et al., 2005). Thus, although it proved costly to implement, this strategy appeared efficient and may be cost effective in long-term.

However, for other authors infrared skin thermometry did not appear an effective tool for the detection of febrile patients (Hausfater et al., 2008). More importantly this technique would *not detect asymptomatic individuals*.

QUANTIFICATION OF ASYMPTOMATIC CASES IN DENGUE VIRUS INFECTIONS

In these diseases, the asymptomatic cases are much more frequent than the symptomatic ones, but their relative number varies according to the geographic area, the epidemiologic context, the immunological status of patients, and the circulating type(s) of DENV. This is clearly exemplified by different surveys.

During a prospective survey carried out in 1980–1981 in Bangkok, Thailand, amongst school children, the authors estimated the ratio of asymptomatic/symptomatic cases to be 6.1:1. This ratio was refined to 5.5:1 for DENV 1 infections and 4.5:1 for DENV 2 cases. In addition, the three DENV 4 infections that were detected during this survey were entirely asymptomatic (Burke et al., 1988). In contrast, another prospective study was conducted in Kamphaeng Phet, Northern Thailand, between 1998 and 2000 and the results were quite different since the ratio was only 1.1:1 (Endy et al., 2002).

However, in Nicaragua, Central America, a similar survey carried out between 2001 and 2003 amongst 4- to 16-years-old school children living in Managua city showed that in 2001, when DENV 2 was prevalent, a ratio of 13:1 was determined. In 2002 this ratio fell to 6:1 when DENV 1 was the most frequently isolated serotype (Balmaseda et al., 2006).

In Singapore, South-East Asia, Health authorities assumed a ratio of asymptomatic/symptomatic infections between 2:1 and 10:1. This was considered to represent a very serious threat for blood transfusion recipients (Wilder-Smith et al., 2009).

Finally, amongst Dutch travelers infected between October 2006 and September 2007 in different tropical countries a ratio of 1.8:1 was observed. In this study the type of infecting DENV was not determined since only serologic testing was performed (Baaten et al., 2011).

Thus, according to these surveys, the ratio of asymptomatic/symptomatic cases of dengue virus infection may extensively vary. These differences might be explained by individual variations in susceptibility or variability in the virulence of DENV strains (Balmaseda et al., 2006). Alternatively the epidemiology of dengue may differ in South-East Asia and the Americas (Halstead, 2006).

WHAT ARE THE RISKS ASSOCIATED WITH ASYMPTOMATIC DENGUE VIRUS INFECTIONS?

In non-endemic regions, it is well established that DENV are being introduced by viremic travelers returning from endemic or epidemic areas, although whether or not they subsequently cause outbreaks depends on a number of crucial requisites: *firstly*, the infected traveler should be sufficiently viremic to be capable of infecting local mosquitoes; *secondly*, this mosquito would need to be a highly competent species and sufficiently abundant at the time of virus introduction to ensure its diffusion; *thirdly*, the local population would need to be highly receptive to DENV. Finally, favorable meteorological conditions represent critic factors for initiating local dengue epidemics in Taiwan (Shang et al., 2010).

Moreover, in both endemic and non-endemic regions, we know that blood transfusion, organ transplantation, and needle stick injuries represent new identified risks of infection by DENV (Hirch et al., 1990; de Wazières et al., 1998; Rigan-Perez et al., 2001; Langgartner et al., 2002; Chen and Wilson, 2004; Nemes et al., 2004; Wagner et al., 2004; Wilder-Smith et al., 2009). *Intrapartum* is another unusual route of contamination by DENV (Hirch et al., 1990; Rigan-Perez et al., 2001; Tran and Chastel, 2008). All these atypical ways of contamination may originate in a silent infection, either pre-clinic, very mild, or totally asymptomatic. In our opinion, these new modes of contamination greatly increase the opportunities for introduction of DENV in non-endemic regions by asymptomatic individuals.

MAGNITUDE OF VIREMIA IN ASYMPTOMATIC CASES OF DENGUE VIRUS INFECTIONS

To accurately appreciate the eventual role of asymptomatic cases for the introduction and spread of DENV in non-endemic regions, it is necessary to try to quantify the level of viremia in such infected persons. This is a basic, presently poorly documented problem.

Indeed, even though asymptomatic cases predominate during epidemics, the precise levels of viremia in such cases remain to be evaluated.

There are two studies using virological methods and taking up the problem of the viremia in asymptomatic cases of dengue infections.

The first was conducted, in 2001–2003, in West Jakarta, Indonesia, and showed that viremia might be detected in asymptomatic cases by RT-PCR or virus isolation: on day 10 during a DENV 1 infection in one individual and in day 4 during a DENV 2 infection in another one. Unfortunately, the RT-PCR method used in this study was only qualitative and thus the precise level of viremia was not established (Beckett et al., 2005).

The second study was performed during the 2006 and 2007 epidemics in Kampong Cham, Cambodia. Using NS1 antigen capture, real time RT-PCR, and MAC–ELISA assays, dengue infection was confirmed in 243 symptomatic patients and in 17 asymptomatic family-related individuals. All four dengue serotypes were identified. As this study was essentially devoted to assess if the NS1 antigenemia might represent a good early marker of the disease severity, it is difficult to precisely evaluate the levels of viremia in asymptomatic cases. In fact, the authors stated that "the level of viremia in asymptomatic cases was not significantly lower in asymptomatic cases than in all dengue confirmed cases ($p = 0.145$)" (Duong et al., 2011).

These scarce and partial results force us to use data established in symptomatic cases to better evaluate the viremia levels in asymptomatic ones.

MAGNITUDE OF VIREMIA IN SYMPTOMATIC CASES OF DENGUE VIRUS INFECTIONS

During a severe outbreak of DF in French Polynesia during 1996 and 1997, the plasma DENV 2 titers ranged from 1.7 to 5.6 log 10 $TCID_{50}$/ml in hospitalized children and the mean duration of the viremia was 4.4 days (Murgue et al., 2000).

In Thailand, viremia titers were determined in 168 children with acute dengue virus infections (DENV 1–4) and two different hospitals (Vaughn et al., 2000). Duration of viremia ranged from 1 to 7 days and the higher mean titer was $10^{7.6}$ MID_{50}/ml median mosquito infectious dose (MID) for patients with DF versus $10^{8.5}$ MID_{50}/ml for patients with hemorrhagic fever. Thus, the severity of the disease was, in this study, clearly associated with the higher titers of viremia.

The same observation was done in Taiwan during a DENV 3 epidemic in 1998. A total of 20 patients, 11 with DF and 9 with dengue hemorrhagic fever (DHF) were studied using a quantitative RT-PCR. The viremia was found under the detection threshold of the method (<600 RNA copies/ml) in five of the DF patients and between 3,900 and 3,710,000 RNA copies/ml in six others. In contrast, in nine DHF patients the viremia levels extended from 46,700 to 14,900,000 RNA copies/ml. These high levels of plasma RNA persisted during defervescence in DHF patients whilst they declined to a level below detection in DF patient. DENV persisting in plasma during defervescence were into immune complexes in most DHF patients (Wang et al., 2003).

Another survey was carried out amongst 80 Thai children using a RT-PCR method able to quantify all the four DENV serotypes. Plasma RNA levels ranged between 5×10^3 and 5×10^9 copies/ml (Sudiro et al., 2001).

In Singapore, Public Health authorities estimate DENV plasma viral RNA levels ranging from $10^{5.5}$ to $10^{9.3}$ copies/ml (Wilder-Smith et al., 2009). They also suggested: "it is likely, although not proven, that viremia is lower and shorter in duration in asymptomatic persons than in symptomatic persons." In the absence of any systematic study in this field such opinion can only be speculative.

On the contrary, it seems reasonable to assume that some asymptomatic cases must achieve viremia levels as high as the lowest estimates for symptomatic cases. In fact, from the above data it is evident that the viremic levels in DF patients are both highly variable and geographically widely dispersed. Therefore it is entirely reasonable to hypothesize that similar dispersion and high variability of virus titers might be present in asymptomatic individuals.

Taking this to the next logical step, if a competent mosquito vector such as *A. albopictus* is already established in non-endemic regions, for instance Southern Europe, the risk that asymptomatic dengue virus infection could provide the source of new foci of disease or eventually epidemics is likely. Moreover, the existence of atypical ways of contamination reinforce the probability of such a scenario.

CONCLUSION

Dengue virus infections cause asymptomatic infections much more frequently than symptomatic cases, sometimes multiplying up to 14 the actual number of infections, but their true role in the introduction and subsequent spread of DENV in non-endemic regions remains to be clarified.

Since very large numbers of individuals are infected and since viremic levels are known to vary by several orders of magnitude in symptomatic patients it is reasonable to argue that at least a proportion of asymptomatic cases might reach levels of viremia sufficient to infect competent mosquito vectors.

In addition, the existence of newly recognized ways of contamination in man such blood transfusion, bone marrow transplantation, and nosocomial infections offer other opportunities for DENV to be introduced and spread in non-endemic regions.

More virological studies are needed on asymptomatic cases of dengue to valid or not these hypotheses.

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Evaluation of 15 local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae)

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The adverse effects of chemical insecticides-based intervention measures for the control of mosquito vectors have received wide public apprehension because of several problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and non-target organisms. These problems have necessitated the need to explore and develop alternative strategies using eco-friendly, environmentally safe, biodegradable plant products which are non-toxic to non-target organisms too. In view of this, 15 plant species were collected from local areas in New Delhi, India. Different parts of these plants were separated, dried, mechanically grinded, and sieved to get fine powder. The 200 g of each part was soaked in 1000 mL of different solvents separately and the crude extracts, thus formed, were concentrated using a vacuum evaporator at 45˚C under low pressure. Each extract was screened to explore its potential as a mosquito larvicidal agent against early fourth instars of dengue vector, *Aedes aegypti* using WHO protocol. The preliminary screening showed that only 10 plants possessed larvicidal potential as they could result in 100% mortality at 1000 ppm. Further evaluation of the potential larvicidal extracts established the hexane leaf extract of *Lantana camara* to be most effective extract exhibiting a significant LC_{50} value of 30.71 ppm while the *Phyllanthus emblica* fruit extract was found to be least effective with an LC_{50} value of 298.93 ppm. The extracts made from different parts of other five plants; *Achyranthes aspera*, *Zingiber officinalis*, *Ricinus communis*, *Trachyspermum ammi*, and *Cassia occidentalis* also possessed significant larvicidal potential with LC_{50} values ranging from 55.0 to 74.67 ppm. Other three extracts showed moderate toxicity against *A. aegypti* larvae. Further investigations would be needed to isolate and identify the primary component responsible for the larvicidal efficiency of the effective plants.

Keywords: *Aedes aegypti*, plant parts, crude extracts, larvicidal agent, toxic component

INTRODUCTION

The importance of mosquitoes as important disease vectors transmitting diseases like malaria, filariasis, Japanese encephalitis, and dengue is well reported (Becker et al., 2003). Their potential to feed on more than one individual, during a single gonotrophic cycle is causing spread of diseases at larger scale (Mackenzie et al., 2004). *Aedes aegypti*, the primary carrier for viruses that cause dengue fever, dengue hemorrhagic fever, chikungunya, and yellow fever is widespread over large areas of the tropics and subtropics (Service, 1983; Yang et al., 2009). At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is by mosquito control, which is frequently dependent on applications of conventional synthetic insecticides (Malavige et al., 2004). According to WHO (2009) about two-fifth of the world's population is now at risk of dengue and the only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. In 2010, a total of 28,292 cases and 110 deaths were reported in India because of dengue (NVBDCP, 2011).

The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either targeting the mosquito larvae at breeding sites through spraying of stagnant water or by killing/repelling the adult mosquitoes using insecticides (Corbel et al., 2004; Joseph et al., 2004). In the past, synthetic organic chemical insecticides-based intervention measures for the control of insect pests and disease vectors have resulted in development of insecticide resistance in some medically important vectors of malaria, filariasis, and dengue fever (WHO, 1992; Singh et al., 2002; Kumar and Pillai, 2010, 2011). Insecticide resistance is increasingly becoming a problem for many vector control programs. Resistance may develop due to changes in the mosquitoes' enzyme systems, resulting in more rapid detoxification or sequestration of the insecticide, or due to mutations in the target site preventing the insecticide-target interaction (Hemingway et al., 2004). The frequent use of chemical insecticides to manage insect pests leads to a destabilization of the ecosystem and enhanced resistance to insecticides in pests (Kranthi et al., 2001). To alleviate these problems, major emphasis has been on the use of natural plant based products as larvicides which can be a safe alternate to synthetic insecticides (Zhu et al., 2008). Biologically active plant extracts have been well documented for evolving an ecologically

sound and environmentally accepted mosquito control program. These provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages. In addition, increasing documentation of negative environmental and health impact of synthetic insecticides and increasingly stringent environmental regulation of pesticides (Isman, 2000) have resulted in renewed interest in the development and use of botanical insect management products for controlling mosquitoes and other insect pests.

A number of such plant products have been used for insect control since time immemorial. Many researchers have reported the effectiveness of plant extracts or essential oils as efficient mosquito larvicides and repellents without posing hazards of toxicity to humans (Amer and Mehlhorn, 2006a,b; Rahuman et al., 2009a,b). More than 2000 plants species have been known to produce chemical factors and metabolites of value in pest control programs (Ahmed et al., 1984), and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes (Sukumar et al., 1991). However, very few plant products have been developed for controlling mosquitoes. Phytochemicals, extracted from the whole plant or specific part of the plant using different solvents may also act against mosquitoes as growth regulators, repellents, and ovipositional deterrent (Amer and Mehlhorn, 2006b; Rajkumar and Jebanesan, 2007).

In view of this, 15 plants were collected from local areas in New Delhi, India and its surrounding regions. The different parts of these plants were evaluated in the laboratory in terms of their larvicidal potential against dengue vector, *A. aegypti*. The assessment of plants for mosquito larval toxicity may help in the formulation of effective strategies for reduction of its population.

MATERIALS AND METHODS

REARING OF MOSQUITOES

The present investigations employ the dengue fever mosquito, *A. aegypti* originated from fields of Delhi and surrounding areas. The colony was maintained in an insectary at $28 \pm 1^{\circ}$C, $80 \pm 5\%$ RH, and 14L: 10D photoperiod (Kumar et al., 2010). Adults were supplied with freshly soaked deseeded raisins. Periodic blood meals were provided to female mosquitoes for egg maturation by keeping restrained albino rats in the cages. The eggs were collected in a bowl lined with Whatman filter paper and were allowed to hatch in trays filled with de-chlorinated water. Larvae were fed upon a mixture of yeast powder and grinded dog biscuits. The pupae formed were collected and transferred to the cloth cages for adult emergence.

COLLECTION OF PLANT MATERIAL

A total of 15 plant species were selected on the basis of their easy availability, uncomplicated cultivation, and possibility of commercialization. It was taken care that these species were not threatened, endangered, and endemic species; and few of them carry some medical importance. The species were gathered from the surrounding areas and brought to the laboratory in polythene bags. Each plant species belonged to a different family. Different parts, with well known fact of non-toxicity to human beings and traditional use in domestic or industrial consumption, were separated from each species and were thoroughly washed with tap water in order to clean dust or any particles stuck to them. The plant parts were observed carefully to find any kind of disease or infection and if found any, those parts were separated and not used for the experiment. The selected parts were kept for drying under shade at room temperature ($27 \pm 2^{\circ}$C) for about 20 days till they dried completely. The 15 plant species and the parts used in the present study are tabulated in **Table 1**.

PREPARATION OF EXTRACTS

The dried parts were mechanically grinded and sieved to get fine powder. The 200 g of each dried and powdered part was extracted with 1000 mL of hexane using soxhlet extraction apparatus for 24 h at a temperature not exceeding the boiling point of the solvent.

Table 1 | Details of the plant species screened and the larvicidal activity of 1000 ppm crude hexane extracts of selected parts of these plant species against dengue vector *Aedes aegypti*.

Name of the plant species	Local name	Family	Part used	% Mortality after 24 h
Abutilon indicum	Indian mellow	Malvaceae	Stem	100
Achyranthes aspera	Prickly chaff flower	Amaranthaceae	Stem	100
Phyllanthus emblica	Amla, Indian gooseberry	Phyllanthaceae	Fruit	100
Cassia occidentalis	Chakunda, coffee senna	Caesalpiniaceae	Leaves	100
Allium sativum	Garlic	Amaryllidaceae	stem	100
Zingiber officinale	Ginger	Zingiberaceae	Stem	100
Momordica charantia	Karela, bitter gourd	Cucurbitaceae	Fruit	100
Lantana camara	Spanish flag, west Indian lantana	Verbenaceae	Leaves	100
Ricinus communis	Castor oil plant	Euphorbiaceae	Leaves	100
Trachyspermum ammi	Ajwain, bishop's weed	Apiaceae	Fruits	100
Putranjiva roxburghii	Putranjiva	Putranjivaceae	Leaves	0
Chrysanthemum indicum	Mums, guldaudi	Asteraceae	Leaves	25
Myristica fragrans	Jaiphal, nutmeg	Myristicaceae	Fruits	10
Bauhinia tomentosa	Kachnar	Fabaceae	Leaves	0
Melaleuca bracteata	Golden bottle brush, mock olive	Myrtaceae	Leaves	15

The extracts, thus obtained, were concentrated using a vacuum evaporator at 45°C under low pressure. After complete evaporation of the solvent the concentrated extract was collected and stored in a refrigerator at 4°C as the stock solution of 1000 ppm for further use.

SCREENING OF EXTRACTS FOR THEIR LARVICIDAL PROPERTIES AGAINST *A. AEGYPTI*

The bioassays were carried out in two phases. The required concentrations of each extract were prepared using ethanol as a solvent. In the first phase, the extracts were tested against early fourth instar larvae of *A. aegypti* to estimate their toxicity. The early fourth instars of mosquitoes were taken, in batches of 20, in plastic bowls containing 99 mL of distilled water and transferred to glass jar containing 100 mL of distilled water and 1 mL of 1000 ppm of extract. Four replicates were carried out simultaneously for each extract making a total of 80 larvae for each test. Controls were exposed to the solvent, i.e., ethanol alone. During the treatment period, the larvae were not provided with any food. The dead and moribund larvae were recorded after 24 h as larval mortality.

The mortality of larvae was determined by observing the movement of the larvae after 24 h of treatment. The larvae were touched gently with the help of a glass rod. The larvae were considered dead if they showed no sign of movements. The larvae were considered moribund if they moved a little but did not show any kind of swimming movement. The moribund larvae were considered dead as these larvae could never revive.

The extracts that failed to give 100% mortality after treatment for 24 h were no longer used, whereas the extracts that provided 100% mortality after treatment for less than 24 h were selected and used for the next stage of the study.

EVALUATION OF SELECTED EXTRACTS AGAINST LARVAE OF *A. AEGYPTI*

Following the results of the first stage, bioassays were conducted to calculate the LC_{50} values of selected extracts against the early fourth instar larvae of *A. aegypti*. The larvicidal bioassay was performed at 28 ± 1°C on *A. aegypti* larvae in accordance with the procedure described by WHO with slight modifications (WHO Report, 2005). The graded series of each of the selected extracts was prepared using ethanol as the solvent. The tests were carried out with each dilution of each extract as described earlier. Four replicates were carried out simultaneously for each dilution making a total of 80 larvae for each concentration of each extract. Controls were exposed to the solvent, i.e., ethanol alone.

DATA ANALYSIS

The tests with more than 20% mortality in controls and pupae formed were discarded and repeated again. If the control mortality ranged between 5 and 20%, it was corrected using Abbott's formula (Abbott, 1925).

$$\text{Corrected Mortality} = \frac{\%\ \text{Test Mortality} - \%\ \text{Control Mortality}}{100 - \%\ \text{Control Mortality}} \times 100$$

The data were subjected to regression analysis using computerized SPSS 11.5 Program. The LC_{50} and LC_{90} values with 95% fiducial limits and chi-square were calculated in each bioassay to assess the significance and measure difference between the test samples.

RESULTS

SCREENING OF EXTRACTS FOR THEIR LARVICIDAL PROPERTIES AGAINST *A. AEGYPTI*

In the first phase of study, 1000 ppm extracts made from different parts of 15 plant species, collected from Delhi and surrounding areas were tested against early fourth instars of *A. aegypti*. The per cent mortality observed after 24 h revealed that out of 15 species, only 10 species could result in 100% mortality. The details of the species collected and mortality data is presented in **Table 1**. Other plant extracts resulted in only 0–25% mortality and thus these species were rejected for further bioassays.

EVALUATION OF SELECTED EXTRACTS AGAINST LARVAE OF *A. AEGYPTI*

The larvicidal efficiencies of the selected extracts were evaluated against early fourth instars of *A. aegypti*. The mortality data is presented in **Table 2**. Bioassays performed with 10 extracts revealed their potency to kill larvae of *A. aegypti*. The treatment resulted in complete mortality with no pupal or adult emergence. The control or untreated group did not exhibit any mortality within 24 h.

Our investigations revealed that hexane extract made from the leaves of *Lantana camara* was the most effective extract exhibiting a significant LC_{50} value of 30.71 ppm. On the other hand the extract made from amla fruit was least effective with an LC_{50} value of 298.93 ppm thus exhibiting 9.4-fold fewer efficacy than *Lantana* leaves (**Table 2**; **Figure 1**). It was also observed that the extracts made from *Achyranthes aspera* (stem), *Zingiber officinale* (stem), *Ricinus communis* (leaves), *Trachyspermum ammi* (fruits), and *Cassia occidentalis* (leaves), though 1.8–2.4 times less effective than *Lantana* leaves possessed significant larvicidal potential with quite low LC_{50} values ranging from 55.0 to 74.67 ppm (**Figure 1**).

Other extracts showed only moderate toxicity against *A. aegypti* larvae. The stem extract of *Abutilon* was found to be 5.98-fold less effective than extract of *L. camara* leaves but 1.6-fold more effective than amla extracts. Likewise, the stem extract of garlic showed 7.1-less efficacy than *Lantana* leaf extract and only 1.4-fold more effective than amla extract; while karela fruit extract exhibited 8.5-fold less toxicity than extract of *Lantana* leaves but only 1.1-fold more effective than that of amla fruits (**Table 2**; **Figure 1**).

The study revealed that the LC_{50} values obtained from the extracts made from *Lantana* leaves and amla fruit were significantly different from that obtained from other extracts. The extracts made from *Achyranthes* (stem), *Zingiber* (stem), *Ricinus* (leaves), *Trachyspermum* (fruits), and *Cassia* (leaves) resulted in mortalities that were not significantly different from each other but were significantly different from those caused by other extracts. Similarly, the extracts from *Abutilon* stem, garlic stem, and karela fruit though significantly different from other extracts did not differ significantly from each other.

DISCUSSION

The control of mosquito-borne diseases can be achieved either by killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the

Table 2 | Larvicidal bioassay of crude hexane extracts of selected parts of 15 plant species against early fourth instars of *Aedes aegypti*.

Name of the plant species	LC_{50} (ppm)*	95% fiducial limits	LC_{90} (ppm)	95% fiducial limits	χ^2	df	SE	Regression coefficient
Abutilon indicum	183.61[c]	149.18–230.07	470.48	350.18–758.39	5.51	4	0.45	3.14
Achyranthes aspera	57.50[b]	50.05–64.66	90.84	78.56–117.97	2.53	3	1.18	6.45
Phyllanthus emblica	298.93[d]	263.27–333.90	454.32	396.57–579.36	2.34	3	1.80	7.04
Cassia occidentalis	74.67[b]	60.47–91.11	202.35	154.00–314.17	5.17	6	0.43	2.95
Allium sativum	218.35[c]	184.37–255.58	434.76	357.22–589.61	4.39	4	0.61	4.28
Zingiber officinalis	55.00[b]	45.41–65.99	129.41	101.26–194.26	5.64	5	0.52	3.44
Momordica charantia	260.14[c]	145.12–447.20	663.29	400.95–927.99	4.54	4	0.46	3.15
Lantana camara	30.71[a]	24.23–38.07	86.36	65.69–131.31	4.75	5	0.39	2.85
Ricinus communis	64.26[b]	53.94–76.46	140.18	110.50–212.00	2.24	5	0.61	3.78
Trachyspermum ammi	65.57[b]	57.29–74.49	108.90	92.21–148.18	1.26	4	1.06	5.81

**Figures in each column followed by the same letter are not significantly different at p = 0.05 (Students' t-test).*

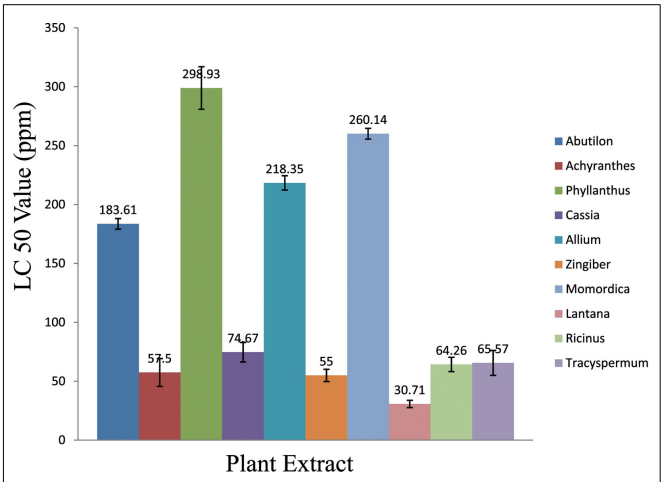

FIGURE 1 | Comparative larvicidal activity of different parts of 10 selected plants against *A. aegypti*.

breeding centers of the vectors in the environment. However, the extensive use of synthetic organic insecticides during the last five decades has resulted in environmental hazards and the development of physiological resistance in the major vector species. This has necessitated the need for search and development of environmentally safe, bio-degradable, low cost, and indigenous methods for vector control, which can be used with minimum care by individual and communities in specific situations (Mittal and Subbarao, 2003).

A number of researches in the field of vector control have revealed the efficacy of different phytochemicals obtained from various plants against different species of mosquitoes. Sukumar et al. (1991) made an extensive review of botanical derivatives tested for mosquito control. A large number of plant extracts have been reported to possess mosquitocidal or repellent activities against mosquito vectors, but very few plant products have shown practical utility for mosquito control. The plant products can be obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, methanol, chloroform, hexane, ethanol, petroleum ether, etc. Plants are rich sources of complex mixtures of bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. It could also be conceived from the review that some phytochemicals act as general toxicants both against adult as well as against larval stages of mosquitoes. The preliminary screening is a good means of evaluation of the potential mosquitocidal activity of plants popularly used for this purpose.

Present study investigates the larvicidal activity of the crude hexane extracts prepared from different parts of 15 local plant species gathered from New Delhi, India and surrounding regions. Our investigations showed that at 1000 ppm only 10 species showed larvicidal activity and thus they were evaluated further for their larvicidal potential. It was revealed that hexane extracts prepared from selected 10 species possess appreciable larvicidal potential against *A. aegypti*. The hexane leaf extract of *L. camara* proved to be most effective extract exhibiting a significant LC_{50} value of 30.71 ppm while the *Phyllanthus emblica* fruit extract was found to be least effective with an LC_{50} value of 298.93 ppm. The extracts made from different parts of *A. aspera*, *Z. officinalis*, *R. communis*, *T. ammi*, and *C. occidentalis* possessed significant larvicidal potential with LC_{50} values ranging from 55.0 to 74.67 ppm. Other extracts showed moderate toxicity against *A. aegypti* larvae.

Our results are comparable with the studies reported by different scientists. Kumar and Maneemegalai (2008) reported that at 1 mg/mL the ethanol extracts of the leaves of *L. camara* caused 84% larval mortality while the methanol extracts showed 48% mortality in the fourth instar larvae of *A. aegypti*. The ethanol extracts of *C. occidentalis* prepared from its leaves showed larvicidal activity against the malarial vector *Anopheles stephensi* at a dose equivalent to LC_{50} of 70.56% for IV instar larvae (Dhandapani and Kadarkarai, 2011). Ethanolic extract of *Allium sativum* bulbs showed significant insecticidal activity against larvae of *Aedes albopictus* exhibiting LC_{50} value of 4.48 g/L (Tedeschi et al., 2011). The toxicity of crude extract of *R. communis* foliage against fourth instar larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* was reported by Elimam et al. (2009). They recorded LC_{50} values as 403.65, 445.66, and 498.88 ppm against second, third, and fourth instar larvae of *A. arabiensis* and 1091.44, 1364.58, and 1445.44 ppm against second, third, and fourth instar larvae of *C. quinquefasciatus*, respectively.

Kamalakannan et al. (2011) reported that methanol extract of the leaves of *A. aspera* caused 50% mortality of *A. aegypti larvae* at 409 ppm. Arivoli and Tennyson (2011) found that the hexane extract of *Abutilon indicum* leaves caused 100% mortality at 1000 ppm with LC_{50} value of 261.31 ppm against the larvae of *A. aegypti* at 24 h. Our results are in agreement with that of Uthayarasa et al. (2010) who reported an LC_{50} value of 202.92 ppm with amla fruit hexane extract against third instars of *A. aegypti*. They also reported that crude extract of amla fruits obtained with dichloromethane proved to be more efficient with LC_{50} value of 166.64 ppm. Singh et al. (2006) carried out investigations with crude aqueous and hexane extracts of *Momordica charantia* against larvae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* and revealed the LC_{50} values of 0.50, 1.29, and 1.45%, respectively with aqueous extracts and 66.05, 96.11, and 122.45 ppm, respectively with hexane extracts.

The botanical extracts from the plant leaves, roots, seeds, flowers, and bark in their crude form have been used as conventional insecticides for centuries. In fact, many researchers have reported the effectiveness of plant extracts against mosquito larvae (Rasheed et al., 2005; Amer and Mehlhorn, 2006a). Warikoo et al. (2012) showed that 24 h exposure to early fourth instars of *A. aegypti* with hexane extract of the leaves of *Citrus sinensis* resulted in 50% mortality at 446.84 ppm. Amusan et al. (2005) revealed the efficacy of the ethanolic extract of the orange peels against the larvae of the yellow fever mosquito, *A. aegypti*. Kumar et al. (2011) found that the peel of *C. limetta* extracted in hexane possessed significant larvicidal potential against *A. stephensi* and *A. aegypti* exhibiting LC_{50} values of 132.45 and 96.15 ppm, respectively.

Our investigations demonstrated the larvicidal potential of various plants extracts of against *A. aegypti*. As the plants are distributed throughout the country and available most of the time, the larvicidal properties of these plant species can be well utilized while planning alternate vector control strategies, based on integrated vector control measures through community-based approaches. The plants are easily available to the local people and multiple medicinal properties, it may be easily acceptable to them, since during application it would neither cause any toxic effect nor any additional economic burden. However, the mechanism causing the larvicidal effect is still unknown and needs to be studied further. Variety of types and levels of active constituents in each extract may be responsible for the variability in their potential against *A. aegypti*. This knowledge may help in designing and implementing an effective strategy from a resistance-management perspective against *A. aegypti*. Further investigations are needed to identify the active ingredient present in the each extract and elucidate their efficacy against *A. aegypti*.

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Plasmodium knowlesi and *Wuchereria bancrofti*: their vectors and challenges for the future

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Malaria and filariasis still continue to pose public health problems in developing countries of the tropics. Although plans are in progress for the elimination of both these parasitic vector borne diseases, we are now faced with a daunting challenge as we have a fifth species, *Plasmodium knowlesi* a simian malaria parasite affecting humans. Similarly in peninsular Malaysia, filariasis was mainly due to *Brugia malayi*. However, we now see cases of *Wuchereria bancrofti* in immigrant workers coming into the country. In order to successfully eliminate both these diseases we need to know the vectors involved and introduce appropriate control measures to prevent the diseases occurring in the future. As for knowlesi malaria it is still uncertain if human to human transmission through mosquito bites is occurring. However, *P. knowlesi* in human is not a rare occurrence anymore and has all the characteristics of a pathogen spreading due to changes in the ecosystem, international travel, and cross border migration. This has created a more complex situation. In order to overcome these challenges we need to revamp our control measures. This paper reviews the vectors of malaria and filariasis in Southeast Asia with special emphasis on *P. knowlesi* and *W. bancrofti* in Malaysia and their control strategies.

Keywords: ***Plasmodium knowlesi, Wuchereria bancrofti,* Southeast Asia, vectors, control**

INTRODUCTION

Malaria and filariasis are the two most important mosquito borne parasitic diseases on a global scale. Currently about 40% of the global population remains at risk of contracting malaria (Hay and Snow, 2006) and 1000 million people in 80 countries are at risk of contracting filariasis (Zagaria and Savioli, 2002). Besides Africa, Asia contributes to a large number of cases (Bockarie et al., 2009). In 1998 World Health Organization (WHO) embarked on a program for global elimination of lymphatic filariasis and this was followed by elimination of malaria program in 2006. This review will be confined mainly to Southeast Asia and with special reference to *Plasmodium knowlesi* and *Wuchereria bancrofti*.

Human malaria has been known to be caused by four *Plasmodium* species namely *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. However, of late *P. knowlesi*, a simian malaria parasite is also affecting humans mainly in Southeast Asia region (Singh et al., 2004; Cox-Singh et al., 2008; White, 2008; Galinski and Barnwell, 2009). The first case of *P. knowlesi* in human was reported in an American surveyor who contracted the disease in the jungles of Pahang in peninsular Malaysia in 1965 (Chin et al., 1965) and subsequently the second suspected case of *P. knowlesi* was reported from Johore (Fong et al., 1971). Extensive studies carried out during the 1960s did not reveal further cases (Warren et al., 1970) and thus the conclusion drawn at that time was that *P. knowlesi* will not affect humans and the first case was just a rare incident. However, laboratory studies carried out then also revealed that monkey to human transmission through mosquito bites can take place (Chin et al., 1968; Contacos, 1970).

About four decades later, Singh et al. (2004) reported 106 cases of *P. knowlesi* that were misdiagnosed as *P. malariae* and in addition there were mixed infection of *P. knowlesi* and *P. falciparum* and *P. vivax* in Sarawak, Malaysian Borneo. Currently knowlesi malaria has been reported from all countries in Southeast Asia with the exception of LAO PDR. (Jongwutiwes et al., 2004; Zhu et al., 2006; Cox-Singh et al., 2008; Luchavez et al., 2008; Vythilingam et al., 2008; Van den Eede et al., 2009; Sulistyaningsih et al., 2010; Khim et al., 2011). Morphologically it is difficult to distinguish between the band stage of *P. malariae* with *P. knowlesi* and the ring stage of *P. falciparum* with *P. knowlesi* (Lee et al., 2009b). Using molecular tools to study the archival blood samples from patients in Sarawak obtained more than a decade ago (in 1996) demonstrated that *P. knowlesi* was occurring there for a long time and was not detected (Lee et al., 2009a). Further studies on blood samples from macaques and the analysis of the mitochondrial DNA showed that *P. knowlesi* underwent a period of population expansion estimated to about 30,000–40,000 years ago (Lee et al., 2011). Studies in Thailand have shown that *P. knowlesi* occurred in humans more than a decade ago (Jongwutiwes et al., 2011).

When the human case was detected in 1965 in peninsular Malaysia, studies were also conducted to elucidate the host and the vectors. This led to many new simian malaria parasites being described (Eyles et al., 1962a,b). *P. knowlesi* was found in *Macaca fascicularis*, *M. nemestrina*, and *Presbytis melalophos*.

Detailed studies on vectors of simian malaria were also conducted in peninsular Malaysia in the 1960s. In the coastal mangrove swamp areas of Selangor, *Anopheles hackeri* was incriminated as the vector of *P. knowlesi* (Wharton and Eyles, 1961). However, this species of mosquito was found biting only monkeys. None came to bite humans. In laboratory studies *A. hackeri* was able

to transmit five species of simian malaria namely *P. cynomolgi*, *P. inui*, *P. coatneyi*, *P. fieldi*, and *P. knowlesi* (Warren and Wharton, 1963). Extensive studies were carried out using monkey baited traps on platforms among foliage of trees at different heights. Detailed records of the vector distribution and studies has been published (Vythilingam, 2010). In the area where the first case was found in Bukit Kertau Pahang, peninsular Malaysia, *A. introlatus* and *A. latens* were obtained from the forest but not in the village (Warren et al., 1970). In the sixties since molecular techniques were not available, in order to confirm species of sporozoites they were inoculated into rhesus monkeys. *A. balabacensis* (=*A. cracens*; Sallum et al., 2005a) a vector of human malaria was incriminated as vector of *P. inui* (Cheong et al., 1965).

Lymphatic filariasis is caused by three species of parasites namely *Brugia malayi*, *W. bancrofti*, and to a lesser extent *B. timori*. Although Asia has an estimated total at-risk population of 68% for LF, it is by far the most important region globally in terms of number of active filarial infections, contributing approximately 59% of the world's burden distributed over 15 countries (Manguin et al., 2010). More than 70% of the LF cases occur on the Indian subcontinent, particularly India, Bangladesh, Maldives, Nepal, and Sri Lanka (Manguin et al., 2010). Migrant workers from these countries go to work in neighboring countries and there is every possibility that microfilariae will be brought along with them.

Rural strain (present only in very remote rural areas of peninsular Malaysia in rural villages of Sabah and Sarawak) of *W. bancrofti* is present in Malaysia but the urban strain is not common (Mak, 1981). Attempts by workers to establish patent infection of *W. bancrofti* in animals have so far been unsuccessful. However, Cross et al. (1974) and Dissanaike and Mak (1980) were able to recover adult worms from experimental infections in Taiwan Monkeys (*M. cyclopis*) and *M. fascicularis* respectively. They found gravid females with active microfilariae in the uterus but none were found in the blood.

Unlike malaria where only few species of the *Anopheles* genus are vectors, vectors of filariasis belong to species of six mosquito genera, such as *Aedes*, *Anopheles, Culex*, *Downsiomyia*, *Mansonia*, and *Ochlerotatus*, in the Southeast Asia region (Mak, 1987).

VECTORS OF SIMIAN MALARIA

Although simian malaria was known to occur in the forest of Southeast Asia way back in the 1960s not much is known about the vectors except in Malaysia. The mosquitoes incriminated as vectors belong to the *Leucosphyrus* group (Wharton et al., 1964) and it was noted that simian malaria parasites developed poorly in the subgenus *Anopheles*. In India, *A. elegans* now known as *A. mirans* (Sallum et al., 2005b; also a member of *leucosphyrus* gr) was incriminated as vector for simian malaria *P. cynomolgi* and *P. inui* (Choudhury et al., 1963).

The first simian malaria vector to be incriminated was *A. hackeri* and five species of plasmodia were isolated namely *P. knowlesi*, *P. coatneyi*, *P. cynomolgi*, *P. fieldi*, and *P. inui* (Wharton et al., 1964). *A. hackeri* was found mainly in the coastal area and was not attracted to humans. It was also found in Southern Thailand and in the Philippine islands of Balabac and Palawan (Sallum et al., 2005a) but its role as vector of simian malaria in those areas have not been investigated.

Within the *Leucosphyrus* complex, *A. latens* was incriminated as vector of *P. inui* in the hill-forest feeding mostly at the canopy level (Wharton et al., 1964). *A. introlatus* was incriminated as vector for *P. cynomolgi* and *P. fieldi* and it rarely feeds on man (Wharton et al., 1964). While in the northern part of peninsular Malaysia *A. cracens* (=*A. balabacensis* = *A.dirus* B) was found infected with *P. cynomolgi* and *P. inui* (Cheong et al., 1965). Laboratory studies also showed that *A. cracens* was a much better vector of malaria parasites compared to other species since 77% of them were infected (Scanlon, 1968).

Recent studies carried out in Pahang incriminated *A. cracens* to be a vector of *P. knowlesi* (Vythilingam et al., 2008). Early studies showed *A. cracens* to be strongly exophilic and exophagic, they avoid contact with sprayed surfaces and contact with humans take place away from the dwellings. The peak feeding was not long after dusk (Scanlon, 1968). After many decades it shows that *A. cracens* has not changed its behavior and in our study we found that it does not enter houses and starts biting humans around 19:00–20:00 h. It also shows preference to bite humans compared to macaques, ratio being 2:1 (Vythilingam et al., 2008).

Besides Malaysia, *A. cracens* occurs in Indonesia (Sumatra) and Thailand (Sallum et al., 2005a). However, their vectorial status in these two countries remains unknown. Currently only the *A. leucosphyrus* group has been incriminated as vectors of simian malaria. In Kapit, Malaysian Borneo, *A. latens* has been incriminated as vector of *P. knowlesi* (Vythilingam et al., 2006; Tan et al., 2008) and four simian malaria parasites namely *P. inui*, *P.coatneyi*, *P. fieldi*, and *P. cynomolgi* (Tan, 2008). *A. latens* has shown different biting times in the forest and farm. In the forest, they are early biters, peak being between 19:00 and 20:00 while in the farm the peak is 01:00–02:00 h (Tan et al., 2008). *A. latens* was the predominant species coming to bite both humans and macaques in the forest.

In other parts of Southeast Asia, besides Malaysia, vectors have been elucidated in Vietnam. Studies have shown *A. dirus* to be the main vector of *P. knowlesi* (Nakazawa et al., 2009; Marchand et al., 2011). The most interesting part of their study was that mixed infection with human malaria and *P. knowlesi* parasites were found in *A. dirus*. This could be due to the fact that malaria parasites are still circulating in the human population living in the forested areas. In Malaysia although mixed infection of knowlesi malaria and other human malaria were found in patients, we never obtained such mixed infection in mosquitoes. This could be due to the fact that our study was confined to two areas in Pahang where human malaria has been eliminated and thus mixed infection could not be detected. Perhaps more extensive surveys need to be carried out in areas where mixed infections of human and simian malaria parasites are being found.

Anopheles dirus is the vector of human malaria in Thailand, Cambodia, Laos, and Vietnam (Chareonviriyaphap et al., 2000; Vythilingam et al., 2003; Trung et al., 2004; Manguin et al., 2008) and it belongs to the Leucosphyrus group which has its main distribution in the Oriental region (Harbach, 2004; Subbarao, 2007; **Figure 1**). In Thailand cases of knowlesi malaria have been reported but the vector has not been incriminated (Seethamchai et al., 2008). The main vectors of human malaria in Thailand are *A. minimus*, *A. maculatus*, and *A. dirus*

FIGURE 1 | Map of South and Southeast Asia showing distribution of an leucosphyrus group of mosquitoes. Insert showing the map of Malaysia. Adapted from Sallum et al. (2005a).

(Chareonviriyaphap et al., 2000). However, recently *A. dirus* has drastically decreased in abundance in major malaria endemic areas of Thailand (Jongwutiwes et al., 2011).

VECTORS OF BANCROFTIAN FILARIASIS

The three variants of *W. bancrofti* found in human are recognized by the periodicity of the microfilaria in the blood. It can be nocturnally periodic, nocturnally subperiodic, and diurnal subperiodic (Mak, 1987). Species of mosquitoes belonging to the six genera of mosquitoes listed above are vectors (Zagaria and Savioli, 2002). It has also been suggested that Southeast Asia may have been the home of this parasite and may have been disseminated to the other continents from here (Hawking, 1976). Thus, Malaysia is vulnerable to the establishment of *W. bancrofti* in both urban and rural areas because the migrant populations coming from filariasis endemic countries work in both urban and rural areas such as plantations. The vectors associated with these parasites are mainly *Anopheles* mosquitoes in the rural areas and *C. quinquefasciatus* in the urban areas. In Banggi Island (north-eastern Sabah, Malaysia), *A. balabacensis* and *A. flavirostris* have been reported as vectors of malaria and Bancroftian filariasis and responsible for maintaining holo- to hyper-endemic levels of both diseases (Hii et al., 1985). In Sarawak (Malaysian Borneo), *A. barbirostris*, *A. donaldi*, *A. letifer*, and *A. latens* (formerly *A. leucosphyrus* A; Sallum et al., 2005a), are considered vectors for malaria and Bancroftian filariasis (Chang et al., 1995; Rahman et al., 1997). In Palau Aur (off the Southern State of Johore in peninsular Malaysia 2° 26′ 57°N 104° 31′ 30°E) *A. maculatus* has been incriminated as the vector of *W. bancrofti* (Cheong and Omar, 1965). *A. maculatus* is also the vector of human malaria in peninsular Malaysia. With the decrease in human malaria cases, and with an influx of migrant workers, it is probable that *A. maculatus* may develop into an efficient vector for bancroftian filariasis. This is based on evidence that majority of mosquitoes that pick up mixed malarial and filarial pathogens do not live long enough for the two parasites to reach the infective stage (Muturi et al., 2006). *Culex quinquefasciatus* is the main vector of urban bancroftian filariasis in countries like India (Das et al., 1992), Sri Lanka (Amerasinghe and Munasingha, 1988), and

also Thailand (Triteeraprapab et al., 2000). Recent studies have shown that the local strain (Malaysian) of *C. quinquefasciatus* is susceptible to the Myanmar strain of *W. bancrofti* (Vythilingam et al., 2005b). This species easily invades suitable habitats and once established it is difficult to control them. Due to changing ecological landscape some species may have been displaced and suitable habitats may have been created for others. Thus, more work is needed to study the species composition of the mosquitoes so that the bionomics, ecology, and epidemiological importance of these vectors will be known.

CONTROL OF VECTORS OF SIMIAN MALARIA AND BANCROFTIAN FILARIASIS

It has been accepted that insecticides treated bed nets (IBN) play an important role in the prevention of malaria. In Africa, it has been shown that with the use of treated nets for every 1000 children protected about 5.5 lives can be saved in children below 5 years (Lengeler, 2004). In Asia and Latin America, it has been shown that the clinical episodes of malaria have been reduced in areas where there is low transmission of malaria and where the entomological inoculation rate is below one. Control activities for the control of malaria vectors, be it adults or larvae were always carried out. For adults, indoor residual spraying (IRS) was conducted using DDT at 2/gm^2. With the use of IRS followed by cases detection and treatment, some countries like Singapore managed to eliminate malaria and obtained malaria free status by WHO in 1983 (Goh, 1983). However, sporadic cases do occur from time to time (Ng et al., 2010).

In Malaysia, anti-larval works for malaria control was actively carried out starting during Watson's time in 1921 and continued during the 1950's. Malaya can claim the honor of being one of the earliest countries in the world to have successfully applied the knowledge of the mode of transmission of malaria to its control (Sandosham, 1984). Many environmental modification techniques were applied to larval habitats to control the breeding of the vectors. Subsoil pipes, automatic siphon, environmental modification techniques were some of the methods that were used so that *Anopheles* mosquitoes would not be able to breed (Singh and Tham, 1988). These were permanent measures that were used to reduce the number of breeding sites. Earth drains were also maintained and these drains were regularly sprayed with malaria GD oil and later with Abate 500E. However, in the hilly areas where *A. maculatus* was the main vector, it was difficult to reduce the number of breeding sites.

Currently *A. latens* is the vector of knowlesi malaria in Kapit, Sarawak (Vythilingam et al., 2006) and is also the vector of human malaria in Sarawak (Seng et al., 1999). Thus, existing control activities for *A. latens* should be able to reduce malaria cases. However, the indoor:outdoor biting activity of *A. latens* shows that they are more exophagic. In our study, infected mosquitoes were not obtained from the long house (where native Sarawakians live; Tan et al., 2008). Thus, perhaps people were getting infected either in the forest or in the farms where they work.

In peninsular Malaysia, *A. cracens* does not enter houses to bite (Scanlon and Sandhinand, 1965) and thus the conventional IBN or IRS will not work. These mosquitoes are also early biters and thus people get bitten while outside their homes in the late evenings or while carrying out forest activities or camping outdoors in forested areas.

However, in Vietnam *A. dirus* has been incriminated as vector of knowlesi malaria and is also the vector of human malaria (Nakazawa et al., 2009; Marchand et al., 2011). It has been documented that the use of IBN and treated hammocks have been instrumental for the reduction of malaria cases (Thang et al., 2009). Thus, this could be one reason why there are only a few knowlesi cases in Vietnam. In Malaysia, existing control measures (IRS and IBN) will not be able to control the vectors of knowlesi malaria due to their exophagic and exophilic behavior. Also in areas where knowlesi malaria is occurring people may have lost their immunity since it is malaria free and thus easily prone to the disease. Perhaps cross-species protection from the established malaria parasites prevented the entry of *P. knowlesi* into the human population (Conway, 2007).

Although vector control measures have been instituted for malaria along with case detection and treatment, for filariasis it has been mainly treatment and Mass Drug Administration (MDA) without any special vector control measures. Control measures have not been instituted specifically for filariasis. In areas where *Anopheles* spp. were vectors of filariasis, it was felt that control measures instituted for malaria vectors will also control filariasis vectors. In the same way where the *Aedes* mosquito is responsible for dengue and filariasis, measures taken for dengue control will also benefit filariasis. In areas where *Mansonia* were vectors for filariasis, very little has been done but with development many of the breeding sites have been destroyed.

In Africa where *A. gambiae* is the vector for both diseases it has been observed that with the Diethylcarbamazine (DEC) there has been a reduction of filariasis and this led to the increase in the infection rate of malaria parasites since the longevity of the mosquito increased (Muturi et al., 2006). It has always been a challenge that MDA alone will not be able to eradicate filariasis and using vector control alone some countries, like Solomon Island, has been successful in the elimination of filariasis (Bockarie et al., 2009). In studies carried out in periodic *B. malayi* areas it was shown that with DEC normal treatment regimen or single dose treatment was able to reduce the prevalence of microfilariae (Hakim et al., 1995). However, the infective bites per month of the vector *A. donaldi* was higher after treatment, though not significantly different before and after treatment (Vythilingam et al., 1996). This shows that perhaps not all people received treatment due to inaccessibility of terrain. Thus, it would be ideal to include MDA and vector control where feasible in a control program.

OPERATIONAL ISSUES REGARDING ELIMINATION OF MALARIA AND FILARIASIS

World Health Organization has now embarked on a global scale to eliminate filariasis and malaria. Thus, countries are now gearing up the activities to eliminate the two diseases. In Southeast Asia, it has been shown that knowlesi malaria is zoonotic, potentially fatal, and widely distributed (Cox-Singh and Singh, 2008). Although in the 1960's it was thought that the first natural infection

was a rare incidence, it was postulated that when human malaria cases reach a low level, the possibility of human population being infected with simian malaria parasites could be significant (Chin et al., 1968). It has also been argued that there is every possibility that other simian malaria like *P. cynomolgi* and *P. inui* can also be transmitted to humans (Baird, 2009). When malaria is eliminated from a place, there lurks danger for simian malaria to be transmitted to humans. This is currently occurring in parts of peninsular Malaysia (Vythilingam et al., 2008).

In most areas in peninsular Malaysia, knowlesi malaria is occurring in malaria free areas. This could be due to two reasons: people have lost their immunity and secondly it is not possible to treat the animal host and thus a pool of parasites is always available for transmission to human host as long as the suitable *Anopheles* vector is present.

Wuchereria bancrofti is responsible for 90% of all human lymphatic filarial infection. India alone accounts for 45.5 million cases (WHO, 2010). In Southeast Asia, Indonesia has about 28.5 million cases (Rusmartini and Yuliantina, 2008) and has both *W. bancrofti* and Brugian filariasis. Filariasis has always been a neglected disease of the poor population with people getting infected during childhood and showing the symptoms during later stages in life (Witt and Ottesen, 2001). Cheap international travel and cross border migration allows the parasites to be easily brought into the countries, and with suitable vectors, it is possible for transmission to take place. Studies have also shown that with MDA there was a reduction in the prevalence of filariasis. However, when MDA was stopped the cases slowly started to increase due to the few that did not undergo treatment and harbored the microfilaria. Besides, since vector control was not carried out, it led to the increase (Cartel et al., 1992; Esterre et al., 2001; Sunish et al., 2002). Thus, multiple rounds of MDA in many countries did not achieve the predicted interruption in transmission since vector control has not been implemented (Bockarie et al., 2009).

As for the vectors there was also evidence where the same mosquito was able to transmit both filariasis and malaria pathogens (Manguin et al., 2010). Studies have shown that co-infection with both parasites was possible and sporozoites were found in higher numbers in *A. gambiae* when third stage bancroftian larvae were present but the survival rate of the mosquitoes were lower in Kenya (Muturi et al., 2006). Other studies in Papua New Guinea have shown that less malaria parasites will develop in mosquitoes with filarial worms (Aliota et al., 2011), thus, there may be a possibility that more cases of malaria will occur once filariasis is eliminated. However, studies are needed in the Southeast Asia to confirm if the vectors will exhibit similar modes of infection as shown in Africa or Papua New Guinea.

Predicting an outbreak is always difficult. Control and surveillance is always carried out after the outbreak and when many lives are lost (Petney et al., 2009). Simian malaria in humans is life threatening and mortality has been reported (Cox-Singh et al., 2008; Barber et al., 2011). Thus, in the case of malaria at least after a few lives have been lost, public health alert will be instituted and the outbreak will be brought under control. It is very difficult to maintain surveillance system once it has been declared that the disease has been eliminated. It needs a lot of commitment from government bodies to have a proper surveillance after elimination. While in the case of filariasis, the disease does not cause mortality and thus people who get infected will only know when they have reached the incurable stage. By this time they would have passed the infection to mosquitoes and silent transmission will be on-going.

When Plasmodium parasites are host specific Anophelism without malaria is possible, but with zoonosis, a pool of parasites will always be available and the elimination of the parasite is not an easy task. With deforestation and changes in the environment it is possible for new vectors to displace the established vectors. One good example is *A. maculatus* which was the predominant mosquito obtained in malaria vector surveys in the past in peninsular Malaysia (Sandosham and Thomas, 1983; Chiang et al., 1991; Vythilingam et al., 1993, 1995; Rahman et al., 1997). However, a survey carried out after the occurrence of knowlesi malaria shows that *A. cracens* is the predominant mosquito and was never reported previously from Pahang (Vythilingam et al., 2008). How far *A. cracens* has spread within peninsular Malaysia is not known. It may be possible that *A. cracens* followed the long tailed macaques from the forest and have now colonized the villages. When these changes occur, the existing control measures may not be sufficient to reduce vector density due to exophagic and exophilic behavior of the vectors. When such changes take place, it is always important to study the mosquito fauna in that area. Changes in land use can sometimes help in the reduction of malaria. One good example is in Thailand where deforestation in the north-eastern part of the country led to a reduction of malaria as *A. dirus* population was reduced since this mosquito needs shaded environment for survival and reproduction (Petney et al., 2009).

In Sabah, Malaysian Borneo, studies carried out in the Kinabatangan area showed that *A. balabacensis* was the main vector for malaria (Hii et al., 1985). However, after two decades, studies carried out in that same area found *A. donaldi* to be the predominant mosquito and was found positive for sporozoites (Vythilingam et al., 2005a). *A. donaldi* is not an efficient vector like *A. balabacensis*. Currently what has been observed is that in areas where human malaria cases have been reduced, knowlesi malaria transmission is occurring in the population. In Kudat, there was a major reduction in slide positivity rate and parasite rate from 1990 to 1991 due to the establishment of primary health care volunteers (Hii et al., 1996). Kudat is a coastal rural farming area with minimal regrowth of forest and in 2009, 137 (87%) cases of *P. knowlesi* was diagnosed in that region and 24 (14%) occurred in children (Barber et al., 2011). Extensive studies are needed to know the vectors involved so that control measures can be instituted where possible. Here is an example of how reduction of human malaria and changes in land use has the potential to facilitate the transmission of knowlesi malaria to humans. Thus, understanding the changes is critical for a control program and also the information on vectorial capacity of the mosquitoes is important and should be included as a criteria in malaria elimination.

Vectors too can change from being primarily zoophilic to primarily anthropophilic. A good example is *A. sinensis* in peninsular Malaysia which was primarily zoophilic and therefore has not

been considered a vector (Reid, 1968). Currently in Singapore *A. sinensis* has been the predominant species attracted to humans in areas where malaria cases were reported (Ng et al., 2010). Due to the changes in the behavior perhaps *A. sinensis* could be a vector for malaria. Changes in temperature also affect the lifecycle of the mosquito and the parasite. It is known that an increase in temperature shortens the lifecycle of the mosquito and also the development of the parasite within it (Gage et al., 2008). Thus, currently with an increase in temperature compared to five decades ago, perhaps a smaller number of mosquitoes may be more efficient in disseminating the parasites since time taken for the development of the infective stage is reduced.

Unlike the malaria parasite, the filarial parasite does not multiply in the body of the mosquito. It has been postulated from a study in Rangoon that a person needs to be bitten 15,500 times by infective mosquitoes before becoming positive (Hairston and De Meillon, 1968). This is for *W. bancrofti* transmitted by *C. quinquefasciatus*. However, 60 million cases are reported in Asia and Southeast Asia (WHO, 2010) and majority being *W. bancrofti*. Extensive vector control will help to reduce the infection rate and should be a priority in countries with large number of cases.

In the filariasis elimination program it is important that the surveys are properly carried out and that coverage of the population should be adequate. Since vector control activities have not been carried out in most countries, any person harboring the parasite or an immigrant with parasites entering the area can lead to silent transmission as infection will not be known for many years to come. The surveillance for this disease would be forgotten as it is more of psychosocial problem (Ottesen et al., 1997; Dreyer et al., 2000).

In Malaysia, currently more than 70% of the filariasis cases are imported into the country (unpublished document). *C. quinquefasciatus* is susceptible to the *W. bancrofti* (Vythilingam et al., 2005b). The migrant workers live both in the urban and rural areas such as plantations and since vectors are present, the dissemination of the parasite to the local population is possible. This is also of concern in Thailand where it has been reported that the prevalence of patent *W. bancrofti* infection in Myanmar immigrants has prompted concern in the public health community that the potential now exists for a re-emergence of Bancroftian filariasis in Thailand (Triteeraprapab et al., 2000).

CHALLENGES FACING SOUTHEAST ASIA AND THE RISK OF THESE TWO DISEASES

Naturally acquired cases of *P. knowlesi* have been reported from travelers visiting this region as shown in **Table 1**. These people have brought back the parasites to Australia, New Zealand, Europe, and USA from Southeast Asia (Kantele et al., 2008; Mali et al., 2008; Bronner et al., 2009; Figtree et al., 2010; Tang et al., 2010; Berry et al., 2011; Hoosen and Shaw, 2011).

Thus, it has still not been established if human to human transmission is taking place. However, it is very clear that cases of knowlesi malaria can no longer be considered single episodic cases but represent true health care emergency (Sabbatani et al., 2011). This goes to show that travel to Southeast Asia can help to spread the parasites to other countries. For example India has common border with Bangladesh, Bhutan, Myanmar, and Nepal. Of these Myanmar is endemic for knowlesi malaria (Jiang et al., 2010) and the *leucosphyrus* group of mosquitoes are present in India. Thus there will be ample occasions for the parasite to be brought into India (Subbarao, 2011). Many countries may still be unaware or do not have molecular tools to detect *P. knowlesi* and thus this parasite may be silently spreading to the region and beyond. It is important for visitors to know about the dangers of getting infected when they are out in the forested areas of Southeast Asia. The most recent report includes cases from the south western border of Thailand with Myanmar (Sermwittayawong et al., 2012).

Table 1 | Distribution of knowlesi malaria cases acquired from Southeast Asia by foreign visitors

No	Country of origin	Place where contracted the disease	Reference
1	New Zealand	Sabah, Sarawak (Malaysian Borneo)	Hoosen and Shaw (2011)
2	USA	Palawan, Philippines	Mali et al. (2008)
3	Finland	North west of peninsular Malaysia	Kantele et al. (2008)
4	Sweden	Bario Highland, Sarawak, Malaysia	Bronner et al. (2009)
5	Spain	Forest areas of Southeast Asia	Tang et al. (2010)
6	France	Thailand	Berry et al. (2011)
7	Australia	Kalimantan, Indonesian Borneo	Figtree et al. (2010)

As for Bancroftian filariasis, immigrant workers from endemic countries will be able to bring the disease as they are not screened at the point of entry. Perhaps now that elimination of filariasis is ongoing it would be prudent to have some surveillance mechanism in place.

CONCLUSION

Although countries in the region are gearing toward elimination of malaria and filariasis, emphasis should include a combination of tools to control the disease and the vectors. It should also be noted that the current control strategies for vectors may not be applicable or appropriate due to changes in vectors and their behavior or changes in parasites. More studies should be carried out in the region to establish if human to human transmission of knowlesi malaria is occurring. Vectors of simian malaria throughout the region should be identified so that appropriate control measures and new strategies can be instituted where possible. Migrant workers need to be screened for filariasis and it is important to establish if silent transmission is occurring in areas where these migrant population live in large numbers. Thus, vector studies should be extensive and focused so as to provide evidence based solutions to these emerging and complex vector borne diseases.

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Mosquitoes as potential bridge vectors of malaria parasites from non-human primates to humans

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Malaria is caused by *Plasmodium* parasites which are transmitted by mosquitoes. Until recently, human malaria was considered to be caused by human-specific *Plasmodium* species. Studies on *Plasmodium* parasites in non-human primates (NHPs), however, have identified parasite species in gorillas and chimpanzees that are closely related to human *Plasmodium* species. Moreover, *P. knowlesi*, long known as a parasite of monkeys, frequently infects humans. The requirements for such a cross-species exchange and especially the role of mosquitoes in this process are discussed, as the latter may act as bridge vectors of *Plasmodium* species between different primates. Little is known about the mosquito species that would bite both humans and NHPs and if so, whether humans and NHPs share the same *Plasmodium* vectors. To understand the vector-host interactions that can lead to an increased *Plasmodium* transmission between species, studies are required that reveal the nature of these interactions. Studying the potential role of NHPs as a *Plasmodium* reservoir for humans will contribute to the ongoing efforts of human malaria elimination, and will help to focus on critical areas that should be considered in achieving this goal.

Keywords: apes, cross-species transmission, host preference, mosquito behavior, *Plasmodium*

NON-HUMAN PRIMATE *PLASMODIUM* PREVALENCE

Non-human primates (NHPs) often serve as reservoir of pathogens of human diseases. The yellow fever virus (Ellis and Barrett, 2008), Chikungunya virus (McCrae et al., 1971; Labadie et al., 2010), and the protozoa *Giardia lamblia* that cause diarrhea (Kowalewski et al., 2011), are all examples of pathogens that originate from NHPs and are infectious to humans. Malaria is caused by parasites of the genus *Plasmodium* that can infect a wide range of vertebrate hosts from reptiles, birds to mammals. Only five *Plasmodium* species are known to cause malaria in humans: *Plasmodium falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*. Of these five species, *P. falciparum* is most common and most lethal (WHO, 2011).

Plasmodium parasites are, however, also found in other primates than humans. *Plasmodium reichenowi* was the first *Plasmodium* species identified in NHPs, isolated from an infected chimpanzee (Coatney et al., 1971; Rayner et al., 2011). Other NHP *Plasmodium* species have been identified (Coatney et al., 1971; Rayner et al., 2011) and their identity can be studied in more detail since the development of molecular tools. A recent study in which mitochondrial, apicoplast, and nuclear gene sequences from 2700 fecal samples from wild-living African apes have been analyzed, showed that *Plasmodium* species are widely distributed in NHPs and with high prevalence (Liu et al., 2010). The detected parasites could be classified into six discrete major clades, all belonging to the subgenus, termed *Laverania*, which discriminates them from more divergent *Plasmodium* species (Liu et al., 2010; Rayner et al., 2011). Interestingly, some of these *Laverania* clades were only associated with chimpanzee (*Pan troglodytes*) samples and others only with samples from western lowland gorilla's (*Gorilla gorilla*).

Although infections with *Plasmodium* parasites appear to be host-specific, chimpanzees, and gorillas were nevertheless found to be infected with mitochondrial DNA similar to that of human *P. vivax* (Liu et al., 2010). Chimpanzees were also found to be infected with *P. ovale* and *P. malariae*, which may have contributed to the perseverance of these human malaria parasites in Africa (Duval et al., 2009, 2010; Hayakawa et al., 2009; Krief et al., 2010; Duval and Ariey, 2012). Several studies performed between 1940 and 1956 showed that these species can be transmitted from apes to humans (Rodhain and Dellaert, 1955a,b; Garnham et al., 1956; Rayner et al., 2011). *P. falciparum* has not been found in wild NHPs but has been detected in captive chimpanzees and bonobos (Liu et al., 2010; Rayner et al., 2011). Interestingly, mitochondrial sequence analysis has revealed that western lowland gorillas probably served as the source of human *P. falciparum* (Liu et al., 2010) and *P. falciparum* diverged between 112,000 and 1,036,000 years ago (Baron et al., 2011).

Little is known about the ability of NHP malaria parasites to infect humans, except for the fifth human *Plasmodium* parasite, *P. knowlesi*, which was long known only as a parasite of monkeys. The parasite was extensively studied for basic immunological, chemotherapeutic, and biological relationships between malaria parasites and their primate hosts (Collins, 2012). A study in 2004 in Malaysian Borneo revealed that 58% of blood samples from people that had been diagnosed with *P. malariae*, were actually infected with *P. knowlesi* after detection by PCR (Singh et al., 2004). These findings have led people to consider *P. knowlesi* to be the fifth human malaria parasite (White, 2008; Collins, 2012). This example indicates that care should be taken when screening for *Plasmodium* by standard blood smears or rapid

diagnostic tests, which will not reveal if a person is infected with ape *Plasmodium*.

In recent years the diversity and origin of malaria parasites has been studied in detail using advanced molecular techniques (Krief et al., 2010; Liu et al., 2010; Baron et al., 2011; Prugnolle et al., 2011). In several reviews on this topic the potential risk of a parasite exchange between NHPs and humans has been identified (Kevin, 2009; Rayner et al., 2011; Duval and Ariey, 2012). The authors of these studies suggest that: (a) Humans living near wild-ape communities should be tested for zoonotic infections using molecular approaches capable of differentiating between human and zoonotic parasites, (b) Wild NHPs should be screened for *P. vivax*, *P. malariae*, and *P. ovale*, because the evidence for cross-transmission is the strongest for these *Plasmodium* species, and (c). Mosquito species should be identified that could transmit *Plasmodium* parasites between NHPs and humans. Techniques for screening for *Plasmodium* parasites in humans and NHPs have advanced in recent years and have been applied in some of the studies on NHP *Plasmodium* mentioned above. The identification of mosquito species that are likely to transmit *Plasmodium* parasites between NHPs and humans has received little attention and will be discussed below.

THE ROLE OF MOSQUITOES

Mosquitoes play a key role in the transmission of *Plasmodium* parasites between humans. Mosquito species characteristics will therefore influence the *Plasmodium* transmission between humans and NHPs. Transmission studies with malaria vectors have mainly focused on those mosquito species that transmit *Plasmodium* between humans. Mosquito species that are likely to facilitate a cross-species exchange of *Plasmodium* have occasionally been studied for their capability of transmitting *P. knowlesi* in Asia (Collins, 2012). Such studies have not been done in Africa, and consequently knowledge about the role of African mosquitoes as *Plasmodium* bridge vectors is lacking (**Figure 1**).

MOSQUITO SUSCEPTIBILITY

About 30 of the approximately 450 species of the insect genus *Anopheles* are vectors of human malaria (White, 1982). The susceptibility of a mosquito to malaria parasites has a great influence on the effectiveness of a mosquito species as a malaria vector. Little is known about the susceptibility of mosquitoes to the different species of NHP *Plasmodia*, mainly because these *Plasmodium* parasites are not available as a laboratory culture. Experiments with a chimpanzee infected with *P. reichenowi* showed that the malaria mosquito species *Anopheles quadrimaculatus*, *A. stephensi*, *A. maculatus*, *A. dirus*, and *A. culicifacies* could be infected with this parasite species. However, *A. gambiae* s.s. and *A. albimanus* were refractory to the infection (Blacklock and Adler, 1922; Collins et al., 1986), which suggests that not all vectors of *P. falciparum* can equally transmit NHP *Plasmodium*.

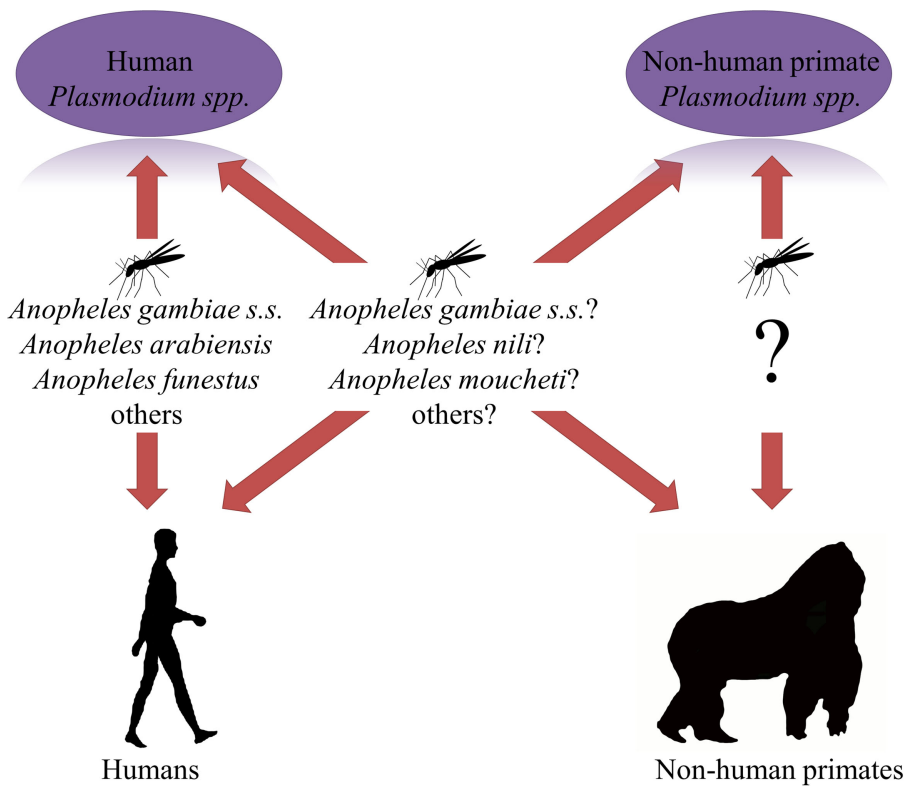

FIGURE 1 | Interactions between mosquitoes, their hosts, and human and non-human primate *Plasmodium species* in Africa. Question marks indicate unidentified vectors.

Studies that nowadays would be considered unethical have shown that mosquitoes infected with NHP *Plasmodia* can transfer the parasites to humans and cause illness (Coatney et al., 1961; Schmidt et al., 1961; Bennett and Warren, 1965; Contacos et al., 1970). Current molecular tools should make it possible to identify mosquitoes in the field that are infected with NHP *Plasmodium* parasites. Detection of parasites in wild apes and humans living near wild-ape communities will support the potential of these mosquitoes as a bridge vector.

MOSQUITO HABITAT

Anopheles gambiae s.s. and *A. funestus* are important African vectors of human malaria partly because they prefer semi-open and open areas and these are the areas that are often more populated by humans. In forested areas other mosquito species become important as malaria vectors for humans. *A. nili*, for example can breed in shaded streams and plays a role in the transmission of malaria in localized rainforest areas (Carnevale et al., 1992; Guerra et al., 2006). *A. moucheti* is considered a forest species and is often reported to be the main vector of human malaria in rainforest areas of Africa (Ollomo et al., 1997; Fontenille et al., 2003; Guerra et al., 2006; Antonio-Nkondjio et al., 2008).

Although studies on mosquitoes that transmit human malaria in rainforest areas are scarce, *A. nili*, *A. moucheti*, and other forest *Anopheles* spp. are possible candidates for malaria parasite transmission from NHPs to humans or vice versa (**Figure 1**). Deforestation, mining, and agriculture may lead to more open areas, thereby giving other mosquito species, like *A. gambiae* s.s. and *A. funestus*, which are known to be effective vectors of human malaria (Gillies and Coetzee, 1987), the opportunity to populate

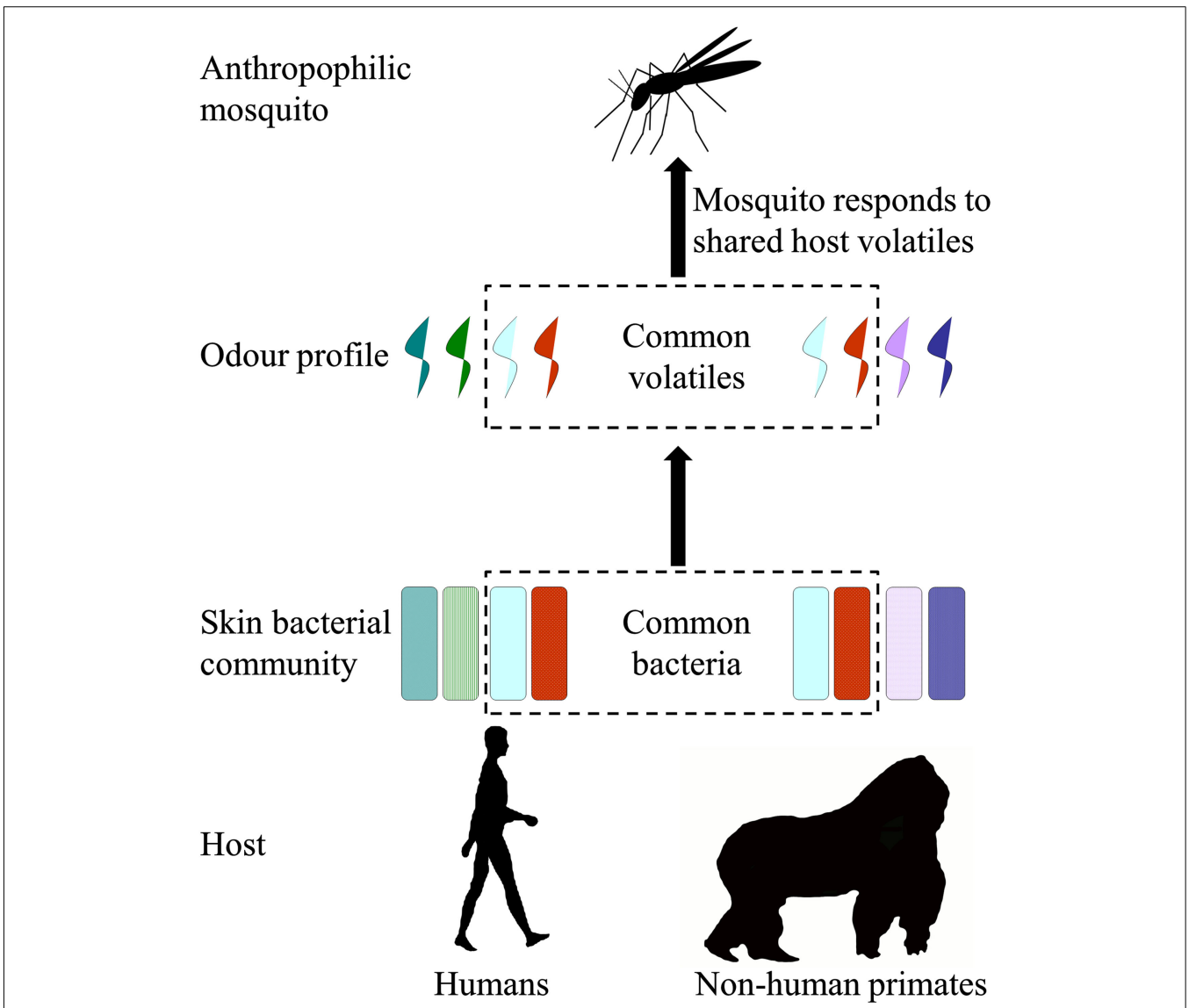

FIGURE 2 | Mosquito-host interactions and the possible response of anthropophilic mosquitoes to volatiles shared by humans and non-human primates.

these regions. These human activities will also lead to closer contact between NHPs and human beings, thereby increasing the risk of disease transmission.

Identification of mosquito species that act as malaria vectors in rainforest areas is essential when trying to eliminate or eradicate human malaria, because these species may possibly transfer *Plasmodium* from NHPs to humans or vice versa. In addition to this, the prevalence of NHP malaria parasites in forest mosquitoes and humans living in these areas should be investigated. This will help to focus on critical areas that should receive additional interventions when trying to eliminate or eradicate malaria.

HOST PREFERENCE

Host selection by mosquitoes drives the transmission of *Plasmodium* parasites between humans, and will play a key role in the frequency and efficiency of an exchange of *Plasmodium* parasite between humans and NHPs. Yet, next to nothing is currently known about the mosquito species that could facilitate such an exchange (**Figure 1**; Rayner et al., 2011).

Identification of mosquito blood meals by multiplex PCR (Garros et al., 2011) may lead to identification of mosquito species that feed on both humans and NHPs. Collected mosquitoes may also be used to determine *Plasmodium* infections in mosquitoes, but the number of mosquitoes needed to confirm such infections is high given the relatively low natural infection rate of human *Plasmodium* infections in mosquitoes (Marchand et al., 2011). Blood-fed mosquitoes can be caught nearby human dwellings with resting boxes, clay pots, or pitfall traps (Service, 1993; Odiere et al., 2007; Qiu et al., 2007). However, the collection of blood-fed mosquitoes in areas where NHPs are naturally abundant may require specific arrangements.

To find their blood host, mosquitoes use physical cues like heat, moisture, vision in addition to chemical cues like carbon dioxide and body odor (Takken and Knols, 1999). Mosquito species with a certain host preference use body odor components to distinguish between different host species (Costantini et al., 1998; Takken and Knols, 1999; Pates et al., 2001). *A. gambiae* s.s. is one of the most important and best studied vectors of human malaria. It is a highly efficient vector because of its restricted, anthropophilic, host range, and preference to feed indoors (Costantini et al., 1998; Takken and Knols, 1999). Interestingly, its sister species, *A. quadriannulatus* and *A. arabiensis*, have a wider host range that is more zoophilic (White et al., 1980; Hunt et al., 1998; Torr et al., 2008) or opportunistic (Costantini et al., 1996, 1998), respectively. Volatiles released by the human skin provide essential cues that guide *A. gambiae* s.s. to its human host (Smallegange and Takken, 2010). Recently, it was found that the human skin microbiota plays a critically important role in producing these volatiles and thereby in mediating mosquito-host interactions (**Figure 2**; Verhulst et al., 2009, 2010, 2011).

The volatiles that are released from the human skin have been studied intensively and this resulted in the identification of more than 350 compounds (Bernier et al., 1999, 2000; Curran et al., 2005; Penn et al., 2007; Gallagher et al., 2008). Less is known about the volatiles released by NHPs. Skin glands and their associated bacteria determine which volatiles are produced and therefore play an important role in the attractiveness of human sweat to mosquitoes

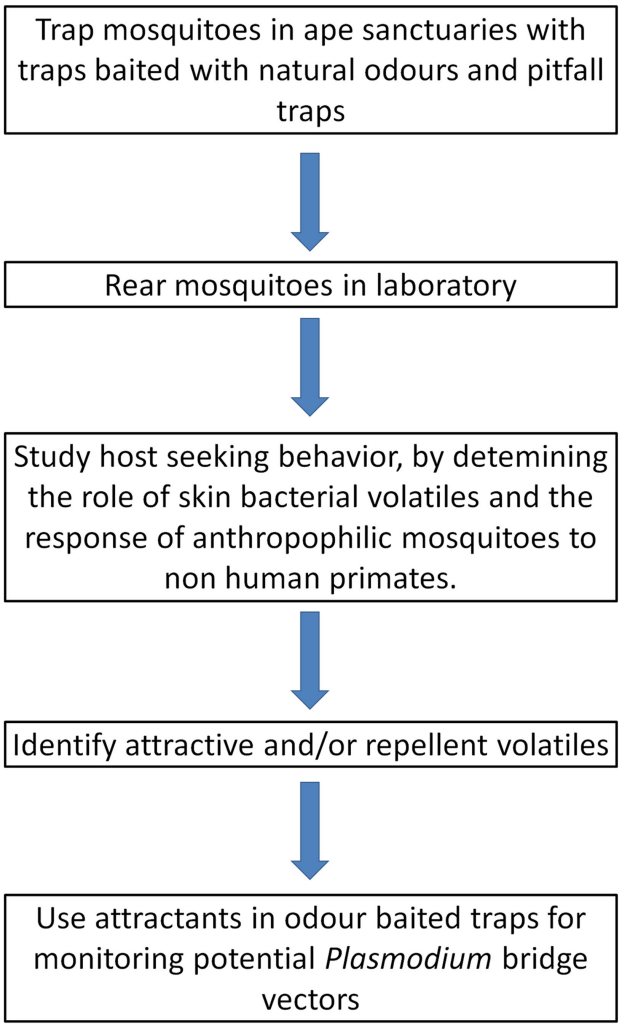

FIGURE 3 | Study design for the identification of mosquito species that may act as bridge vectors of *Plasmodium*.

(Smallegange et al., 2011). Mosquitoes also vary in their attraction to different parts of the human body (Dekker et al., 1998). The type of skin glands and associated bacteria determine the difference between, for example, foot odor (eccrine glands) and axillary odor (apocrine glands). In most mammals, eccrine glands are limited to the friction surfaces of the hands, feet, and tail. By contrast, the eccrine glands of great apes and humans are distributed over the general body surface (Smallegange et al., 2011). Also the extensive aggregation of both apocrine and eccrine glands in the axillae is only found in humans and great apes (Folk and Semken, 1991; Smallegange et al., 2011).

While the distribution of sweat glands is relatively similar between humans and great apes, little is known about the body odor profiles of great apes and other NHPs and how these compare to human body odor. It also remains to be investigated if the similarities or differences between ape and human odor are mediated by their skin microbiota (Smallegange et al., 2011). Mosquitoes caught near NHPs in sanctuaries in Africa may be used in the

laboratory to understand how mosquitoes are attracted to NHPs. It is not known if anthropophilic mosquitoes like *A. gambiae* s.s. are also attracted to odorants from NHPs and thereby may bite both humans and NHPs (**Figure 2**). Studying the bacteria that play a role in the attractiveness of humans and NHPs to mosquitoes may lead to the identification of volatiles that specifically attract or repel mosquitoes that may form a bridge between humans and NHPs. Next, these volatiles may be used to trap mosquitoes near wild populations of NHPs to identify potential vectors and the malaria parasites they carry (**Figure 3**). Studying their host-seeking behavior in the laboratory will also reveal which mosquito species would readily bite both humans and NHPs and how restricted this host preference is.

CONCLUSION

In 2007 the Bill and Melinda Gates Foundation, followed by the World Health Organization (WHO) and the Roll Back Malaria (RBM) Partnership declared that the paradigm of malaria control and elimination has been extended to encompass an ultimate goal of malaria eradication (Roberts and Enserink, 2007; Roll Back Malaria Partnership, 2008; Alonso et al., 2011). Since that declaration the discussion on the probability of malaria eradication has intensified (Roberts and Enserink, 2007; Greenwood, 2008; Ferguson et al., 2010; Kappe et al., 2010; Alonso et al., 2011; The malERA Consultative Group on Vector Control, 2011). However, in 2010 there were still an estimated 216 million human cases of malaria and 655,000 deaths and the consequences of malaria may decrease gross domestic product by as much as 1.3% (WHO, 2011).

The possibility that NHPs act as a reservoir for human *Plasmodium* or are a source of NHP *Plasmodium* that may affect human health should be investigated before malaria eradication is considered. Current vector control efforts are focused on human habitats, mainly because the most important vector of human malaria *A. gambiae* s.s. is highly anthropophilic and found only around human settlements (Costantini et al., 1998; Takken and Knols, 1999; Pates et al., 2001). In the process of malaria elimination in any country, emphasis is often laid on monitoring parasite prevalence in humans. The case of *P. knowlesi* has shown that conventional methods of screening for *Plasmodium* parasites will not reveal human infections with NHP *Plasmodia* and therefore humans living near wild-ape communities will need more detailed testing to detect these parasites (Kevin, 2009; Rayner et al., 2011).

The significance of NHPs as a potential source for human infection will largely depend on the vector species that transmit *Plasmodium* between apes, and whether their behavior facilitates transmission to humans. By understanding how mosquito species are attracted to NHPs, and using this knowledge to identify the vectors that may transmit *Plasmodium* between apes and humans, we can understand the zoonotic potential of NHP *Plasmodium* parasites. Identification of the bacterial volatiles that specifically attract mosquitoes that may form a bridge between humans and NHPs may lead to the development of selective odor-baited mosquito traps (Mukabana et al., 2012). These traps can be used for monitoring or mass trapping of mosquito species that facilitate such a cross-species exchange of *Plasmodium* parasites (**Figure 3**). Detailed screening for *Plasmodium* parasites and trapping of vectors in areas where humans and NHPs coexist will help to focus on critical areas that should receive additional interventions when attempting to eliminate or eradicate malaria.

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Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination

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Existing malaria vector control measures such as Long Lasting Insecticidal Nets (LLIN) and Indoor Residual Spraying (IRS) combined with Artemisinin Based Combination Therapy (ACT) drugs have significantly reduced the malaria burden in many parts of Africa (Battarai et al., 2007; Sharp et al., 2007a; Ceesay et al., 2008; O'Meara et al., 2008; WHO, 2009; Chizema-Kawesha et al., 2010; Ngomane and de Jager, 2012). The benefit of these interventions extends beyond the personal protection of the households that use them to protect entire communities by reducing either the infectiousness of blood stage parasites in human populations (Killeen et al., 2006a), or the abundance and survival of mosquito vectors (Killeen et al., 2007). It has been shown both theoretically (Killeen and Smith, 2007; Killeen et al., 2007) and in the course of operational control (Hawley et al., 2003; Klinkenberg et al., 2010) that significant community-wide reductions in transmission can be obtained even when intervention coverage levels are modest (35–75%). However, it is unlikely that these packages of interventions of their own will be sufficient to achieve malaria elimination in the most endemic settings where transmission rates are extremely high (Gillies and Smith, 1960; White, 1969; Oyewole and Awolola, 2006; Bayoh et al., 2010; Van Bortel et al., 2010; Bugoro et al., 2011a; Reddy et al., 2011; Russell et al., 2011). Even should the considerable financial, logistic, and behavioral obstacles that currently limit attainment of 100% coverage be overcome (Vanden et al., 2010; Larson et al., 2012), the combined use of effective anti-malarial drugs and these vector control interventions are not predicted to be sufficient for elimination in these settings (Killeen et al., 2000; Griffin et al., 2010) Because they do not cover the full spectrum of all locations where mosquito exposure occurs, and even if only a small percentage of mosquitoes remain and bite outside, their existence could be enough to prevent the transition from very low to zero transmission.

The World Health Organization (WHO) defines malaria elimination as meaning the permanent reduction "to zero incidence of locally contracted cases, although imported cases will continue to occur and continued interventions measures are required" (WHO, 2008b). Achieving this goal will require full understanding of where and when persons are most exposed to the bites of mosquito vectors in order to target interventions where they can achieve maximum impact. While elimination is possible in some settings with low malaria transmission intensity (WHO, 2009; Griffin et al., 2010), and where the dominant vectors exhibit the stereotypical behaviors of biting indoors and late at night where they can be targeted by LLIN and/or IRS (Mabaso et al., 2004; Sharp et al., 2007b; John et al., 2009; WHO, 2009), it is unlikely that these methods will be sufficient to push prevalence below the WHO-defined pre-elimination threshold (<1 case/1000 population/year) in areas of high transmission (Molineaux and Gramiccia, 1980; Kleinschmeidt et al., 2009; Russell et al., 2010) and where the majority of human exposure to transmitting mosquitoes occurs outside human dwellings to which most current interventions are restricted (Taylor, 1975; Pates and Curtis, 2005; Tirados et al., 2005; Oyewole and Awolola, 2006; Geissbühler et al., 2007; Griffin et al., 2010; Van Bortel et al., 2010; Bugoro et al., 2011a; Yohannes and Boelee, 2012). To date, probably the most comprehensive attempt to achieve local elimination within an endemic region of Africa was made in the Garki region of northern Nigeria in the 1970's (Molineaux and Gramiccia, 1980; WHO, 2008b). Before initiating this campaign, malaria transmission within this region was extremely high (example as indexed by an estimated annual entomologic inoculation rate, EIR of 20–120 infectious mosquito bites per person per year). During the elimination program which ran from 1972 to 1973, near complete coverage of all households (97–99%) with propoxur-based IRS combined with mass drug administration of the anti-malarials sulfalene and pyrimethamine (73–92% coverage) was attained throughout the study area. Although these intense efforts led to a drastic reduction in malaria prevalence within the region from 70 to 1%, the threshold for local elimination was not even approached (Molineaux and Gramiccia, 1980). A critical factor in the failure of elimination was incomplete suppression of vector populations due to the existence of low level outdoor-feeding (exophagy) and resting (exophily) behaviors within a small proportion of locally important vector populations (Molineaux and Gramiccia, 1980). This small proportion of mosquitoes with atypical behaviors was sufficient to prevent elimination even across a short period of time in which the potential for insecticide resistance was not recorded. Furthermore, by preventing the rapid achievement of elimination, the existence of these vectors may enhance the likelihood and spread of Insecticide Resistance by necessitating the continued application of high dose formulations over longer periods of time.

While historically transmission in much of Africa has been dominated by vector species that primarily feed and rest indoors where they can be efficiently targeted with domestic insecticides (Gillies and DeMeillon, 1968; White, 1974; Gillies and Coetzee, 1987), there is growing evidence from across the continent that the widespread use of LLINs and IRS is driving vector species composition toward those with more flexible behaviors (Braimah et al., 2005; Pates and Curtis, 2005; Tirados et al., 2005; Antonio-Nkondjio et al., 2006; Oyewole and Awolola, 2006; Geissbühler et al., 2007; Bayoh et al., 2010; Reddy

et al., 2011; Russell et al., 2011). For instance *Anopheles gambiense sensu stricto* has been historically viewed as the most significant vector of malaria in Africa (Gillies and DeMeillon, 1968; White, 1974; Gillies and Coetzee, 1987; Kiszewski et al., 2004). However in the wake of the widespread deployment of domestic insecticidal interventions, this species is in significant decline in many areas; with the majority of remaining transmission now being dominated by *An. arabiensis* (Braimah et al., 2005; Tirados et al., 2005; Bayoh et al., 2010; Russell et al., 2011); a closely related sibling species that can exhibit much more flexible behaviors including biting people and resting outdoors, and switching their biting between humans and common domestic animals such as cattle (Gillies and DeMeillon, 1968; White, 1974; Gillies and Coetzee, 1987; Tirados et al., 2005).

Even low levels of exophagy, exophily, or zoophagy (**Table 1**) may substantially attenuate the impact of LLIN and IRS because this allows mosquitoes to obtain blood while avoiding fatal contact with insecticides (Smith and Gillies, 1960; Taylor, 1975; Molineaux and Gramiccia, 1980; Pates and Curtis, 2005; Geissbühler et al., 2007; Killeen and Smith, 2007; Govella et al., 2010; Bugoro et al., 2011a). Furthermore, the deployment of insecticides inside houses is likely to place strong selection on even highly endophilic species to switch their behaviors, which could not only prevent the achievement of elimination but undermine the continued effectiveness of these interventions (White, 1974; Pates and Curtis, 2005; Van Bortel et al., 2010; Bugoro et al., 2011a). For example in Bioko Island, Equatorial Guinea, *An. gambiae s.s.* were historically documented to feed almost entirely indoors (Molina et al., 1996). However, following initiation of mass coverage campaigns with indoor-insecticide based interventions, the behavior of this vector species has switched to an almost 50:50 split between indoor and outdoor biting (Reddy et al., 2011).

With the shift in policy from targeted to universal distribution of LLINs to each sleeping space in many parts of Africa (Teklehaimanot et al., 2007; WHO, 2008a; Kilian et al., 2010), it has been increasingly observed that mosquitoes can re-adjust their biting activity so that much of it occurs outdoors and before bed time across different settings (Rubio-Palis and Curtis, 1992; Trung et al., 2005; Bayoh et al., 2010; Van Bortel et al., 2010; Bugoro et al., 2011a,b; Reddy et al., 2011; Russell et al., 2011). This is due to the fact that indoor biting species (Smith and Gillies, 1960; Gillies and Furlong, 1964; Gillies and DeMeillon, 1968; Pates and Curtis, 2005; Killeen et al., 2006b) and subpopulations (Coluzzi et al., 1979; Molineaux and Gramiccia, 1980; Bendesky and Bargmann, 2011) will be more affected, leaving predominantly the outdoor-feeding, "early biting" residual populations and individuals (Gillies and Smith, 1960; Taylor, 1975; Bayoh et al., 2010; Bugoro et al., 2011a,b; Russell et al., 2011) to maintain residual transmission. Evidence from the previous Global Malaria Elimination campaign (1950s) suggests this is not only a recent phenomenon. Following the widespread implementation of IRS in the South Pare Region of Tanzania (1955–1959), the highly endophilic vector *An. funestus* disappeared leaving only an *An. gambiae s.l.* population which exhibited extremely exophilic behavior (Smith and Gillies, 1960). Similarly, *An. funestus* was also replaced by the highly zoophagic and exophilic species *An. rivulorum* and/or *An. parensis* on at least three distinct occasions following the implementation of IRS in South Africa, Kenya, and Tanzania (Gillies and Smith, 1960; Gillies and Furlong, 1964). Note that not all of the mosquito vector behavioral shifts mentioned above may necessarily hinder malaria control. For example, while a shift in vector behavior to biting people outside or before bedtime would clearly allow them to evade control, the impacts of others such as increased zoophagy are not clear cut. For example, while shifting their behavior from biting people to livestock would allow vectors to avoid insecticides, but it would concurrently prevent them from becoming infected or transmitting. Thus if interventions prompted increased zoophily in vector populations, it could actually help enhance control and prospects for elimination if it resulted in a long term, stable shift away from biting humans, and did not just provide them with a short term strategy to maintain their populations in the face of insecticide use, before later returning to feeding on humans when insecticide coverage dropped and/or resistance emerged (see reference Ferguson et al., 2010 for more review).

More recently, the long term use (~10 years) of ITNs at high coverage in western Kenya and south eastern Tanzania has been accompanied by a substantial shift in malaria vector species composition as manifested by the progressive diminishment of the importance of the highly endophagic/endophilic *An. gambiae s.s.* (Bayoh et al., 2010; Russell et al., 2011). Examples of similar phenomenon have also been observed outside Africa. In the Solomon islands, for example, intense IRS campaigns conducted in the 1960s appeared to have eliminated the major vectors *An. punctulatus* and *An. koliensis* which predominantly rest indoors, but recent programs combining ITNs and IRS have had negligible impact upon human biting rates because the remaining, current primary vector species *An. farauti* feeds and rests predominantly outdoors (Bugoro et al., 2011a). Similar phenomena may be in Asia where human exposure to mosquito bites predominantly occurs outside houses and before bed time (Van Bortel et al., 2010).

Table 1 | Definition of mosquito behavioral choices.

Exophagy: is a tendency for mosquitoes to prefer biting outside
Endophagy: is a tendency for mosquitoes to prefer biting indoor
Exophily: is a tendency for mosquitoes to prefer resting outside
Endophily: is a tendency for mosquitoes to prefer resting indoor
Zoophagy: is a tendency for mosquitoes to prefer feeding on animal hosts

CONCLUSION

As mosquito feeding behaviors critically determine the effectiveness of most current front-line vector control intervention measures, there is a critical need to establish systematic monitoring of these phenotypes and how they are changing as part of the drive toward elimination. Furthermore, although malaria control experts must undoubtedly continue to deliver interventions that tackle indoor transmission in Africa, a considerable investment of resources in methods that target mosquitoes outside of houses and before sleeping hours is urgently required to sustain existing levels of malaria control and

make further inroads to achieve elimination (Ferguson et al., 2010; Griffin et al., 2010). To date, there is no intervention that specifically targets outdoor biting mosquitoes in common use throughout Africa. The only currently operational approach that could provide these benefits is larviciding (Killeen et al., 2002a, 2006c; Fillinger et al., 2008, 2009; Worrall and Fillinger, 2011), which by killing larval mosquitoes in their aquatic habitats may be assumed to efficiently target both the endophilic- and exophilic proportion of vector populations. Recent analysis suggests this method may be cost effective and practical in a much wider range of ecological settings than previously considered (Worrall and Fillinger, 2011). Even so, there is unlikely to be one "silver-bullet" approach to controlling outdoor biting mosquitoes and there is an urgent need to develop and assess a variety of complementary measures. Encouragingly there has been upsurge in interest in such methods in recent years, with progress being made toward assessing the potential use of large-scale spatial repellents (Moore et al., 2007), the application of insecticides to alternative hosts such as livestock (Rowland et al., 2001), the development of odor-baited traps for use outdoors (Knols et al., 2010; Okumu et al., 2010), the enhancement, and expansion of larvicide-based approaches (Soper and Wilson, 1943; Shousha, 1948; Kitron and Spielman, 1989; Killeen et al., 2002a,b; Fillinger and Lindsay, 2006; Gu and Novak, 2006; Gu et al., 2006; Fillinger et al., 2008, 2009; Chaki et al., 2009) and environmental management (WHO, 1982; Castro et al., 2004, 2009, 2010).

While malaria control experts must not stop to deliver interventions that tackle indoor transmission, we argue that further sustained investment not only into systematic monitoring of mosquito behavioral phenotypes and how they are changing but also on rapid case detection and treatment and substantial investment into the development and translation of outdoor-based interventions into wide-scale use must be prioritized a fundamental strategy for achieving elimination in mainland Africa.

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Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe – new distribution trends

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Cardiopulmonary dirofilariasis is a cosmopolitan disease caused by *Dirofilaria immitis*, which affects mainly canids and felids. Moreover, it causes zoonotic infections, producing pulmonary dirofilariasis in humans. Heartworm disease is a vector-borne transmitted disease, thus transmission depends on the presence of competent mosquito species, which is directly related to favorable climate conditions for its development and survival. Cardiopulmonary dirofilariasis is mainly located in countries with temperate and tropical climates. Europe is one of the continents where animal dirofilariasis has been studied more extensively. In this article we review the current prevalence of canine and feline cardiopulmonary dirofilariasis in the European continent, the transmission vectors, the current changes in the distribution and the possible causes, though the analysis of the epidemiological studies carried out until 2001 and between 2002 and 2011. The highest prevalences have been observed in the southern European countries, which are considered historically endemic/hyperendemic countries. Studies carried out in the last 10 years suggest an expansion of cardiopulmonary dirofilariasis in dogs toward central and northern Europe. Several factors can exert an influence on the spreading of the disease, such as movement of infected animals, the introduction of new species of mosquitoes able to act as vectors, the climate change caused by the global warming, and development of human activity in new areas. Veterinary controls to prevent the spreading of this disease, programs of control of vectors, and adequate protocols of prevention of dirofilariasis in the susceptible species should be carried out.

Keywords: heartworm disease, *Dirofilaria immitis*, dogs, cats, prevalence, vectors, Europe

INTRODUCTION

Dirofilaria immitis is a parasitic nematode responsible of canine and feline cardiopulmonary dirofilariasis in both domestic and wild hosts, and the causal agent of human pulmonary dirofilariasis. It is a zoonotic parasitic disease mainly located in temperate, tropical, and subtropical areas of the world (Simón et al., 2009b). Different species of culicid mosquitoes (*Culex* spp., *Aedes* spp., *Anopheles* spp.) act as an intermediate stage in order to complete their life cycle. When taking a blood meal from a microfilaremic host, the mosquitoes become infected and the microfilariae develop to the third-stage larvae (L3) in the malpighian tubules of the mosquitoes (Cancrini and Kramer, 2001), which are deposited on the host while the mosquito is taking a blood meal, becoming sexually mature within a few months in the main pulmonary arteries and right ventricle.

Heartworm infection is a severe and life-threatening disease. Initially the pulmonary vasculature is affected, and the lung itself and, finally, the right chambers of the heart (Furlanello et al., 1998). Feline infection is diagnosed with increasing frequency in areas where the disease is endemic in canines. However, the development of the parasite in cats takes longer compared to dogs and most infections are amicrofilaraemic. Additionally, many cats tolerate the infection without any noticeable clinical signs or with signs manifested only transiently and sometimes sudden death may arise without warning (Genchi et al., 1992; McCall et al., 2008).

The presence of *D. immitis* in dogs constitutes a risk for the human population. In the human host is the causative agent of the pulmonary dirofilariasis and in many cases produces benign pulmonary nodules which can initially be misidentified as malignant tumors (Simón et al., 2005).

The transmission of infectious diseases is influenced by many factors, including climatic and ecological elements. It is widely anticipated that climate change will impact the spread of vector-borne diseases in Europe, since arthropod vectors are especially sensitive to climatic factors. Weather influences the development and maintenance of the vectors, but climate change is one of many factors that influence vector habitat. Several studies have described the effects of the climate change in the spreading of other vector-borne diseases in Europe (Semenza and Menne, 2009; Genchi et al., 2011a,b).

The aim of the present work is to review the current epidemiological situation of the cardiopulmonary dirofilariasis in dogs (the main host) and cats in Europe, its vectors and evaluate the possible causes of the changes in the distribution of the disease by conducting a retrospective analysis of the epidemiological situation.

EPIDEMIOLOGICAL DISTRIBUTION OF ANIMAL DIROFILARIASIS IN EUROPE UNTIL 2001

Until 2001, cardiopulmonary dirofilariasis was mainly found in the southern European countries, such as Spain, Portugal, Italy, and France. Greece, Turkey, and some Eastern countries reported a few scattered studies while in central and northern European countries only isolated cases were reported (**Figure 1**).

In Italy, the area of highest prevalence values for dogs and cats was along the Po River Valley in northern Italy, where the prevalence rate for dogs ranged from 50 to 80% in animals no treated with preventive drugs (Genchi et al., 2001). A study of 1986 defined as infected by *D. immitis* 50% of the Northern provinces and only 15% of the provinces of central and southern Italy (Pampiglione et al., 1986). In the 1980s and 1990s, *D. immitis* showed a relevant prevalence increase in endemic areas (Genchi et al., 2001) and it was also recorded outside the main endemic area of the Po Valley, in provinces of north-eastern Italy previously regarded as non-endemic (Poglayen et al., 1996). Similarly, in Piedmont, an extensive survey carried out in the 1990s reported a spread of *D. immitis* westward and south-westward of the traditional endemic area, where *D. immitis* infection successfully established in hilly and pre-alpine areas as well in urban areas (Rossi et al., 1996). The disease was also present northwards into the provinces of Friuli-Venezia-Giulia (Pietrobelli et al., 1998). Heartworm disease has also been reported in central areas of the country, which showed lower infection rates for *D. immitis*, i.e., Toscana and Umbría (Magi et al., 1989; Pietrobelli et al., 1998; Genchi et al., 2001), except the Tuscan coast, where Magi et al. reported prevalences higher than 28% in 1989. Epidemiological data on the occurrence of dirofilariasis by *D. immitis* in southern Italy are scant (Cringoli et al., 2001) and limited to sporadic case reports, though no infection or very low prevalence were found in these areas, where *D. immitis* prevalence (microfilaremic dogs) was 0.01% in Sicily (Giannetto et al., 1997) and 0.5% in Campania (Cringoli et al., 2001). In Sardinia a prevalence of 4.1% in the southern area of the island was reported (Arru et al., 1968). The infection by *D. immitis* in cats is documented in northern Italy, where the prevalence rate in the hyperendemic areas resulted to be up to 24% (Genchi et al., 1992, 1993, 2001), while the presence of heartworm in feline populations of other areas of Italy was not investigated so far. Cases of foxes infected by *D. immitis* in Tuscany were described (Gradoni et al., 1980; Marconcini et al., 1996).

In Spain, *D. immitis* was found in large areas of the country, although the prevalence of the disease was higher in the southern areas, where the reported prevalence was 8.5% in Andalucia, 6.7% in Extremadura, or 6.3% in Murcia; indeed, in the Iberian peninsula, the highest prevalence was 36.7% in the southern province of Huelva (Guerrero et al., 1989; Ortega-Mora et al., 1991). Other southern areas with high prevalences were found in Cadiz (12%), Córdoba (18%), Badajoz (8–14%), or Alicante (13%; Anguiano et al., 1985; Guerrero et al., 1989; MSD-AGVET, 1991; Ortega-Mora et al., 1991). In the rest of the Iberian Peninsula, the higher prevalences were associated with irrigated areas. In the central areas of Iberian Peninsula low prevalences of canine dirofilariasis were reported. In Madrid, several studies stood the canine prevalence between 1.1 and 2% (Ortega-Mora et al., 1988; Guerrero et al., 1989; Rojo-Vázquez et al., 1990), except in the area of Aranjuez (population under the influence of the river Tajo), where Guerrero et al. (1992) reported a prevalence of 6.8%. In the northwest of Spain, the prevalence reported in Salamanca was 12%, rising to 33.3% in irrigated areas close to the river Tormes (Pérez et al., 1989). In the northeast of Spain, the highest prevalences were found in Zaragoza (13.5%; Castillo et al., 1989), where high prevalences in irrigated areas were found in foxes (31.5%; Castillo et al., 1989). In Catalonia the global prevalence remained low (2.17% region of Catalonia; 1.2% district of Barcelona; Rojo-Vázquez et al., 1990; Gutiérrez et al., 1995); Guerrero et al. (1995) reported an increase of the prevalence of *D. immitis* infection in dogs in Catalonia from 0.38% in 1989 to just over 5% in 1995. Besides, the prevalences in this area raised noticeably in the irrigated areas,

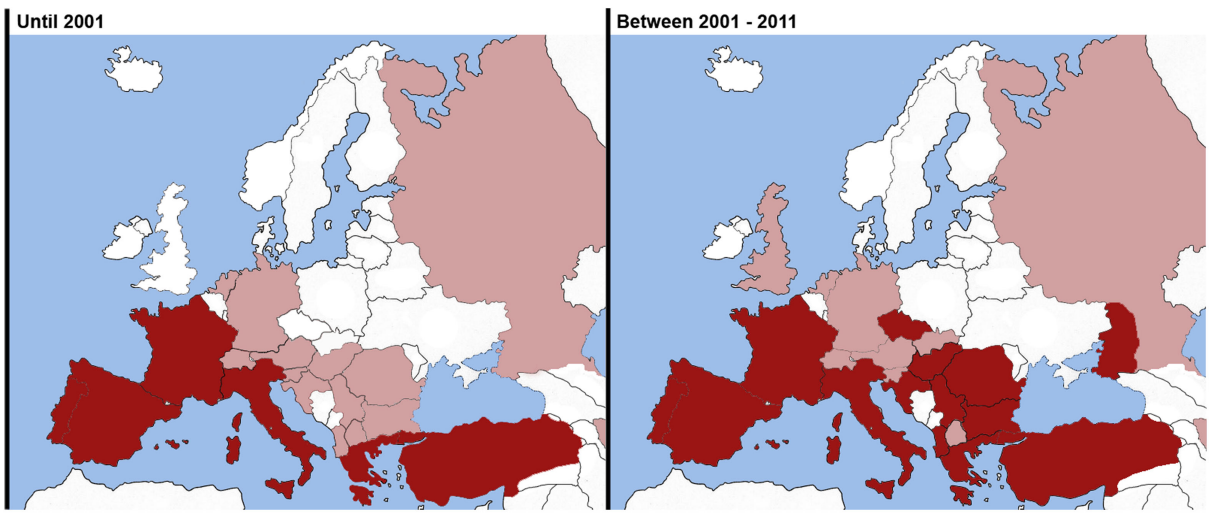

FIGURE 1 | Comparison of geographical distribution in Europe of heartworm disease observed in dogs between 2001 and 2011. Endemic areas (Red). Sporadic cases reported (Pink). *Illustrated map taking into account Genchi et al. (2005, 2009) and data from the referenced literature.

such as River Ebro Delta (35.8%; Anguera-Galiana, 1995) and Bajo Llobregat (12.8%; Aranda et al., 1998). Canary Islands, sited in front of the north-western African coast, were considered an endemic area of the disease. On the island of Gran Canaria, serial epidemiological studies carried out in different years allowed monitoring the evolution of the canine dirofilariasis. These studies showed an increase in the prevalence from 36.7% in 1989 to 58.89% in 1998, reaching the highest prevalence (67.02%) in 1994. This is the highest prevalence reported in Spain so far (Montoya et al., 1998). The island of Tenerife, on the other hand, showed a drop of prevalence from 41.8% in 1984 to 23% in 1999 (Valladares et al., 1987; Guerrero et al., 1989; Stenzenberger and Gothe, 1999). Infections by *D. immitis* in foxes, were reported with the highest prevalence found in foxes from irrigated areas (32%), while in semiarid regions the prevalence was much lower (1.7%) and in mountain foxes heartworm was absent (Gortázar et al., 1994, 1998). Finally, in the northern areas of the Iberian Peninsula no cases of canine dirofilariasis were reported (Guerrero et al., 1989, 1992) although exist a report of *D. immitis* infection in a wolf (Segovia et al., 2001).

In Portugal, the only study published showed that canine heartworm infection was prevalent in several southern regions of Portugal, including Ribatejo (16.7%), Alentejo (16.5%), and Algarve (12%). The Island of Madeira had the highest prevalence with 30% of the dogs tested being positive for *D. immitis* microfilaremia (Araujo, 1996).

In France, *D. immitis* occurred mainly in the South, along the Mediterranean coast, predominantly in Bouches du Rhône, Vancluse, and Corse Island (5–15%), and to a lesser extent, in Haute-Garonne and Dordogne (Doby et al., 1986a; Guerrero et al., 1992). The overall prevalences of canine dirofilariasis reported in France were 0.74% in 1986, 1.48% in 1988, and 0.8% in 1989 (Ducos de Lahitte, 1990; Guerrero et al., 1992). *D. immitis* infections were reported beyond the Mediterranean, in field studies with positive findings in Normandy and Brittany in the Northwest of France, as well as in the department of Dordogne in the southwest. Doby et al. reported in 1986 canine prevalences in Brittany of between 3.7–10%, and 5% in Normandy (Doby et al., 1986b; Ducos de Lahitte, 1990). Guerrero et al. (1992) reported in 1992 prevalences in 7.3% of the dogs tested in Alpes Maritimes and 2.5% in dogs examined in Pyrenees Atlantique; nevertheless, most of these dogs had lived in french-administered territories outside of the European continent. The parasite was also diagnosed in northern France (Cherburg area, just below 50°N latitude), showing a prevalence of 3.7%, apparently as consequence of autochthonous infection (Doby et al., 1986a). In cats, microfilariae of *D. immitis* were detected in the urine, but not in the blood, of a cat in Sommieres, in southern France. The cat also showed radiographic evidence of *D. immitis* infection (Beaufils et al., 1991).

In Greece a study carried out between 1987 and 1991 showed microfilariae of *D. immitis* in the 10% of the studied dogs (Papazahariadou et al., 1994) while in 1999 in dogs from the area of Macedonia the reported prevalence was 34.13% (Founta et al., 1999). In general, in Greece during the 90s decade the incidence of the disease increased rapidly and at the end of the millenium was considered endemic in the central and northern parts of the country (Polizopoulou et al., 2000). In a study carried out in 2001 in the Attiki region (south of Greece) low canine *D. immitis* prevalence (0.7%) was observed (Diaku, 2001).

In Turkey, the first case of dirofilariasis reported in a dog date from 1951 (Güralp, 1981). Until 2001 only a few studies regarding the distribution and prevalence of animal dirofilariasis are published. In the Army Veterinary Research and Training at Gemlik, Bursa, 2.98% of dogs were infected (Coskun et al., 1992), and in Ankara, between 2000 and 2001 a prevalence of 9.3% was reported (Öge et al., 2003).

In Switzerland, border country with Italy and France, Arnold et al. (1994) reported a case of an infected dog and suggested a possible autochthonous infection, which confirmed in 1998 (Genchi et al., 1998), moment at which Switzerland became an endemic country. In the south of Switzerland, between 1995 and 1998, the reported prevalences ranged from 0.6 to 1.07% (Deplazes et al., 1995; Bucklar et al., 1998). Deplazes et al. (1995) diagnosed another autochthonous case in a dog from the south of the country (Cantón de Ticino) close to Como and Varese (northern Italy). In the same region, in 2001 the prevalence raised considerably, reporting 10.7% of microfilaremic dogs and 3.2% of amicrofilaremic dogs, some of them also infected by *D. repens* (6%; Petruschke et al., 2001).

In Germany, between 1993 and 1996 a total of 80 dogs were diagnosed, of which 45 were amicrofilaremic (Zahller et al., 1997).

In the Netherlands, seven cases of infected dogs were recollected between 1992 and 1993, the same amount of cases of canine dirofilariasis reported in the previous years (Meyer et al., 1994).

In the only study published in Macedonia, the prevalence reported was 0.9% (Ježic and Simic, 1929).

In Rumania, at least four dogs were diagnosed between 1903 and 1935 (Genchi et al., 2001). Later, the average prevalence was 35% rising to 67% in some areas (Olteanu, 1996).

In the former Yugoslavia, in Croatia between 1987 and 1989 several cases of canine dirofilariasis were reported but no considered autochthonous (Brglez and Senk, 1987; Genchi et al., 2001). In Serbia the first report data from 1999 by Dimitrijevic (1999). In Slovenia and Bulgaria, the reported prevalence of *D. immitis* was around 4–5% (Olteanu, 1996), being in Bulgaria 1.4% in pet dogs and 12.5% in stray dogs (Georgieva et al., 2001). In Albania, a study carried out between 1995 and 1996 with samples from the coastal western area showed infection in 13.5% of the dogs (Rapti and Rehbein, 2010).

In the former USSR (Russia) there are only 3–4 reports of isolated incidents of infection in dogs from the Republics of Azerbaijan and Turkmenistan, situated in the center of the country, in Ussuri Region in the Far East and in Abkhazia (Artamonova et al., 1997).

Finally, Switzerland, Netherlands, Germany, Austria, United Kingdom, and Hungary also reported dirofilariasis in imported dogs, or dogs previously living in endemic areas of Europe, the south of United States of America and/or Middle or Far East, fact that could distort the real prevalences of these countries (Stokhof and Wolvekamp, 1978; Boros et al., 1982; Hinaidy et al., 1987; Arnold et al., 1994; Meyer et al., 1994; Deplazes et al., 1995; Wohlsein et al., 1996; Zahller et al., 1997).

EPIDEMIOLOGICAL SITUATION OF ANIMAL DIROFILARIASIS IN EUROPE BETWEEN 2002 AND 2011

In 2011, cardiopulmonary dirofilariasis remains endemic and spreading out the southern European countries; this disease has spread to countries in Eastern and Center of Europe where its presence and distribution were only reported by sporadic cases or not reported at all (**Figure 1**).

In Italy, *D. immitis* is endemic in northern Italy and has now spread all over the country, which shows a current change of distribution of this parasite throughout the Italian territory while canine and feline heartworm infection is more frequently diagnosed in southern regions of Italy (Otranto et al., 2009; Traversa et al., 2010a). While the last studies report the lower prevalences published in the endemic area of northern Italy (6.12%; Piccinini and Carreri, 2010), autochthonous foci of canine dirofilariasis have been described in central regions such as Tuscany and Umbria, which were considered non-endemic until 1999 (Piergilli-Fioretti et al., 2003; Mortarino et al., 2008). Nowadays, heartworm disease infection has become endemic in these areas (Piergilli-Fioretti et al., 2003; Mortarino et al., 2008; Magi et al., 2011). In Umbria (hilly central region), where only imported cases have been previously reported, the prevalence ranges between 5 and 15% (Piergilli-Fioretti et al., 2002, 2003; Genchi et al., 2005; Mortarino et al., 2008). In Tuscany a prevalence of canine dirofilariasis of 12.5% was reported (Mortarino et al., 2008; Magi et al., 2011). Furthermore, *D. immitis* has been detected for the first time in autochthonous dogs living in another previously *Dirofilaria* free region of central Italy, i.e., Abruzzo, close to Umbria and Lazio regions (Paoletti et al., 2008). In a recent study carried out in 2008 and 2009, in the Abruzzo region of central Italy prevalences of 2.3 and 0.3% were found in native dogs and cats, respectively (Traversa et al., 2010a). In a recent survey dogs from four different areas of southern Italy were sampled (Apulia and Calabria regions), and prevalences between 0.24 and 2.57% were found in Apulia region while prevalence of 3.43% was found in Calabria region (Otranto et al., 2009). In Sardinia, where the prevalence was very low in the past (<2%), there has been an increasing pattern of prevalence, rising to 17% in the central west area of the island (Scala et al., 2004). *D. immitis* infections in cats has been diagnosed mostly in northern Italy where prevalences between 7 and 27% have been found, depending on location, in the hyperendemic area of the Po River valley (Kramer and Genchi, 2002; Genchi et al., 2008). In the central area of Italy a prevalence of 23.5% is reported in cats living in Tuscany (Magi et al., 2002). In Tuscany too, epidemiologic studies observe the presence of adults of *D. immitis* between 6.06 and 7.1% of the red foxes studied (Magi et al., 2008, 2009). For the first time, has been described the first diagnosis of mature heartworm infection and presence of microfilariae in an exotic felid (*Panthera pardus pardus*) in north-eastern Italy (Mazzariol et al., 2010). Furthermore, a case of *D. immitis* in a wolf in the south of Italy was reported (Pascucci et al., 2007).

In Spain, a epidemiological study carried out in 2006, reports high prevalences in the Mediterranean coast (18% in Alicante, 9% in Murcia) and on the island of Ibiza (39%; Rodes, 2006), while in Mallorca recently was reported a case of dirofilariasis in a dog which never traveled outside the island (Makowski et al., 2010). Another study shows prevalences of 2% in Barcelona, 0.85% in Tarragona, and 0.3% on the island of Mallorca (Solano-Gallego et al., 2006). In the center of the Iberian Peninsula high prevalences are reported in Arganda del Rey (8%), Azuqueca de Henares (24%), and Guadalajara (10%), areas of influence of the Henares, Jarama, and Tajuña rivers (Gómez-Bautista and Ortega-Mora, 2002). In a study carried out in Salamanca between 2008 and 2009, a prevalence of 29.08% is observed, similar to that reported 20 years ago (Morchón et al., 2011b). Besides, for the first time significant *D. immitis* prevalences are found in two Northern provinces: La Rioja (12%) and La Coruña (4.2%; Simón et al., 2009a; Morchón et al., 2010). On the island of Gran Canaria, the prevalence of canine dirofilariasis has been gradually dropping, from 23.87% in 2002 (Sosa et al., 2002) to 19.2% in 2010 (Montoya-Alonso et al., 2011). On the island of Tenerife, the prevalence remains constant ranging from 22.3% in 2001 to 21% in 2006 (Morales et al., 2001; Montoya et al., 2006). Regarding feline dirofilariasis, on the island of Gran Canaria two seroepidemiologic studies show an increase of the prevalence from 18.3 to 33% between 2004 and 2011 (Morchón et al., 2004; Montoya-Alonso et al., 2011). In 2006, the first diagnosis of *D. immitis* infection in an African lion (*Panthera leo*) born and living in Alicante is described (Ruiz de Ybáñez et al., 2006). Besides, exists a report of *D. immitis* infection in a fox from the north-eastern of Spain (Mañas et al., 2005). Finally, a study carried out in Eurasian otter (*Lutra lutra*), 48 Eurasian otters from different regions of the Iberian Peninsula were examined, finding *D. immitis* prevalence of 2.1% (Torres et al., 2004).

In Portugal, in 2011 the overall canine prevalence in the north and north center of Portugal is 2.1% (Balreira et al., 2011), with the higher prevalences found in Aveiro (6.8%) and Coimbra (8.8%). In the last 15 years there is not published any study of prevalence in the south of Portugal, although a study carried out on five Eurasian otter (*Lutra lutra*) from Alentejo, found *D. immitis* in two of them (Torres et al., 2004). In cats, a study carried out in the north and north-center of Portugal reports a *D. immitis* seroprevalence of 17.51% (Vieira et al., 2011).

In France, in 2009, a canine prevalence of 0.22% was found in an epidemiologic study carried out in most of the national territory; the positive results came from dogs from Corsica and Boches-du-Rhône, in the south of France; local prevalence in the department Bouches du Rhône is 2.1% and even higher local prevalence is determined for Corsica (12.5%; Pantchev et al., 2009). Simultaneously, a study was done over dogs with clinical signs compatible with *D. immitis* infection. In this group, *D. immitis* is confirmed in the 6.87% of the dogs. Of these, most of them are from the South of France, where local prevalences are 27.3% for Corsica and 22.2% for Boches-du-Rhône, and the only two positive cases from the northern part of the country likely came into France from abroad: one was brought from Martinique and other one from French Guyana (Pantchev et al., 2009). The authors of the study suggested that an expansion of the Southern endemic areas of *D. immitis* into the North has not occurred. They also indicated that dogs in the Southern areas of the country are still at a high risk of heartworm infection.

In Greece, although in 2001 the prevalence found in the Attiki region, in the south of the country, was 0.7% (Diaku, 2001), other study carried out in 2003, reported *D. immitis* infections in 13.1%

of the studied dogs in the environs of Athens, in the province of Attiki (Jensen et al., 2003). Since then the disease is considered endemic in the south of the country too. Lefkaditis and Koukeri (2005) reported in that dirofilariasis is a common parasitic disease in Thessaloniki and the most recent study shows a *D. immitis* prevalence of 17.9% in dogs living on the eastern foothills of Mt Olympus in Northern Greece (Lefkaditis et al., 2010).

In Turkey the disease is widely spread showing prevalences between 1 and 27%. In Istanbul the infection affects the 1.52% of the dogs (Öncel and Vural, 2005), 2% in the area of Gemlik of Bursa (Civelek et al., 2007), 9.6% in the province of Kayseri (Yildirim et al., 2007), and 26% in the province of Hatay. A study carried out by Simsek et al. (2008) reports prevalences of 14.8% in Ankara, 12.3% in Sakarya, 10.5% in Mersin, and 18.3% in Kocaeli. Finally, in the region of Kirikkale positive cases were also reported (58% of the infected dogs were microfilaremic and 27.46% were amicrofilaremic; Yildiz et al., 2008).

In Germany there is an increase in the number of infected dogs reported, most of them imported from endemic areas. Between 2005 and 2006, the prevalence was 1.2% (5.483 samples included in the study; Hirsch and Pantchev, 2008), between 2008 and 2010 was 1.49% (8.545 samples included in the study), of which 30% were microfilaremic, and between 2009 and 2010 the prevalence was 2.6% (Pantchev et al., 2009, 2011). It is not considered an endemic country since, up to date, all reported cases corresponded to imported animals, most of them coming from Corfu, Sardinia, Bulgaria, Hungary, and Canary Islands.

In United Kingdom isolated cases are still being diagnosed. Recently a dog was diagnosed and not registered as autochthonous so far, since it is not clear if the dog have previously been in endemic areas (Traversa et al., 2010b).

In Rumania the only study found, published by Sofía et al. (2007) reported the presence of *Dirofilaria* spp. in 23.07% of the studied dogs, which provides evidence of the current presence of the parasite in the country, which is considered an endemic country.

In Bulgaria between 2001 and 2006, 6.6% of the studied dogs were infected and, besides, 8.62% were microfilaremic (Kostadinov, 2007). In this country, canine dirofilariasis is not currently considered a rare disease but an endemic disease. Exceptionally, the presence of *D. immitis* has been reported in foxes (3%) and jackals (8.9%; Kirkova et al., 2007).

In Hungary two studies have been published, reporting two infected dogs; one of them constitutes the first autochthonous diagnosis which is why it is currently considered an endemic country (Farkas, 2003; Jacsó et al., 2009).

In Croatia and Serbia several studies demonstrate the presence of dirofilariasis as an endemic disease in these countries and its constant spreading. In Serbia, between 2006 and 2007 the global prevalence reported was 7.2% and, specifically, in the regions of Vojvodina and Branicevo was 7.2 and 3.17% respectively (Zivicnjak et al., 2006; Dimitrijevic et al., 2007; Tasic et al., 2008). In the region of Belgrade, a few years later the prevalence was 22.01%, showing co-infections with *D. repens* in 3.97% of the dogs. In Kosovo, the global prevalence is 9% and in the northern areas of the country increases up to 6.57% reaching 16.1% in some areas (Lazri et al., 2008). In the Istria Peninsula in Croatia, the canine dirofilariasis reaches prevalences of 16 and 8% in the southern areas. In the south of Slovakia, between 2007 and 2008, two studies showed *D. immitis* infection in 10 dogs co-infected by *D. repens* (Miterpáková et al., 2010).

In the Czech Republic, Svobodová et al. (2002) detected the first endogenous case of heartworm disease in dogs therefore is currently considered an endemic country. Two years later 89 infected dogs were detected, being one of them imported from an endemic area (Svobodová and Misonova, 2005). Finally, Dobesova et al. (2007) reported a prevalence of 6.7% with co-infection with *D. repens* in 2.7% of the dogs, all of them collected near Austria and Slovakia.

In Albania, between 2007 and 2008 prevalence was 3% in the district of Tirana and 7% in samples collected between Albania and Kosovo (Lazri et al., 2008; Hamlet et al., 2009).

In Russia, dirofilariasis is currently considered an emerging disease. Lately, numerous cases are appearing in the center and southern areas of the country. In the region of Moscow, canine dirofilariasis has been reported in 33 districts, all of them co-infected by *D. repens* (Supriaga et al., 2011) and in the region of Rostov (south of Russia), between 2002 and 2009, 6.1% of the studied dogs presented *D. immitis*, 5% of them co-infected by *D. repens* and microfilaremic (Kartashev et al., 2011); currently, the region of Rostov is considered an endemic area.

VECTOR TRANSMISSION OF DIROFILARIASIS

Several studies carried out in endemic areas researched which vector species are transmitting agents of cardiopulmonary dirofilariasis. To that end, vector mosquitoes have been captured through field studies using animal-bait traps; besides, these studies allowed the evaluation of the different mosquito species attracted to the hosts and the evaluation of the effectiveness of the transmission of the parasite (Cancrini and Kramer, 2001). These studies have been carried out in several areas of the globe (Unites States of America, Brazil, Italy, Iran. . .); animals (dog and cat) and/or humans have been used as bait. Approximately 70 species of culicid mosquitoes mainly from the genera *Culex* spp., *Aedes* spp., *Anopheles* spp., *Culiseta* spp., and *Coquilletidia* spp. have been identified, and are considered potential vectors of animal and human dirofilariasis, although only in a few cases its real vectorial capacity could be proven (Cancrini and Kramer, 2001; Cancrini et al., 2006).

Various studies in Europe had reported several species of mosquitoes infected by *D. immitis* larvae such as *Cx. pipiens* in Spain (Morchón et al., 2007), Italy (Cancrini et al., 2006), and Turkey (Yildirim et al., 2011); *Cx. theileri* in Madeira, Portugal (Santa-Ana et al., 2006), and on the Canary Islands, Spain (Morchón et al., 2011a); *Ae. vexans* in Turkey (Biskin et al., 2010; Yildirim et al., 2011) and *Ae. albopictus*, *Ae. caspius*, *An. maculipennis*, and *Cq. richiardii* in Italy (Cancrini et al., 1995, 2003, 2006).

In Europe, the activity of these species is limited to the period of time between spring and summer, whereas the behavior of the mosquitoes when search a host to feed follows different patterns depending on the species. Some are active only during the night, such as *Cx. pipiens*, *Anopheles* spp. while others are active predominantly at dawn or during the day (*An. maculipennis*, *Ae. albopictus)* and some other species show two peaks of activity: at dusk and at

dawn, such as *Ae. caspius* (Mattingly, 1969; Di Sacco et al., 1992; Pollono et al., 1998).

Mosquito development and activity are regulated by climate, primarily temperature and humidity, just as L3 development depends on the ambient temperature. It has been demonstrated experimentally that infectious L3 development requires 8–10 days at 28–30°C, 11–12 days at 24°C, and 16–20 days at 22°C. Below 14°C, development arrests, although it can be restarted when the ambient temperature increases above this threshold (Cancrini and Gabrielli, 2007). Consequently, the climate and its changes determine the transmission and presence of dirofilariasis in temperate regions (Genchi et al., 2005). A good example of the impact of climate on the distribution and prevalence of dirofilariasis is illustrated by the island of Gran Canaria. With only 40 km of diameter, this hyperendemic island is divided in four different isoclimatic areas depending on altitude, with marked temperature and humidity differences among them. As consequence, the prevalence of *D. immitis* is significantly different between the canine populations of each zone, varying from 12 to 32% (Montoya-Alonso et al., 2010b).

There is currently a scientific consensus regarding the existence of anthropogenic climate change, which is ascribed to natural processes and human activity altering atmospheric conditions with an increase of the worldwide mean surface temperature by 0.74°C (Threnberth, 2005; Semenza and Menne, 2009). Global warming affects host-parasite systems by influencing the amplification and emergence of parasite populations, inducing changes in the development and survival rates of both parasite and vector and altering seasonal transmission dynamics (Brooks and Hoberg, 2007). With respect to dirofilariasis, climate change is lengthening annual periods of mosquito activity, shortening larval developmental stages, and increasing transmission across multiple geographical regions, which means more suitable conditions for its spread toward new areas.

Table 1 | Distribution of animal dirofilariasis in Europe until 2011 and between 2002 and 2011.

	Until 2001	2002–2011	Vectors
Portugal	●	●	*Cx. theileri*
Spain	●	●	*Cx. pipiens, Cx. theileri*
Italy	●	●	*C. pipiens, Ae. albopictus, Ae. caspius, An. maculipennis, Cq. richiardii*
France	●	●	
Greece	●	●	
Turkey	●	●	*Cx. pipiens, Ae. vexans*
Switzerland	≤10.7%, □	?	?
Austria	□	?	?
Germany	□	≤2.9%	?
United Kingdom	□	■	?
Netherlands	■, □	?	?
Macedonia	≤0.9%	?	?
Rumania	≤67%	●	?
Croatia	■	●	?
Serbia	■	●	?
Slovenia	≤5%		?
Bulgaria	≤12.5%	●	?
Albania	≤13.5%	≤7%	?
Slovakia	?	■	?
Czech Republic	?	●, □	?
Hungary	□	?	?
Republic of Azerbaijan	■	?	?
Turkmenistan	■	?	?
Russia – region of Rostov	■	■, ●	?

Endemic area (●), isolated cases (■), imported cases (□) and no data (?).

FACTORS CONTRIBUTING TO THE SPREADING OF CANINE CARDIOPULMONARY DIROFILARIASIS

The analysis of the epidemiologic studies carried out until 2001 and between 2002 and 2011 show a change in the pattern of distribution of the disease, with a spreading toward north and Eastern Europe. The results of these epidemiologic analyses are summarized in the **Table 1**. Between 2002 and 2011 there are reports of canine dirofilariasis in countries and regions previously considered free of the disease (Sofía et al., 2007; Simón et al., 2009a; Morchón et al., 2010; Kartashev et al., 2011) and there have been reported autochthonous cases of dirofilariasis in countries where previously only imported cases had been reported (Svobodová et al., 2002; Farkas, 2003; Jacsó et al., 2009; Makowski et al., 2010). The cause of this spreading might be multifactorial, and in this review we discuss the different factors affecting the changes of the distribution of cardiopulmonary dirofilariasis in Europe.

There are several factors which exert a big influence on the spreading of the disease (Genchi et al., 2001). In the first place, the presence and movement of microfilaremic reservoirs, as well as the increasing number of dogs traveling for holidays or commerce of dogs from endemic areas being relocated, are key factors for the maintenance of the infection in endemic regions and the spread into new areas. It is important to mention the role as reservoir of other animals, such as the coyote in California (Sacks, 1998) and the fox in Australia (Marks and Bloomfield, 1998); in Europe, the role of foxes or wolves as reservoirs of dirofilariosis could be important factors to consider when studying the factors contributing to the maintenance and spreading of the disease. Another fundamental factor is the presence of mosquitoes able to act as vectors, as well as the existence of adequate climate conditions for its correct development. Furthermore, it is necessary take into account the introduction in a given area of new species of competent mosquitoes; *Aedes albopictus* represents an example of this, which being native from southeastern of Asia and western Pacific has spread to Europe, Africa, and America in the last decades. This quick spreading has been facilitated by international transport nets of used tires and gardening products, as well as by accidental transport of adults in vehicles from close affected areas (Reiter and Sprenger, 1987; Madon et al., 2002; Flacio et al., 2004; Roiz et al., 2007). Besides, *A. albopictus* is a highly adaptable species; in temperate areas its activity period is limited to summer, surviving during the winter in egg-stage, a fact not observed in the mosquito colonies from the tropical areas (Hawley et al., 1987; Mitchell, 1995). In general, a region is susceptible to be colonized by stable colonies of *Ae.*

albopictus when the average temperature is 0°C in winter, 20°C in summer and presents at least 50 cm of annual rainfall (Knudsen et al., 1996). In Europe was found for the first time outside its area of origin in 1979 in Albania (Adhami and Reiter, 1998); later was detected in Italy, where have become a plague (Romi, 2001). Currently it is also present in France, Montenegro, Switzerland, Greece, Spain, Croatia, Bosnia-Herzegovina, Slovenia, Belgium, Netherland, and Germany (Schaffner and Karch, 2000; Pétric et al., 2001; Flacio et al., 2004; Schaffner et al., 2004; Samanidou-Voyadjoglou et al., 2005; Aranda et al., 2006; Kloblucar et al., 2006; Scholte and Schaffner, 2007; Pluskota et al., 2008; Scholte et al., 2010). There is a concern about the marked anthropophilia observed in *Ae. albopictus* regarding its involvement on the transmission of several diseases to the human population (dirofilariasis amongst them; Cancrini et al., 1995). Its vectorial capacity has been proved in the transmission of Dengue fever and Yellow fever, is a potential vector for several arbovirus (Mitchell, 1995) and its capacity to transmit infective larvae of *D. immitis* has been confirmed in its area of origin, in Italy and some areas of North America (Comiskey and Wesson, 1995; Cancrini et al., 2003; Gratz, 2004). In a recent study of the nutritional habits of the mosquito carried out in the province of Rome, it was observed that in the urban areas *Ae. albopictus* fed almost exclusively on humans, while in rural areas they show a mixed feeding pattern (human-horse and human-dog; Valerio et al., 2010).

Other potential vector species of heartworm disease is *Ae. aegypti.* This species was vey abundant in the early twentieth century in Southern Europe and in harbor cities of the Mediterranean Basin, mainly in Syria, Lebanon, Turkey, Greece, former Yugoslavia, Italy, Corsica (France), and Spain. Currently, constitutes a potential vector of the disease in America (Vezzani et al., 2006, 2011b); however, in Europe it has not been proven as a vector of the disease except in experimental infections (Scholte et al., 2010).

The environmental conditions constitute another important factor affecting the distribution of the disease; these play an essential role in the distribution of the dirofilariasis. Because of the climate change, influenced by the global warming, the vector population has more suitable conditions for its development, increasing the geographical distribution of vectors and the number of mosquitoes able to transmit the disease, as well as the suitable temperatures allow to expand the risk season for the transmission of the disease by favoring the development of infectious larvae in the vector (Genchi et al., 2009), as well as the insertion and spreading of vectors from another areas (Hendrickx et al., 2004; Rogers and Randolph, 2006). Other studies have demonstrated the influence of the climate change in the spreading of other vector-borne diseases in Europe (Semenza and Menne, 2009). On the other hand, it is also important to take into consideration the changes in ecology and the habitat, alterations in the system and water storage, the pollution and the development of resistances to insecticides (Harrus and Baneth, 2005). Building construction and human activity in new areas play an important role given that increases the density of potential hosts and develops a suitable environment for the proliferation of certain species of mosquitoes; the development of residential settlements of non-endemic areas and areas of low incidence led to the spread and increased prevalence of heartworms by altering drainage of undeveloped land and by providing water sources in new urban home sites. Besides, urban sprawl has led to the formation of "heat islands," as buildings retain heat during the day and subsequently radiate it during the night, which can potentially create microenvironments that support development of heartworm larvae in mosquito vectors during colder months, thus lengthening the transmission season (Arnfield, 2003). Considering these factors, the most favorable environments for the spreading of the dirofilariasis are characterized by high humidity and temperature, which allow the adequate development and activity of the vector. It has been demonstrated that the irrigated lands for farming present higher prevalences that those areas close to them (Gortázar et al., 1998; Montoya-Alonso et al., 2010a). All the mentioned factors might be determinant for the spreading, as well as the rise of the prevalence in new areas (i.e., south of Greece and Italy) where, due to the unawareness of this disease, not adequate prophylactic measurements were taken. On the other hand, due to the increase of the incidence of canine dirofilariasis, a greater attention by the scientific community toward these areas is being paid, resulting in more accurate and numerous epidemiologic studies.

It is also important to notice that, based on the results of the epidemiological studies carried out until 2001 and between 2002 and 2011, a decrease of the prevalence of canine dirofilariosis in hyperendemic areas has been observed. This is clearly seen in the northern Italy, where the prevalence has decreased from 50 to 80% to nearby 6% in the last published study (Piccinini and Carreri, 2010); also, the island of Gran Canaria (Spain) has experienced a decrease of canine dirofilariasis from 67% in 1994 to 19% in 2010 (Montoya et al., 1998). This might be caused by the fact that preventive therapy had begun to be administered continuously to the canine population thanks to the education of the pet owner and a better understanding of the disease (Montoya-Alonso et al., 2010a). Similar trends have been observed in other endemic areas outside Europe, where a decrease in canine dirofilariasis prevalences have been described recently (Labarthe and Guerrero, 2005; Vezzani et al., 2011a); the authors conclude that this trend might be due to effective chemoprophylaxis, the abusive use of injectable ivermectin and the extensive use of tetracyclines to control other infections.

CONCLUSION

Finally, on the basis of results in this review, it is possible to confirm that the prevalence of canine and feline dirofilariasis is increasing and spreading to the north-eastern and center European countries, being reported new cases of canine dirofilariosis in countries previously considered free of the disease, or being diagnosed more often, as well as the first reports of autochthonous cases in countries where previously only imported cases were described. In general, the increased movement of infected dogs across Europe, a greater attention toward the disease, the climate change, the emergence of new species of vectors and changes in ecosystem due to human activity, lifestyle of people, are the possible causes of this increase. In these countries, this should be controlled by periodic heartworm antigen test done in veterinary clinics for detection of *D. immitis* infection and a correct prevention protocol should be carried out in all traveling

dogs, in the same way that correct protocols of early diagnosis and prevention of heartworm disease has been performed in hyperendemic areas for years, resulting in a decrease of the prevalence of the disease in those areas and contributing to the change in the distributions pattern of the canine cardiopulmonary dirofilariasis.

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Animal viral diseases and global change: bluetongue and West Nile fever as paradigms

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Environmental changes have an undoubted influence on the appearance, distribution, and evolution of infectious diseases, and notably on those transmitted by vectors. Global change refers to environmental changes arising from human activities affecting the fundamental mechanisms operating in the biosphere. This paper discusses the changes observed in recent times with regard to some important arboviral (arthropod-borne viral) diseases of animals, and the role global change could have played in these variations. Two of the most important arboviral diseases of animals, bluetongue (BT) and West Nile fever/encephalitis (WNF), have been selected as models. In both cases, in the last 15 years an important leap forward has been observed, which has lead to considering them emerging diseases in different parts of the world. BT, affecting domestic ruminants, has recently afflicted livestock in Europe in an unprecedented epizootic, causing enormous economic losses. WNF affects wildlife (birds), domestic animals (equines), and humans, thus, beyond the economic consequences of its occurrence, as a zoonotic disease, it poses an important public health threat. West Nile virus (WNV) has expanded in the last 12 years worldwide, and particularly in the Americas, where it first occurred in 1999, extending throughout the Americas relentlessly since then, causing a severe epidemic of disastrous consequences for public health, wildlife, and livestock. In Europe, WNV is known long time ago, but it is since the last years of the twentieth century that its incidence has risen substantially. Circumstances such as global warming, changes in land use and water management, increase in travel, trade of animals, and others, can have an important influence in the observed changes in both diseases. The following question is raised: What is the contribution of global changes to the current increase of these diseases in the world?

Keywords: global change, climate change, emerging diseases, bluetongue, West Nile virus

INTRODUCTION

Infectious disease can be viewed as a play involving at least two characters: the pathogen and the host. While both roles can be represented by a great variety of performers, pathogens exhibit by far the highest variety and complexity. This review is about viral infections in animals. It aims first to give an idea of the enormous complexity and diversity of the existing infectious agents, emphasizing their extraordinary capacity for change and adaptation, which eventually leads to the emergence of new infectious diseases. Secondly, it focuses on the influence of the environment in this process, and on how environmental (including climate) changes occurring in recent times, have precise effects on the emergence and evolution of infectious diseases, some of which will be illustrated with specific examples. Finally, it describes the recent and dramatic expansion of two of the most important emerging animal viral diseases at present, bluetongue (BT) and West Nile fever/encephalitis (WNF), dealing with their relationship to climate and other environmental changes, particularly those linked to human activities, collectively known as "global change," and that can be at least in part seen a consequence of the "globalization" phenomenon.

EMERGING VIRAL DISEASES OF ANIMALS

COMPLEXITY AND DIVERSITY OF ANIMAL VIRUSES

To illustrate the enormous complexity of animal viruses, consider the following example: take any animal species, e.g., bovine. There are five known species of herpesviruses that could infect bovines specifically. Similarly, there are nine different known equine herpesviruses, eight human herpesviruses, and so on and so forth (Pellet and Roizman, 2007). Bearing in mind that there are approximately 5,400 different species of mammals (Wilson and Reeder, 2005) and that most of them have yielded at least one, most frequently several distinct herpesviruses on examination, the number of existing mammalian herpesvirus species would be huge, probably in the range of thousands. But there are also herpesviruses specific for birds, for reptiles, for amphibians, etc., so the above number would be increased consequently with the number of other vertebrate species (for the moment viruses of invertebrates, a world largely to be discovered, will not be considered). Likewise, let's bear in mind that there are other taxonomic families of viruses besides the *Herpesviridae* family, like the *Poxviridae* (e.g., smallpox and myxomatosis), *Flaviviridae* (e.g., yellow fever and dengue), *Orthomyxoviridae* (e.g., influenza), *Picornaviridae*

(e.g., foot-and-mouth disease, polio, and hepatitis A), *Reoviridae* (e.g., BT and African horse sickness), etc., and for each family of viruses one can reason about in the same way. In light of this example, a first conclusion is that we only know a fraction of the viral pathogens that actually exist. To these, we must add the non-pathogenic viruses that circulate silently, which obviously are less known, and which probably exist in far greater numbers and variety than their pathogenic counterparts. The complexity of viruses of plants, bacteria, fungi, and parasites is not lower than that of animal viruses. This gives a rough idea of the real complexity of the virus world in which only a small part is known.

THE GENESIS OF INFECTIOUS DISEASES

Despite the distress and alarm that the emergence of a new infectious disease causes, the fact is that organisms change over time in adaptive processes that determine their evolution, and this implies the emergence of new infectious diseases and the disappearance or change of the existing ones. These processes are therefore normal and expected to occur with some frequency, as a result of the high generation rates and enormous capacity for mutation and adaptation exhibited by microorganisms in general, and viruses in particular.

The term *emerging infectious diseases* applies to those diseases in which any of the following situations is applicable: (1) a known infection spreading to a new geographic area or population, (2) a new infection that occurs as a result of evolution or change of an existing pathogen, and (3) a previously unknown disease or pathogen that is first diagnosed (Brown, 2004; OIE, 2011). Factors determining the emergence of an infectious disease are largely unknown, although they are related to the adaptation of infectious agents to new species (Lederberg, 1997) and/or to the concomitant appearance of changes in the environment that offer new opportunities for pathogens to thrive (Smolinski et al., 2003). With regard to adaptation to new species, first it is worth reminding that every virus has its own "host range," that is, the variety of species susceptible to be infected by a particular virus. There are, on one hand, viruses with a broad range of hosts, for example, West Nile virus (WNV), that is capable of infecting hundreds of species of birds, as well as many species of mammals, reptiles, and amphibians (McLean et al., 2002), and, on the other hand, viruses with a narrower range of hosts such as, for instance, classical swine fever virus, which only infects suidae (pigs and wild boar; OIE, 2008), or the aforementioned herpesviruses, each of which usually infect specifically one or a few related species (Pellet and Roizman, 2007).

Two cases can be distinguished with regard to pathogen adaptation to new host species: in the first, the pathogen completes its cycle and survives normally in a given species which acts as reservoir host, but occasionally infects other(s), causing disease in these "incidental hosts." This occurs in a number of zoonoses. A good example is given by highly pathogenic avian influenza virus subtype H5N1 of Asian origin (H5N1 HPAIV), now present on three continents. Different wild birds act as reservoir hosts for this virus, which affects severely different species of domestic poultry, causing enormous losses to the poultry industry (Alexander, 2000). However, besides birds, it occasionally affects some species of mammals, including humans (causing a zoonosis), cats, and mustelids like ferrets and martens, in which it is extremely pathogenic. Nevertheless, transmission of H5N1 HPAIV between individuals in mammal species is not effective at all (Imai and Kawaoka, 2012), a fact that luckily prevents by now a possible pandemic of disastrous consequences for humankind. In the second case, the adaptation to new species is more "genuine" in the sense that the pathogen has indeed crossed the "species barrier," that is, has established a complete cycle of transmission in a new species. It can be assumed that all viruses currently known went through a process like this in their past evolution to adapt to the species which are their current natural hosts. Influenza viruses themselves were adapted to their avian hosts in remote times. Some of them gave rise, through analogous adaptation processes, to the current swine, equine, and human influenza viruses, which are able to complete an infectious cycle in these species, independently of birds (Suarez, 2000). Among the viruses that have undergone a process of adaptation to a new species, it is worth reminding the case of swine vesicular disease virus, which causes an economically important disease in pigs (Escribano-Romero et al., 2000). This virus is closely related genetically to Coxsackie virus B5, a human enterovirus, and current evidence suggests that it arose as a result of a relatively recent adaptation from human to swine (Jimenez-Clavero et al., 2005a), providing a good example that adaptations and crossing the species barrier can go either to or from humans, and that the human being is just one more species in this regard. Other viruses that have recently emerged to affect new species are several variants of the genus Henipavirus, infecting bats in Southeast Asia (Nipah virus) and Oceania (Hendra virus; Eaton et al., 2006). In recent years an increase in fatal cases by these viruses in humans and livestock (pigs and horses) has been observed, but an inefficient transmission between individuals of these species has prevented further spread in the human and/or livestock populations. However, in a recent outbreak of Nipah virus occurring in Bangladesh it appeared that an efficient transmission occurred between humans (Gurley et al., 2007). Finally, it is important to note that there is probably a species barrier crossing after every first diagnosis of a viral emerging disease, even though the natural reservoir might remain unknown. In 2003, the first cases of what appeared to be a new disease entity with fatal consequences for affected humans, were diagnosed in Hong Kong. It received the name of severe acute respiratory syndrome (SARS) and caused a great social concern worldwide, particularly when clusters of cases were detected in up to a dozen countries in three continents around the world, although a rapid response prevented further spread. The causative agent of this disease, named SARS virus, was identified during earlier investigations (Drosten et al., 2003; Kuiken et al., 2003), but its natural host remained unknown until *Rhinolophidae* (horseshoe bats) species were identified as reservoir hosts (Li et al., 2005; Cui et al., 2007).

INFLUENCE OF THE ENVIRONMENT IN INFECTIOUS DISEASES

The environment of viruses is constituted essentially by the cells they infect. Viruses must parasitize cells to ensure their propagation. Outside cells, viruses lack of activity, although to allow transmission to another individual they need to survive outside the cell. The way a virus survives in the environment is the result

of an adaptation that largely determines how it is transmitted. Viruses can be transmitted through direct contact between individuals, or through other ways such as, for instance air, water, feces, body fluids, food, or fomites. In some cases, transmission needs the participation of other living organisms, so-called vectors, in which the virus is equally propagated. These vectors are often blood-sucking insects that inoculate the infectious pathogen through their bites, thus spreading the infection in a population. A virus may preferably use a single transmission route, but viruses using more than one way of transmission are frequent.

The progress of a viral infection in a population is a multifactorial process, depending on a range of both biotic and abiotic factors. These factors and their influence on the development and transmission of the infection at the population level constitute the eco-epidemiology of an infectious disease. There is a strong relationship between the route of transmission and the eco-epidemiology of a given infectious disease. For example, the spread of infections that are transmitted by direct contact largely depends on the population density, which determines the distance between infected and susceptible individuals. For airborne infections, temperature, humidity, and wind can have a significant influence on the progress of the epidemic. Waterborne and foodborne viruses are usually highly resistant to adverse environmental conditions. A particular case of this type of transmission is represented by the fecal–oral route (often water, food, or objects are contaminated by fecal waste). Viruses that are transmitted through this route have a characteristic resistance to low pH, allowing them to pass through the animal's digestive tract, overcoming the natural barrier that represents the acid secretion of gastric parietal cells. Often, these viruses produce diarrhea, thus being shed in large amounts and returning to the environment, where they can remain infectious for a variable period (up to several months in some instances), depending mainly on environmental temperature (the colder the longer), but also on the presence of salts, organic matter, moisture, solar radiation, etc., until they reach another host and begin the infectious cycle again (Jimenez-Clavero et al., 2005b).

In the case of vector-borne diseases, in addition to pathogen and host, infectious cycle requires a third player: the vector. This fact results in a more complex eco-epidemiology. Habitats of arthropod vectors depend on a number of environmental conditions, including range of temperature, humidity, water availability, etc. Arboviruses (i.e., viruses transmitted by arthropod vectors), are distributed necessarily in areas where populations of competent vectors (i.e., vectors which are able to spread the disease to new hosts) are abundant enough. Each arthropod vector species occupies a particular ecological niche within specific environmental conditions, so that its distribution can be greatly affected by changes in temperature, rainfall, humidity, plant coverage, etc. (Randolph and Rogers, 2010; Weaver and Reisen, 2010).

In summary, environmental variations constitute an important factor in the occurrence and spread of infectious diseases in general. This is particularly important for arthropod-borne diseases, where environmental changes can influence both their geographic range and the risk of introduction (Sutherst, 2004; Gould and Higgs, 2009; Tabachnick, 2010).

EMERGING INFECTIOUS DISEASES AND GLOBAL CHANGE

The Planet Earth is in continuous change. Environmental changes are driven by both abiotic and biotic factors. Of these, impact caused by humans has gained importance as the human population has risen, from the local level to reach a global scale (Meadows et al., 1972). Global change is defined as the impact of human activity on the fundamental mechanisms operating in the biosphere (Duarte, 2006). Global change comprises not only impacts on climate, but also on the water cycle, land use, biodiversity loss, invasion of alien species into new territories, introduction of new chemicals in Nature, etc. The influence of global change on emerging infectious diseases has been the subject of different revisions (Fayer, 2000; Weiss and McMichael, 2004; Wilcox and Gubler, 2005; Patz et al., 2008). As noted above, environmental changes largely determine the evolution of many emerging infectious diseases, most notably arthropod-borne ones (see reviews by Sutherst, 2004; Gould and Higgs, 2009; Tabachnick, 2010), Therefore, climate changes will impact necessarily on these diseases (Rosenthal, 2009). However, there are many other driving factors – not only those related to climate changes – that have or have had a powerful influence on the occurrence or change of many infectious diseases. **Table 1** shows some of these drivers, identified as relevant in the emergence and re-emergence of infectious diseases in a recent study (Woolhouse and Gowtage-Sequeria, 2005). It is not the purpose of this article to review systematically all these factors, which have been reviewed elsewhere (Smolinski et al., 2003; Woolhouse and Gowtage-Sequeria, 2005) but to briefly expose some examples, in order to highlight their relative importance in the emergence of infectious diseases in an overall context of globalization.

For instance, intercontinental transport by air makes it possible to move persons and goods thousands of miles away within few hours. For a person who has just acquired an infection it is possible to arrive to destination even within the incubation period,

Table 1 | Main categories of factors associated to the emergence and re-emergence of human pathogens. Seventy-five percent of them are zoonotic (from Woolhouse and Gowtage-Sequeria, 2005).

Rank*	Driver
1	Changes in land use or agricultural practices
2	Changes in human demographics and society
3	Poor population health (e.g., HIV, malnutrition)
4	Hospitals and medical procedures
5	Pathogen evolution (e.g., antimicrobial drug resistance, increased virulence)
6	Contamination of food sources or water supplies
7	International travel
8	Failure of public health programs
9	International trade
10	Climate change

**Ranked by the number of pathogen species associated with them (most to least).*

and develop (and transmit) the disease upon arrival, resulting in an "imported disease." This not only applies to people, but occurs similarly as a result of trade of live animals and their products, an important economic activity worldwide, which is subjected to strict regulations (OIE, 2011) that must be implemented in coordination with all countries, precisely to prevent the spread of infectious diseases harmful to livestock, which in this context are called "exotic" or "transboundary" diseases. However, these rules have been insufficient to halt the spread of many infectious animal diseases of tremendous economic impact, partly because control and surveillance systems do not always work effectively. As an example of what an effective control can achieve, it is worth to mention what constituted the first detection in Europe of highly pathogenic avian influenza virus H5N1 from Asia, which occurred in 2004 at a border checkpoint in the airport of Brussels (Belgium). Customs officers detected in the luggage of a traveler from Thailand two mountain hawk eagles (*Spizaetus nipalensis*) alive, apparently brought as a gift. The two birds were found to be infected with the virus (Van Borm et al., 2005). This case also reminds that, in addition to the above, illegal trafficking of animals, including exotic species, must also be considered as a relevant factor involved in importation of transboundary diseases. A good example was given in 2003 by the occurrence of outbreaks of monkeypox in humans in the U.S., originated as a result of illegal import of infected exotic rodents from Ghana (Guarner et al., 2004). The disease has reached local populations of rodents (prairie dogs, *Cynomys* spp.).

Regarding arboviral diseases, the impact of trade and transport on the distribution of vectors is reflected by the global expansion of the tiger mosquito (*Aedes albopictus*), associated with trade in used tires (Reiter and Sprenger, 1987) or in *Dracaena* plants ("lucky bamboo"; Madon et al., 2002). Rain causes small pools of water inside the tires stored outdoors. This constitutes an excellent breeding habitat for this mosquito, because it mimics the hollow trunks of rainforest trees that are its natural habitat. Through transport of used tires containing *A. albopictus* eggs, this mosquito has reached a worldwide distribution. This mosquito is a competent vector of many pathogens, including dengue, yellow fever, chikungunya, Venezuelan equine encephalitis, and WNF (Paupy et al., 2009; Weaver and Reisen, 2010).

Other factors derived from human activities linked to the emergence of infectious diseases are those related to land use: a growing human population is demanding more resources necessary for its supply and welfare, particularly food, but also energy, raw materials, water, urban land, etc. New agricultural plantations and livestock grazing plots on deforested lands promote the emergence and/or spread of infectious diseases hitherto confined to the forest habitat (Smolinski et al., 2003; Woolhouse and Gowtage-Sequeria, 2005).

Deforestation and biodiversity loss are often cited as causes of increased incidence of emerging infections in certain regions. An example can be found in the emergence of Junin virus in Argentina (Charrel and de Lamballerie, 2003). Changes in water use are, nonetheless, one of the factors most clearly related to the modification of eco-epidemiological patterns accompanying emerging infectious diseases. New irrigation plans, construction of dams, etc. have a direct effect on the abundance of competent vectors for transmission of various arboviruses. The new irrigation in northwestern Australia seem to be the main cause of the recent expansion of Murray Valley fever, an emerging zoonotic arbovirus in that country (Mackenzie et al., 2004). The construction of dams may have caused an important impact on the recent emergence of Rift Valley fever in East Africa, causing highly virulent outbreaks for both man and livestock, by allowing a dramatic increase in mosquito populations involved in transmission (Martin et al., 2008).

Healthcare is another area of human activity that, somehow paradoxically, is linked in many instances to infectious disease emergence. Iatrogenic transmission of infectious diseases, through transfusions, transplants, and other medical interventions, has undoubtedly had an effect on the expansion of certain pathogens such as hepatitis C virus (Alter, 2002; Prati, 2006). Fortunately, current medical practice has reduced this risk very significantly (Alter, 2002; Prati, 2006). The administration of biologicals derived from animals or animal cell cultures, such as vaccines and therapeutic products, can also act as a vehicle for the transmission of pathogens (Parkman, 1996). Despite strict controls on each batch of vaccine for the presence of certain pathogens, there have been cases of transmission of adventitious viruses in contaminated vaccines. One of the best known cases of this kind is represented by bovine viral diarrhea virus (BVDV), a pestivirus affecting cattle. In the manufacturing process of many vaccines, cell cultures are widely employed, and fetal calf serum is used as an additive common to cell culture media. BVDV is highly prevalent in cattle and certain variants of the virus remain unnoticed in cell cultures. In some cases, batches of fetal calf serum from BVDV-infected animals were used inadvertently, resulting in viral contamination of vaccines. Some of them (those addressed to the bovine, such as vaccine against bovine herpesvirus type 1) eventually resulted in outbreaks of viral diarrhea in vaccinated cattle, which alerted for the presence of the virus (Makoschey et al., 2003). Currently, regulatory agencies, such as the European Medicines Agency, have modified their safety requirements to control specifically the risk of contamination by animal pestiviruses in drugs and vaccines.

CLIMATE CHANGE AND EMERGING INFECTIOUS DISEASES

Global warming is the rise in average surface temperature of the earth observed in the last decades (+0.6°C in the last half century; IPPC, 2007). This observation applies also specifically to Europe (EEA, 2012) and North America (Karoly et al., 2003), territories particularly concerned to WNV and BT virus (BTV) emergences, as it will be discussed later. The increase has accelerated in recent years and is expected to go faster, so that in the twenty-first century the average surface temperature of the earth is expected to rise in a range between +1.6 and +6°C, depending on the different scenarios considered (IPPC, 2007). Although still a contentious issue, most scientists now accept that human activity has contributed to the observed global warming (Oreskes, 2004), mostly through emissions of greenhouse gases produced by industrial activity and consumption of fossil fuels for transport, energy production, etc. (IPPC, 2007). The impact of global warming on the environment is the subject of numerous recent studies, based on predictive modeling scenarios depending on the

level of emissions. It is not the purpose of this article to review these models, which are described extensively elsewhere (IPPC, 2001, 2007), but to highlight some future climate trends, mainly in Europe, the scenario for some recent unusual observations regarding arboviral diseases (Purse et al., 2008; Wilson and Mellor, 2009; Zientara et al., 2009; Calistri et al., 2010; MacLachlan and Guthrie, 2010; Sotelo et al., 2011a). For this, I will rely on data from the European project PRUDENCE (Prediction of Regional scenarios and Uncertainties for Defining EuropeaN Climate change risks and Effects; Christensen, 2005). **Table 2** summarizes the most marked trends for Europe in the next 100 years according to this study. In addition to an increase in mean annual air temperature, predicted to be between 1.4 and 4.5°C, depending on the areas (the highest rise is expected to occur in the Iberian Peninsula), the study suggests there will be more droughts, more wildfires, heat waves will be more frequent, and all this will be particularly noticeable in southern Europe. Winters will become milder, and this will occur more rapidly in northern latitudes. As a result, frost will decrease, and minimum temperatures will rise. These circumstances favor living cycles of certain arthropod vectors, which in these conditions will be able to overwinter more easily in latitudes and areas beyond their current geographic ranges. Other remarkable trends include rising temperatures and unusually hot summers, but also greater interannual variability, particularly in central Europe, which will make adaptations more difficult. Waves of extreme temperatures (both cold and heat) will become more frequent. With regard to rainfall, the tendency is to increase in northern Europe and decrease in the South. Therefore, increasingly severe droughts are expected in southern Europe, with a considerable impact on agriculture and water resources. However, torrential rains, especially in summer, will become more common throughout Europe, causing flooding to occur more frequently. Snow will become rarer. River flows will decrease in the South, and increase in northern Europe. Extreme wind events (hurricanes, cyclones) will also be more frequent.

Table 2 | Long-term climatic trends in Europe, based on data from PRUDENCE* (Prediction of Regional scenarios and Uncertainties for Defining EuropeaN Climate change risks and Effects; Christensen, 2005).

Parameter	Trend
Air temperature	Rise in air temperature
Winter temperature	Milder winters
Seasonal variation	Less seasonal variation
Drought stress	Higher drought stress
Forest fires	More forest fires
Night-time temperatures (frost)	Warmer night-time temperatures
Heat waves	More days of extreme heat and heat waves in summer, and more year-to-year variability
Very hot summers	Unusually hot summers more frequent
Rainfall	Rainfall: increase in the North and decline in the South
Snow	Snow: decline
Floods	Floods: more frequent
Strong winds (hurricanes/cyclones)	Higher frequency of hurricanes/cyclones with extreme strength

**PRUDENCE was a project funded by the European Commission under its fifth framework programme. It had 21 participating institutions from a total of 9 European countries. For further information, see PRUDENCE: http://prudence.dmi.dk/*

How will these climate changes impact on emerging infectious diseases? The consequences of global warming are expected to be diverse and highly variable in different geographical locations. For example, as noted above, the effects of climate change in Europe will differ significantly between northern and southern latitudes, and these differences could even be greater at the local level, although they will not be easily distinguished from weather variations. These changes may affect disease emergence not only through direct effects on populations of vectors, reservoirs, and hosts, but also indirectly, through, for instance, induced changes in human activities. For instance, in a scenario like that depicted in the predictive climatic models above cited for southern Europe, lower rainfall, increased temperature, and reduced water availability will have important consequences not only on agricultural activities which, as already noted, have a major impact on infectious diseases, but for example on tourism, which is the main source of wealth in these regions. A significant decline in economic activity could lead to a decrease in population, food demand, and hence livestock. In this changing context, plenty of uncertainties, it is difficult to predict an overall trend: some infectious diseases will probably emerge, some existing ones will spread, and others may disappear. Even in specific cases a disease may be exacerbated transiently and disappear later. For example, the cease of agricultural production in irrigation areas, which can likely occur in a scenario of extreme drought, could lead at long-term to local extinction of mosquito-borne diseases, whose breeding habitat depends on the infrastructure dedicated to this type of agriculture. However, disuse and neglect could favor the accumulation of organic matter in certain points of these infrastructures, turning them into optimal habitats for breeding of vectors, something that the normal maintenance of the network usually avoids. This would likely lead to a transient increase in the incidence of certain diseases, although prolonged drought would eventually lead these vectors to extinction.

The effects of climate change are beginning to be perceived, and at the same time remarkable changes in the geographic range and incidence of some infectious diseases are being observed, suggesting some kind of relationship, which is difficult to ascertain. As remarked above, climate change is not the only factor influencing infectious disease emergence, as there are other components in the global change which have also important effects on disease emergence. The following sections deal with two of the emerging arboviral diseases of animals that have changed more radically their epidemiological patterns in recent years: BT and WNF (Zientara et al., 2009). Climate, weather and other factors related to global change which are potentially involved in disease emergence will be examined specifically for each of these two diseases.

BLUETONGUE

THE VIRUS AND THE DISEASE

Bluetongue is a non-contagious infectious disease of ruminants (reviewed in MacLachlan, 1994; Wilson and Mellor, 2009; MacLachlan, 2011). Mainly affects sheep, particularly some selected European breeds. Cattle and goats act as reservoirs of the virus, which infect these animals usually producing a milder disease, often asymptomatic. The disease can affect also wild ruminants, severely in some species. BT is an economically important disease for livestock, included in the list of notifiable diseases to the OIE (World Organization for Animal Health) and is therefore subject to strict regulations regarding the trade of animals and their products (OIE, 2011), causing severe economic losses in the affected countries.

Clinical manifestations range from subclinical infection to acute illness that can be fatal. Expression of BT disease reflects a variety of virus, host, and vector factors (MacLachlan, 2004). Clinical signs in sheep include pyrexia, tachypnea, nasal discharge, and lethargy. Pathology is characterized by generalized edema, hemorrhage, especially in lymph nodes, lungs, heart, and skeletal muscle and necrosis on the surface of the oro-nasal mucosa and gastro-intestinal tract (reviewed in MacLachlan et al., 2009).

The causative agent, BTV, belongs to the family *Reoviridae*, genus *Orbivirus*, has a genome of segmented double-stranded RNA (dsRNA), contained in a non-enveloped capsid shell (reviewed in Schwartz-Cornil et al., 2008). Its main route of transmission is through the bite of midges of the genus *Culicoides*. The BTV genome is divided into 10 dsRNA segments which encode seven structural (VP1–VP7) and four non-structural (NS1, NS2, NS3, and NS3a) proteins. BTV is closely related to other orbiviruses such as African horse sickness virus (AHSV) and epizootic hemorrhagic disease of deer virus (EHDV), to which it shares not only physico-chemical characteristics but also eco-epidemiology and vector type, although differing in host range. As for now, 26 serotypes of BTV have been described (two of them very recently; Hofmann et al., 2008; Maan et al., 2011), with no cross-protective immunity between them. Within each serotype there is also a great variability, largely facilitated by the segmented genome, allowing genetic "reassortment." This enables these viruses to generate multiple variants that may differ in important characteristics or phenotypes, such as pathogenesis, host range, vector competence, or transmissibility (MacLachlan, 2004).

Viremia is transient, starting at 3–5 days after infection in sheep somewhat later in other ruminants, peaking at about 7–10 days, declining slowly thereafter (Darpel et al., 2007). The duration of viremia is variable, depending on the species affected, and can last for 5–6 weeks in sheep, up to 8 weeks in cattle (MacLachlan et al., 1994; Bonneau et al., 2002). Virus detection in blood is commonly used as a proof of infection in diagnostic tests based in specific reverse transcription-polymerase chain reaction (RT-PCR) methods (Jimenez-Clavero et al., 2006). The host's immune system responds to infection by generating serotype-specific neutralizing antibodies, which are widely recognized as key factors in the long-term protective response against infection (Schwartz-Cornil et al., 2008). Seroconversion occurs between 1 and 2 weeks after infection. Serogroup-specific antibodies are directed primarily to the VP7 protein of the virus capsid, which are commonly detected by enzyme immunoassay (ELISA) employing recombinant VP7 antigen (Afshar et al., 1992; Mecham and Wilson, 2004), while serotype-specific neutralizing antibodies (which are detected by specific neutralization tests) recognize epitopes in the VP2 capsid protein (DeMaula et al., 1993; Pierce et al., 1995). The presence of specific antibodies in serum samples is another common diagnostic test, which indicates that infection has occurred, or that the animal has been vaccinated. There are live attenuated and inactivated vaccines, which are specific for each serotype.

BLUETONGUE ECO-EPIDEMIOLOGY

Survival and transmission of BT in an area requires the presence of *Culicoides* vectors. To be transmitted, BTV must infect a competent *Culicoides* vector. In this process the vector acquires the infection by feeding on blood from a viremic host, then the virus multiplies and disseminates throughout the vector body, reaching the salivary glands, ready to be inoculated in the next host after biting on a further blood feed. There is no transovarial transmission of the virus. There are approximately 1,500 known species of *Culicoides* midges, of which about 50 have been shown to be competent for BTV transmission (Wilson and Mellor, 2009). In different endemic areas, BTV is transmitted through one or a few distinct local *Culicoides* species. For example, in North America this species is *Culicoides sonorensis*, while in southern Europe and Africa *Culicoides imicola* is the dominant vector. The distribution and abundance of *Culicoides* species involved in BTV transmission matches with the distribution of the disease in endemic areas (Mellor and Wittmann, 2002). It also depicts the maps of areas at risk for its introduction. Similarly, seasonality strongly influences vector populations throughout the year, and this determines the periods of occurrence of disease cases (Mellor and Wittmann, 2002). For instance, in temperate zones of the northern Hemisphere most cases of disease occur between August and November, coinciding with the period of greatest abundance of vectors. Among the key factors linking weather to BTV epidemiology, temperature has a crucial influence on vector survival, which, as noted above, directly affects disease occurrence, transmission, and spread. For example, the mean survival time of a *Culicoides* midge depends on the temperature, i.e., survive longer at lower temperatures (Veronesi et al., 2009). By contrast, higher temperatures promote breeding and feeding of the vectors, which increases virogenesis and transmission of BTV (Wilson and Mellor, 2009). The optimal range of temperature lies between 13 and 35°C. However, excessively high (above 40°C) or low (<0°C) temperatures are lethal in a short period of time. Between 0 and 13°C *Culicoides* midges remain in a state of dormancy that allows them to survive until more favorable temperatures allow resuming their activity. Relative humidity is also a key factor in *Culicoides* life cycle (Wittmann et al., 2002). The mentioned temperature ranges are valid only above a minimum relative humidity. Winter survival (overwintering) is crucial for the maintenance and consolidation of the presence of BTV in a geographic area. Midge survival is favored by mild, frost-free, winters. Wind is another important factor that links weather to BTV eco-epidemiology. Wind blows can drag swarms of *Culicoides* midges, and bring them hundreds

of kilometers away. This mechanism has been widely recognized as an important way *Culicoides* (and BTV, if present in the vectors) spread, bridging distances up to 700 km under favorable conditions (Carpenter et al., 2009; MacLachlan and Guthrie, 2010). This form of dispersal is the most likely way BTV has reached the shores of southern Europe from Africa in many occasions in the last years (Wilson and Mellor, 2009).

As noted above, though primarily pathogenic in sheep (particularly in some selected breeds) BTV can infect a variety of ruminants, where the disease often remains asymptomatic. For the transmission to occur the virus must replicate in the host to reach a minimum level of viremia which enables it to be ingested in sufficient amount in a blood feed of a *Culicoides* vector to be able to replicate in it. Camels also seem to act as reservoirs (Batten et al., 2011), possibly playing a role in the spread of the disease by facilitating the virus to get through the "sand barrier" represented by the Sahara desert, which stands between the tropics and subtropics (where BTV is endemic), and North Africa and Europe where it causes epizootics periodically. This role for camels not only for BT, but also for other epizootic arboviral diseases is supported by recent serological evidence (El-Harrak et al., 2011; Touil et al., 2012). South American camelids are susceptible to the infection, but develop only a mild form of the disease (Schulz et al., 2012). A wide range of wild ruminant species are susceptible to BTV infection, but only a few suffer from severe disease, including white-tailed deer (Falconi et al., 2011) and mouflon (Fernandez-Pacheco et al., 2008; Lopez-Olvera et al., 2010). The role of wild ruminants in the eco-epidemiology of BTV is less known.

BLUETONGUE: PAST AND PRESENT

Bluetongue was first described in South Africa in the eighteenth century, affecting imported merino sheep (MacLachlan and Guthrie, 2010). Outside Africa, BTV was detected for the first time in Cyprus in 1943. Shortly after it was found in North America, Middle East, Australia, and Asia, as an endemic disease affecting tropical and subtropical areas with epizootic incursions in temperate zones where the presence of competent vectors allowed its transmission (MacLachlan, 2004). Traditionally, its natural geographic range was considered to be located between latitudes 35°S and 40°N. In Europe, regions with suitable conditions for incursions of BT were the south of the Iberian and Italian Peninsulas and some islands of the Aegean Sea, where climate and presence of competent vectors, along with the proximity to endemic areas (Middle East and Africa) contributed to create an area at high risk of entry of the disease (reviewed in Mellor and Wittmann, 2002; Mellor et al., 2008). In 1956, BT burst into the Iberian Peninsula from Africa, causing major losses in sheep. However, apart from this episode and some subsequent incursions in Cyprus and the Greek islands of Lesbos and Rhodes, Europe had remained free of this disease, which was considered "exotic" in this continent. This situation began changing after 1998, with increasingly frequent outbreaks in the Mediterranean islands and in southern continental Europe (Mellor and Wittmann, 2002; Mellor et al., 2008). A parallel upsurge was observed meanwhile in the Middle East and the North of Africa. Between 1998 and 2001, BT outbreaks were declared in the territories of Greece, Bulgaria, Turkey, Macedonia, Serbia, Croatia, Montenegro, Bosnia-Herzegovina, Albania, Italy, France, and Spain. BTVs involved belonged to at least five different serotypes (1, 2, 4, 9, and 16). Four of them (1, 2, 9, and 16) came from the East (Middle East), and two more introductions from the South (Africa) involved serotypes 2 and 4 (Mellor et al., 2008). Even during this period the disease showed some expansion to the North, breaking the northern limit of the disease in Europe, represented by latitude 40°N, with some outbreaks occurring near 45°N in the Balkans. Since then, BT is considered an emerging disease in Europe (Wilson and Mellor, 2009). But the quantum leap in the epidemiology of the disease in Europe was to take place in August 2006 when an outbreak of BT was declared in the Netherlands at the Maastricht region. The virus isolated in this outbreak belonged to serotype 8, and was the first occurrence of this serotype in Europe (Wilson and Mellor, 2009). The disease has since spread with unprecedented speed and virulence, affecting first Holland, Belgium, Luxembourg, France, and Germany, and expanding rapidly into the UK, Austria, Czech Republic, Switzerland, Denmark, Italy, and Spain (Saegerman et al., 2008). Since then the virus showed its ability to overwinter in unusually high latitudes, remaining present in most of Europe, and even spreading to other European countries such as Hungary, Norway, and Sweden. The number of outbreaks produced by this BTV8 in Europe since its first detection in 2006 is 89,136 (EUBTNET, 2011). The incidence of the disease peaked in 2007 with 50,479 outbreaks declared. Massive vaccination campaigns, initiated in 2008, contributed largely to control the disease, so that the number of outbreaks were drastically reduced since 2009, and practically disappeared in 2010 and 2011 (EUBTNET, 2011). The epidemic of BTV8 in Europe was unusual not only because of the latitude in which it occurred, immediately suggesting that vectors involved in transmission should be different from those "expected" and more common in southern Europe (*C. imicola*). In fact, it appears that the BTV8 responsible for the epidemic was adapted to midge species more common in northern Europe, such as those of the *C. obsoletus* complex (Wilson and Mellor, 2008). Another remarkable characteristic of this virus is its special virulence and pathogenicity, which also affected cattle. In addition, this virus seems able to be transmitted to the fetus through the placenta, a feature that had only been observed in infections with attenuated BTV vaccine strains, and never in BTV field strains (MacLachlan et al., 2009). These unusual characteristics illustrate perfectly the principle of emerging viruses arising from an underlying pool of varying viruses, with new properties that may result in new ecological and epidemiological patterns of a viral disease.

But recent occurrence of BT in Europe has not been limited to the, otherwise outstanding, BTV8 epizootic. Other serotypes have reached the shores of the southern European countries and caused severe harm to livestock in these years (Mellor and Wittmann, 2002; Saegerman et al., 2008). Of particular relevance were the incursions of BTV serotype 4 in Spain, Italy, and the western Mediterranean islands in 2003–2004, and the entry of a particularly highly pathogenic strain of BTV serotype 1 first in Sardinia (Italy) in 2006, and then in Spain in 2007, which extended rapidly to Portugal and France, causing also many thousands of outbreaks in these countries. The same strategy adopted to fight against the BTV8 epizootic, based on vaccination campaigns, was used

simultaneously against BTV1 and BTV4, with the same success at reducing disease incidence to almost negligible (Wilson and Mellor, 2009). Currently, new incursions of different BTVs continue threatening Europe, a continent that will no longer be considered out of reach of BT.

BLUETONGUE: FUTURE TRENDS

In the past 15 years very significant qualitative changes have occurred with regard to the epidemiology of BT, which has reached unprecedented severity in Europe (Wilson and Mellor, 2008, 2009; Zientara et al., 2009). Some important trends observed in this period are: (1) a major increase in the frequency of BTV incursion events in Europe, (2) a geographic expansion of the disease, reaching latitudes close to parallel 55°N, i.e., 15° beyond the classic northern distribution limit for BT in Europe, (3) an ability to overwinter in locations *a priori* unsuitable for known competent vectors, (4) changes in some strains with regard to their pathogenicity (BTV1 strain is highly pathogenic in sheep; unpublished observation) and host range (BTV8 strain is pathogenic in European bovine), and (5) new ways of transmission (BTV8 strain can be transmitted transplacentally, affecting fetuses and newborn cattle).

What is the explanation for all these changes? Undoubtedly, the rising temperature in Europe in recent years (EEA, 2012) is a factor having a major influence in this situation (Purse et al., 2008; Wilson and Mellor, 2008). Particularly, unusually mild winters that occurred in 2006–2007 and 2007–2008 in the affected areas have probably favored the habitat of certain *Culicoides* species that could act as vectors in these regions, promoting their geographical expansion. Some studies indicate that the distribution of *C. imicola*, the principal vector of BTV in southern Europe, has undergone a geographic expansion, reaching areas where it had never been observed, for example the North of Spain (Goldarazena et al., 2008), although some authors have not observed expansions of this vector in Italy in recent years (Conte et al., 2009). Nevertheless, in northern and central Europe, competent vector species must be different, because *C. imicola* is not present in these areas (Saegerman et al., 2008). Other likely competent vectors for BT which are present in Europe are *C. obsoletus*, *C. pulicaris*, *C. scoticus*, and *C. dewulfii* (Saegerman et al., 2008). For some of these vector species, their vectorial capacity for BT transmission was considered secondary or even irrelevant, as compared to *C. imicola*. However, changes in circulating viruses involving adaptations to some of these vectors may have improved this capability. On the other hand, it is a well-known fact that the temperature can affect vector competence, effectively increasing it above a certain temperature threshold (Wilson and Mellor, 2009).

But climate change alone is insufficient to explain the whole picture regarding the current rise of BT in Europe. First, I have already mentioned how the wind can drag swarms of infected *Culicoides*, spreading the disease to considerable distances, and that this is likely route of entry of BT in southern Europe (Ducheyne et al., 2007), though animal movements could also play a role. I have also mentioned how camels could act as carriers for these viruses from sub-Saharan Africa to North Africa, where BT is often detected some time before affecting Europe (Touil et al., 2012). But how was BTV8 introduced into northern Europe? Unlike other strains that have invaded Europe in recent years, this BTV-8 strain has not previously been detected in the North of Africa or the Middle East. Among the few sequence data available to date on relevant BTV strains, it has been shown that the most similar virus found is a strain from sub-Saharan Africa (Nigeria) isolated in 1982 (Maan et al., 2008). This "gap" in space and time indicates clearly that more sequence data from relevant BTV sources are needed to draw any conclusion about the origin of the European BTV-8 strain. As already discussed in the previous section, the trade of animals and animal products, as well as of wildlife (either legal or illegal), may be behind many accidental introductions of infectious diseases, and this hypothesis should be taken into account in this case, although thus far, no evidence has been obtained neither for nor against it (Mintiens et al., 2008). Other questions remaining where climate change does not provide a full explanation are (1) the virus ability to overwinter in extremely cold conditions such as those found in certain areas of northern Europe, (2) the role that vertical transmission may have on its survival, and (3) phenotypic changes perceived in some recent BTV variants, which are more pathogenic and/or affect other species such as cattle. More research is needed to find answers to these questions.

Bluetongue is no longer a tropical disease, exotic, typical of warm countries, but it has come to stay in Europe. This radical change cannot be understood without taking into account global changes that are taking place in the world of which climate change plays an important role. Current knowledge indicates that this climatic change will become increasingly intense in the future, which, at least in theory, would favor the spread of BT, although other factors may also modulate this trend.

WEST NILE FEVER/ENCEPHALITIS

THE VIRUS AND THE DISEASE

West Nile virus is the etiological agent of an emerging zoonotic disease whose impact on animal and public health is considerable, being the most widespread arbovirus in the world today (reviewed in Hayes et al., 2005a; Kramer et al., 2008; Brault, 2009). A percentage of WNV infections result in severe encephalitis, and it is a communicable disease both for human and animal health. WNV taxonomically belongs to the family *Flaviviridae*, genus *Flavivirus*. Virions are spherical in shape, about 50 nm in diameter, and consist of a lipid bilayer that surrounds a nucleocapsid that in turn encloses the genome, a unique single-stranded RNA molecule, which encodes a polyprotein that is processed to give the 10 viral proteins. Of them, three (C, E, and M) form part of the structure of the virion, and the rest (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) are so-called "non-structural" and play important roles in the intracellular processes of replication, morphogenesis, and virus assembly. Inserted into the lipid bilayer are two proteins, E (from "envelope") and M ("matrix"), which participate in important biological properties of the virus, such as its host range, tissue tropism, replication, assembly, and stimulation of cellular and humoral immune responses. E protein contains the major antigenic determinants of the virus.

As far as we know, there are no serotypes of WNV, but two main genetic variants or lineages can be distinguished, namely lineages

1 and 2. While the former is widely distributed in Europe, Africa, America, Asia, and Oceania, the second is found mostly restricted to Africa and Madagascar, although it has recently been introduced in Central and Eastern Europe (Bakonyi et al., 2006; Platonov et al., 2008) and has further extended to southern Europe (Bagnarelli et al., 2011; Papa et al., 2011). In addition, other viral variants closely related phylogenetically to WNV have been described, which are different from lineages 1 and 2, and have been proposed as additional WNV lineages. One of them, known as "Rabensburg virus," isolated form mosquitoes in the Czech Republic in 1997, shows low pathogenicity in mice (Bakonyi et al., 2005). Similarly, other viruses closely related to WNV have been isolated in India (Bondre et al., 2007), Russia (Lvov et al., 2004) Malaysia (Scherret et al., 2001), and Spain (Vazquez et al., 2010). All these viruses have been proposed to represent different genetic lineages of WNV. Except for the Indian variant, which has been involved in outbreaks of encephalitis in humans, the rest are of unknown relevance for animal and human health.

West Nile fever/encephalitis is a disease transmitted mainly by mosquitoes, while wild birds are its natural reservoir. WNV is capable of infecting a wide range of bird species. Nevertheless, birds were considered less susceptible to the disease until the recent epidemic of WNV in North America, affecting many species of birds lethally, made to re-examine this concept (Komar et al., 2003). Occasionally it may affect poultry species, mainly geese and ostriches. Other domestic birds like chickens and pigeons, are susceptible to infection but do not get sick, and are often used as sentinels for disease surveillance. In addition to birds, WNV can also affect a wide range of vertebrates species, including amphibians, reptiles, and mammals, and it is particularly pathogenic in humans and horses, which act epidemiologically as "dead end hosts," that is, they are susceptible to infection but do not transmit the virus (McLean et al., 2002; Kramer et al., 2008).

The first case of WNF was described in Uganda (West Nile district, hence the name of the virus) in a feverish woman, from whose blood the virus was first isolated in 1937 (Smithburn et al., 1940). It was considered a mild disease, endemic in parts of Africa (an "African fever"). However, since around 1950s, the occurrence of disease outbreaks with neurological disease, lethal in some cases, caused by WNV, especially in the Middle East and North Africa, made necessary to rethink this concept. In humans, the majority of WNV infections are asymptomatic, about 20% may develop mild symptoms such as headache, fever, and muscle pain, and less than 1% develop more severe disease, characterized by neurological symptoms, including encephalitis, meningitis, flaccid paralysis, and occasionally severe muscle weakness (Hayes et al., 2005b). Advanced age is considered a risk factor for developing severe WNV infection or death. The mortality rate calculated for the recent epidemic of the disease in the U.S. is 1 in every 24 human cases diagnosed (Kramer et al., 2008).

In horses (reviewed in Castillo-Olivares and Wood, 2004) neurological disease is manifested by approximately 10% of infections, and is mainly characterized by muscle weakness, ataxia, paresis, and paralysis of the limbs, as a result of nerve damage in the spinal cord. They may also suffer from fever and anorexia, tremors and muscle stiffness, facial nerve palsy, paresis of the tongue, and dysphagia, as a result of affection of the cranial nerves. A proportion of horses infected with WNV die spontaneously or is slaughtered to avoid excessive suffering. The mortality rate can vary between outbreaks. For example, in the outbreak in 2000 in the Camargue (France), 76 horses were affected, of which 21 died (Zeller and Schuffenecker, 2004). In 1996 in Morocco, a WNV outbreak affected 94 horses, of which 42 died (Zeller and Schuffenecker, 2004). Severe equine cases do not seem to predominate in older horses, as occurs in humans (Castillo-Olivares and Wood, 2004). Other mammals may also suffer from the disease. Rodents such as laboratory mice and hamsters are highly susceptible, so they can be used as experimental model of WNV encephalitis. Lemurs and certain types of squirrels appear to be the only mammals capable of maintaining the virus in local circulation (Rodhain et al., 1985; Root et al., 2006). WNV can also infect other mammals, including sheep, in which it causes abortions, but rarely encephalitis (Hubalek and Halouzka, 1999). WNV has been isolated from camels, cows, and dogs in enzootic foci (Hubalek and Halouzka, 1999). The virus has been shown to infect frogs (*Rana ridibunda*), which in turn are bitten by mosquitoes, so that the existence of an enzootic cycle in these amphibians is postulated, at least for some variants of the virus (Kostiukov et al., 1986). Outbreaks of severe WNF with high mortality have been reported in captive alligators and crocodiles, presumably transmitted through feeding of contaminated meat (Miller et al., 2003). It has been shown experimentally that WNV can infect asymptomatically pigs (Teehee et al., 2005) and dogs (Blackburn et al., 1989; Austgen et al., 2004). However, guinea pigs, rabbits, and adult rats are resistant to infection with WNV (McLean et al., 2002). Among non-human primates, rhesus and bonnet monkeys (but not *Cynomolgus* macaques and chimpanzees), inoculated with WNV develop fever, ataxia, prostration with occasional encephalitis and tremor in the limbs, paresis or paralysis. The infection can be fatal in these animals.

The virus is propagated in the reservoir hosts, resulting in a viremic phase that usually lasts no more than 5–7 days (Komar et al., 2003). The duration and level of viremia depends on the species infected (Komar et al., 2003). The detection of the virus or its genetic material in serum or cerebrospinal fluid in a laboratory test is a proof of diagnostic value (De Filette et al., 2012). The virus is evidenced by virological (virus isolation) or molecular (RT-PCR-conventional and real-time, NASBA) techniques. In epidemiological surveillance it is useful to detect the presence of WNV in mosquitoes, for which they are homogenized and analyzed using the same methods mentioned above (Trevejo and Eidson, 2008). Specific antibodies against the virus are detectable in blood few days after infection (Komar et al., 2003; De Filette et al., 2012). Antibody detection is performed by serological tests (enzyme immunoassay or ELISA, hemagglutination inhibition or HIT) which can be confirmed by more specific serological techniques (virus-neutralization test; Sotelo et al., 2011c). Serological diagnosis of acute infection should be done by detection of IgM antibodies in serum and/or cerebrospinal fluid using an immunocapture ELISA together with the detection of an increase in antibody titer in paired sera taken one in the acute phase and the other, at least 2 weeks later (Beaty et al., 1989).

The fight against this disease is not straightforward because there are no vaccines licensed for human use, and even though

there are some available for veterinary use, they are efficacious to prevent disease symptoms and outcome at the individual level but do not prevent the spread of the infection, mainly due to the establishment of an enzootic cycle among wild birds and mosquitoes (Kramer et al., 2008; De Filette et al., 2012). Control methods are mainly based on prevention and early detection of virus spread through epidemiological surveillance and targeted application of insecticides and larvicides (Kramer et al., 2008).

WEST NILE ECO-EPIDEMIOLOGY

West Nile virus is maintained in nature in an enzootic ("rural" or "sylvatic") cycle between its natural reservoirs, wild birds, and ornithophilic mosquitoes acting as vectors. WNV is a generalist pathogen, as exemplified by the fact that in North America the virus has been found infecting 284 different species of birds and 59 species of mosquitoes, although of these not more than 10 have a relevant role as vectors (Hayes et al., 2005a). This wide host and vector ranges probably facilitate the colonization of vast areas (Kramer et al., 2008). Primary enzootic vectors are most often mosquitoes belonging to the genus *Culex*, but the virus can be transmitted by mosquitoes of other genera (e.g., *Aedes* sp.) Transovarial transmission of the virus has been shown to occur in at least some *Culex* species (Mishra and Mourya, 2001), and this may provide an overwintering mechanism in very cold climates. However, it is not clear whether transovarial transmission takes place as effectively as to allow overwintering. The virus has been repeatedly isolated from ticks, and transmission through tick bites has been shown experimentally (Abbassy et al., 1993; Hutcheson et al., 2005; Formosinho and Santos-Silva, 2006). This has led to the postulation of a role for ticks in overwintering, though this issue needs further studies to be ascertained. WNV can also be transmitted in the absence of vector, using different routes. Firstly, there is experimental evidence of direct transmission in poultry (geese; Banet-Noach et al., 2003). Secondly, carrion birds found infected during periods of absence of vector suggests oral transmission, likely through feeding on contaminated carrion (Garmendia et al., 2000; Dawson et al., 2007). In humans, WNV transmission routes such as intrauterine, lactogenic, and iatrogenic (through transfusions and transplants), are well documented (Hayes et al., 2005b). Occupational exposure of laboratory workers handling contaminated samples has also led to some cases of disease, mostly through cuts or punctures with contaminated material (Hayes et al., 2005b; Venter et al., 2010).

Birds are the natural reservoirs of WNV. Once infected, they are able to replicate the virus in sufficient quantity to enable its transmission to a blood-sucking mosquito. This is not the case of mammals, in general poorly effective as hosts for the virus (Blitvich, 2008). Nevertheless, mammals can be susceptible to the disease to varying degrees. Birds maintain the virus in a rural cycle. In some instances a spillover from this cycle occurs which enables the establishment of a urban cycle, producing outbreaks, sometimes of epidemic character, especially in equines and humans, but also affecting susceptible birds.

West Nile virus is endemic in large parts of Africa, Australia, and India, and more recently (as discussed below) arrived to North America, where since then became endemic. In Europe, North Africa, and the Middle East the virus has produced occasional outbreaks in areas close to river basins and large wetlands where the presence of vectors (mosquitoes) and reservoirs (birds) provide the optimal conditions for the maintenance of the viral cycle. Short distance spread of the virus to neighboring territories occurs most likely by birds (not necessarily migratory) acting as carriers. The virus can occasionally be spread to long distances by wild bird migrations (Malkinson et al., 2002), although this is not likely a frequent event (Sotelo et al., 2011b), neither it explains all transcontinental translocations of the virus, and significantly, it does not explain the arrival of WNV to North America. Long distance geographic dispersal of WNV by migrating birds is a hypothesis based mainly on circumstantial evidence, such as the discovery of an infected flock of storks in Israel in summer 1998 on their migration back to Africa from central Europe (Malkinson et al., 2002). This hypothesis is supported by molecular phylogenetic studies between isolates from recent outbreaks in Europe and isolates from central Africa, suggesting that birds that migrate between continents may act carrying the virus (Charrel et al., 2003). However, although translocation of WNV (and also other flaviviruses alike, for instance Usutu virus) by bird migration is likely to occur, its frequency does not seem to be as high as to explain every WNV outbreak found in Europe. On the contrary, recent phylogenetic evidence supports that all WNV strains isolated in the western Mediterranean area since 1996 are a monophyletic group arising from a single common ancestor, a strain which could have arrived to this area in 1996 or even earlier, and since then it has been maintained in endemic circulation, evolving and spreading throughout the area (Sotelo et al., 2011b). Finally, one must not assume that the flow of WNV between Europe and Africa operates only in one direction (i.e., from Africa to Europe). The example previously mentioned on migrating storks (Malkinson et al., 2002) as well as specific studies on bird migrations and risk of introduction of pathogens (Jourdain et al., 2007) show that WNV can be translocated from Europe to Africa.

WEST NILE FEVER/ENCEPHALITIS: PAST AND PRESENT

As already mentioned, in the first half of the twentieth century WNF was considered of little importance due to the benign pathology it caused in endemic areas in equatorial Africa, where it was first isolated and diagnosed. However, after 1951 this concept began to change, as WNV was associated to severe cases of encephalitis, some fatal, in the first epidemic of WNV outside Africa, which occurred in Israel between 1951 and 1957 (Paz, 2006). The virus circulated in Egypt in the same period, where the first eco-epidemiological studies on WNV were carried out. These studies characterized the enzootic cycle of WNV between birds and mosquitoes, identifying men and horses as susceptible to the disease but not transmitting the infection (Taylor et al., 1956). Between 1962 and 1965 there were several outbreaks of severe illness due to WNV in the Camargue, France, affecting both horses and humans. In 1971, WNV was isolated from mosquitoes in southern Portugal, in the course of an epidemiological study starting after an outbreak of encephalitis in horses, in which 29% of the surviving horses showed seropositivity against WNV. These sporadic outbreaks occurred not only in Mediterranean countries,

but also in South Africa, Russia, Romania, and India. After that, WNV in Europe remained silent for almost three decades. Its re-emergence took place almost simultaneously in the western Mediterranean (Algeria, 1994; Morocco, 1996; Tunisia, 1997; Italy, 1998, 2008; France, 2000; and Portugal, 2004), in central and eastern Europe (Romania, 1996; Czech Republic, 1997; Russia, 1998; Hungary, 2004; and Austria, 2008), and in the Middle East (Israel, 1998; revisions Hubalek and Halouzka, 1999; Murgue et al., 2001; Sotelo et al., 2011a). Very recently, another lineage of WNV (lineage 2) was detected in central Europe (Hungary, 2004), extended to Austria (2008) and reached the Balkans and Greece in 2010, where it continues its spread (Bakonyi et al., 2006; Papa et al., 2011; Wodak et al., 2011). Almost simultaneously, in Russia (Volga basin) a new epidemic caused by a lineage 2 WNV which is genetically different from the variant affecting central Europe, was observed, causing an increasing number of human cases since 2004 to date (Platonov et al., 2008). This virus has extended westward reaching Romania in 2010 (Sirbu et al., 2011).

This new wave of WNV in Europe differed from the former in several aspects. Firstly, its duration: this wave has extended further in time, to the point that it is still ongoing with increasing intensity, Since the late 1990s sporadic outbreaks have occurred in the same locations in consecutive years. For example, in the Camargue, France, after the first outbreak of 2000, new outbreaks occurred in 2003, 2004, and 2006 (Zientara et al., 2009), and virus isolates available from these outbreaks were very closely related phylogenetically (Sotelo et al., 2011b). This fact supports that the virus is circulating endemically in the area since 2000 or before. In Morocco, an outbreak occurred in 2003 affecting horses, similar to that produced in 1996, and again, WNV isolates from both outbreaks show a striking similarity at the genetic level (Sotelo et al., 2011b). In Italy an outbreak occurred in horses in Tuscany in 1998, and 10 years later, in 2008, a serious epidemic took place in the Po river delta, which affected hundreds of horses and produced some human cases. Since then the same area has been affected by epidemic waves of WNF every year to date, and again, the viruses isolated in consecutive years are genetically closely related (Sotelo et al., 2011b). In Spain the first WNV isolation took place in 2007 from golden eagles (Jimenez-Clavero et al., 2008). Later, an outbreak affecting horses and humans occurred in 2010, which continued in 2011 (OIE, 2010). Apparently, all the viruses isolated since 1996 from western Mediterranean countries (France, Italy, Morocco, Portugal, and Spain), have a close phylogenetic relationship, which, as noted above, supports a single introduction in the area of a virus which has been able to remain endemic and spread in the area during all these years (Sotelo et al., 2011b). Secondly, the affected territory is more extended in the present epidemic wave than in the previous one. In fact there are more countries affected than ever in Europe. Moreover, the number of clinical cases is higher (Sotelo et al., 2011a). Exceptionally severe, with unusually high mortality in humans, were the epidemics on an "urban cycle" that occurred in 1996 in Bucharest, Romania (about 1,000 human cases, 396 severe, 17 deaths), in 1999 in Volgograd, Russia (approximately 1,000 human cases, 40 deaths), in 1998–1999 in Israel (about 400 human cases, 35 deaths) and in Greece in 2010–2011 (336 human cases, 40 deaths; Anonymous, 2011). Finally, the range of WNV variants circulating in Europe is presumably higher than in the previous wave: While all isolates analyzed from European outbreaks occurring between 1951 and 1971 belonged to lineage 1, more recent European outbreaks have revealed not only two "classic" WNV lineages (1 and 2), but also up to three "unusual" WNV variants, including Rabensburg strain or lineage 3, Krasnodar strain or lineage 4 and putative lineage 7 from Spain.

However, the most striking change in WNV epidemiology occurred when the virus reached the American continent in 1999. This year the virus appeared in New York in an unexpected and not yet well explained way. This event initiated the largest WNV epidemic in history. The virus spread relentlessly throughout the continent, reaching the Pacific coast and Canada in 2002. Only in the U.S. the virus has caused to date about 30,000 clinical cases in humans of which more than 1,000 were fatal. The disease incidence peaked in 2004 and since then a slow decline in clinical cases has been observed (reviewed in Murray et al., 2010). WNV is now considered endemic in North America, after continuous circulation for 12 consecutive seasons and in its southward advance has produced sporadic disease cases in Central America and the Caribbean. In South America the virus has remained essentially unnoticed, except for an outbreak in horses in Argentina in 2006 (Morales et al., 2006). The reason why WNV circulates with such great intensity in North America, compared with other regions of the world, currently has no explanation.

WEST NILE FEVER/ENCEPHALITIS: FUTURE TRENDS

The past 15 years have witnessed an unprecedented expansion of a disease caused by an arbovirus, WNV, shared by animals and man. This expansion has taken place in parallel with an increased incidence of the disease in susceptible hosts, mostly birds, horses, and humans (Brault, 2009). While in the Old World warning signs were observed at the end of the last decade of the twentieth century, the emergence of WNV in the New World triggered all alarms. There the virus found a vast territory plenty of new naïve hosts, and initiated one of the major arbovirus epidemics known so far. WNV arrived in America to stay, as evidenced by the fact that the numbers of human cases diagnosed in the last years have stabilized at around 1,000 per year (CDC, 2011).

With regard to the relationship between climate change and the observed expansion and increase in cases of WNF some climatic patterns influencing local WNV circulation can be pointed out. Firstly, high temperatures enhance virogenesis in vectors (Reisen et al., 2006), which, along with vector abundance and competence, promote the occurrence of WNV outbreaks. Therefore, abnormally dry and hot summers, accompanied by mild winters, favor WNV circulation, while extremely cold winters break WNV cycle and disrupt its transmission, as illustrated in the following example: in southern Russia (Volgograd region) after several seasons (1997–2002) with extremely hot summers followed by mild winters, the circulation of WNV was enhanced, but two upcoming harsh winters disrupted this circulation and caused that lineage 1 WNV, responsible of the outbreaks thus far, ceased its activity and extinguished, Two years later (2004), once more favorable climatic conditions were restored, WNV re-emerged and caused new clinical cases in humans, but, as mentioned above, this time

the virus circulating did not belong to the classic lineage 1, but to a newly introduced variant, which was identified as lineage 2 (Platonov et al., 2008). This example illustrates quite well how WNV epidemiology is determined by climatic factors, and year-to-year variations can lead to WNV enhancement or extinction from an endemic area. Other well-known episodes of WNF epidemics occurred in scenarios of abnormally hot summers, such as in Israel in 1998 (Paz, 2006) or New York in 1999 (Epstein, 2001). Secondly, it is known that a prolonged drought after a heavy rainy season promotes the habitat of *Culex* vectors by enhancing accumulation of organic matter in suspension in standing water. Severe drought episodes preceding by few months the onset of WNF outbreaks have been documented in the United States (Shaman et al., 2005). Thirdly, episodes of heavy rains, hurricanes, floods, and overflow of river basins have been implicated in the onset, or increase in outbreaks, of WNF. The WNV outbreak that occurred in the Czech Republic in 1997 was preceded by significant flooding, including severe flooding of the river Vltava in central Bohemia. In another instance, a few weeks after Hurricane Katrina affected Louisiana and Mississippi a significant increase of cases of WNV neuroinvasive disease was observed in the area (Caillouet et al., 2008).

Significantly, all weather conditions favoring the occurrence of WNF outbreaks, mentioned above, such as extremely hot summers, mild winters, droughts, floods, hurricanes, etc., tend to increase in frequency and intensity according to predictions on climate changes described in Section "Climate Change and Emerging Infectious Diseases" and listed in **Table 2**. However, current WNV emergence cannot be easily explained by a single factor such as climate change, but, more likely, a number of factors, both abiotic and biotic, might contribute. Among these factors, the emergence of new virus phenotypes, more virulent and/or more transmissible and/or more adapted to their hosts and/or vectors, could play an important role (Brault, 2009). Also, other factors related to globalization mentioned above, such as increase of transport, animal movements, etc., are likely to be involved in the observed geographical expansion of WNV. Taking these factors into account, and the observed climate trends mentioned above, WNF will only be expected to move forward in the twenty-first century.

CONCLUSION

Certain arboviral animal diseases such as BT and WNF have undergone significant changes in their known epidemiology, gaining unprecedented importance in the past 15 years. It is plausible that this increase has been influenced by climate changes, although their importance relative to other environmental factors (changes in agriculture, land uses, etc.) is difficult to estimate. Predictive models on climate in the coming years foresee an intensification of the circumstances favoring the activity of these arboviral pathogens. BT and WNF constitute paradigms of emerging infectious diseases whose fate is linked to global changes.

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Integrated strategy for sustainable cattle fever tick eradication in USA is required to mitigate the impact of global change

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The ticks *Rhipicephalus* (*Boophilus*) *annulatus* and *R.* (*B.*) *microplus*, commonly known as cattle and southern cattle tick, respectively, impede the development and sustainability of livestock industries throughout tropical and other world regions. They affect animal productivity and wellbeing directly through their obligate blood-feeding habit and indirectly by serving as vectors of the infectious agents causing bovine babesiosis and anaplasmosis. The monumental scientific discovery of certain arthropod species as vectors of infectious agents is associated with the history of research on bovine babesiosis and *R. annulatus*. Together, *R. microplus* and *R. annulatus* are referred to as cattle fever ticks (CFT). Bovine babesiosis became a regulated foreign animal disease in the United States of America (U.S.) through efforts of the Cattle Fever Tick Eradication Program (CFTEP) established in 1906. The U.S. was declared free of CFT in 1943, with the exception of a permanent quarantine zone in south Texas along the border with Mexico. This achievement contributed greatly to the development and productivity of animal agriculture in the U.S. The permanent quarantine zone buffers CFT incursions from Mexico where both ticks and babesiosis are endemic. Until recently, the elimination of CFT outbreaks relied solely on the use of coumaphos, an organophosphate acaricide, in dipping vats or as a spray to treat livestock, or the vacation of pastures. However, ecological, societal, and economical changes are shifting the paradigm of systematically treating livestock to eradicate CFT. Keeping the U.S. CFT-free is a critical animal health issue affecting the economic stability of livestock and wildlife enterprises. Here, we describe vulnerabilities associated with global change forces challenging the CFTEP. The concept of integrated CFT eradication is discussed in reference to global change.

Keywords: cattle, tick, babesiosis, integrated eradication, global change, climate, modeling, sustainability

INTRODUCTION

Global change makes human and animal populations vulnerable to emerging and re-emerging tick-borne diseases. Environmental and climatic changes ascribed to human activity are collectively referred to as global change (Sutherst, 2001; Camill, 2010). The accelerated rate of global change alters the epidemiology of tick-borne diseases. Tick-borne diseases are complex systems subject to shifts in ecological processes influencing tick biology and consequently the epidemiology of pathogens transmitted by ticks (Randolph, 2010; Reisen, 2010; Tabachnick, 2010; Olson and Patz, 2011). This review explores the ramifications of global change on the Cattle Fever Tick Eradication Program (CFTEP) of the United States of America (U.S.) and how these challenges threaten the ability to keep the national cattle herd free of bovine babesiosis by suppressing the invasive cattle fever tick (CFT) vectors, *Rhipicephalus* (*Boophilus*) *annulatus* and *R.* (*B.*) *microplus*. The reader is referred to the review by Aubry and Geale (2011) for details on bovine anaplasmosis, which is another economically important infectious disease of cattle caused by *Anaplasma marginale* and transmitted by CFT.

Research on bovine babesiosis and CFT involves a notable discovery in the history of medical science. Elegant studies by Smith and Kilbourne (1893) revealed that the apicomplexan protozoon *Babesia bigemina* transmitted by *R. annulatus* caused bovine babesiosis in susceptible cattle. We now know that *R. microplus* also transmits *B. bovis*, which causes a more virulent form of bovine babesiosis. Smith and Kilborne's monumental work was soon followed by others reporting the role of various

blood-feeding arthropod species, such as lice and mosquitoes, transmitting pathogens to humans and animals causing severe maladies like malaria, yellow fever, typhus, and dengue (Schultz, 2008). Cooper Curtice also made invaluable contributions to the history of CFT and bovine babesiosis research. His detailed investigations describing the life history of CFT laid the foundation for the CFTEP (Logue, 1995). The interaction between Smith, a physician, and Kilborne, a veterinarian, is a fine example of what we now term the One Health (Bulloch, 1935). The One Health concept defines the collaborative effort of multiple disciplines to attain optimal health for people, animals, and our environment (Welburn, 2011). Such approach identified knowledge gaps in human and bovine babesioses and the benefits of its application to the CFTEP are reflected in progress to translate research into tools that can be integrated to minimize the impact of CFT outbreaks on U.S. agriculture, which are described here (Pérez de León et al., 2010).

Bovine babesiosis is a devastating tick-borne disease of cattle that was eradicated from the U.S. by eliminating CFT populations. This significant event in the history of disease eradication campaigns was accomplished through the efforts of the CFTEP managed by state and federal agencies in collaboration with livestock producers from 1907 until 1943. A permanent quarantine zone established in Texas extending over 800 km from Del Rio to Brownsville and delimited in the south by the Rio Grande, known in Mexico as Río Bravo, is managed by the U.S. Department of Agriculture-Animal Health Inspection Service (USDA-APHIS) and the Texas Animal Health Commission (TAHC) to buffer tick and stray animal incursions from Mexico where CFT and bovine babesiosis are endemic (**Figure 1A**).

Keeping the U.S. cattle herd free of bovine babesiosis and CFT is an important animal health issue. Previous estimates converted to today's currency rate indicate that the livestock industry realizes annual savings of at least 3 billion dollars since the U.S. was declared free of bovine babesiosis and CFT (Graham and Hourrigan, 1977). The dynamics of global change have increased the risk for re-emergence of CFT and bovine babesiosis in the U.S. (Bram et al., 2002; Guerrero et al., 2007; George et al., 2008). This risk poses significant challenges to future efforts by the CFTEP. Portions of the U.S. still provide suitable habitat for CFT (Estrada-Peña et al., 2005), which correspond with the CFT historical range before the CFTEP was initiated particularly in the 13 states comprising the Southern Region (**Figure 1A**). Cattle and calves rank among the top five agricultural commodities in nine of the 13 Southern Region states with cash receipts ranging from 5.3% (Florida) to 46% (Oklahoma) of all agricultural commodities. The cow-calf inventory of the 13 Southern Region states is more than 42% of the entire U.S. beef cattle inventory (McBride and Mathews, 2011). Approximately 1/3 of all U.S. fed-cattle originate from Southern Region ranches operated by over 400,000 producers representing nearly 49% of all U.S. cow-calf producers. The economic stability of the cattle industry is vital to the nation.

Global change involves disruptive processes that augment instability in ecosystems, and the services they provide, that are or can be inhabited by ticks (Hanson et al., 2008). These processes add uncertainty to future outcomes of current policies to manage ticks and tick-borne diseases. The disruptive processes driving

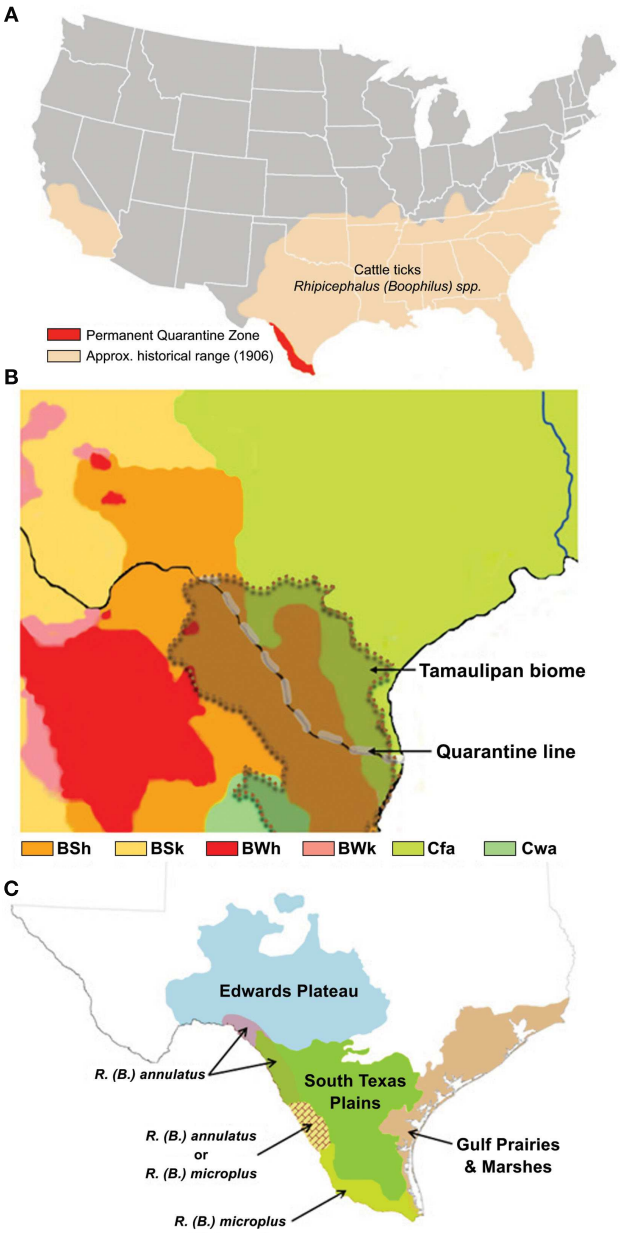

FIGURE 1 | (A,C) The U.S. distribution of cattle ticks in 1906 depicts the zone of risk for tick re-establishment and the permanent quarantine zone along the Texas–Mexico international boundary and quarantine line **(A)**, as adapted from Graham and Hourrigan (1977); the Tamaulipan biome of the Texas–Mexico border area (Scifres, 1985), indicated by the overlay with dotted boundary **(B)**, is the major ecoregion comprising the quarantine zone that consists of four Koppen–Geiger climate classifications, as adapted from Peel et al., 2007, where the majority of the biome is of an arid-steppe (BSh) classification linked with the Temperate (Cfa) classification that extends throughout the historical range of cattle fever ticks, whereas in Mexico the biome includes small areas with climate generally classified as Arid desert (BWh) or Temperate (Cwa); the distribution of ecoregions, as adapted from Correll and Johnston (1970), reflects the diversity of ecological and environmental resources **(C)**, and is integrated with an overlay showing the quarantine zone distribution of recorded incursions of *R. annulatus* and *R. microplus*, as adapted from Lohmeyer et al. (2011).

emergence and re-emergence of tick-borne diseases affecting livestock linked to global change include the alteration of ecosystems and agroecosystems, incursion of natural habitats by humans and animals, shifts in land-use, movement of people and animals, climate change, selection of acaricide–resistant tick populations, increased international trade and travel, civil unrest, expansion of tick and pathogen host range, and governmental or management failure (Pegram et al., 2000; Harrus and Baneth, 2005; George, 2008; De Meeûs et al., 2010; Brouqui, 2011; Perry et al., 2011; Food and Agriculture Organization of the United Nations (FAO), 2011). The deterioration of public safety on the Mexican side of the border in south Texas related to illegal drug activity has complicated efforts by the CFTEP (Aguilar, 2011). Such effects are compounded by the zoonotic nature of some tick-borne diseases and the vulnerability of public health and central veterinary authorities trying to deal with the unexpected consequences of global change (Hoberg et al., 2008; Jones et al., 2008; Black and Nunn, 2009; Munderloh and Kurtti, 2011). Tick species other than *R. microplus* and *R. annulatus* serve as vectors of zoonotic babesioses (Vannier et al., 2008).

Our ability to assess immediate and future impacts of global change on animal pests and diseases needs to be enhanced. Bovine babesiosis and CFT are among the diseases and pests requiring attention (Cumming and Van Vuuren, 2006; Food and Agriculture Organization of the United Nations (FAO), 2008). Agri-intelligence and foresight are two provocative methodologies that have been applied to better understand and proactively recognize threats and risks to national security associated with agriculture to ensure today's decisions anticipate and incorporate adaptive strategies related to an uncertain future (Munroe, 2007; Munroe and Willis, 2007; Willis, 2007; Suk et al., 2008; Pretty et al., 2010). The incorporation of inputs from diverse disciplines and organizations with broad mandates is a goal of agri-intelligence, which enables analysis through networks aimed at synergizing efforts to deliver a value-added output decision makers can use to make more informed policy development and superior operational outcomes (Munroe, 2007). Foresight is a process that looks at the time horizon to facilitate forward preparedness efforts through critical thinking focused on exploring scenarios including those that may appear unlikely today (Smith, 2007). Forecasting approaches, including modeling, are advocated as a means to facilitate anticipation and enhance prevention, preparedness, and the management of disease outbreaks (Baylis and Githeko, 2006; Garner et al., 2007; Munroe and Willis, 2007; Estrada-Peña, 2008; Woolhouse, 2011).

The principles of agri-intelligence and foresight are applied here to dissect the forces of global change and discern the interconnectedness between environmental and anthropogenic factors driving the temporal and spatial variation of CFT outbreaks in south Texas. This exercise was framed by the historical record of CFT outbreaks in the U.S. since 1959 (**Figure 2**), and it provides further assessment of CFT and bovine babesiosis research needs identified through a process that applied the One Health concept (Pérez de León et al., 2010). The historical situation with CFT is outlined to reveal critical challenges the CFTEP is facing. Such aspects unify the past, present, and future. This perspective provided a lens to recognize and appreciate the complexity of, and the relationships between issues the CFTEP must contend with to contain CFT within the permanent quarantine zone. The correlates and trends with global change described below formed the basis to suggest a framework for the integration of CFT eradication strategies that could be used to enhance the ability of regulatory agencies in the U.S. to anticipate, prepare for, mitigate, and prevent the burden of CFT outbreaks thereby contributing to the benefits society derives from having a sustainable livestock production system (**Figure 3**). Sustainability in this context means continued productivity into the indefinite future through the use of sound scientific evidence to inform decision making and guide policy makers involved with the CFTEP while maximizing agroeconomic opportunities for livestock producers (National Research Council of the National Academies, 2010; Simmons, 2011; Walker, 2011).

ARRIVAL AND SPREAD OF CATTLE FEVER TICKS IN THE U.S.

The CFT are not native to the Americas. The origin of *R. annulatus* includes the arid and temperate climates of southern Russia, the Near- and Middle-East, and the Mediterranean basin whereas the origin of *R. microplus* is the tropical climates of the Indian subcontinent (George, 1990; Estrada-Peña et al., 2006). Evidence indicates that Spanish colonialists served as the pathway that brought livestock infested with CFT and infected with bovine babesiosis to Mexico and Cuba during the 1500s (García-García et al., 1999). The introduction and spread of CFT into what is now the U.S. followed the pattern of North American settlement and commerce. It is suspected that by the end of the eighteenth century bovine babesiosis and *R. annulatus* had emerged in the U.S. because in 1795 North Carolina prohibited driving cattle into the state from South Carolina or Georgia between 1 April and 1 November of each year due to an undetermined disease of livestock (George, 1989). By the late 1800's, CFT were distributed throughout the southern states and southern California (**Figure 1A**). Bishopp (1913) first reported cattle infestations with *R. microplus* after collecting the ticks in 1912 from animals in Key West, Florida; he speculated that this CFT arrived from the Caribbean islands through commerce. The ticks and bovine babesiosis, known earlier as Texas, Mexican, Spanish, or splenetic fever, were significant economic impediments to cattle industry development throughout the Southern U.S. (Malone, 1989; Strom, 2009). High mortality rates in non-immune animals brought into the Southern states rendered efforts to develop cattle production through improved breeding and management useless. Before eradication, CFT infestation alone reduced the weight of a 500 kg steer by 100 kg in a year and reduced milk production of dairy cattle by 25% (Agricultural Research Service, 1968).

The common name "cattle tick" was derived in part from the devastating economic impact these parasites had on cattle, and in part from their adaptation to this domestic host (Hoogstraal and Aeschlimann, 1982). However, new parasitic relationships have evolved as CFT invaded various parts of the world. Non-bovine hosts of these parasites include buffalo, ox, horse, mule, donkey, sheep, goat, dog, swine, antelope, and several species of deer (Cooley, 1946; Barré et al., 2001; Ghosh et al., 2007; Cançado et al., 2009; Pound et al., 2010). Ticks spend most of their life in the environment off the host in the egg and larval stages (**Figure 4**), a period that may last 6–9 months depending on microclimates produced by vegetation communities and rainfall (Newell and Daugherty,

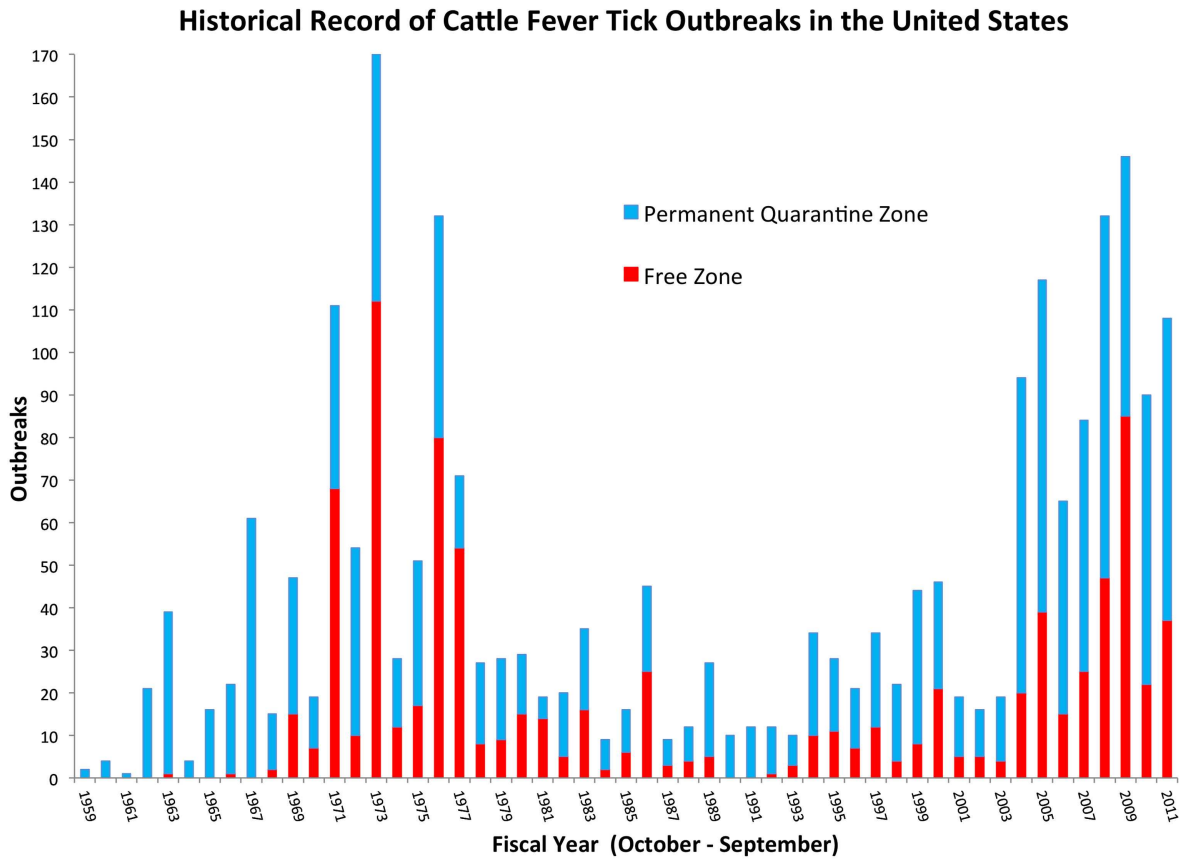

FIGURE 2 | Historical record of CFT outbreaks in the U.S. Each fiscal year shows the contribution by the Permanent Quarantine and Free Zones to the total number of outbreaks. The Free Zone comprises the area in the 48 contiguous states outside the Permanent Quarantine Zone, which is located along the Rio Grande in southern Texas. The period shown in the graph depicts records maintained by USDA-APHIS-Veterinary Services and it covers fiscal years 1959 through 2011. Data updated after Pérez de León et al. (2010).

1906; Cotton and Voorhees, 1911; Graybill, 1911). Larvae quest on vegetation for, and eventually attach to a passing host, seek a predilection site to attach and then blood feed. The parasitic portion of their life is completed on the same animal. Therefore, CFT have a one-host life cycle (**Figure 4**). Blood-fed larvae molt into nymphs and the feeding and molting process is repeated to produce male and female adult CFT. Adults mate and gravid, blood-engorged females detach, drop, and oviposit approximately 3,500 eggs in suitable microclimates at the soil-vegetation interface before dying (Davey et al., 1994). The parasitic phase can be completed over 3–4 weeks providing a substantial period for tick dispersal by foraging animals or human transport of infested animals (Walker et al., 2003). Between three and six generations are produced per year depending on tick species and climatic variables of rainfall and temperature (Mount et al., 1991). In wet-dry season climates, populations generally tend to increase during rainy periods and decline during dry periods (McCulloch and Lewis, 1968; Rawlins, 1979; Daynes and Gutierrez, 1980; Cardozo et al., 1984). Additional information on CFT can be accessed through the TickApp[1] developed to provide educational information on tick biology, ecology, associated diseases, prevention, protection, control, and management.

HISTORICAL OVERVIEW OF ERADICATION EFFORTS

Early workers observed that separation of CFT from cattle effectively prevented transmission of babesiosis pathogens, and eventually reduced or eliminated tick populations (Curtis and Francis, 1892; Newell and Daugherty, 1906). Tick elimination tactics included cultural practices and evolved with the development of the plunge dip vat to treat cattle with acaricides (Francis, 1894; Graham and Hourrigan, 1977). Regulations for CFT eradication used surveillance to define the spatial boundaries of infestations and quarantine of cattle and premises to implement treatments. Two strategies were employed for tick elimination. A "pasture vacation" strategy first removed ticks from infested cattle by acaricide dipping allowing removal of animals to alternate non-infested premises, then leaving the infested premises vacated for 6–9 months for tick larvae to perish without a host. A "systematic dipping" strategy treated 100% of cattle by dipping at 7–14 day intervals for the 6–9 month period. Acaricide concentrations set to kill 99% of the ticks and effective dip vat management stopped cattle tick development. These procedures required knowing the exact

[1]http://tickapp.tamu.edu

FIGURE 3 | Suggested framework toward integrated cattle fever tick eradication strategies depicting interconnectedness of critical elements for sustainability of bovine babesiosis-free status in the U.S.

cattle inventory at every dipping session and assuring all animals were confined inside fenced premises. Variations on systematic dipping included double fencing, cross fencing, overstocking, and rotations to improve pasture coverage and increase tick-host contact, in effect "sponging" ticks from the premises (Gray et al., 1979). The CFTEP was declared successful in 1943, and the last outbreak of bovine babesiosis in the U.S. occurred in 1949 (Malone, 1989).

Surveillance and detection were critical to identify and contain CFT incursions as well as to assess success of operational tactics. Probabilities of detecting infested animals or premises is the product of many interacting abiotic and biotic factors influencing spatial and temporal distributions of CFT (Palmer et al., 1976; Teel et al., 1997, 2003). When the resident population is low or widely dispersed due to weather conditions, early stage of establishment, or other factors, the probability of detection is low. Continuous active surveillance by well trained personnel and improvements in detection sampling methods and technologies are essential.

IMPACTS OF GLOBAL CHANGE ON OPERATIONS OF THE CATTLE FEVER TICK ERADICATION PROGRAM

Two spatial and climatic characterizations are relevant to the discussion and interpretation of CFTEP challenges centered in the Tamaulipan scrubland biome, defined as a region whose climate produces similar climax associations of flora and fauna. The Köppen–Geiger climate classification system has recently been updated (Rubel and Kottek, 2010) and provides a common global system for interpretation. A nomenclature for ecological regions, denoted "ecoregions," was developed in the U.S. to identify similarities in type, quality, and quantity of environmental resources as a spatial framework for ecosystem research, assessment, and management (Bryce et al., 1999). This system incorporates variation in biotic and abiotic patterns and composition including geology, physiography, vegetation, climate, soils, land-use, wildlife, and hydrology. Ecoregion names for Texas as defined by Correll and Johnston (1970) will be used in this discussion. We recognize that climate classification continues to be refined through more robust algorithms and this has implications on our understanding of the ways global change shapes the landscape epidemiology of CFT (Rubel and Kottek, 2010; Cannon, 2012). Major aspects related to global change impacting CFTEP operations in the permanent quarantine zone involve shifts in regional ecology, including wildlife fauna as alternative hosts for CFT. Potential future risks include: climate change, human population impacts on land-use and fractionation, acaricide resistance, and infection of wildlife with strains of *B. bovis* and *B. bigemina* pathogenic to cattle.

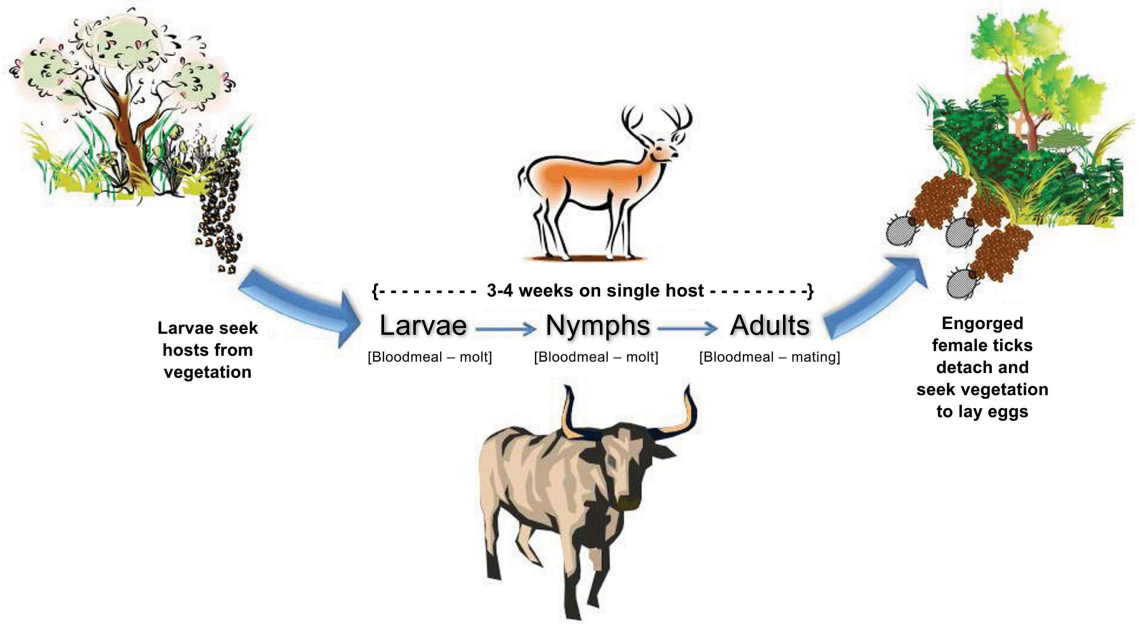

FIGURE 4 | One-host life cycle evolved by cattle fever ticks that comprises an on-host phase where larvae and nymphs sequentially blood feed and molt, and adults feed and mate, and an off-host phase in which mated engorged females drop from the host, oviposit, and die. Larvae hatching from the eggs initiate the next generation. In the absence of hosts, the off-host phase may constitute 87–93% of the life cycle's duration. Used by permission (Teel et al., 2011; http://tickapp.tamu.edu).

SHIFTS IN REGIONAL ECOLOGICAL CHARACTERISTICS

The Rio Grande marks the U.S.–Mexico boundary in south Texas and this river flows according to rainfall and reservoir containment as it makes its way toward the Gulf of Mexico. With adequate flow, it provides a physical barrier to slow livestock and wildlife movement between countries, while during drought low-water levels permit increased animal and human movement. The river and CFT quarantine zone cross the Tamaulipan scrub biome (**Figure 1B**). Based upon the updated Köppen–Geiger climate classification (Rubel and Kottek, 2010), the biome climate includes four categories (**Figure 1B**). The majority of the biome is classified as an arid-steppe (BSh), which changes to a temperate classification (Cfa) in Texas that extends throughout the historical range of CFT in the southern U.S. (**Figure 1A**), whereas in Mexico it includes small areas with climate generally classified as arid desert (BWh), or temperate (Cwa). The region is comprised of four ecoregions including Gulf Prairies and Marshes, South Texas Plains (also known as Rio Grande Plains), Edwards Plateau (north east of the river and quarantine line), and the Tamaulipan brushlands (south west of the river) (**Figure 1C**; Correll and Johnston, 1970). Elevation ranges from sea-level to 1000 feet near the most inland locations. Annual rainfall may be 30–50 inches along the Gulf Coast, but declines to 14–16 inches in the inland reaches of the South Texas Plains-Edwards Plateau. Along the Gulf Coast summers are long, hot and humid (subtropical; Archer et al., 1988) and winters are very mild (>300 frost free days), while progressively more inland locations have hotter, drier summers, and winters subject to more freezing temperatures (Correll and Johnston, 1970). The region is subject to the vagaries of tropical storms from the Caribbean and Gulf of Mexico, as well as periodic and sometimes extended periods of drought. An overlay showing the distribution of CFT species intercepted along the quarantine line reflects the distribution of *R. microplus* in the higher rainfall and more humid areas of the Gulf Prairies and Marshes and South Texas Plains, and the distribution of *R. annulatus* in the drier inland reaches of the South Texas Plains-Edwards Plateau (**Figure 1C**; adapted from Lohmeyer et al., 2011). Both species have been intercepted in the narrow area near the middle of the quarantine line. Retrospective climate evaluations found suitable habitat for *R. microplus* along Gulf and Atlantic coastal states that included a narrow area at the southern end of Texas (Estrada-Peña et al., 2005). The derived variables of vapor pressure deficit, evaporation, and total rainfall were the principal determinants explaining variation among the 5-year periods of high suitability. Though no climate or habitat suitability studies have been conducted for *R. annulatus*, it is expected that this species could re-establish throughout its original range. A recent risk assessment of hypothetical CFT outbreaks in the temperate zone (Cfa; **Figure 1B**), east of the South Texas Plains, concluded that significant additional infrastructure and personnel would be needed to meet operational demands, and treatment costs and production losses to producers would likely exceed the economic viability of their operations under current tick elimination options (Anderson et al., 2010).

Descriptions of early landscapes on what is today the Tamaulipan scrubland were of vast grasslands with woody plants limited to sites such as water ways and drainages (Scifres, 1980, 1985). Natural fires, cyclical wet and dry periods, and infrequent grazing by migratory herbivores (e.g., bison, antelope, and deer) minimized overgrazing, and all contributed to the natural management shaping this grassland ecology. Eventual settlement of the area brought about cessation of natural fire and fencing with more intense and long-term grazing by confined herbivores (e.g., cattle, horses, sheep, goats), the combination of which permitted woody plants to expand from their refuges and invade the expanse (Archer et al., 1988). Many areas of South Texas and Northern Mexico eventually gained a continuous cover of brush mottes comprised of large central primary species, such as honey mesquite, *Prosopis glandulosa*, and as many as 15 secondary species in the community. The anthropogenic alteration of the grassland resulted in brush invasion and the transformation to scrubland. Brush encroachments decreased forage production, the primary resource for rangeland cattle (Scifres, 1980), while concurrently providing more favorable habitat for tick survival (Teel, 1991). Consequently, tactics to control brush were developed into integrated brush management strategies to optimize forage production on fertile sites (Scifres and Hamilton, 1985), and these strategies would ultimately include management for wildlife species as well as cattle (Inglis, 1985). Another pathway for the impact of global change is the facilitative ecological interaction between stands of the non-native giant reed (*Arundo donax*) and CFT. The giant reed is an invasive weed that consumes economically significant amounts of water in Mexico and the U.S. Giant reed stands along the Rio Grande were shown to provide abiotic and biotic conditions that are favorable for CFT survival (Racelis et al., 2012). Cooperation between the U.S. and Mexico resulted in a control program to control the giant reed using biocontrol agents (Goolsby et al., 2011), which is likely to alleviate complications this invasive weed presents to the CFTEP.

Efforts to improve rangeland forage production also led to the introduction of drought tolerant buffelgrass, *Cenchrus ciliaris*, from Africa. Rangeland vegetation communities including brush mottes that provide canopy and grasses and forbes of interstitial zones influence the microclimate at the soil-vegetation interface. Spatial distribution of habitats with optimal microclimates interacts with host-landscape behavior to disperse and sustain CFT populations. Management practices for brush control, forage production, grazing, drought, and wildlife have all been developed for land owners/managers engaged in cattle or wildlife production systems in the South Texas Plains. The USDA, Natural Resource Conservation Service, has offered financial and technical assistance for land owners/managers in 17 Texas counties impacted by CFT since 2009. The program funds practices that control brush and improve fences, animal handling facilities, water distribution, and wildlife management[2].

Land-use and fractionation

Landscape fragmentation resulting from changes in vegetation cover, property size, use of natural resources, and wildlife diversity and abundance is acknowledged to impact the ecology of tick-borne disease systems, including Lyme disease (Brownstein et al., 2005) and Crimean-Congo hemorrhagic fever (Estrada-Peña et al., 2010). Two CFT outbreaks in 2009 provide a case study in contrasting land-use and fractionation and the impact of a growing human population. An outbreak involving *R. annulatus* occurred in the northern portion of the quarantine zone in country largely devoted to cattle production on large pastures (typically 500–2000 acres) where white-tailed deer (WTD) and human populations are relatively low. In contrast, an *R. microplus* outbreak in the southern portion was characterized by many landowners having small parcels of land (typically 50–200 acres), a comparatively high human population with widely variable land-use and a high concentration of WTD estimated to be one animal per four acres. The South Texas Plains ecoregion (approx. 20 million acres) is comprised of 67% native pasture (rangeland), 10% improved pasture, 4% wildlife management, 13% cropland (dryland/irrigated), and the remainder in a variety of uses (Gilliland et al., 2010). Crop and citrus enterprises are aggregated in the Rio Grande Valley at the very southern portion of the permanent quarantine zone, while the vast majority of land-use to the north is cattle and wildlife on rangeland and pasture. Rural land prices and sales in the South Texas Plains steadily increased from 1990 with sales peaking in 2004 and prices peaking in 2008, before economic recession brought about declines in both parameters (Gilliland et al., 2010). Over recent decades landowners-managers have integrated predominantly cow-calf operations with native and/or exotic game operations to diversify economic enterprises (Inglis, 1985). Increasing fragmentation of farms and ranches is resulting in rural property size decline driven by social and economic factors including profitability of agricultural enterprises, environmental concerns, and intergenerational land transfers. An evaluation of rural property patterns in Texas (Kjelland et al., 2007) found the strongest predictor of land fragmentation to be non-agricultural value. Changes in land-use and fragmentation are expected to continue to alter the ecology of CFT and the risks associated with bovine babesiosis. The operational impact of these changes on the CFTEP program is extended to the regulatory logistics and public relations associated with the elimination of outbreaks in smaller premises where livestock and wildlife ranching is now practiced. This requires a renewed effort to engage and raise awareness of program goals and procedures among diversified livestock producers.

WILDLIFE AS ALTERNATIVE CATTLE FEVER TICK HOSTS

White-tailed deer

The deer *Odocoileus virginianus*, commonly known as WTD, was abundant and widespread prior to pioneer settlement (Young, 1956; Rue, 1978). Substantial WTD populations were a resource for meat and hides, and these became significant objects of trade and commerce. One central Texas trading house is reported to have baled and shipped 75,000 deer hides between 1844 and 1853 (Young, 1956). By the late 1800's WTD were at their lowest in many regions (Rue, 1978). The bovine-centered CFT eradication campaign made steady progress until the 1930's when deer infested with *R. microplus* were deemed responsible for persistent infestations in Florida (George, 1990). Though controversial, WTD population reductions were conducted in six counties after other

[2]http://www.tx.nrcs.usda.gov

tactics failed, and ticks were subsequently eliminated (George, 1990; Strom, 2009). It is noteworthy that *R. annulatus* was first described from specimens collected from WTD, then known as *Cervus virginianus*, in Florida (Say, 1821).

The esthetic and recreational hunting value of WTD remained, and conservationists working with state-federal agencies re-established and conserved WTD populations throughout the U.S. In the Llano Basin of the Edwards Plateau ecoregion (**Figure 1C**), an area of approximately 525,000 acres, the average deer density in 1954 was 14.4 per 100 acres, ∼75,600 animals, and by 1961 deer density increased to 18.9 per 100 acres with a census of 99,750 animals (Rue, 1978). Successful WTD population growth in Texas through the 1960's was influenced by three factors: beneficial cover and browse was provided by brush vegetation communities; a more defined hunting season was established with conservation goals; and, elimination of the primary screwworm, *Cochliomyia hominovorax* (Rue, 1978). The Texas population in 2004 was estimated at 3.8 million animals (Pound et al., 2010). Range expansion for the Texas WTD subspecies included the Tamaulipan brushlands of Mexico where they are managed for overabundance today (Taylor, 1956; Martinez and Hewitt, 2001).

Most WTD responded to bi-weekly cattle gathering and dipping activities on a CFT infested ranch by temporarily moving to adjacent premises, then returning to the infested and quarantined pastures after activities subsided, while a small number moved into new home ranges (Hood and Inglis, 1974). The behavior of WTD on landscapes has primarily been investigated for deer management and conservation goals (Felix et al., 2007). Robust modeling and field studies are needed on the interactions of cattle and deer (and/or other wildlife species) behavior on tick-infested landscapes to define dynamic relationships of host diversity on tick dispersal and maintenance and to improve tactics for tick suppression and elimination as this aspect has been identified as a gap in our ability to develop effective control strategies to control parasites affecting livestock and wildlife (Morgan et al., 2004). The physiology and grooming behavior of WTD reduced both the number of *R. annulatus* successfully feeding and their subsequent fecundity (Cooksey et al., 1989). Nevertheless, the frequency of both CFT species infesting WTD in both Texas and Mexico increased (Gray et al., 1979; George, 1990). Movement of tick-infested WTD from Mexico across the Rio Grande is often the suspect, or confirmed source of infestations in Texas. Expanses of game fencing were erected as physical barriers to deer, and the previously successful bovine-centric eradication strategies were challenged. Infested premises where the pasture vacation option was implemented often resulted in prolonged infestations and quarantines suggesting WTD were capable of sustaining tick populations in the absence of cattle (George, 1990). Of the two CFT species, *R. microplus* is known to sustain populations on deer in the absence of cattle (Kistner and Hayes, 1970; George, 1990). Reliance on cattle dipping as a means of sponging ticks from infested land (Gray et al., 1979), and the implementation of WTD-specific treatment tactics have been integrated into the CFTEP (George et al., 2008; Pound et al., 2010). Efforts to eradicate CFT infesting alternative wildlife hosts such as WTD present a challenging situation for the CFTEP. The process of issuing quarantines and treating livestock and deer is a significant financial burden on federal and state agencies that also impacts livestock producers. Estimates indicated that the expense to systematically treat every cattle could average $250 per head and lead to an 80% decline in net cash farm income (Anderson et al., 2010). During 2011, approximately 9% of total CFTEP expenditures were allocated to CFT mitigation for deer. Future challenges include optimal integration of these tactics and other emerging technologies as discussed below in adaptable ways to different and changing landscape types for environmentally sound and sustained CFT eradication.

Exotic ungulates

Interests in foreign wildlife conservation, recreational hunting, and marketing exotic meats provided incentives for private landowners to import exotic ungulates and develop management programs. Initial importations date to the 1920's, and today Texas has more species and greater numbers of exotic game than any other state in North America (Mungall and Sheffield, 1994). Statewide surveys conducted by the Texas Parks and Wildlife Department in 1963 estimated 13 species established and 13,000 animals, and by the final survey in 1996 estimated 76 species and 190,000 animals. These surveys indicate approximately 2/3 are confined by high game fencing and 1/3 are free-ranging. The most successful introductions have been of hoof stock, most originating from Asia. The success of these animals in Texas is due in part to the similarity of respective foreign climates and lack of predators.

The South Texas Plains and Edwards Plateau ecoregions (**Figure 1C**) have the highest concentration of exotic animals adjacent to the international boundary and permanent quarantine zone (Mungall and Sheffield, 1994). Axis (*Cervus axis*), fallow (*Cervus dama*), and sika deer (*Cervus nippon*), nilgai (*Boselaphus tragocamelus*) and black buck antelope (*Antilope cervicapra*), mouflon (*Ovis musimon*), and aoudad sheep (*Ammotragus lervia*) comprise the most abundant species, and the range of several of these species are known to extend into Mexico. Exotic species discovered with infestations of either *R. microplus* or *R. annulatus* include nilgai, aoudad sheep, wapiti (*Cervus canadensis*), red deer (*Cervus elaphus*), fallow deer, and axis deer (Sheffield et al., 1983; Pound et al., 2010). Like *R. microplus*, the original home of nilgai is the Indian subcontinent. Wapiti and red deer were introduced in Mexico where free-ranging populations live along the border with south Texas (Gallina and Escobedo-Morales, 2009); there are anecdotal reports from Mexico of heavy *R. microplus* infestations in red deer. While the suitability of other exotic hoof stock as potential hosts for CTF is unknown, the adaptability of *R. microplus* to new wildlife hosts has been demonstrated in Brazil and New Caledonia (Cançado et al., 2009; De Meeûs et al., 2010).

Invasive feral swine (*Sus scrofa*) are found throughout the southern region and they have invaded the 10 ecoregions of Texas (Taylor et al., 1998; Rollins et al., 2007; Campbell and Long, 2009). The Texas feral swine population has recently been estimated to be at least 2 million animals (Campbell et al., 2008), and their success credited to high reproductive rate, omnivory, and adaptability (Mungall and Sheffield, 1994). They are significant pests in rural and urban environments, yet also considered valuable for recreational hunting (Rollins et al., 2007). Seven non-CFT species of Ixodid ticks have been found parasitizing feral swine in Texas ecoregions east and north of the border area (Sanders, 2011;

Schuster, 2011). Six tick species are three-host ticks and one species, *Dermacentor albipictus*, is a one-host tick. The potential for feral swine to serve as a host for CFT is raised by the recovery of adult *R. microplus* from domestic pigs in Bangladesh (Islam et al., 2006; Ghosh et al., 2007). Additionally, feral swine create frequent breaches in game fences compromising barrier integrity to wildlife containment (Bodenchuk, 2010). Anthropogenic interests in native and exotic wildlife add host diversity for *R. microplus* and *R. annulatus* in this region, which provides new avenues for tick dispersal and maintenance. Future efforts to eliminate CFT in outbreaks involving exotic hosts will require integration of such factors as habitat use modification, home range management, nutrition, behavior, and the expertise of wildlife specialists to develop best practices (**Figure 3**).

CLIMATE VARIABILITY AND CHANGE AS DRIVERS OF CFT OUTBREAK SURGES

Solar cycles and global climate influence the dynamics of ecosystems at the macro level (Lean, 2010). A glance at global and national weather/climate events for 2011 provides context for the pathways of global change impacting CFTEP operations. Globally, 2011 was the second wettest year on record since 1900 and this was accompanied by the warmest annual above-average temperatures registered in the southern U.S. The drought in northern Mexico was ranked ninth in a list of top global climate/weather events and Texas experienced its driest year on record in 2011 (National Climatic Data Center, 2012).

Climatic factors limit the geographic distribution of tick species and consequently the epidemiology of tick-borne diseases is also susceptible to the influences of climate (Sonenshine and Mather, 1994; Gray et al., 2009). Where they are endemic, the abundance of tick species is influenced by changes in temperature and moisture. Climate variability is one of the drivers for the emergence and re-emergence of tick-borne diseases (Lafferty, 2009; Randolph, 2010).

In addition to solar cycle activity, the interplay of oceanic and atmospheric processes results in cyclical phenomena that impact regional climates (Gurney and Sapiano, 2006). Examples of such phenomena include the El Niño/Southern Oscillation (ENSO), the Accumulated Cyclone Energy Index (ACE; Enfield and Cid-Serrano, 2010), and other oceanic indicators, which are linked to indices used to forecast weather conditions. Global environmental events influence the population ecology of arthropod-vectors and vector-borne diseases (Baylis and Githeko, 2006; Linthicum et al., 2008; Anyamba et al., 2012). Anomalies in these phenomena alter the ecological balance, which can result in outbreaks of pests and vector-borne diseases. A warning system based on temporal and spatial predictions of Rift Valley fever outbreak activity derived from analysis of ENSO-related climate anomalies measured through remote sensing of environmental parameters facilitated disease outbreak and mitigation activities (Anyamba et al., 2009). The ENSO, for example, was found to influence CFT populations in North America (Estrada-Peña et al., 2005). Areas in the southern U.S. are subject to sudden changes in habitat suitability for the CFT that are linked to ENSO. This trend highlights a risk for the CFTEP as divergent changes in climate can augment the threat of invasion by *R. microplus* or *R. annulatus* (Estrada-Peña and Venzal, 2006).

At the regional scale, it is noted that the number of tick events recorded within the permanent quarantine zone and in the free zone of south Texas covering fiscal years 1959 through 2011 shows distinct, recurring surges of CFT activity (**Figure 5A**). By averaging the first (1960–1980) and second (1990–2011) surges, a characteristic tick cycle was revealed (**Figure 5B**). Significantly, the rapid onset of the surge and equally rapid subsidence, or recovery,

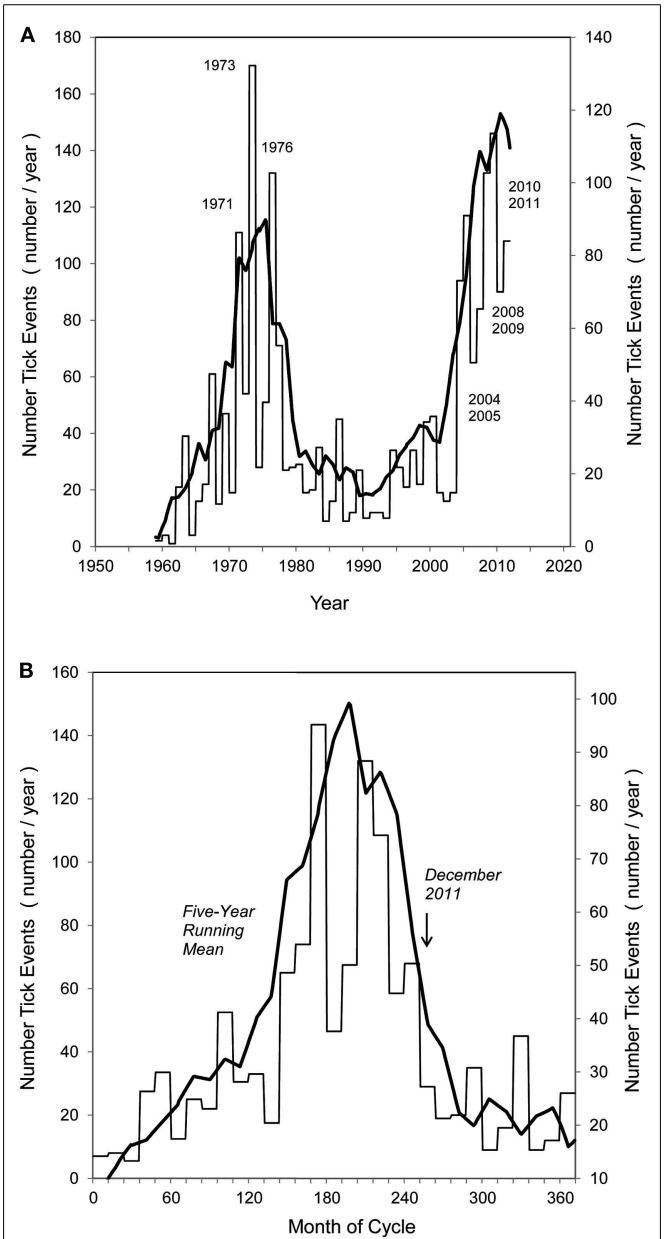

FIGURE 5 | (A,B) Pattern of tick surges in quarantine + free zones of south Texas, 1959–2011, with 5-year running mean (heavy line): **(A)** yearly level of tick events, showing major years of outbreak in 1970 and 2000 decades; **(B)** averaged surge periods of 1959–1989, and 1991–2011, showing sharp onset and subsidence of characteristic surge dynamic. December 2011 identifies last sample date and expected drop of tick levels over following year.

suggests that this is a representative and recurring dynamic of CFT outbreaks in this region.

Fast Fourier Transform analysis was applied to test the hypothesis of recurring cycles of CFT outbreak activity (SeaSolve, 2004). In addition to identifying principal, recurring CFT events, Fast Fourier Transform analysis enables the recognition and quantification of embedded patterns, which can be important clues to other factors driving CFT population surges such as solar, oceanic, and/or precipitation cycles. **Figures 6A–C** show a dominant interval lasting 35 years for increased CFT activity in the permanent quarantine + free zone, 33 years in the permanent quarantine zone, and 36 years for outbreak activity in the free zone, respectively. In the latter, the pattern appears simple and likely the result of CFT infestations spreading beyond the permanent quarantine zone that includes a lag interval of about three years. It appears that other factors are operating in the permanent quarantine + free, and permanent quarantine zones (**Figures 6A,B**). Beyond annual variations, there seems to be a pattern that might include a 4–6 or 8.5 years cycle, which could repeat approximately every 17 years. This observation suggests the operation of ENSO effects with recurrences at those frequencies. It is hypothesized that the 33–36 year interval reflects the influence of the ACE operating at a multidecadal frequency. These spectral intervals are early approximations at this point, and it is recognized that the Fast Fourier Transform analysis is based on only two surges of CFT activity with the most recent surge apparently still in process.

A reconstruction of the wavelet analyses as shown in **Figure 7** depicts how the results of the Fast Fourier Transform analysis can be applied to forecast future surges of CFT activity in the region. Should the pattern of 1959–2011 repeat itself, one could anticipate a third surge of tick activity in the 2040 decade; uncertainty on its timing and severity needs to be resolved through a better understanding of oceanic signals driving high rainfall intervals in the region, as explored below. Assessing future impacts of climate oscillations and climate perturbations, from tropical hurricanes to acute drought, by modeling normal and extreme conditions would better inform regulatory officials and policy makers on operational needs and regulatory changes as suggested for other disease systems influenced by global change (Garner et al., 2007; Woolhouse, 2011; Anyamba et al., 2012). In particular, strategic forecasting by the CFTEP would enable the adaptation of integrated protocols involving the use of different technologies to minimize the number of, and expedite the elimination of outbreaks. Examples of emerging technologies for integrated eradication are mentioned in the following section.

The field data show the first surge peaked in 1973 when 112 of 170 outbreaks were recorded in the free zone. It took 6 years to reduce the total number of outbreaks at a significant cost for producers and the CFTEP and TAHC, but CFT incursions into the free zone continued to be detected until 1990. The second surge perhaps peaked in 2009 and it appears to be subsiding now. Another *a priori* observation suggests that there is a lag interval where outbreaks in the permanent quarantine zone increase before a transient invasion of the free zone occurs. Thus, the historical record documents recurring, distinct periods of CFT outbreak activity in the south Texas–Mexico border region. The likelihood of recurring episodes is supported by statistical analysis and reconstruction of cycle frequencies in the CFT record. Climate warming was identified as a trend that can alter habitat suitability for *R. microplus* in the Americas including the southeastern U.S. (Estrada-Peña, 2001).

A shift in mean temperature at the global and century scale is primarily a phenomenon of changes at high latitudes of the northern and southern hemispheres. Mean temperature changes in specific regions such as south Texas may or may not coincide with this global trend. The regional trend, for example, decreased from 1895 through about 1975, but then warmed to levels tracking the global increase. Of note, the temperature increases since

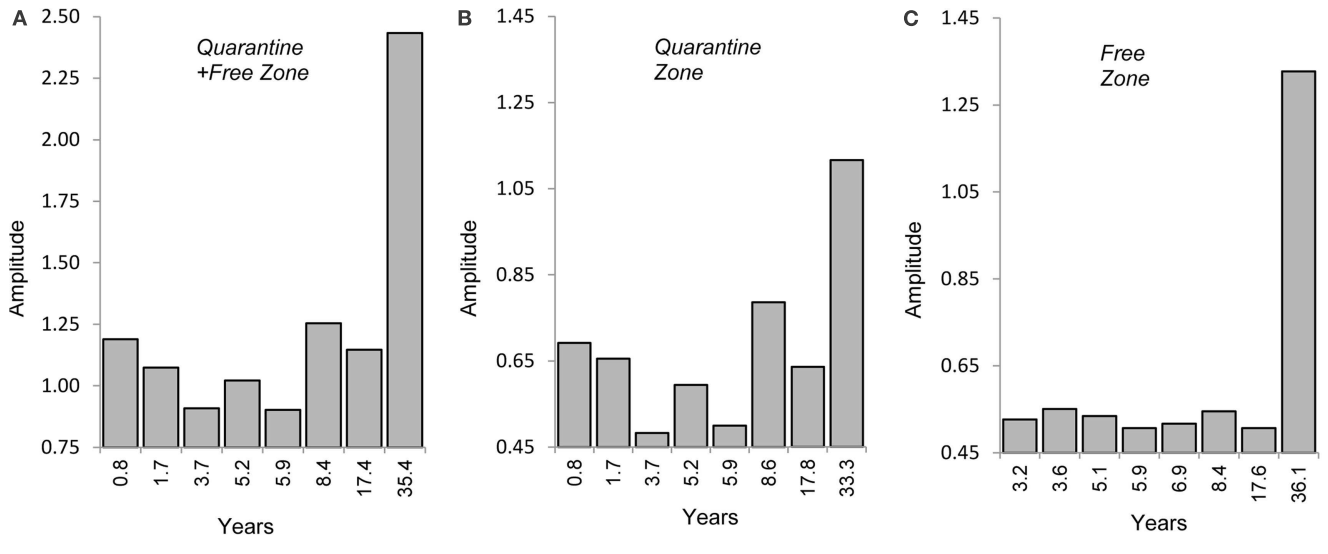

FIGURE 6 | (A–C) Fast Fourier Transform analysis showing strength (amplitude) of main and sub-frequencies (years) of tick outbreak patterns: **(A)** Quarantine + Free Zone; **(B)** quarantine zone; **(C)** Free zone.

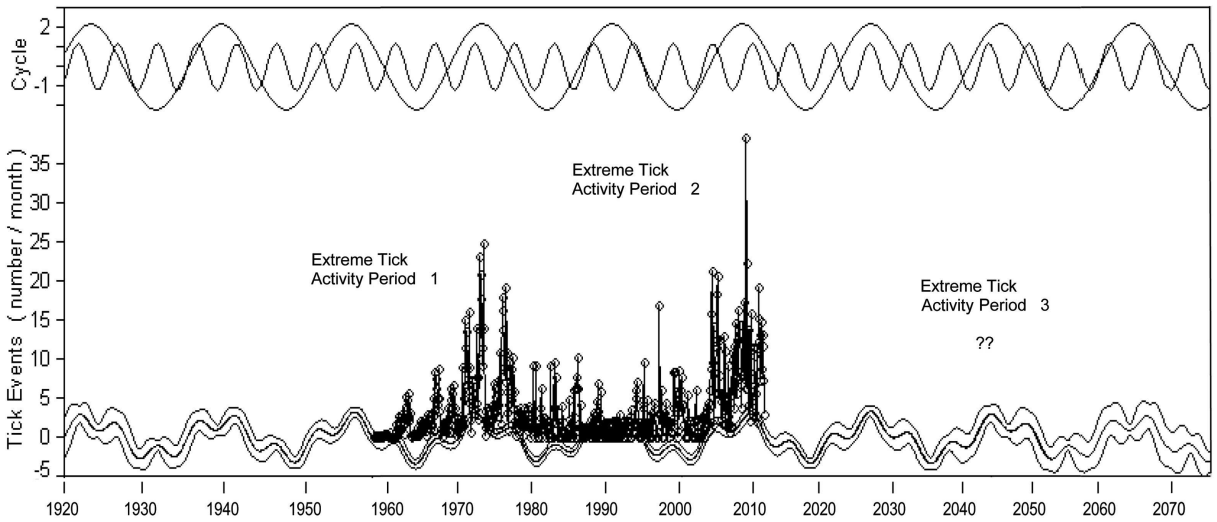

FIGURE 7 | Fast Fourier Transform wavelet analysis of tick events in Quarantine + Free Zone. Two main cycle frequencies are shown at top of graph. Tick outbreak data (1959–2011) on which analysis is based is shown in vertical line and point format. Tick cycles are backcast to 1920 and forecast to 2077 with 90% confidence limits. A period of increased CFT outbreak activity is posited to occur during the 2040 decade.

1975 occurred in all south Texas NOAA Climate regions from the coast (Region 10) inland to NOAA Climate Region 5, west of the Big Bend border area along the Rio Grande. Moreover, the high plains of the U.S. continental interior, i.e., Platte Drainage, CO, also showed this post-1975 warming pattern, as did Caribbean Region sea surface temperatures. Hence, it appears that a widespread regional temperature trend consistent with, and possibly driven in part by changing global conditions could be influencing CFT outbreak activity, especially in the case of the tropically adapted *R. microplus* as suggested by Estrada-Peña (2001).

Because relative humidity is an important determinant of tick survival (Estrada-Peña, 2001; Corson et al., 2004; Estrada-Peña and Venzal, 2006), we probed for a connection between outbreak surges and long-term regional rainfall. We also queried the possibility that ocean indicators such as the ACE not only drive the rainfall patterns in south Texas (Enfield et al., 2001), but offer an advanced signal of pending major precipitation shifts. The analysis of rainfall in the quarantine + free zones revealed two marked wet periods when the 10-year running precipitation average was applied (**Figure 8A**). The 10-year averaging was required because any monthly pattern was masked by especially high variability and no apparent regression trend. Notably, these prolonged wet intervals coincide closely with the two CFT outbreak surges (**Figure 8A**). Moreover, the high rainfall interval appears driven by the ACE, which is a measure of North Atlantic and Caribbean Region hurricane severity that brings rain into south Florida and south Texas. Since 1975, the lag between the intervals of high rain periods in south Texas and increase in ACE is ∼3–5 years (**Figure 8B**). Hence, a simple climatic algorithm to track outbreak surges that offers the additional possibility of anticipating such surges years in advance is presented here. The Atlantic Multidecadal Oscillation, thought to correlate with and drive the ACE (Enfield and Cid-Serrano, 2010), also provides advance warning of changes in the south Texas rainfall.

ACARICIDE RESISTANCE RISK AND TECHNOLOGICAL OPPORTUNITIES FOR INTEGRATED ERADICATION

The CFT *R. microplus* is ranked as the sixth most pesticide-resistant arthropod in the world (Whalon et al., 2008). This situation reflects the historical reliance on chemical acaricides, which are pesticides that kill ticks, to control and eradicate CFT. Arsenical dips were introduced in 1911 and they were used by the CFTEP until the 1960s (Graham and Hourrigan, 1977). Organophosphate acaricides replaced arsenical dips in 1967, and in 1971 the CFTEP started using coumaphos to dip cattle and spray horses. Additionally, an injectable macrocyclic lactone acaricide formulation is used on a need-basis by the CFTEP during outbreaks where dipping alone presents difficulties for eradication. The two technologies employed to eliminate CFT infesting WTD are ivermectin-medicated corn and topical permethrin self-treatment using a bait station fitted with the "2-poster" adapter (Pound et al., 2010). Corn medicated with ivermectin was used to eradicate long-term CFT infestations maintained by wapiti and WTD (Pound et al., 2009).

In Mexico, organophosphate acaricides were used for the national tick eradication program between 1974 and 1984 (Trapaga, 1989). Pyrethroids and amitraz were authorized for CFT control in Mexico since 1985 (Rosario-Cruz et al., 2009). The use of amitraz gained popularity after CFT became refractory to treatment with organophosphates and pyrethroids, but resistance to amitraz was eventually reported in 2001 (Domínguez-García et al., 2010). The ticks have also evolved resistance to fipronil and multiple resistance to organophosphates, pyrethroids, amitraz, and ivermectin in Mexico (Miller et al., 2008; Fernández-Salas et al., 2012). Resistance to organophosphates and pyrethroids has been detected in CFT collected in Texas (Miller et al., 2005, 2007). Surveillance of acaricide resistance in CFT from outbreaks and other sources is an ongoing effort by the Cattle Fever

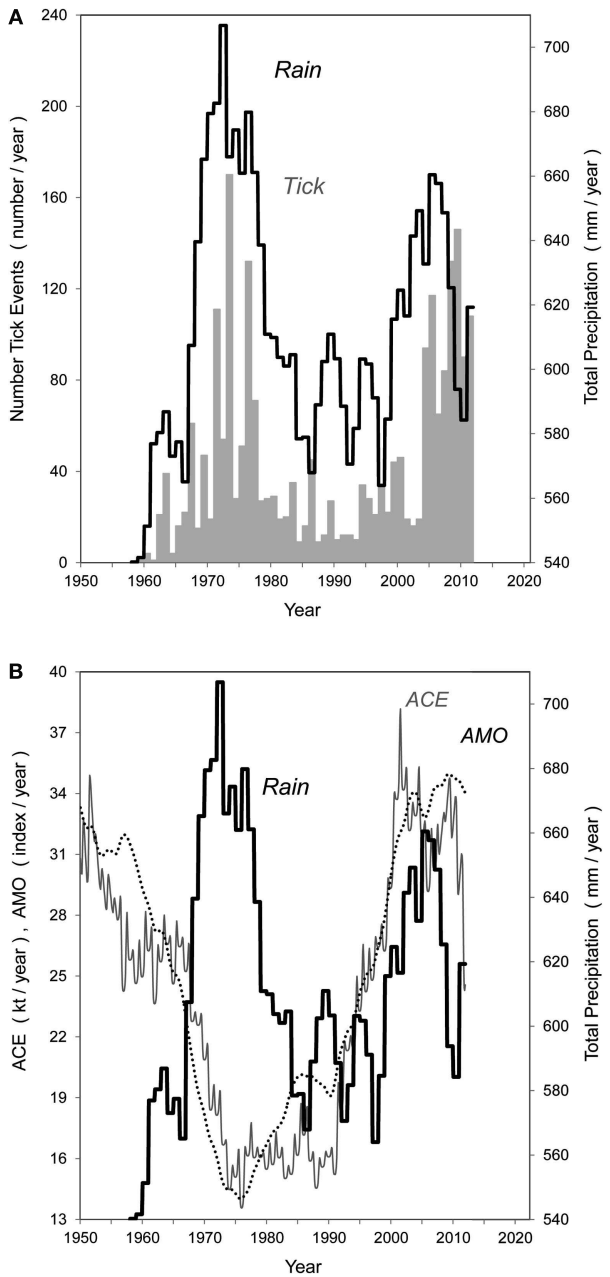

FIGURE 8 | (A,B) Comparison of annual total precipitation (rain, heavy line) in south Texas, 1959–2011 to: **(A)** number of tick events in quarantine + free zones (light gray); **(B)** magnitude of the annual Accumulated Cyclone Energy Index (ACE) and Atlantic Multidecadal Oscillation (AMO). The AMO at $1/(45x+25)$ of scale shown. No lags are applied.

Tick Research Laboratory of the USDA-Agricultural Research Service because this is a constant threat to CFTEP operations.

Anti-CFT vaccination in cattle and wildlife, and the passive administration of systemic acaricides for cattle are two technologies that could be used in an integrated fashion by the CFTEP (Pérez de León et al., 2010). Vaccines against *R. microplus* based on the protective antigen Bm86 were developed and marketed under the names TickGARD (Willadsen et al., 1995), and Gavac (Canales et al., 1997). Their efficacy against that CFT species generally ranges from 10 to 89% (Willadsen, 2008). The level of efficacy reported for vaccines containing a recombinant form of Bm86 varies according to, among other things, the expression system used for vaccine production, characteristics of the tick population targeted, and host factors (de la Fuente et al., 1999; Patarroyo et al., 2002; Sitte et al., 2002; Casquero Cunha et al., 2012). It has been postulated that sequence variation in the *bm86* locus is associated with low susceptibility of certain *R. microplus* populations infesting cattle immunized with commercial Bm86-based vaccines (García-García et al., 1999). Interestingly, the efficacy of Bm86-based vaccines against *R. annulatus* is >99%; this level of efficacy is equivalent to that obtained with commercial acaricides in the absence of resistance. Anti-CFT vaccines based on Bm86 play an important role in integrated tick management systems (de la Fuente et al., 2007; Willadsen, 2008). Strategies combining anti-CFT vaccination and acaricides were shown to reduce the number of acaricidal applications necessary for tick control (de la Fuente et al., 2007), which also extends the useful life of acaricides by delaying the development of acaricide resistance. Gavac is not commercially available in the U.S. This vaccine was 99% efficacious against an outbreak strain of *R. annulatus* (Miller et al., 2012).

The post-genomic era offers the opportunity to take a systems biology-based approach to antigen discovery and vaccine development (Oberg et al., 2011). A web-based repository of *R. microplus* genomic data is helping with the identification and selection of sequences coding for molecules that can be tested as protective antigens (Bellgard et al., 2012). Ecologically based modeling can also be applied to evaluate and adjust various factors to maximize vaccine efficacy against CFT invading the U.S. (Miller et al., 2012). All these approaches are being applied to discover novel antigens that protect cattle in the permanent quarantine zone against *R. microplus* infestation, which could also be used to vaccinate WTD (Guerrero et al., 2012). Carreón et al. (2012) proved the feasibility of protecting WTD against CFT infestation through vaccination. The utility of vaccinating WTD for CFT eradication in south Texas could be tested initially in high-fenced premises. Combining vaccination with the existing CFT treatments for WTD will eliminate their role as reservoirs for the ticks.

As compared to other suitable hosts, populations of CFT realize their full reproductive potential when parasitizing cattle. Although it is likely that CFTEP strategies will remain focused on eliminating CFT infesting cattle, the use of methods applied to wildlife is needed because it appears that pasture vacation represents an anachronism from the time when cattle, horses, mules, and asses were the only hosts of concern (George et al., 2008). Therefore, it is proposed that the combined use of emerging technologies like vaccination of cattle and WTD against CFT and the use of an ivermectin-medicated feed block be explored. The integration of these tools with existing treatment protocols could lessen the economic and operational impacts of quarantines on producers.

RISK FOR WILDLIFE WITH STRAINS OF *B. BOVIS* AND *B. BIGEMINA* PATHOGENIC TO CATTLE

Attempts to infect WTD with the agents of bovine babesiosis were unsuccessful (Kuttler et al., 1972). A study investigating the possibility that WTD might serve as a reservoir of bovine babesiosis revealed that the addition of bovine serum was required for *in vitro* culture of *B. bovis* in WTD erythrocytes (Holman et al., 1993). Serologic evidence and results from PCR tests indicated that WTD in Mexico could be infected with *B. bovis* and *B. bigemina* (Cantu-Covarrubias et al., 2009). Similar evidence exists for WTD in Texas (Holman et al., 2011). However, the data thus far suggests that WTD bitten by CFT infected with *B. bovis* or *B. bigemina* function as diluting hosts because these piroplasms are unable to undergo the asexual portion of their life cycle in WTD and become infective for CFT. The deer may be having a zooprophylactic effect by reducing the prevalence of infected CFT in the environment.

The nilgai is a tropically adapted bovid that poses a risk for the re-emergence of bovine babesiosis in the U.S. because, in addition to its host ability for CFT, this species adapts well to agricultural areas and commingles with cattle in south Texas (Sheffield et al., 1971; Davey, 1993; Leslie, 2008). *Babesia* infection in a nilgai from India was reported (Baviskar et al., 2009). There are approximately 30,000 nilgais along the south Texas–Mexico border and a population in Mexico was polymerase chain reaction-positive for *B. bovis* and *B. bigemina* (Cárdenas-Canales et al., 2011).

PERSPECTIVES

The predominant CFT species at the beginning of the eradication campaign was *R. annulatus*. By comparison *R. microplus* is a more invasive species. Records indicate CFT outbreaks in south Texas are frequently caused by *R. microplus*. The invasion and re-invasion of other parts of the world by *R. microplus* highlight the danger this tick poses to U.S. animal agriculture. Climate change could augment the economic impact of CFT by influencing habitat suitability and shifts in potential distribution of both species. Climate oscillations of wet and dry periods will undoubtedly continue to impact CFT and the preparedness of the CFTEP to respond to these changes. Additional hypothesis-driven research is required to effectively understand and manage the risk of CFT and bovine babesiosis re-emergence in the U.S. The complicated nature of current and future problems driven by global change requires that societal, environmental, scientific, and policy considerations be integrated to develop the most effective and sustainable strategies and goals to prevent re-establishment of CFT and/or outbreaks of bovine babesiosis (**Figure 3**).

Analyses of climate change effects presented here demonstrate that North Atlantic oceanic indicators, i.e., AMO and ACE, and region-scale rainfall shifts have a marked impact on periodic, CFT outbreak activity in south Texas, and may provide opportunity to develop responsive surveillance strategies. Here, it was documented that the collection and analysis of meaningful data allowing assessment and prediction to identify early warnings of pending changes in CFT outbreak activity is possible. It is recognized that future developments in monitoring and interpreting important ocean signals such as the AMO and ACE will continue to improve predictive power. Ongoing and future efforts to carefully systematize CFT records and put them into electronic format for pre-structured models designed to share with and fully inform decision makers at all levels is justified.

Comprehensive studies are advocated to explore thoroughly climate impacts on CFT, as well as other ticks, to design and integrate best management practices to evaluate ecosystem shifts affecting emergence of tick-borne diseases, and to inform policy makers for rational and effective decision making. This knowledge base could be used as the foundation to create and implement an international CFT monitoring and prediction program for the assessment and analysis of changes in tick distribution, ecology, host utilization, and disease epidemiology to mitigate the economic impact of these ticks and the pathogens they transmit. Such systems have been applied successfully to other arthropod-borne diseases and they could be consolidated in existing databases (Anyamba et al., 2009; Gale et al., 2010; Vesco et al., 2011). Worldwide coordination to adapt and refine prediction systems for ticks and tick-borne diseases will enable the enhancement of public health and veterinary services programs to anticipate, prepare, and respond to these emerging and re-emerging human and animal health threats (Institute of Medicine, and National Research Council, 2009).

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A renewed way of malaria control in Karnataka, South India

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INTRODUCTION

Malaria still causes a major public health problem in tropical and sub-tropical countries. Globally, World Health Organization (WHO) reports approximately 225 million malaria cases and 781,000 deaths each year, mostly in African children (WHO, 2010). In the past decade, several efforts have been initiated to scale-up malaria control, especially under Roll Back Malaria of WHO. Most of the interventions are indoor residual sprays (IRS), and use of insecticide treated nets (ITNs)/long-lasting impregnated nets (LLINs). This has resulted in significant reductions on malaria-associates morbidity and mortality in most parts of Africa. In spite of massive initiatives undertaken by various agencies, malaria still continues to haunt (O'Meara et al., 2010). Here, we describe how two larvivorous fish guppy (*Poecilia reticulata)* and mosquito fish *Gambusia affinis* are used in malaria control in villages in Karnataka, south India for more than a decade.

MALARIA SITUATION IN INDIA

National Vector Borne Disease Control Program (NVBDCP) is the central nodal agency, which takes care of all major vector borne diseases in India. Six diseases namely malaria, lymphatic filariasis, Japanese Encephalitis, kala-azar, dengue, and chikungunya are the major vector borne diseases in India [National Vector Borne Disease Control Program (NVBDCP), 2012]. Lymphatic filariasis and kala-azar are undergoing elimination process.

Currently, India contributes about 70% of malaria and 50% of mortality in the South East Asian Region of WHO. In most geographical settings, malaria transmission is heterogeneous having variable paradigms (Dash et al., 2008). It has witnessed several phases of malaria. In 1950s under the Global Malaria Eradication Program of WHO, malaria was almost reached at the eradication phase with the extensive use of DDT as the incidence declined from an estimated 75 million cases and 800,000 deaths in 1947 to a merely 49,151 cases in 1961 with no deaths. Recent trends show that each year India reports approximately two million malaria cases with 1000 deaths. Most of the cases are reported in the ethnic tribes living in the forested pockets of the states of Odisha, Jharkhand, Madhya Pradesh, Chhattisgarh and the North Eastern states which contribute bulk of morbidity and mortality (Dash et al., 2008).

Of the 10 known vectors, *Anopheles culicifacies* is the main rural vector mainly breeds in wells, streams and ponds, and responsible for 60–70% of rural malaria transmission. It has a complex of five sibling species designated as A, B, C, D, and E. *Anopheles fluviatilis*, vector of local importance breeds in slow running streams and has four sibling species namely S, T, U, and V. Species S is the main malaria vector in most of the tribal belts (Dash et al., 2008).

STRATEGY FOR VECTOR CONTROL UNDER NATIONAL PROGRAM

The main and effective control strategy to interrupt transmission is the use of synthetic insecticides such as organochlorine, organophosphate compounds, and synthetic pyrethroids applied under the national program guidelines. The major component of rural malaria control relies on IRS, whereas in the urban settings the main component is on larval control. Several chemical insecticides, insect growth regulator compounds, and also bio-pesticides such as *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* are being employed in vector control.

Insecticide method has not been very successful due to various factors of human behavior, resistance to vectors, operational, administrative, and prohibitive costs. Moreover, there are negative impacts on the non-target species. These are some of the reasons to go for other alternative methods of vector sanitation, draining, and environmental managements. In the national program, use of larvivorous fish has been advocated as an alternate strategy. A detailed description has given in the manual [National Vector Borne Disease Control Program (NVBDCP), 2009].

LARVAL SOURCE MANNGEMENT

Larval source management (LSM) in vector control has not got much importance due to various factors. The major reason was the lack of confidence in employing at the grass root level. In rural settings, besides scattered human settlements, there are vast breeding habitats of mosquitoes. In the beginning of the twentieth century, LSM was the only method of vector management, and got less importance after the arrival of DDT. It lasted for almost four decades, and it was realized the need of the alternate strategy when there was a resurgence of malaria in the mid 1970s. Even today LSM has received a low profile in vector management. Fillinger and Lindsay (2011) have given a detailed account on the use of this strategy, and pointed out that most of the developed nations are using this strategy to manage mosquito control. They questioned why this strategy has not got due importance in the African countries where the real problems exist.

LARVIVOROUS FISH IN MALARIA CONTROL

Among all the biocontrol agents, larvivorous fish are widely used in vector control. Approximately 315 fish species under seven genera are reported to have larvivorous nature (Ghosh and Dash, 2007). Two poeciliid fish *P. reticulata* and *G. affinis* are being used extensively. In India, fish are used in the public health program since 1903. In 1908, top minnow *Poecilia* a native of Caribbean Island was brought to British India for fish-based mosquito control. Another minnow *Gambusia* (generally known as mosquito fish) was introduced intentionally in most parts of the world out its native South America in 1905 (Gerberich, 1985). In 1928, Dr. B. A. Rao, brought this fish to India form Italy through sea route, and was released in the famous Lalbagh tank in Bangalore. This began the era of fish-based malaria control in India (Ghosh and Dash, 2007; Chandra et al., 2008).

WHAT LED TO FISH INTRODUCTIONS IN KARNATAKA?

Karnataka state produces 70% of high quality mulberry silk in India. District Kolar and adjoining areas produce major share of this

variety of silk. Malaria was posing a serious public health problem in these areas, and the routine IRS for malaria control was hampering the rearing of the silk worms (Lepidopteran larvae). The local farmers thus resisted to such spray operations, and malaria control operation never got a local community support. Under such prevailing conditions, we took a challenge to control malaria without insecticide. Initially, we did not know how to solve such problem as there were no guidelines available for non-insecticide method of malaria control in rural settings.

To begin with, in 1993 one highly malaria problematic primary health center (PHC) Kamasamudram (population over 37,000) was selected as a demonstration site to evolve a non-insecticide method of malaria control. This PHC is situated 100 km east of Bangalore city. Entomological surveillance of vector species showed the presence of *A. culicifacies* and *A. fluviatilis*. After long deliberations, it was decided to undergo LSM against these vectors. Geographical reconnaissance was carried out to identify and locate the potential vector breeding habitats. Among various breeding sources, wells, ponds, and streams were the main breeding habitats of the two vector species. It was found that malaria was comparatively numerous in six sub-centers of this PHC. Interestingly, there were very less malaria in the remaining five sub-centers. This area is connected with the Kolar Gold Field which was operational from 1896. Further analysis of malaria distribution in the six sub-centers showed that villages having more number of malaria cases are surrounded by wells and ponds. On the contrary, villages located on streams had less number of malaria. Based on this information, genetic analysis of the vector species was carried out. Two sibling species A and B of *A. culicifacies* and, species T of *A. fluviatilis* were present. Species A was more numerous in villages having wells and ponds, whereas species B in villages located on streams (Ghosh et al., 2005). Based on laboratory experiments, species A is an efficient carrier of malaria parasites, while species B exhibited partial refractoriness to *P. falciparum* and complete refractoriness to *P. vivax* infections (Adak et al., 2006). All *A. fluviatilis* belonged to species T, and were zoophagic indicating non-participation in transmission (Ghosh et al., 2005). This information was very important that helped in designing the LSM of the vector species. A pre-plan for channelization of the streams with a huge cost was thus abandoned (Ghosh et al., 2005).

Subsequent surveys in the non-malarious sub-centers detected the presence of *Poecilia* in most of the breeding habitats. Entomological surveillance also indicated the absence of the vector species. This was a great breakthrough in our search for planning a suitable vector control strategy. Accordingly, *Poecilia* was introduced in all the breeding habitats especially in ponds and wells in 1994. This resulted in almost 50% reduction on malaria incidence in 1995 from an average annual parasite index (API; cases per 1000 population per year) of over 40. Entomological surveillance and *Poecilia* monitoring indicated the ineffectiveness of this fish in ponds. In late 1995, approximately 500 *Gambusia* were released in a ditch connected with the main channel of Kamasamudram tank. In 1996 a heavy flood had occurred, and these fish were dispersed in the entire area along with the flood waters. Subsequently, this resulted in a total elimination of malaria in this area in the subsequent years for over 15 years. Entomological monitoring also showed very level of vector abundance that did not support malaria transmission.

In 2001, the trial was extended in a highly malaria-endemic area having a population of 1.3 million. Here the average API was over 130 and malaria has reached to a near elimination phase. Now, the program is being extended to the entire state.

DISCUSSION

Larvivorous fish in malaria control is a renewed strategy (Ghosh and Dash, 2007). In our study we have combined the use of two fish for better sustainability and effect on the vector population. *Poecilia* is very effective in closed eco-system mainly in wells, whereas *Gambusia* in open eco-systems namely ponds and streams. Another edible fish Grass carp *Ctenopharyngodon idella*, a large cyprind native of eastern Asia is useful in clearing aquatic weeds in ponds where mosquito larvae hide. Combination of *C. idella* with *Gambusia* effectively controlled larvae of *A. culicifacies* in village ponds that contained aquatic vegetations. *C. idella* cleaned the vegetations, and subsequently *Gambusia* eliminated the mosquito larvae. Thus malaria was eliminated in the affected villages (S. K. Ghosh, personal observation).

We have observed that *Gambusia* can be cultured along with other edible fish without any impact on the local fish fauna. Local fish species *Puntius* sp., *Rasbora daniconius*, *Glossogobius giuris*, *Chanda nama*, *Channa* sp. along with common edible carps were collected in *Gambusia* introduced ponds (S. K. Ghosh, personal observation). Haq et al. (1991) reported that *Gambusia* did not alter the edible fish production in village ponds in north India. They recommended both these fish can be cultured to get the dual benefits.

Fish-based malaria control is very cheap and sustainable. As per our estimate only INR 1.00 (US $ 0.02) per capita per year is required for the entire operation. Kusumawathie et al. (2006) estimated application of *P. reticulata* was 2.67 times less costly than that of temephos (an organophosphorus anti-larval insecticide).

Several reports are now available on the use of fish in malaria control. In Somalia, Mohamed (2003) reported larval control in *barkits* (reservoirs) with *Oreochromis spilurus spilurus*. Fletcher et al. (1992, 1993) also mentioned the use of local fish *Aphanius dispar* and *O. spilurus spilurus* in Ethiopia. Matias and Adrias (2010) reported the effectiveness of *Nothobranchius guentheri*, a native of Tanzania, in temporary mosquito breeding habitats. This fish can be applied in arid zones where malaria vectors breed in temporary habitats.

There are some reports on negative impacts of the non-native poeciliid fish on the local fish fauna especially *Gambusia* (Rupp, 1996). However, we have not observed such impacts either on the local edible fish production or on the fish fauna. Work is underway to address this issue.

CONCLUSION

Our study has clearly shown that fish can be introduced in malaria control program. *Poecilia* introductions in indoor water storing cement tanks also contained *Aedes aegypti* larval infestation in Karnataka (Ghosh et al., 2011). This can be incorporated in the integrated vector management program (WHO, 1997). Use of global positioning systems and remote sensed data will enhance the progress of the fish-based malaria control program (Boswell et al., 2005). This work needs full

dedication, and all potential breeding habitats must be covered with fish. Routine monitoring of fish survival and reintroduction are important for this strategy. Community participation and health education is an integral part of this strategy to achieve a malaria free world (Ghosh et al., 2006).

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Global climate change and its potential impact on disease transmission by salinity-tolerant mosquito vectors in coastal zones

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Global climate change can potentially increase the transmission of mosquito vector-borne diseases such as malaria, lymphatic filariasis, and dengue in many parts of the world. These predictions are based on the effects of changing temperature, rainfall, and humidity on mosquito breeding and survival, the more rapid development of ingested pathogens in mosquitoes and the more frequent blood feeds at moderately higher ambient temperatures. An expansion of saline and brackish water bodies (water with <0.5 ppt or parts per thousand, 0.5–30 ppt and >30 ppt salt are termed fresh, brackish, and saline respectively) will also take place as a result of global warming causing a rise in sea levels in coastal zones. Its possible impact on the transmission of mosquito-borne diseases has, however, not been adequately appreciated. The relevant impacts of global climate change on the transmission of mosquito-borne diseases in coastal zones are discussed with reference to the Ross–McDonald equation and modeling studies. Evidence is presented to show that an expansion of brackish water bodies in coastal zones can increase the densities of salinity-tolerant mosquitoes like *Anopheles sundaicus* and *Culex sitiens*, and lead to the adaptation of fresh water mosquito vectors like *Anopheles culicifacies*, *Anopheles stephensi*, *Aedes aegypti*, and *Aedes albopictus* to salinity. Rising sea levels may therefore act synergistically with global climate change to increase the transmission of mosquito-borne diseases in coastal zones. Greater attention therefore needs to be devoted to monitoring disease incidence and preimaginal development of vector mosquitoes in artificial and natural coastal brackish/saline habitats. It is important that national and international health agencies are aware of the increased risk of mosquito-borne diseases in coastal zones and develop preventive and mitigating strategies. Application of appropriate counter measures can greatly reduce the potential for increased coastal transmission of mosquito-borne diseases consequent to climate change and a rise in sea levels. It is proposed that the Jaffna peninsula in Sri Lanka may be a useful case study for the impact of rising sea levels on mosquito vectors in tropical coasts.

Keywords: *Aedes, Anopheles,* brackish water habitats, climate change, coastal zones, mosquito-borne diseases, preimaginal development, sea level rise

INTRODUCTION

Mosquito vectors transmit important human parasitic and arboviral diseases. Malaria caused by protozoan parasites of the genus *Plasmodium*, and lymphatic filariasis caused by the nematodes *Wuchereria bancrofti* and *Brugia malayi*, have been estimated to have a recent worldwide prevalence of 247 and 120 million cases respectively (World Health Organization, 2010a,b). Dengue, the most common human arboviral disease, is reported to have a prevalence of 50 million cases in more than 100 countries, with about 500,000 persons requiring hospitalization each year for dengue hemorrhagic fever/dengue shock syndrome that has overall case fatality rate of 2.5% (World Health Organization, 2009a). Minimizing human-mosquito contact and reducing vector populations by the application of insecticides and through managing and eliminating preimaginal development sites are important components of mosquito-borne disease control programs. Reducing human – mosquito contact indoors through the use of bed nets, particularly insecticide impregnated bed nets, has successfully helped reduce malaria prevalence over the past decade in many afflicted countries (World Health Organization, 2011). However this preventive measure is less effective against dengue because the *Aedes* vectors of dengue, unlike important *Anopheles* malaria vectors, tend to bite outdoors and during daytime. Reducing vector density by eliminating or managing preimaginal development habitats, larviciding, and space and residual spraying near infection foci are therefore central to the control of *Aedes aegypti* and *Ae. albopictus*, the principal vectors of dengue (World Health Organization, 2009b).

Ongoing global changes attributable to human activities, e.g., changes in climate, healthcare, land use, pollution, population

movements, and urbanization, can significantly alter the rates of transmission of mosquito-borne diseases in most parts of the world (Sutherst, 2004). The United Nations Framework Convention on Climate Change (UNFCCC) described global climate change as long term changes in commonly measured meteorological parameters, over and above natural variations, that are directly or indirectly attributable to human activity altering the atmospheric composition. Climate change parameters most often considered for their impact on mosquitoes are temperature, rainfall, and humidity, but others such as atmospheric particle pollution and wind can also have an impact. Primary changes in such parameters, caused principally through the increased emission of greenhouse gases into the atmosphere, can alter the bionomics of mosquito vectors and therefore the rates of transmission of mosquito-borne diseases (Sutherst, 2004 and see Dynamics of Disease Transmission by Vector Mosquitoes in the Context of Global Climate Change in Coastal Zones below). These primary changes in global climate can produce further alterations in the biosphere and geosphere that can additionally affect mosquito vector bionomics. Prominent among such secondary changes are the global distribution and characteristics of plants and animals, the frequency and severity of extreme weather events, and a global rise in sea levels. Many studies have examined the impacts of global climate change involving temperature, rainfall, and humidity on common mosquito-borne diseases like malaria and dengue (Lindsay and Martens, 1998; Githeko et al., 2000; Rogers and Randolph, 2000; Reiter, 2001; Hunter, 2003; McMichael et al., 2006; Confalonieri et al., 2007; Paaijmans et al., 2009). However, they did not consider the possible impacts of rising sea levels due to global warming on mosquito-borne disease in coastal zones. We recently proposed that a rise in sea levels can increase the prevalence of many vector-borne diseases in coastal zones (Ramasamy and Surendran, 2011).

A precise definition of the landward boundary of a coastal zone is not possible as this will depend on local characteristics. The island of Sri Lanka legislatively regards it to extend 300 m inland from the mean high water level. For the purpose of this article, the coastal zone is considered to be the land area extending inland from the sea-land interface where sea water salinity has a significant influence on its biological and physical characteristics. In this article we provide an overview of the possible effects of global climate change and rising sea levels on mosquito-borne diseases in coastal zones, with dengue and malaria as particular examples. It is expected that this will stimulate further consideration of a hitherto neglected aspect of global climate change and human health, and lead to the development of appropriate mitigating measures worldwide.

DYNAMICS OF DISEASE TRANSMISSION BY VECTOR MOSQUITOES IN THE CONTEXT OF GLOBAL CLIMATE CHANGE IN COASTAL ZONES

The rate of spread of a mosquito-borne disease in a non-immune population can be represented in a simple form by the Ross–MacDonald equation (MacDonald, 1957).

$$R_o = \frac{ma^2\alpha\beta p^n}{r\left[-\log_e(p)\right]}$$

where R_o is the number of secondarily infections generated from a single infected human in a non-immune population

m = ratio of the number of vector mosquitoes to the number of humans

a = average number of human blood meals taken by a mosquito in a day

α = probability of transmission of pathogen from an infected human to a biting mosquito

β = probability of transmission of pathogen from an infected mosquito to a non-immune human during feeding

p = daily probability of survival of the mosquito vector

n = duration in days from infection of a biting mosquito until the mosquito becomes capable of infecting humans after the pathogen undergoes obligatory development in the mosquito. This is also termed the extrinsic incubation period

r = recovery rate in humans (inverse of the average duration of infectiousness in days)

The Ross–McDonald formula is fundamentally important for determining the effects of climate change on the transmission of mosquito-borne diseases in coastal and inland areas. A qualitative analysis may be made by considering the impact of the predicted changes on parameters that determine R_o.

TEMPERATURE

Adult and preimaginal forms of mosquitoes have an optimal range of temperature for survival and development and this closely matches the climate where each vector species is found. A change in the ambient temperature will tend to affect p. Mosquito survival in areas with less than optimal temperatures will be increased if climate change results in warming to temperatures closer the optimum for the mosquito species concerned. Thus a mosquito vector whose optimal survival temperatures are found in lowland areas of the tropics may spread to higher latitudes of the sub-tropical and temperate zones and to the higher altitudes in tropical countries. It has been suggested that a latitudinal range of shift of about 200 km is possible per °C rise in global temperature (Sutherst, 2004). A limited rise in temperature will also favor an increase in the human biting rate a, hasten mosquito development and therefore increase the relative vector density m, and reduce the extrinsic incubation period n (Lindsay and Birley, 1996). Because of the exponential relationship of the extrinsic incubation period n to R_o, it is a dominant variable determining R_o. However, the dependence of mosquito survival and development, human biting rates and the extrinsic incubation period on temperature is likely to show different optima for each of the parameters, which in turn will generate a complex variation of R_o with changes in ambient temperature. The impacts of global temperature change on disease transmission by mosquito vectors are likely to be broadly similar in coastal and inland areas.

RAINFALL AND HUMIDITY

Climate change alters rainfall which has a direct effect on humidity. An optimal humidity significantly increases mosquito survival p. Furthermore, rainfall, rate of evaporation, and humidity will influence the availability of habitats for oviposition and preimaginal development of the mosquito vectors and therefore influence

m, the ratio of mosquitoes to humans. An expansion of habitats for preimaginal development as a result of climate change will therefore tend to increase vector density in relation to the human population, favoring disease transmission. However the relationship between rainfall and mosquito larval habitats is a complex one. Peak malaria transmission closely follows the rainy season in tropical countries, e.g., Sri Lanka (Ramasamy et al., 1992a,b). Rainfall forms surface pools of fresh water that are favored preimaginal development habitats for the major fresh water *Anopheles* vectors in Sri Lanka and other tropical countries (Ramasamy et al., 1992a,b; Surendran and Ramasamy, 2010). However, excessive rainfall can wash away larvae and eggs and reduce the numbers of small puddles thereby temporarily lowering the rates of malaria transmission. Less than normal rainfall in tropical wet zones results in the drying up of rivers and formation of pools in river beds which can also increase malaria transmission. *Aedes aegypti*, the principal urban vector of dengue, can develop indoors in water containers, and its development is therefore less dependent on rainfall (Barraud, 1934; World Health Organization, 2009b). *Aedes albopictus*, the alternative vector of dengue in mainly peri-urban and rural settings, tends to undergo larval development in water collections outdoors and is therefore more dependent on rain-fed habitats, e.g., water collections in leaf axils, tree holes, and discarded containers (Barraud, 1934; World Health Organization, 2009b). *Aedes albopictus* densities increase during the monsoon season in the Jaffna peninsula, a coastal zone in Sri Lanka (Surendran et al., 2007a).

Coastal zones, depending on their aridity, are likely to be affected similarly to inland areas by rainfall. However, an additional consideration in coastal areas is that a drier climate can favor salinity-tolerant vectors, e.g., *Anopheles sundaicus* in Southeast Asia and *An. merus* and *An. melas* in Africa. Conversely, higher rainfall can expand the habitats of fresh water vectors like *An. culicifacies* and *An. gambiae* in the coastal zones of Asia and Africa respectively. Alterations in vector composition as discussed in Section "Variations in Mosquito and Pathogen Populations" can influence disease transmission rates.

VARIATIONS IN MOSQUITO AND PATHOGEN POPULATIONS

The variables α and β in the Ross–MacDonald equation are dependent partly on the intrinsic genetic characteristics of the vector. The innate immune mechanisms in the midgut that prevent infection of the gut and further development of the pathogen are genetically determined. The subsequent ability of pathogens to disseminate through the hemocoele to the salivary glands and then be transmitted to humans during a blood meal is also influenced by genetic factors. Therefore α and β will vary with vector species and pathogen strain. Both factors contribute to vector competence, which is a measure of the intrinsic ability of a particular species of vector to transmit disease.

The strain of pathogen can also influence α and β and thereby alter R_o. The replacement of a local strain of dengue virus III subtype by a more virulent III subtype in Sri Lanka in the 1980s increased the incidence of dengue hemorrhagic fever. Although both dengue III strains multiplied equally well in cultured cell lines, and infected an equal proportion of mosquitoes, the virulent strain of the virus multiplied to higher titers and disseminated to the salivary glands more efficiently than the local strain (Hanley et al., 2008). These findings are consistent with the two viral strains possessing the same α but different β values. Global climate change therefore can potentially influence α and β by causing changes in vector populations and pathogen strains that are better adapted to the altered climate.

The implications of these considerations for coastal zones are that changes in vector composition as a result of alterations in the extent and salinity of larval habitats can modify disease transmission dynamics. Furthermore, genetic changes in pathogens that result in better adaptation to salinity-tolerant mosquitoes can increase disease transmission rates in coastal areas.

HUMAN IMMUNITY

A population exposed to a mosquito-borne disease will over time generate protective immune responses to the pathogen that can result in complete or partial immunity to reinfection. Near complete immunity may develop against a particular dengue virus serotype (Guzman and Vazquez, 2010) while partial immunity resulting in milder disease is more characteristic of malaria (Ramasamy, 1998). Either partial or complete immunity will alter the recovery rate *r* and effectively reduce β in the Ross–MacDonald equation. Individuals in malaria-endemic areas develop antibodies to the surface antigens on gametes that develop in the mosquito midgut from ingested gametocytes and these are able to reduce the infectivity of the parasite to mosquitoes (Peiris et al., 1988). This phenomenon, termed transmission blocking immunity, tends to reduce the value of α and consequently R_o in malaria-endemic areas. However it has also been observed that antibodies to the sexual stages of malaria parasites, depending on their concentration, sometimes have the opposite effect, i.e., they enhance transmission to mosquitoes (Peiris et al., 1988). Therefore immunity, and its qualitative and quantitative aspects as well as its temporal change in the population, introduces considerable complexity into modeling disease transmission. Generally, the expansion of vector populations, as a result of climate change, into disease-free areas or areas where disease endemicity is insufficient to elicit good protective immunity, will often lead to initial high rates of disease transmission that will decrease in time as the population develops immunity. Similar considerations on population immunity apply to the transmission of mosquito-borne diseases in coastal zones.

MODELED PREDICTIONS ON THE IMPACTS OF PRIMARY GLOBAL CLIMATE CHANGE ON MOSQUITO-BORNE DISEASES

Models have been developed for forecasting the impact of global climate change on mosquito-borne diseases, notably the global distributions of malaria (Lindsay and Martens, 1998; Githeko et al., 2000; Rogers and Randolph, 2000; Paaijmans et al., 2009) and dengue (Hales et al., 2002). One model used current temperature, rainfall, and humidity ranges that permit malaria transmission to forecast malaria distribution in 2050 in a global climate change scenario (Rogers and Randolph, 2000). This model found surprisingly few changes, but predicted that some parts of the world that are presently free of malaria may be prone to a greater risk of malaria transmission while certain malaria-endemic areas

will have a decreased risk of malaria transmission (Rogers and Randolph, 2000). Larger areas of northern and eastern Australia are expected to become more conducive for the transmission of dengue (McMichael et al., 2006) and a greater proportion of the global population at risk of dengue (Hales et al., 2002) as a result of global climate change. While these models did not specifically address changes in coastal zones, the transmission of malaria (Rogers and Randolph, 2000) and dengue (Hales et al., 2002; McMichael et al., 2006) were generally predicted to increase in coastal areas of northern and eastern Australia. Many modeling forecasts are limited by uncertainties in the extent of global climate change as a result of the inability to accurately predict major drivers such as future emission rates of greenhouse gases. Other factors such as the resilience of the geosphere and biosphere that are difficult to estimate precisely, and regional characteristics, can also influence climate change parameters. Furthermore, the considerable adaptability of mosquito vectors and their pathogens to changing environments are difficult to model. Models however have an important role in highlighting potential problems and the need to develop measures to counter possible increases in disease transmission.

Global climate change has led to observable alterations in the global distribution of plants and animals with species adapted to warmer temperatures moving to higher latitudes (Root et al., 2003). However there is no unequivocal evidence yet that global climate change has already affected the distribution of a mosquito-borne disease in inland or coastal areas. The reports of increased incidence of malaria epidemics related to warmer temperatures in the Kenyan highlands have been controversial as changes in many other factors could have influenced malaria transmission in this area, and perhaps even masked an increase in transmission due to higher temperatures (Githeko et al., 2000; Alonso et al., 2011; Omumbo et al., 2011; Chaves et al., 2012). However it is clear that the incidence of malaria has decreased over the last decade in many countries due primarily to better case detection and treatment, the use of insecticide treated mosquito nets and indoor residual spraying of more effective insecticides (World Health Organization, 2011). It seems quite likely that such improvements in malaria control measures worldwide have masked any tendency for the incidence of malaria to increase as a result of global climate change (Gething et al., 2010).

On the other hand, there is evidence that short term changes in global climate can influence the incidence of mosquito-borne diseases. The El-Nino Southern Oscillation (ENSO) entails multi-annual cyclic changes in the temperature of the eastern Pacific Ocean that influences air temperature and rainfall in large areas of the bordering continents, spreading as far as Africa. ENSO has been associated with a higher incidence of dengue in some countries, notably in parts of Thailand in recent times (Tipayamongkholgul et al., 2009). Global warming due to the greenhouse effect may increase the frequency of ENSO events (Timmermann et al., 1999) and therefore cause more numerous epidemics of dengue. The warming of surface sea temperatures in the western Indian Ocean due to short term fluctuations known as the Indian Ocean Dipole (IOD) is associated with higher malaria incidence in the western Kenyan highlands (Hashizume et al., 2009). The effects of short term ENSO and IOD events are a likely indication of the potential impacts of long term global climate change on mosquito-borne diseases that can also affect coastal zones.

There have been very few studies on other primary climate changes like wind and atmospheric pollution that can also affect mosquito populations in coastal areas. Changes in wind patterns as a result of climate change are difficult to predict and likely to be locality-specific. It can be expected that higher onshore wind velocities will tend to disperse mosquito populations further inland. Atmospheric pollution will be higher in the vicinity of urban coastal areas, and it may be anticipated that mosquitoes will adapt to pollution with time. The gaps in knowledge in these areas need to be addressed.

EFFECTS OF SECONDARY CHANGES ON THE TRANSMISSION OF MOSQUITO – BORNE DISEASES IN COASTAL AREAS

The more important secondary changes caused by climate change that can influence disease transmission in coastal areas are alterations in the distribution and types of plants and animals, and a rise in sea levels. The frequency and severity of extreme weather events will affect coastal zones but their impact on mosquito-borne disease transmission is generally likely to be short-lived. The possible impact of a rise in sea levels is considered in Section "Rising Sea Levels Due to Global Warming Can also Influence the Transmission of Mosquito-Borne Diseases in Coastal Zones." The nature and type of vegetation is related to the availability of larval habitats. A measure of vegetation that can be assessed by remote sensing light reflectance is the Normalized Difference Vegetation Index (NVDI). A recent study in Paraguay that measured forest cover over a period of time by the NVDI showed that the incidence of malaria was associated with deforestation (Wayant et al., 2010). Vegetation associated with water, e.g., rice fields, are positively correlated with larval habitats and this has for example been demonstrated by recent remote sensing studies in Burkina Faso (Dambach et al., 2009). NVDI measurements also showed a positive correlation between vegetation and anopheline larval density in a coastal town in Kenya (Eisele et al., 2003). These findings suggest by analogy that changes in the nature and types of vegetation in coastal zones, as a result of climate change, can influence the transmission of malaria and most likely other mosquito-borne diseases. Changes in vegetation and agricultural practices driven by climate change can affect the prevalence and distribution of wild animals and livestock that provide alternatives to humans as sources of blood meals for mosquitoes. This can also influence the transmission of mosquito-borne diseases in coastal zones.

A case study from Guyana involving a malaria epidemic in the 1950s illustrates the complex interactions between some of these factors on malaria transmission in a coastal zone (Giglioli, 1963). *Anopheles darlingi*, an anthropophagic (females preferring to feed on human blood) and endophilic (preferring to rest indoors) freshwater species, was the primary malaria vector in the Demerara river estuary in Guyana. It was eliminated, together with malaria, in the estuary by an indoor DDT spraying campaign in 1946–1950. The salinity-tolerant, zoophagic (preferring to feed on animal blood) and exophilic (preferring to rest outdoors) *An. aquasalis* was a minor vector in the estuary, but increased in numbers during dry seasons due to saline water intrusion in the estuary. The elimination of *An. darlingi* in the area was accompanied by the

conversion of pastures into rice fields, and increased human settlement. An outbreak of *Plasmodium vivax* malaria that occurred in 1960–1961 was accompanied by a marked increase in *An. aquasalis* collection indoors. The evidence suggested that there were two main causes for these changes, *viz.* adaptation of *An. aquasalis* to become more anthropophagic and endophilic as a result of diminishing numbers of livestock and an increase in the human population density, and the immigration of infected persons into the area. These changes were sufficient to re-establish endogenous malaria transmission (Giglioli, 1963).

RISING SEA LEVELS DUE TO GLOBAL WARMING CAN ALSO INFLUENCE THE TRANSMISSION OF MOSQUITO-BORNE DISEASES IN COASTAL ZONES

Approximately 5% of mosquito species are adapted to undergo preimaginal development in brackish and saline waters (water with <0.5 ppt or parts per thousand, 0.5–30 and >30 ppt salt are termed fresh, brackish, and saline respectively). Many salinity-tolerant mosquitoes are important vectors of human diseases as shown in **Table 1**.

Salinity-tolerant mosquito larvae possess cuticles that are less permeable to water than freshwater forms, and their pupae have thickened and sclerotized cuticles that are impermeable to water and ions (Bradley, 1987). Salinity-tolerant mosquito larvae also possess varying physiological mechanisms to cope with salinity. *Aedes taeniorhynchus* drink the surrounding fluid and excrete Na^+ and Cl^- from the posterior rectum to produce hyperosmotic urine (Bradley, 1987). *Culex tarsalis* larvae accumulate proline and trehalose in hemolymph to maintain isoosmolarity in brackish waters in a process termed osmoconformation (Garrett and Bradley, 1987). *Anopheles albimanus* larvae are able to differentially localize sodium-potassium ATPase in rectal cells in fresh or saline water for osmoregulation through ion excretion (Smith et al., 2008).

We hypothesize that mosquito-borne disease transmission in coastal areas are not only influenced by global climate change causing alterations in temperature, rainfall, and humidity, but also rising sea levels (Ramasamy and Surendran, 2011). The Intergovernmental Panel for Climate Change has predicted that global warming will raise sea levels by 18–59 cm by the end of the twenty-first century through the melting of glaciers and polar ice as well as the thermal expansion of seawater (Nicholls et al., 2007; United Nations Intergovernmental Panel on Climate Change, 2007). Rising sea levels will affect the extent of saline or brackish coastal water bodies including estuaries, lagoons, marshes, and mangroves that provide preimaginal development sites for salinity-tolerant mosquito species in coastal areas. Models suggest that the salinity of estuarine systems will rise and their boundaries move further inland with more pronounced tidal water flows into rivers (Nicholls et al., 2007). A proportion of coastal wetlands such as salt marshes and mangroves will become inundated by the sea but this will be compensated for by additional saline wetlands being formed further inland (Nicholls et al., 2007). Rising sea levels, and higher water withdrawal rates from freshwater aquifers near the coast by expanding populations will increase saltwater intrusion in the aquifers (Food and Agricultural Organisation, 2007). These changes in turn will cause ponds, lakes, and wells in coastal areas to become more brackish. The potential impact of rising sea levels, as opposed to climate change involving temperature, rainfall, and humidity, on the prevalence of mosquito-borne diseases in coastal areas was not recognized (Lindsay and Martens, 1998; Githeko et al., 2000; Rogers and Randolph, 2000; Reiter, 2001; Hunter, 2003; McMichael et al., 2006; Confalonieri et al., 2007; Paaijmans et al., 2009), until we proposed that an expansion of brackish and saline water bodies in coastal areas due to rising sea levels can increase the density of salinity-tolerant mosquito vectors and cause freshwater mosquito vectors to adapt to brackish water habitats (Ramasamy and Surendran, 2011). Such developments can lead to an increase in the density of vectors relative to humans (m in the Ross–MacDonald equation, see Dynamics of Disease Transmission by Vector Mosquitoes in the Context of Global Climate Change in Coastal Zones) and therefore to an increase in R_o. Increased transmission of mosquito-borne diseases in coastal areas due to salinity-tolerant vectors can also cause the diseases to be propagated to inland areas through bridging vectors that may be fresh water or euryhaline (possessing the ability to undergo

Table 1 | Common salinity-tolerant mosquito vectors of human disease adapted with permission from Ramasamy and Surendran (2011).

Species	Distribution	Transmitted pathogens
Aedes dorsalis	Temperate Eurasia, N America	West Nile virus and Western equine encephalitis virus
Ae. (*Ochlerotatus*) *taeniorhynchus*	N & S America	Eastern equine encephalitis virus
Ae. togoi	North Pacific rim	Japanese encephalitis virus and filarial parasites
Ae. (*Ochlerotatus*) *vigilax*	Australasia, SE Asia	Filarial parasites, Ross River virus, Barmah forest virus
Anopheles albimanus and *An. aquasalis*	N & S America, Caribbean	Malaria parasites
An. atroparvus	Coast of W Europe	Malaria parasites
An. farauti and *An. annulipes*	Australasia	Malaria parasites
An. melas and *An. merus*	Africa	Malaria parasites
An. multicolor	N Africa, Middle East	Malaria parasites
An. sacharovi	Russia, S Europe	Malaria parasites
An. subpictus	Asia	Malaria and filarial parasites
An. sundaicus	S Asia, SE Asia, China	Malaria parasites
Culex sitiens	Indian ocean rim countries	Japanese encephalitis virus and Ross River virus
Cx. tritaeniorhynchus	Russia, Middle East, Africa, India	Japanese encephalitis virus

preimaginal development over a wide range of salinity) species. On the other hand, it is possible that rising salinity in coastal habitats, where important fresh water mosquitoes undergo preimaginal development, may reduce disease transmission. However, as discussed below, fresh water mosquitoes are capable of adapting to an expansion of brackish water habitats in coastal areas.

There is very good historical evidence that changes in the extent of brackish water coastal habitats of anopheline mosquitoes has influenced the distribution of malaria. The association of malaria with the Pontine Marshes near Rome is one such example. Draining of the marshes in the early twentieth century with water pumps had greatly reduced malaria incidence. The flooding of the marshes with sea water toward the end of World War 2 was accompanied by a resurgence of malaria which was reversed once again by draining the marshes (Geissler and Guillemin, 2010). A reduction in the extent of the habitat of a brackish water vector has also historically been associated with a lower incidence of malaria in England and the Netherlands. *An. atroparvus* was primarily responsible for transmitting *vivax* malaria until the early 1900s in marshland areas of England (Dobson, 1994) and the river deltas of the Netherlands (Takken et al., 2002). A reduction of the breeding sites for *An. atroparvus* as a result of the draining of coastal marshes helped eliminate malaria from these areas (Dobson, 1994; Takken et al., 2002).

Because of the slow rate of rise in sea levels due to global warming, and confounding factors such as improvements in disease prevention and treatment, it has not yet been possible to observe an impact of rising sea levels on mosquito vectors and their transmitted diseases. However the December 2004 Asian tsunami provided relevant examples that suggest that such effects can indeed occur. The density of *An. sundaicus* s.l., a widespread malaria vector along Asian coasts (Surendran et al., 2010; Sinka et al., 2011), increased in the Andaman and Nicobar islands following the intrusion of sea water inland, and this was accompanied by a rise in the incidence of falciparum malaria in the islands (Krishnamoorthy et al., 2005). Higher densities of *Culex sitiens*, an established vector of arboviruses (Weaver and Reisen, 2010), and *An. sundaicus* s.l. were also observed in an area of Thailand that was affected by the tsunami (Komalamisra et al., 2006). New brackish water habitats that were created by the tsunami led to freshwater breeding mosquitoes adapting to undergo preimaginal development in them, e.g., larvae of typical freshwater mosquitoes *An. stephensi* and *An. culicifacies*, were found in newly formed brackish water bodies immediately after the 2004 tsunami in India (Gunasekaran et al., 2005). *An. culicifacies* larvae were also observed for the first time in brackish water bodies near the coast in eastern Sri Lanka, 5 years after the tsunami although a relationship to the inundation caused by the tsunami could not be established (Jude et al., 2010). Mosquitoes are highly adaptable as shown by their ability to exploit a variety of ecological niches and rapidly develop insecticide resistance. It is therefore likely that, given adequate selective pressure most, if not all, fresh water mosquito vector species can adapt to oviposit and undergo preimaginal development in brackish water.

Human-induced ecological changes provide additional examples that suggest that an expansion of brackish water mosquito habitats can increase malaria transmission. Large-scale shrimp farming in the Mekong delta of Vietnam locally increased the density of *An. sundaicus* s.l. (Trung et al., 2004) and similar trends have been seen elsewhere in Southeast Asia. Also, higher densities of *Aedes* (*Ochlerotatus*) *camptorhynchus*, a known vector of Ross River virus, have been associated with increasing salinization of freshwater bodies caused by large-scale and intensive wheat farming in Western Australia (Jardine et al., 2008; Carver et al., 2009, 2010; van Schie et al., 2009).

AEDES AEGYPTI AND *AEDES ALBOPICTUS*, THE MAJOR VECTORS OF DENGUE, CAN UNDERGO PREIMAGINAL DEVELOPMENT IN BRACKISH WATER

Development of a vaccine against dengue is hampered by the existence of four virus serotypes and because a suboptimal immune response to any one of the serotypes can exacerbate disease caused by a subsequent infection with that serotype (Halstead, 2003; Chun et al., 2007). Only drugs that provide symptomatic relief are presently available to treat dengue and therefore there is considerable concern internationally about the currently observed global spread of dengue, chikungunya, and other arboviral diseases (Cavrini et al., 2009; Schwartz and Albert, 2010; Weaver and Reisen, 2010). Hence the control of mosquito vector populations is crucial for reducing dengue and arboviral diseases for which vaccines are not available.

Aedes aegypti is the principal tropical mosquito vector of arboviruses causing yellow fever, dengue, and chikungunya (Cavrini et al., 2009; Weaver and Reisen, 2010; Walter Reed Biosystematics Unit, 2011). *Ae. aegypti* is also able to transmit other arboviruses, including Ross River and Murray Valley Encephalitis viruses, in laboratory experiments (Ramasamy et al., 1990), and is a natural vector of *B. malayi* that causes filariasis in Asia (Erickson et al., 2009). The closely related *Ae. albopictus* is an alternate vector of dengue and chikungunya (Rezza et al., 2007; Cavrini et al., 2009; Weaver and Reisen, 2010; Walter Reed Biosystematics Unit, 2011). Unlike *Ae. aegypti*, *Ae. albopictus* has developed a diapausing egg stage that has enabled it to survive winters and spread to temperate regions, causing for example a chikungunya epidemic in northern Italy in 2007 (Rezza et al., 2007) and two autochthonous cases of dengue in southern France in 2010 (La Ruche et al., 2010).

Importantly, larval source management and reduction strategies are presently directed exclusively toward freshwater habitats, because of the long and widely held view that the two *Aedes* species only develop naturally in fresh water (Barraud, 1934; Chan et al., 1971; Kulatilaka and Jayakuru, 1998; Ooi et al., 2006; World Health Organization, 2009a,b). We recently showed however that *Ae. aegypti* and *Ae. albopictus* are able to oviposit and undergo preimaginal development in collections of brackish water in unused wells, abandoned boats, disposable plastic, and glass food and beverage containers (**Figure 1**) in coastal Sri Lanka (**Figure 2**) and Brunei Darussalam (Ramasamy et al., 2011). We hypothesized that brackish water development may be an adaptive response to the almost exclusive application of *Aedes* larvae control measures (with insecticides such as temephos and *Bacillus thuringiensis* toxin) to freshwater habitats and the elimination of such habitats in the urban and peri-urban environment (Ramasamy et al., 2011). Furthermore, the brackish water *Ae. aegypti* larval sites were found close to areas of high dengue incidence in the city of Jaffna

FIGURE 1 | Brackish water development habitats of *Ae. aegypti* and *Ae. albopictus* larvae in Sri Lanka. The photographs show the brackish water collections containing larvae in: **(A,B)** – disused boats; **(C,E)**: abandoned wells; **(D,F)** discarded food and beverage containers (reproduced with permission from Ramasamy et al., 2011).

in the Jaffna peninsula of northern Sri Lanka (**Figures 2** and **3**) suggesting that they may play a role in the transmission of dengue in coastal zones (Ramasamy et al., 2011). In a limited survey of domestic brackish water wells in a coastal division of Jaffna city, ~25% of brackish water wells ($n = 110$) were found to have *Ae. aegypti* larvae (Surendran, S. N., Jude, P. J., Thabothini, V., Raveendran, S., Ramasamy, R., unpublished data). Household wells are usually exempt from dengue control measures because they are not considered to be significant preimaginal development sites (World Health Organization, 2009b). Our findings are the first to show that brackish water domestic wells are a habitat for the development of mosquito vectors of dengue. The *Aedes* larval positivity rates in brackish water we recorded in Sri Lanka are higher than the House Index (% of houses positive for *Aedes* larvae) or Breteau Index (number of containers with larvae per 100 houses) for fresh water habitats that have been typically associated with dengue epidemics elsewhere (Sanchez et al., 2006). We therefore hypothesize that the *Aedes* mosquitoes emerging from such hitherto unrecognized habitats, that are not targeted by larval source reduction programs, may at least be partly responsible for the failure to eliminate dengue in Sri Lanka and other island states like Singapore and Cuba where the dengue control programs exclusively target fresh water larval habitats (Chan et al., 1971; Kulatilaka and Jayakuru, 1998; Ooi et al., 2006; Sanchez et al., 2006; World Health Organization, 2009b).

We have suggested that global warming, leading to an expected 18–59 cm rise in sea levels by the end of this century (Nicholls et al., 2007; United Nations Intergovernmental Panel on Climate Change, 2007), and a consequent expansion of coastal brackish water habitats, can increase disease transmission by salinity-tolerant *Aedes* vectors in coastal areas that can then spread disease to inland areas through bridging vectors (Ramasamy and Surendran, 2011; Ramasamy et al., 2011). *Aedes albopictus* since the 1980s has spread from Asia to Africa, America, and Europe (Rezza et al., 2007; Cavrini et al., 2009; La Ruche et al., 2010; Weaver and Reisen, 2010). We further hypothesize that salinity-tolerant and diapausing *Ae. albopictus* will increase the potential for disease transmission in coastal areas of the temperate zone.

AEDES AEGYPTI AND *AEDES ALBOPICTUS* MAY BE ADAPTING TO BRACKISH WATER HABITATS

There is evidence to suggest that the larvae of *Ae. aegypti* and *Ae. albopictus* in the Jaffna peninsula, where there is greater salinization of ground water compared to Batticaloa in mainland east Sri Lanka, are more tolerant of salinity than in Batticaloa (Ramasamy et al., 2011). These conclusions were drawn from examining the

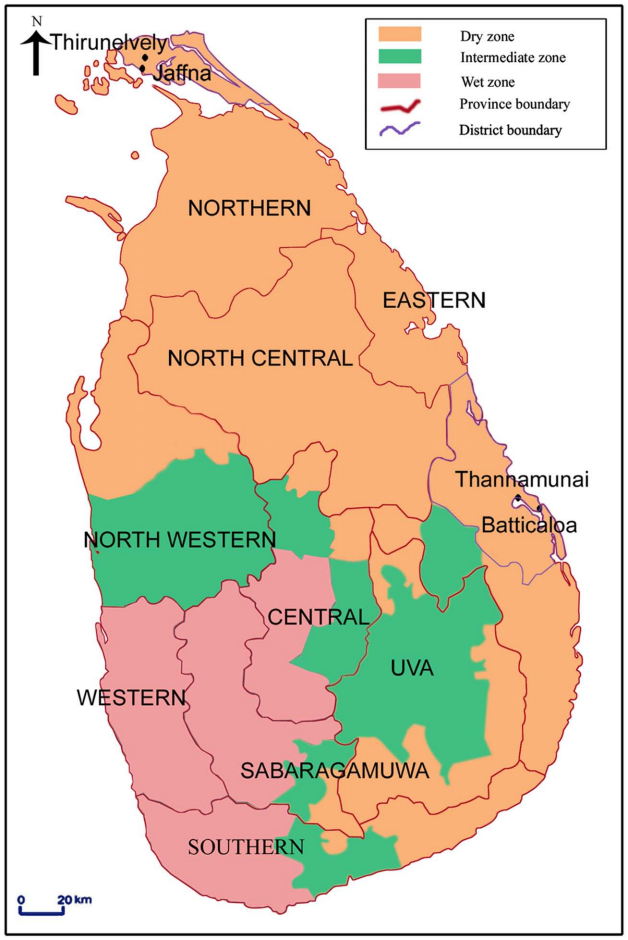

FIGURE 2 | Map of Sri Lanka showing the different provinces and the *Aedes* larvae collection sites in Jaffna and Batticaloa districts. Sri Lanka is an island in the Indian ocean with an area of 65525 km^2 located between latitudes 5′55 and 9′50 North of the equator The central hills of the island divide the surrounding plains into two distinct rainfall zones: the wet and dry zones. The wet zone receives an annual rainfall exceeding 2500 mm in two main rainy seasons: the North-East monsoon in October-December and the South-West monsoon in May-July. Inter-monsoonal rains also occur in the wet zone. The dry zone, with an annual rainfall below 2000 mm, receives maximal rainfall during the North-East monsoon and little or no rain during the rest of the year. An intermediate zone, with mixed characteristics, lies between the dry and wet the intermediate zone. The beige, green, and pink shaded areas show the dry, intermediate, and wet rainfall zones respectively (reproduced with permission from Ramasamy et al., 2011).

tolerance of first and third instar larvae, derived from eggs in freshwater ovitraps, to different salinities, with emergence of adults as the end point (Ramasamy et al., 2011). The greater salinization of ground water in Jaffna peninsula is the result of a combination of factors – its predominant limestone geology (Rajasooriyar et al., 2002), a high and increasing population density, and growing use of water from inland limestone aquifers for agriculture and domestic consumption. Rising sea levels are expected to further exacerbate ground water salinization in the relatively flat peninsula.

Aedes aegypti larvae in laboratory studies have been shown to osmoconform in the short term to a limited increase in the salinity of the surrounding fluid by accumulating ions and amino acids in their hemolymph (Edwards, 1982). There is presently no data to differentiate between such a reversible physiological mechanism and irreversible genetic changes as causes for the adaptation of *Ae. aegypti* and *Ae. albopictus* to brackish water. If genetic changes are responsible, then it is possible that the terms α and β in the Ross–MacDonald equation may be different between the fresh water and salinity – tolerant forms of the two vector mosquitoes, resulting in a differential capacity to transmit dengue virus. Analogous considerations also apply to malaria transmission. Variations in salinity tolerance between sibling species within the many anopheline species complexes are known (Ramasamy and Surendran, 2011; Surendran et al., 2011) and these may have different abilities to transmit malaria. The possible adaptation of *An. culicifacies* to brackish water in eastern Sri Lanka (Jude et al., 2010) and India (Gunasekaran et al., 2005) has been discussed in Section "Rising Sea Levels due to Global Warming can also Influence the Transmission of Mosquito-Borne Diseases in Coastal Zones." In the long term, such adaptation can lead to speciation and this is exemplified in Africa by the evolution of the salinity-tolerant coastal vectors *Anopheles merus* and *Anopheles melas* from the fresh water vector *Anopheles gambiae* (Coluzzi and Sabatini, 1969). Changes in the relative proportions of closely related but genetically different vector populations resulting from adaptation to an increased availability of brackish water habitats in coastal areas can therefore alter the rates of disease transmission.

JAFFNA PENINSULA AS A CASE STUDY FOR THE IMPACTS OF CLIMATE CHANGE AND RISING SEA LEVELS ON MOSQUITO VECTORS IN TROPICAL COASTS

The Jaffna peninsula is located at the apex of northern Sri Lanka (**Figures 2** and **3**). Jaffna is traditionally an agricultural area with an extensive coastline. It is largely composed of sedimentary limestone of the Miocene period (Rajasooriyar et al., 2002), has a maximum altitude of 10.4 m and contains many lagoons and other sea water inlets. Almost all locations in the peninsula are <10 km from the sea, lagoon, or other sea water inlets. Therefore the entire peninsula may be considered to be a coastal zone. Open wells sunk in the limestone aquifers in Jaffna are normally recharged during the North-East monsoon rains in the months from October to December. Water from wells is used for drinking and domestic, agricultural, and industrial purposes at an increasing rate. Many areas in Jaffna city have piped fresh water derived from deep artesian wells from Thirunelvely in the center of the peninsula. However brackish water from wells in the coastal areas of the city is used for watering gardens and washing. Jaffna has a high and increasing population density estimated presently to be 700 persons per km^2 in a total peninsular area of 1130 km^2. Increasing salinization and nitrate pollution of ground water in the peninsula, and salinization in the outlying populated islands, is a serious problem in the Jaffna district (Nagarajah et al., 1988).

The Jaffna district has traditionally been an endemic area for malaria. There was a high incidence of malaria in the 1990s with an estimated peak of ∼10,000 cases per 100,000 persons per year in 1998 (**Figure 4**). Population estimates for this period were not

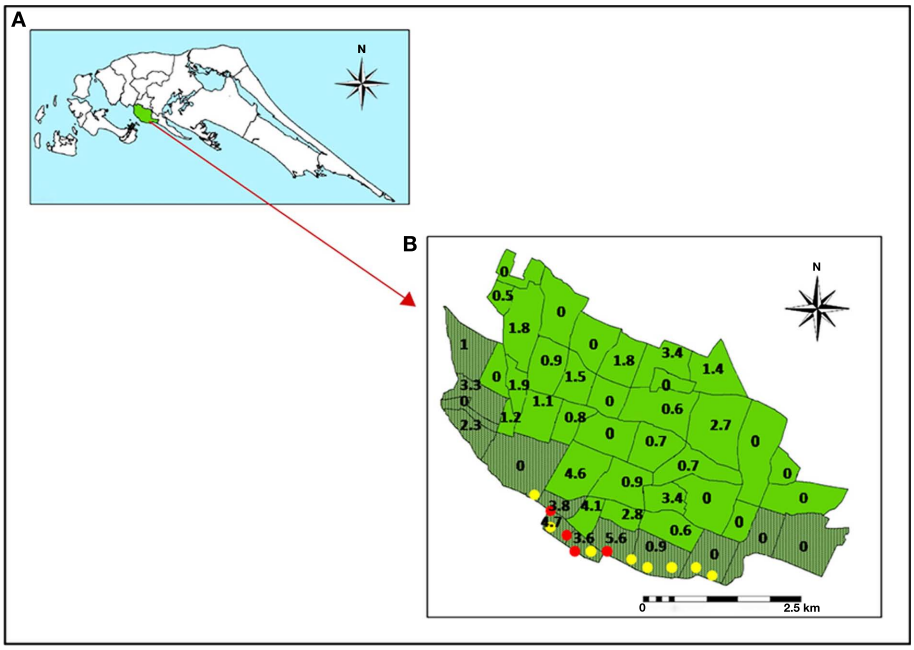

FIGURE 3 | Relationship between dengue incidence and brackish water sites with larvae of *Ae. aegypti* in Jaffna. (A) Map of Jaffna peninsula in northern Sri Lanka. **(B)** Map of Jaffna city showing its administrative divisions with coastal divisions shaded in dark green. The numbers indicate the incidence of dengue per 1000 persons for the 7 months October 2010 to April 2011 in each division. Red and yellow filled circles show brackish water sites along the Jaffna coastal area that were respectively positive and negative for *Ae. aegypti* larvae. Each circle had one or more container, well or boat that was sampled (reproduced with permission from Ramasamy et al., 2011).

accurate due to large-scale displacement and migration caused by civil war. There was a sharp decline in malaria cases after 2002 and no local transmission has been reported since 2007. A study of land use patterns, socio-economic status, and vector breeding identified certain coastal areas to have a high risk of malaria in the peninsula (Kannathasan et al., 2009). The anopheline mosquito species distribution in cattle-baited collections in the district in 2005–2006 was *An. culicifacies* 0.5%, *An. subpictus* 46%, *An. varuna* 4%, *An. nigerrimus* 44%, and *An. pallidus* 5.5% (Kannathasan et al., 2008). Of the three *An. subpictus* sibling species, B, C, and D, collected in the peninsula at the time, the more salinity-tolerant species B was predominant accounting for ≥65% of the *An. subpictus* collection (Kannathasan et al., 2008). It was particularly prevalent in coastal sites (Kannathasan et al., 2008). However, we have recently shown that most, if not all, *An. subpictus* species B identified on morphological characteristics in Sri Lanka are genetically closer to the well-known salinity-tolerant vector of Asia, *An. sundaicus* s.l. (Surendran et al., 2010). The results therefore suggest that the salinity-tolerant *An. sundaicus* s.l. has been the major vector of malaria in the Jaffna peninsula in the past, and its abundance and *Plasmodium* infection rates need to be monitored to prevent a recurrence of malaria.

A large number of dengue cases with several deaths have been reported in Jaffna in recent years with a peak of incidence in 2010 of 490 cases per 100,000 persons (**Figure 5**). Dengue transmission occurs during and soon after the North-East monsoon rains in October–December in Jaffna. The Jaffna peninsula also experienced an epidemic of chikungunya during 2006–2007 (Surendran et al., 2007b). The spread of chikungunya was rapid and resulted in much morbidity in Jaffna due possibly to the lack of prior immunity in the population. It was estimated that over 10,000 people were treated at the out-patient department of government hospitals in November–December, 2006 (Surendran et al., 2007b). *Aedes aegypti* and *Ae. albopictus*, the known vectors of dengue and chikungunya, are present in the Jaffna peninsula and are able to oviposit in indoor and outdoor ovitraps with mixed infestation throughout the year (Surendran et al., 2007a). There was a seasonal variation in the prevalence of the two mosquito species, with *Ae. aegypti* predominating during the pre-monsoon period and *Ae. albopictus* during the monsoon (Surendran et al., 2007a).

There are no reports on the local transmission of Japanese encephalitis and filariasis in the Jaffna peninsula in recent times although the respective primary vectors *Culex tritaeniorhynchus* and *Culex quinquefasciatus* are present (Rajendram and Antony, 1991). Furthermore, larvae of *Culex sitiens*, a known vector of arboviruses including the Japanese encephalitis virus, were collected from domestic wells with salinity ranging from 10 to 20 ppt in the islands off the peninsula (Surendran, S.N., unpublished observations).

The major malaria control activities in the peninsula involve the early detection of cases and prompt treatment, indoor residual spraying of insecticides, and the supply of insecticide treated bed nets to the population. For the control of dengue, an active source reduction campaign along with public education and focal thermal fogging are undertaken by health authorities in Jaffna. A study carried out during the 2006 chikungunya epidemic in Jaffna targeting 162 families revealed that although they were generally aware of the involvement of mosquitoes in the transmission of the disease,

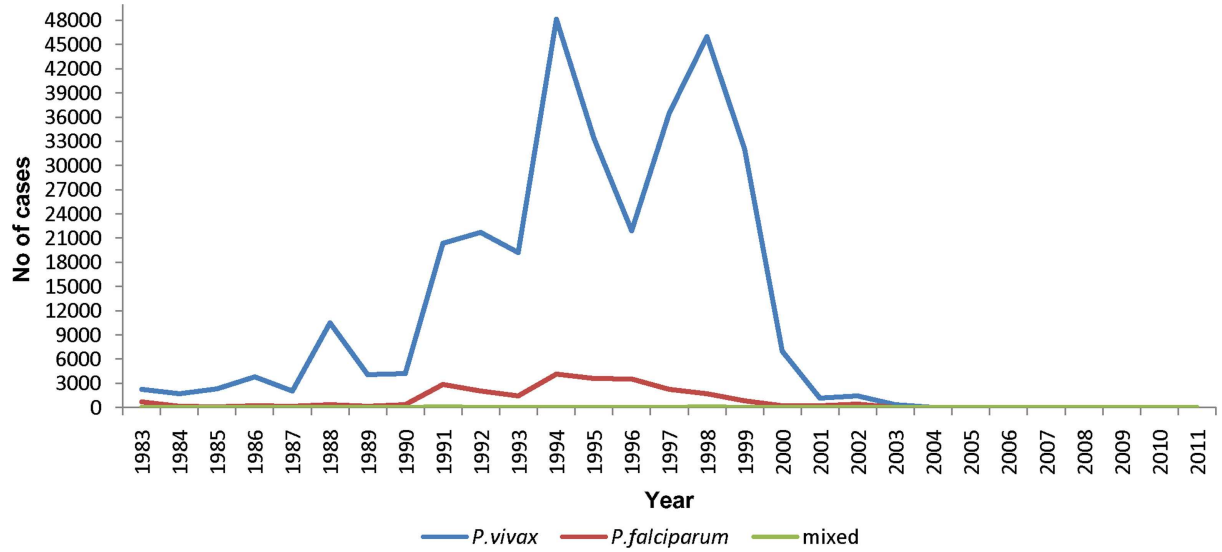

FIGURE 4 | Number of malaria cases in the period 1983–2011 in the Jaffna district. Data from the Anti-Malaria Campaign of the Ministry of Health, Jaffna.

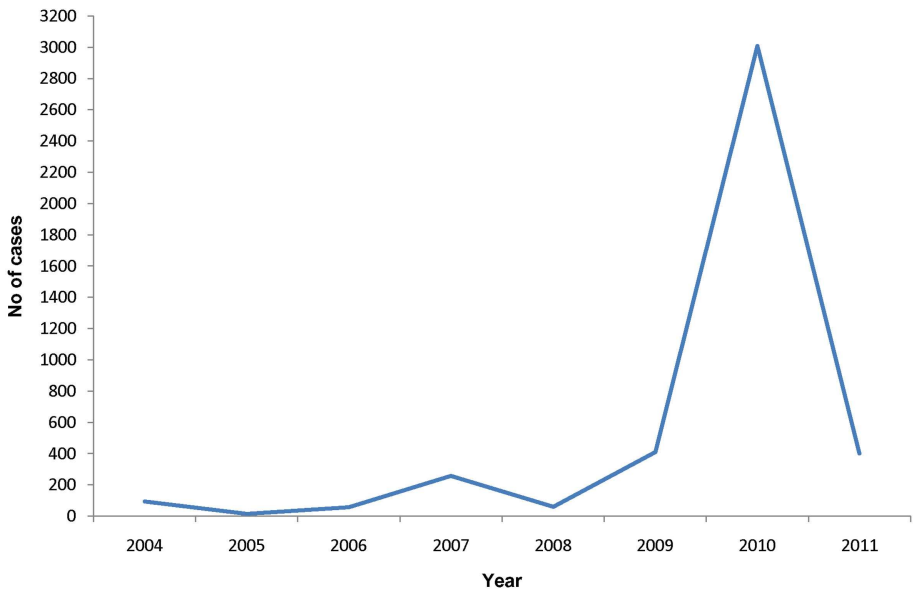

FIGURE 5 | Number of dengue cases in the period 2004–2011 in the Jaffna district of Sri Lanka. Data from the Office of the Regional Director of Health, Jaffna.

82 of the 162 houses inspected were found to have *Aedes* larvae (Surendran et al., 2007b). A similar study carried out to assess public perception toward malaria, targeting 157 households living in high risk and low risk areas, showed that knowledge of the involvement of mosquito in malaria was high among all populations (95%; Kannathasan et al., 2008). Knowledge of preimaginal mosquito development habitats was greater in high risk (90%) than low risk (70%) malarial areas. It may be surmised that disease burden and the public awareness programs have significantly influenced public perceptions on the mode of transmission of chikungunya and malaria. The impacts of the present dengue control measures and public education programs on in Jaffna have not yet been similarly evaluated. Furthermore, the recent findings that malaria and dengue vectors are able to tolerate salinity variations and undergo preimaginal development in brackish waters (Jude et al., 2010; Surendran et al., 2010, 2011; Ramasamy et al., 2011) have yet to lead to the development of appropriate new vector control strategies by health authorities. Specific issues regarding insecticidal control may arise in the context of its application to brackish water larval habitats. Larvicides successfully used in fresh

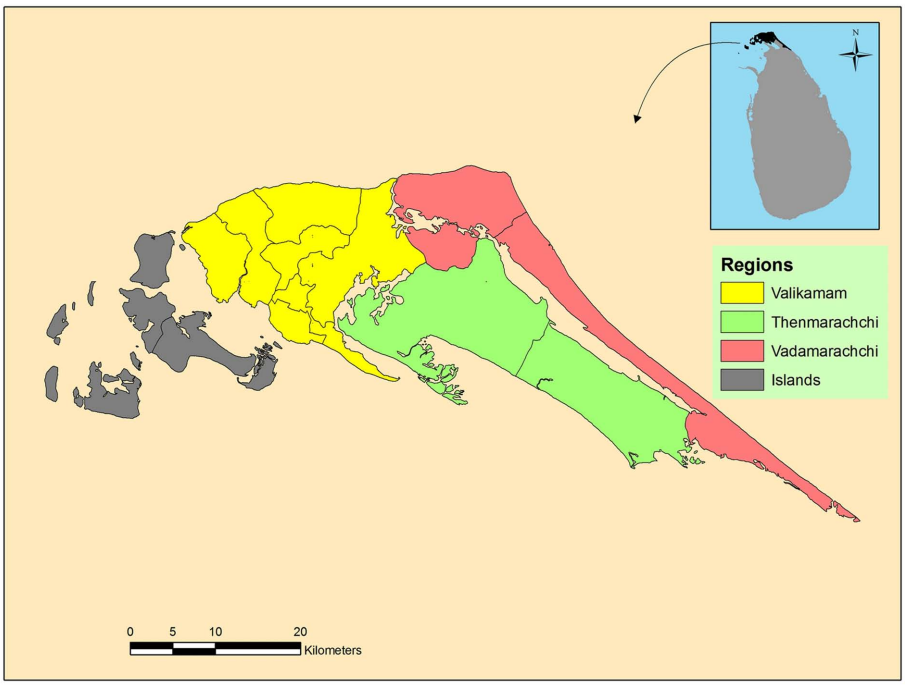

FIGURE 6 | Map of the different administrative divisions in the Jaffna peninsula.

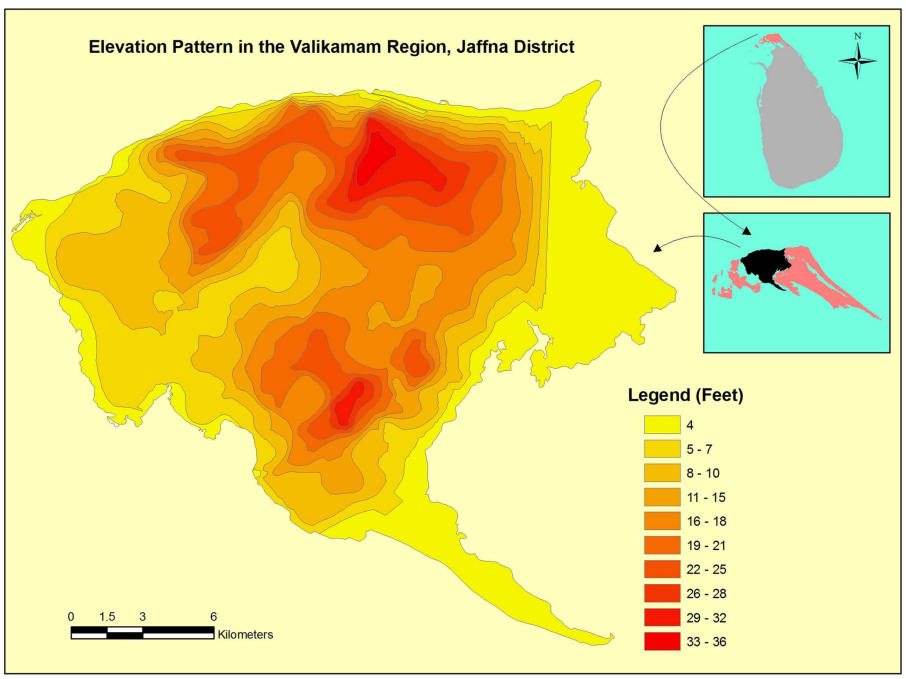

FIGURE 7 | Map showing elevations above mean sea level in the Valikamam division of the Jaffna district.

water habitats may not always have the same efficacy in brackish water. For example, *Bacillus thuringiensis* a soil dwelling bacterium commonly used in the dengue vector control program in Jaffna, is less effective at salinities in the range of 8–14 ppt against *Ae. aegypti* larvae (Gobika, S., Tharmatha, T., Jude, P.J., Senthilnanthanan, M., Kannathasan, S., Surendran, S.N. and Ramasamy, R., unpublished

data). This is relevant because *Ae. aegypti* and *Ae. albopictus* larvae were detected in brackish water of up to 15 ppt salinity in coastal zones of Sri Lanka (Ramasamy et al., 2011).

The limited data available in Sri Lanka suggest that the temperature in the country has increased by 0.14°C per decade in the period 1930–2000. Rainfall has shown a decreasing trend, with 2% decrease in Jaffna in this period (Premalal, 2010). Decreasing rainfall is expected to increase the ground water salinization by reducing the rate of recharge with fresh water. The Jaffna peninsula is composed of four administrative divisions (**Figure 6**) with Valikamam being the most populous. A relief map of Valikamam showing the elevation above mean sea level is presented in **Figure 7**. This suggests that the predicted 18–59 cm (∼0.5–2 feet) rise in sea levels by the end of this century (Nicholls et al., 2007; United Nations Intergovernmental Panel on Climate Change, 2007), may markedly increase salinization of inland freshwater aquifers and wells in the peninsula (Food and Agricultural Organisation, 2007). Domestic wells and ponds near the coast will become more brackish. These developments can influence mosquito vector prevalence, distribution and ecology, and promote further adaptation of fresh water vectors to undergo preimaginal development in brackish waters. Such changes will need to be monitored closely by health authorities.

CONCLUSION

More than half the global population live on land that is <60 km from a seashore. Population density in coastal areas is expected to increase from 87 persons per km^2 in the year 2000 to 134 persons per km^2 in 2050 (United Nations Environment Programme, 2007), and this trend is likely to be particularly pronounced in tropical developing countries where many mosquito-borne diseases are endemic. Therefore growing numbers of people will be placed at risk by an increase in mosquito vector populations in coastal zones. Hence there is an important need to raise awareness among the health authorities and other relevant government sectors, e.g., environmental management, on the health risks associated with mosquito vectors developing in brackish water, particularly in the context of rising sea levels due to global warming. This is relevant not only to the Jaffna peninsula but similar coastal zones of tropical and semi-tropical countries worldwide, where mosquito-borne diseases are endemic. The development of appropriate preventive and mitigating measures will be necessary in local, national, and global levels. More research into salinity-tolerant mosquito vectors, particularly with regard to their changing bionomics is needed to develop better control measures. We propose that the Jaffna peninsula constitutes a useful case study for the impact of global climate change and rising sea levels on mosquito vector populations and disease transmission in tropical coastal zones. A systems-based approach encompassing the effects of different primary climate change parameters and their secondary effects, changing salinity, and other ecological and socio-economic factors on mosquito populations and disease transmission is possible in the Jaffna peninsula.

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The emergence and maintenance of vector-borne diseases in the Khyber Pakhtunkhwa Province, and the Federally Administered Tribal Areas of Pakistan

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Human populations throughout much of the world are experiencing unprecedented changes in their relationship to the environment and their interactions with the animals with which so many humans are intimately dependent upon. These changes result not only from human induced changes in the climate, but also from population demographic changes due to wars, social unrest, behavioral changes resulting from cultural mixing, and large changes in land-use practices. Each of these social shifts can affect the maintenance and emergence of arthropod vectors disease or the pathogenic organisms themselves. A good example is the country of Pakistan, with a large rural population and developing urban economy, it also maintains a wide diversity of entomological disease vectors, including biting flies, mosquitoes, and ticks. Pathogens endemic to the region include the agents of piroplasmosis, rickettsiosis, spirochetosis, and viral hemorrhagic fevers and encephalitis. The northwestern region of the country, including the Khyber Pakhtunkhwa Province (KPK), formerly the North-West Frontier Provence (NWFP), and the Federally Administered Tribal Areas (FATA) are mountainous regions with a high degree of habitat diversity that has recently undergone a massive increase in human population density due to an immigrating refugee population from neighboring war-torn Afghanistan. Vector-borne diseases in people and livestock are common in KPK and FATA regions due to the limited use of vector control measures and access to livestock vaccines. The vast majority of people in this region live in abject poverty with >70% of the population living directly from production gained in animal husbandry. In many instances whole families live directly alongside their animal counterparts. In addition, there is little to no awareness of the threat posed by ticks and transmission of either zoonotic or veterinary pathogens. Recent emergence of Crimean–Congo hemorrhagic fever virus in rural populations, outbreaks of Dengue hemorrhagic fever have been reported in the region, and high prevalence of cattle infected and co-infected with multiple species of hemoparasites (*Theileria*, *Babesia*, *Anaplasma*). The emergence of which has followed the increased density of the rural population due to an influx of refugees from violent conflicts in Afghanistan and is exacerbated by an already impoverished society and wide diversity of potential arthropod vectors. These human outbreaks may be exacerbated by episodes of social upheaval but are also tied to the historically close association of people in the region with their livestock and subsequent zoonosis that result from spillover from co-habitation with infected domestic animals.

Keywords: *Anaplasma, Babesia*, Crimean-Congo hemorrhagic fever, dengue virus, Eid Islamic festival, hemoparasites, emerging and re-emerging disease

BACKGROUND

Recent estimates report that infectious diseases account for 26% of the global burden of morbidity and mortality to the world's population, with lower and middle income countries suffering to a much higher degree than higher-income countries (Pinheiro et al., 2010). Vector-borne diseases specifically have been one of the leading causes of morbidity and mortality of human and animal populations since such records have been kept and in 2004 accounted for >50 million disability-adjusted life years (DALYs) (Townson et al., 2005). Mosquito-borne diseases such as malaria and dengue alone accounted for 46 million DALYs in the world. An analysis of 335 emerging infectious disease (EID) events between 1940 and 2004 demonstrated a marked increase in the number of EIDs, the majority of which were of zoonotic origin (60.3%), 71.8% from wildlife, and 22.8% of which were vector-borne (Jones et al., 2008). This study also showed that EID events are significantly associated with socioeconomic and ecological factors where regions that have lower income and in lower latitudes had a higher

probability of experiencing an EID event (Jones et al., 2008). The authors generated a predictive model of EID events and generated "hot-spots" and showed that human population density correlated well to areas where EIDs are expected to result. Both Pakistan and neighboring India retained some of the highest estimates of relative risk for an EID event from all four predictive categories including vector-borne diseases, zoonosis from wildlife, zoonosis from non-wildlife, and the emergence of drug-resistant pathogens.

Vector-borne diseases at the beginning of the twentieth century often resulted in widespread epidemics affecting thousands of people throughout the world (Gubler, 1998b). In response to disease outbreaks and coinciding with a better understanding of vector-borne disease dynamics, disease control measures focusing on the elimination or reduction of vector populations were highly effective at blocking the transmission of diseases. New vaccines, insecticides, and a combination of approaches termed Integrated Pest Management (IPM) or Integrated Vector Management (IVM) have proved effective methods of reducing transmission of vector-borne pathogens (Weidhaas and Focks, 2000). Ironically, the apparent success of these efforts resulted in a reduction of control programs for malaria, yellow fever, and dengue as early as the 1970s and were largely disbanded or the diseases were no longer considered severe enough public health concerns (Gubler, 2010).

Pathogens within populations emerge for many reasons including introduction via infected humans and livestock, lack of public health resources (poverty), and changes in climate that allow for expansions of vector ranges due to changes in precipitation or temperature regimes. These interactions result not only from human induced changes in the climate, but also from population demographic changes due to wars, social unrest, behavioral changes resulting from cultural mixing, and large-scale changes in land-use practices. Changes in socio-economic status and the concentration of humans in urban environments were shown in Eastern Europe to lead to an increase in the incidence of tick-borne encephalitis (TBE) cases (Sumilo et al., 2008). The authors report that although an increase in infected wildlife or *Ixodes* ticks was not necessarily detected, other societal changes (change in land-use, reduced amount of pesticide usage, and an increase in human density) were factors responsible for bringing humans into closer contact with infected ticks (Sumilo et al., 2008). Each of these social shifts can affect the maintenance and emergence of arthropod vectors of disease or the pathogenic organisms themselves. One of the most important drivers of EID emergence will continue to be increases in human population density and the ecological shifts associated with that change.

We use Pakistan as an example of a country with a large rural population and developing urban economy that is also endemic for a wide variety of insect and tick vector species (Reisen and Boreham, 1979; Ghosh et al., 2007). Since independence in 1947, Pakistan has had a volatile history, starting with a massive influx of people from neighboring India due to religious differences (Pakistan is an Islamic Republic while India is majority Hindu). Additionally, the northwestern region of the country, including the Khyber Pakhtunkhwa Province (KPK), formerly the North-West Frontier Provence (NWFP), and the Federally Administered Tribal Areas (FATA) has recently undergone an increase in human population density due to an immigrating refugee population from neighboring war-torn Afghanistan. The migration has occurred over a period of 30 years, beginning with the Soviet invasion in 1979 and then more recently with the influx of International Security Assistance Forces (ISAF) and NATO forces into the region. This has lead to a net increase of 3–5 million refugees and their animals in and around Peshawar and the KPK (Yusuf, 1990). Peshawar is the closest large city to the Afghanistan border, directly across from the Khyber Pass, an important trade route and a porous mountainous border populated by tribal peoples from the area. The increase in population density and the poor living conditions provide two important prerequisites for the establishment of an EID or for the amplification of diseases already endemic.

The majority of the population of the KPK and FATA are Pashtun and speak the pashto language with a high diversity of dialects. Originally, their ancestors are migrants from Afghanistan, and central Asian countries that settled mostly in and around the rocky and mountainous regions of northwestern Pakistan and Afghanistan. This region stretches from the Himalayas in the north to the Sulaiman Mountain Range and Waziristan in the South. The whole province is an amalgam of lush green planes, rocky mountains, pine forests, and deserts. Agriculture is limited by the lack of arable land and irrigation water limiting the socio-economic growth for the people of province. Most of the population of the KPK and FATA depend on raising livestock for their livelihood (>70%). This province has recently suffered from natural disasters including a massive earthquake in 2010 in the northern regions of the country and a flood in the same year that affected a majority of the people and livestock living there. Agricultural land was damaged, crops were destroyed, and livestock were killed. The scenario also paved the way for EID and endemic diseases to flourish including Dengue virus (DENV), malaria, and cholera (http://www.promedmail.org/).

Vector-borne diseases in people and livestock are common in the KPK and FATA regions due to the limited use of vector control measures and access to vaccines. Human outbreaks may be exacerbated by episodes of social upheaval but are also tied to the historically close association of people in the region with their livestock and subsequent zoonosis that can result from spillover from infected domestic animals. The goals of this review are to discuss the current distribution of vector-borne diseases in KPK and FATA including the emergence and maintenance of zoonotic viruses and hemoparasites of livestock, discuss hypotheses for emergence and subsequent endemism in the region, and finally to suggest preventative measures to block transmission in humans and domestic animals.

DENGUE VIRUS, DENGUE FEVER, AND DENGUE HEMORRHAGIC FEVER

Dengue virus, a member of the Flaviviridae (genus *Flavivirus*) group of viruses, is considered one of the world's foremost EIDs (Jelinek, 2010). The virus is a single stranded, enveloped, positive strand RNA virus with four antigenically distinct serological subtypes (DEN-1–4). DENV is transmitted by mosquitoes in the genus *Aedes* (*Ae. aegypti* and *Ae. albopictus*), who preferentially feed on humans and thrive in peri-domestic environments (Kyle and Harris, 2008). In humans, the disease presents as an acute and

benign febrile disease or more severely with cephalalgia, retro-ocular pain, photophobia, and muscle and joint pains commonly referred to as "bone-break fever." In tropical Asia a severe and fatal hemorrhagic form of the disease occurs mainly in children. The current pandemic has resulted in an estimated 50–100 million cases of Dengue fever (DF) and ∼250,000–500,000 cases of dengue hemorrhagic fever per year (DHF; Guzman and Kouri, 2003). The first case of DF was identified in Manila, Philippines, and since has been reported throughout Southeast Asia and then globally. In Southeast Asia the number of cases of DHF has increased from ∼10,000 to more than 200,000 cases per year since it is initial discovery in the 1950s (Gibbons and Vaughn, 2002). While the exact mechanism for the emergence of DHF is still not understood, most DHF cases have been associated with either DEN-2 and DEN-3 (Gubler, 1998a), both of which have been reported circulating in Pakistan and the Indian sub-continent (Raheel et al., 2011).

The Indian sub-continent has experienced outbreaks of DF with increasing frequency since the initial discovery of the disease. While DF may have been endemic in the Indian sub-continent previously the first recorded cases of DHF occurred in Delhi in 1989 associated with DEN-2 subtype (Dar et al., 1999). Several DENV outbreaks have occurred periodically, occurring every 2–3 years following the initial event (Vijayakumar et al., 2005; Raheel et al., 2011). The first recorded outbreak of DHF in Pakistan occurred in 1994 in Karachi (Chan et al., 1995). The following year a second outbreak occurred in Balochistan in which ∼1800 cases were reported. The virus may have been present before the recorded 1994 outbreak though. A prospective sero-epidemiological study of apparently healthy Pakistani residents of Karachi conducted in 1982 showed that 50–60% of the sample population had Hemagglutination Inhibition (HI) antibodies to three flaviviruses including Japanese encephalitis virus, West Nile virus, and Dengue virus (DEN-2; Hayes et al., 1982). The status of the serotypes of the outbreaks that occurred in Pakistan were not well defined, although antibodies for DEN-1 and DEN-2 subtypes were detected in patient sera from 1994 and again in 1998 (Chan et al., 1995; Akram et al., 1998). In more recent outbreaks (2005 and 2006) antibodies and PCR positive samples together detected the co-circulation of DEN-2 and DEN-3 viral subtypes (Jamil et al., 2007; Khan et al., 2008). In a 2008 outbreak in Punjab, DEN-2–4 subtypes were detected in patient sera and was associated with DHF cases (Humayoun et al., 2010).

In the Fall of 2011, DENV outbreaks in Pakistan surged once again causing over 15,000 cases in Lahore alone and >200 in Peshawar and the KPK (Rai, 2011). The magnitude of that year's epidemic followed a year where most of the country was affected by massive flooding creating unprecedented mosquito habitat. The vector of DENV, *Ae. aegypti* and *Ae. albopictus*, are common human bitters and survive well in peri-domestic environments including shipping containers, human detritus, and potted house plants. Little information is available for the distribution of DENV vectors in the FATA, although one study identified *Ae. albopictus* and four other *Aedes* spp. mosquitoes from the KPK, formerly NWFP (Suleman and Shafqat, 1993). In most endemic areas, DENV outbreaks are associated with the seasonally wet and warm periods and do not typically occur during cold dry periods. However, evidence suggests that transovarial transmission of the virus can occur in both *Ae. aegypti* and *Ae. albopictus* allowing for mosquitoes eggs that survive the cold dry periods to serve as the following years infected cohort (Gunther et al., 2007). This is however dependent on a permissive climate. Both mosquito species, once infected remain infected for life (Kyle and Harris, 2008). However, in tropical areas the longest-lived *Aedes* mosquito lasted 174 days and the average life-span of a single adult mosquito is approximately 2 weeks (Kyle and Harris, 2008) making overwintering of infected adult mosquitoes unlikely. In the KPK we hypothesize that outbreaks in the large cities of the tropical areas of the country (Lahore and Karachi) spillover into more rural populations via human and animal travel along established trade routes into the KPK. DENV can then infect local *Ae. albopictus* and transient populations of *Ae. aegypti* leading to outbreaks in humans. Now that both serotypes occur in the region we will most likely observe an increase in the amount of DHF cases.

Outside of the KPK, multiple control strategies have been employed in Pakistan including pesticide treatment, larval mosquito habitat removal, and community education campaigns. While pesticide treatment is commonly used to combat mosquito populations in endemic regions, current evidence suggests resistance of *Ae. albopictus* to a number of agrochemicals in Pakistan (Khan et al., 2011). Although larval habitat removal and public health education have served as valuable tools to combat the disease in other countries (Itrat et al., 2008), this may be an unrealistic in the KPK and FATA where there is limited access for vector control agencies. Itrat et al. (2008) identified that education programs disseminated via television may be a more effective method of public education regarding larval habitat control than earlier efforts using newspapers due to a high illiteracy rate. People in the area are willing to participate in eradication efforts but unfortunately education programs are effective at educating only a limited proportion of society and there for less effective. This is especially true in KPK and FATA where most individuals are largely agrarian, migratory, and illiterate.

CRIMEAN–CONGO HEMORRHAGIC FEVER VIRUS

Crimean–Congo hemorrhagic fever virus (CCHFV, family Bunyaviridae, genus *Nairovirus*) is a tick-borne RNA virus and causes severe illness throughout Africa, Asia, Southeast Europe, and the Middle East (Whitehouse, 2004). It was first described in Crimea in the Soviet Union in 1944 and shortly thereafter an identical virus was described in the African Congo (Casals, 1969). The virus mainly affects mononuclear phagocytes, endothelial cells, and hepatocytes, causing severe hemorrhagic fever with case fatality rates ranging from 3 to 30% (Ergonul, 2006). Additionally, case fatality estimates following nosocomial infections in people who provide primary care are especially dangerous, and may reach up to 80% in some areas (Swanepoel et al., 1989; Fisher-Hoch et al., 1992; Ergonul, 2006). People become infected with the virus by exposure to blood and body fluids of infected livestock, by nosocomial infections, or by the bite of an infected tick (Burney et al., 1980; Whitehouse, 2004). The primary tick vectors of the virus are ticks in the genus *Hyalomma* and the distribution of the disease closely resembles that of the tick vector (Hoogstraal, 1979). It is hypothesized that livestock, small mammals (hares), and migrating birds serve as the reservoir hosts for the disease and display

asymptomatic infections even when they have high viremic loads circulating in the blood (Hoogstraal, 1979; Swanepoel et al., 1983). *Hyalomma* is a generalist feeder and may readily feed on infected livestock or birds during one blood meal and then on humans during a subsequent feeding. The virus can be maintained via transstadial and limited transovarial transmission within the tick, and therefore once established in a region may remain, circulate, and become endemic within tick populations (Gonzalez et al., 1992).

Although the distribution of CCHFV is worldwide, with the exception of the Americas, there has been a recent emergence of the virus in areas where infection has not historically been common, including Greece (2008), Georgia (2009), Turkey (2002), Iran (2003), and Pakistan (2005; Saleem et al., 2009; Carroll et al., 2010; Mild et al., 2010). Pakistan has had 14 CCHFV outbreaks to date and of those, half have occurred since the year 2000 (Rai et al., 2008). In addition to an increased outbreak frequency there also appears to be an increase in the virulence of clinical disease with mortality rates since 2002 reaching as high as 75%, as opposed to earlier reports of 5–30%. Nosocomial transmission reportedly has a much higher mortality rate and many of the severe outbreaks in Pakistan have included infection amongst health care providers (Rai et al., 2008). Causes of emergence of CCHFV are varied and include changes in vector and reservoir host distribution due to changing climactic conditions, reservoir host migration patterns, and especially the movement of people and their animals from endemic to non-endemic regions (Mild et al., 2010).

In Pakistan, CCHFV outbreaks have occurred sporadically since it is initial discovery in 1976 in Rawalpindi (Burney et al., 1980). While the virus is considered enzootic in Balochistan, other areas in the north of the country including the cities of Abbottabad and Peshawar (KPK) have recently experienced deadly outbreaks (Saleem et al., 2009). CCHFV outbreaks typically occur following the migrations of nomadic people and livestock to district centers where they bring animals to sell and slaughter. This occurs following times of social upheaval (i.e., immigration from neighboring Afghanistan and following the 2005 earthquake) as well as during the culmination of the Islamic festival of Eid-ul-Azha (Smego et al., 2004; Rai et al., 2008; Saleem et al., 2009). In both cases livestock and people leave the rural country-side to travel to the large city centers of each district where animals are sold and slaughtered. For example, in the village of Bannu, the average number of sheep, goats, and cattle within the city can increase 100-fold during the few days preceding the Eid-ul-Azha festival. Abattoir workers are commonly infected during slaughter of viremic animals and during the Eid-ul-Azha, animal slaughter in individual homes can serve as a source of transmission to those who may not normally be at increased risk of infection (Rai et al., 2008). In addition, due to the porous nature of the Pakistan-Afghan border livestock and people from neighboring Afghanistan also bring their animals to market in Pakistan or graze their animals in the Pakistani countryside. This disease has been report in Afghanistan where in 2009 a US soldier acquired a CCHFV infection (Olschlager et al., 2011). The effect of increased movements of people and livestock due to the war in Afghanistan may have resulted in the establishment of infected ticks and/or livestock to a previously naïve region, namely the KPK and FATA Provinces in northwestern Pakistan.

The risk of CCHFV infection is directly related to the density of vectors available for human contact and the prevalence of CCHFV in the vector populations (Hoogstraal, 1979). The prevalence in the vector population is indirectly related to the prevalence of CCHFV in wild animal reservoir populations and the density of livestock that can maintain the vector-pathogen-host cycle. Tick populations are regulated by the availability of hosts for feeding and by individual tick survival both of which vary depending on climactic conditions and differential habitat quality (Sonenshine, 1992). Outbreaks of CCHFV in Pakistan appear to occur following the migration of rural people and their animals into the cities during religious celebrations or strife exposing urban populations to either exposure to viremic fluids from slaughtered animals or through the bites of infected ticks transported by infested livestock. CCHFV human cases primarily occur during the months of October and November (Jamil et al., 2005), which often corresponds to the season where the Eid-ul-Azha celebration and can result in large numbers of animals brought to the cities from rural areas for sacrifice (Rai et al., 2008). In both the KPK and FATA the timing of DHF outbreaks occur most frequently during August, September, and October (Riaz et al., 2009). The outbreak of DHF is associated with an increase in mosquito breeding habitat following the monsoon rains of these months (Riaz et al., 2009), which often ends just prior to the Eid celebration. The clinical presentation of both diseases is also similar. Both diseases are reported most commonly in young males, presenting with fever, vomiting, and diarrhea (Jamil et al., 2005; Butt et al., 2008). CCHFV may also lead to bleeding from body orifices (∼50% cases) while DHF results in mucosal bleeding and a rash in 80% of patients although early on in the infection may appear the same (Butt et al., 2008; Riaz et al., 2009; Saleem et al., 2009). These subtle differences in presentation may lead to dangerous consequences for primary care givers as CCHFV may be transmitted directly. Diagnosis of either virus infection can be quickly accomplished with molecular methods however there is limited availability of this type of diagnosis in Pakistan, especially in the FATA and KPK regions (Smego et al., 2004).

EMERGING VECTOR-BORNE HEMOPARASITES

Hemoparasitic diseases caused by vector-borne parasites (protozoans and bacteria) constitutes a disease entity of considerable public and veterinary health, and economic importance in tropical and subtropical regions (Colwell et al., 2011). While many are of obvious importance in Pakistan and globally (malaria, leishmania, and borreliosis), a large number of novel pathogens are being identified in animal populations that have not been reported previously due to improved diagnostic analysis made possible by molecular genetic tools. In fact, hemoparasitic pathogens serve as a limiting factor in maintaining exotic cross-bred cattle, local cattle breeds, buffaloes, and small ruminants in many subsistence agricultural communities. Economic losses result both directly and indirectly from the morbidity and mortality caused by the high incidence of these diseases and from the animals serving as a reservoir of zoonotic vector-borne agents (Irwin and Jefferies, 2004; Otranto et al., 2009). In Pakistan, the increased immigration of people resulted in a concomitant increase in the number of livestock. There for accidental transportation of pathogen vectors

on livestock and infected animals could account for the spread of zoonotic pathogens and their establishment in an area in which it had not been present previously. Additionally, the importation of naive animals to a region where an endemic disease occurs could result in the exposure of new hosts and amplification of a pathogen. This scenario has been realized in piroplasmosis epidemics previously (Caracappa, 1999; Hofle et al., 2004) although little information is available in Pakistan.

In the KPK and FATA, livestock can be infected by multiple blood-borne parasites including those that cause piroplasmosis (*Babesia* and *Theileria* spp) and Anaplasmosis (caused by *Anaplasma marginale, A. central*, and *A. phagocytophilum* in sheep and goats), all of which can cause severe disease in animals and potential production losses (Khan, 1991). *Babesia* spp. and *A. phagocytophilum* are recognized as human zoonotic pathogens in the region and diagnosis in the human cases can be subtle and difficult. Heamoparasitic disease occurs sporadically and cases are identified throughout the year (Buriro et al., 1994; Khan et al., 2004). The prevalence of *Babesia* and *Anaplasma* in the people of KPK and FATA is unknown, but given the widespread prevalence in animals humans are surely affected.

Intraerythrocytic parasites in the genus *Babesia* infect domestic animals and causes severe anemia and hemoglobinuria. The pathology of this disease is due to the dividing parasites within erythrocytes that produce rapid destruction of the cells and accompanying hemoglobinemia, hemoglobinuria, fever, and lethargy (Beattie et al., 2002). Acute cases lead to death in 20% of untreated infected animals, parasitemia may involve between 0.2 and 45% of erythrocytes (Mahoney, 1977). *Babesia* spp. are transmitted by the bite of a tick vector, namely due to hard tick vectors (Ixodidea) and is maintained transovarially (Ruebush et al., 1981). The disease babesiosis, appears particularly severe in naïve animals when they are introduced into an endemic area, and is a considerable constraint on livestock development in many parts of the world. In Pakistan, *B. bovis, B. bijemina*, and *B. ovis* are most commonly identified infecting livestock (Khan, 1991). In North America and Europe disease in humans has been caused by *B. microti* and *B. divergens* respectively (Hunfeld et al., 2008). The reservoir hosts include wild rodents, wild ungulates, and Bovids (Mahoney, 1977). Babesiosis has increasingly been identified in North America and Europe as a zoonotic disease due to an increased ability to diagnose infection with molecular methods. Because of the subtle effects of anemia, disease in humans can be misdiagnosed, but with the emergence of immunosuppressive pathogens like HIV, infection with *Babesia* spp. may become more common (Harrus and Baneth, 2005; Rai et al., 2007).

Anaplasmosis caused by the rickettsial bacteria, *A. marginale* and *A. centrale* in large ungulates and *A. phagocytophilum* in sheep and goats, has also been identified in livestock in Pakistan. *Anaplasma* spp. belong to the family Rickettsiaceae bacteria that are obligately intracellular and also difficult to diagnose. In fact, 53% of the EID events that have occurred over the past 50 years were caused by *Rickettsia* (Jones et al., 2008). The parasites are cosmopolitan in nature, and the disease often co-exits with babeseosis due to a shared Ixodid vector (*Ixodes persulcatus* throughout Asia), but single species infections predominate. This disease is characterized by acute anemia, fever, jaundice and the degeneration of the internal organs. Naturally buffaloes, bison, various antelope species, deer, elk, camels, goats, and sheep are all susceptible to anaplasmosis while *A. phagocytophilum* usually has a natural reservoir in wild rodents (Foley et al., 2008). The emergence has been associated with an increase in the presence of wild rodent hosts in regions where the tick vector is already abundant (Chen et al., 1994). Again while the pathogen does exist in sheep no evidence of infection has been identified, although as mentioned earlier, with the emergence of HIV we expect the emergence of these other tick-borne pathogens (Harrus and Baneth, 2005; Rai et al., 2007).

CONCLUSIONS

Control of vector-borne disease in regions such as the KPK and FATA is made more difficult due to an already frail health infrastructure and because these diseases manifest with a wide variety of symptoms making misdiagnosis or inappropriate clinical treatments more likely. At the same time, a diversity of pathogens exist in a region that has suffered from political instability, refugee immigration, and poverty for decades which often results in limited access to health care and, presentation of patients to medical facilities late in the course of disease resulting in higher mortality rates (Rai and Khan, 2007). The majority of the human population in the KPK and FATA live in poverty with >70% of the population living directly from production gained in animal husbandry. In many instances whole families live directly alongside their animal counterparts. The increase in human density has lead directly to an increase in livestock density in the area. These conditions allow for the establishment of pathogens and potentially lead to increased transmission risk of zoonotic and vector-borne diseases through this close association between people and their livestock.

Residents of the KPK and FATA have limited access to vaccinations and acaracides due to the relatively high cost of these preventatives and because of the difficulties associated with the transportation and distribution of these products when available. Control efforts are hampered by the lack of public awareness in local residents to the threat posed by tick bites and the transmission of zoonotic or veterinary pathogens. This is compounded with the region's endemicity for a variety of pathogenic agents including those that cause piroplasmosis, rickettsiosis, viral hemorrhagic fevers, and encephalitis. Although few resources are currently available to stop transmission allowing many problems to flourish, we suggest a combination of preventative and surveillance measures can be used to decrease the risk to human and animal residents of the region. Beginning with vector control efforts based on protecting livestock from acquiring infection via the bites of infected ticks. Veterinarian and public health agencies can use relatively inexpensive methods to eradicate ticks from domestic livestock through the use of topical acaricides and insecticidal drenches or dip tanks. Protecting livestock from tick bites can also serve to lessen the number of infected ticks potentially available to transmit disease to human populations. Surveillance programs tied closely with communication between medical doctors, veterinarians, and public health officials are necessary in order to develop predictive models of disease occurrence that enable public health agencies to focus their limited resources during control efforts. For many of these diseases public education at

local markets and in local mosques in regards to proper biosafety precautions would be a valuable addition to most vector-borne disease control programs.

The rapid evolution of molecular genetic technologies has enabled diagnostic laboratories outside of developed countries to provide quick and accurate testing of clinical samples but their use in surveillance and disease prevention programs has not kept pace (Harrus and Baneth, 2005). These technologies can and should be applied to address the health and economic needs of the people in this region. Simple preventative measures such as these may not be sufficient to eradicate these diseases themselves but may help to alleviate human suffering in the endemic regions and possibly slow future transmission into regions of the country that are not currently affected.

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In search for factors that drive hantavirus epidemics

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In Europe, hantaviruses (*Bunyaviridae*) are small mammal-associated zoonotic and emerging pathogens that can cause hemorrhagic fever with renal syndrome (HFRS). Puumala virus, the main etiological agent carried by the bank vole *Myodes glareolus* is responsible for a mild form of HFRS while Dobrava virus induces less frequent but more severe cases of HFRS. Since 2000 in Europe, more than 3000 cases of HFRS have been recorded, in average, each year, which is nearly double compared to the previous decade. In addition to this upside long-term trend, significant oscillations occur. Epidemic years appear, usually every 2–4 years, with an increased incidence, generally in localized hot spots. Moreover, the virus has been identified in new areas in the recent years. A great number of surveys have been carried out in order to assess the prevalence of the infection in the reservoir host and to identify links with different biotic and abiotic factors. The factors that drive the infections are related to the density and diversity of bank vole populations, prevalence of infection in the reservoir host, viral excretion in the environment, survival of the virus outside its host, and human behavior, which affect the main transmission virus route through inhalation of infected rodent excreta. At the scale of a rodent population, the prevalence of the infection increases with the age of the individuals but also other parameters, such as sex and genetic variability, interfere. The contamination of the environment may be correlated to the number of newly infected rodents, which heavily excrete the virus. The interactions between these different parameters add to the complexity of the situation and explain the absence of reliable tools to predict epidemics. In this review, the factors that drive the epidemics of hantaviruses in Middle Europe are discussed through a panorama of the epidemiological situation in Belgium, France, and Germany.

Keywords: Belgium, France, Germany, hantavirus, HFRS, NE, biotic factors, abiotic factors

INTRODUCTION

Hantaviruses (*Bunyaviridae*) are carried by rodents, insectivores, and – as recently confirmed – by bats (Kim et al., 1994; Weiss et al., 2012) and transmitted to humans by inhalation of infected excreta (Heyman et al., 2009c). So far, only some rodent-borne hantaviruses have been found to be pathogenic to humans. The relationship between the rodent population density, the hantavirus prevalence in the rodent population, and the number of human hantavirus cases, including the hemorrhagic fever with renal syndrome (HFRS) cases in Eurasia, has been suggested worldwide (Tersago et al., 2011a). It is thus important to know whether or not hantavirus-carrier rodent populations are peaking or not. The factors that drive rodent population dynamics are of prime importance for predicting hantavirus epidemics (Linard et al., 2007a,b; Jonsson et al., 2010), but at the same time highly complex and largely unknown (Krebs, 1999).

The annually recorded numbers of clinically apparent hantavirus infections in Europe has been steadily increasing during the last 20 years (Heyman et al., 2011). In general, the awareness of public health authorities and the availability of diagnostics is supposed to have improved (Faber et al., 2010; Heyman et al., 2011), but one of the most important reasons might be that we have been ignoring a historical truth: human well-being and good health depends in the long-run on the stability of earth's ecological and physical systems. It was easy to overlook or ignore this dependency in the nineteenth and twentieth century, when the human species began to grow exponentially, its environment was increasingly becoming urbanized and industrialized and natural systems became increasingly under pressure (Ramalho and Hobbs, 2012).

The human impact on ecosystems is indeed immense; man-made agricultural ecosystems dominate much of Europe's landscape and, due to the intensification of agriculture and subsequent use of fertilizers and pesticides, biodiversity in particular has changed significantly in almost all agricultural areas (Sanchez et al., 2011; Shochat and Ovadia, 2011). The vast majority of farmland wildlife has suffered greatly and bird populations have decreased by 50% or more since the 1980s in Europe (Donald et al., 2006). Recently, the reduced use of both pesticides and fertilizers and environmental friendly farming (e.g., organic farming) mark positive changes that can be seen across Europe (Noyes et al., 2009). Forest ecosystems in Europe have also experienced dramatic declines, deforestation has however been reversed or management

has changed in the last two decades and in some areas forests have been expanded significantly (Bezirtzoglou et al., 2011). Moreover, the often overlooked mountain ecosystems possess a high diversity of habitats and species and are important for water supply and its regulation toward lower altitude areas, but are also especially vulnerable to impacts from changes in agriculture, infrastructure, tourism, and climate (DeGraaf et al., 1992; Huitu et al., 2003).

Urbanization was and still is a significant factor that is changing Europe's biodiversity, mainly because of the inevitable rural to urban land-use change (Franklin, 1993). Urban ecosystems are therefore almost never integrated into wider biodiversity considerations, in the past two decades the increasingly renewed contact of people with nature (de-urbanization) has been found to relieve urban stress and to help fight climate change through increased awareness, but it has also made people – through increased risk for contact with wildlife – more vulnerable for zoonotic pathogens (Franklin, 1993). Adverse health effects need however not be exclusively related to infectious diseases. One of the most striking recent examples of detrimental health effects related to climate was the heat wave during the summer of the year 2003, which was probably the hottest summer in Europe for 500 years. Between 20,000 and 40,000 heat-related deaths occurred across Europe in August 2003 alone (Schär et al., 2004; Laaidi et al., 2011).

Ironically, while human pressure onto the environment had detrimental effects for the entire ecosystem, recent observed change for the better might – in collaboration with changes in human behavior and climate – increase the chances for infectious diseases transmission. Their reservoirs and vectors – ticks, fleas, insects, and mammals mostly – are in general not amongst the many species that appear on the IUCN red list[1]. But although rodents – the reservoirs for hantaviruses – are generally not regarded as threatened mammals, there is ample historical evidence of the extinction of several rodent phylogenetic lineages. Rodent species represent about 50% of mammalian extinctions in the last 500 years (Amori and Gippoliti, 2003). Rodents will probably remain unpopular, despite increasing evidence that many rodent species in fact sustain ecosystems structures and functions (Amori and Gippoliti, 2003).

As this paper aims to define factors that drive hantavirus epidemics, an important question is to what extent certain parameters influence hantavirus activity and whether these parameters occur solely in one region or on multi-country level. Perhaps the most difficult question is how factors that facilitate and those that inhibit the hantavirus transmission mechanism interact and what the net outcome of this equation is and will be in the future. The following chapters summarize the present knowledge on hantaviruses and disease transmission in Belgium, France, and Germany. These three countries were chosen as examples for discussing the epidemiology, and possible factors having an impact on changes in the oscillations of hantaviruses in Middle Europe.

THE HANTAVIRUS SITUATION IN BELGIUM

Belgium is situated in the temperate deciduous forest biome, it consists of three main geographical regions; a highly industrialized coastal plain in the northwest and central plateau, and the mainly forested Ardennes in the southeast. Belgium's highest point (694 m) is located in this region. The Ardennes extend into N-France and in W-Germany.

Although scattered data concerning clinically apparent human hantavirus infections are available from 1976 on in Belgium, this was only reliably and on a national level recorded from 1996 on by the Reference Laboratory for Vector-Borne Diseases (RLVBD) in Brussels, that was appointed National Reference Laboratory for Hantavirus Infections; the data were regularly reported to the Scientific Institute of Public Health (SIPH), the reporting organism to the public that is usually – but erroneously – cited as the source of the data (**Figure 1**). A total of 3,124 cases have been diagnosed according to the case-definition of the European Network for Imported Viral Diseases (ENIVD[2]) until end of 2011 in Belgium.

Puumala virus (PUUV), carried by the bank vole (*Myodes glareolus*) is the only hantaviral serotype that is known to infect humans in Belgium, it induces a mild form of HFRS called nephropathia epidemica (NE); on average 150 cases occur yearly. From 2005 on there is a definite increase in the yearly number of NE cases in Belgium (**Figures 2A–D**). Although increased awareness and improved diagnostic tools were originally argued for the increasing number of yearly cases, the RLVBD data (Heyman, personal communication) show that the total number of serology demands is more or less constant between the years but that the number of submitted samples that showed positivity for hantaviral antibodies increased from 25 to 56%. Thus, increased awareness and improved diagnostic tools do not seem to be responsible for the increase in cases, a more targeted diagnostic strategy applied by clinicians is however possible. Under-diagnosis is probably also important as only 5–10% of the infected individuals display clinical symptoms (Heyman et al., 2009c).

From the data collected by the Belgian reference laboratory we learn that there existed –from 1993 on – a 3-year epidemic cycle up until 1999, between 1999 and 2005 a 2-year cycle occurred and from 2005 on all years – i.e., seven in total – showed an increased hantavirus activity (**Figure 1**). This unique hantavirus epidemiology pattern that only occurs in Belgium and not in the surrounding countries raises questions whether the parameters that drive hantavirus epidemiology apply only to Belgium or also to other European countries (Heyman et al., 2011).

Here we discuss a set of parameters that could drive hantavirus epidemics in Belgium.

CARRIER AND HOST BEHAVIOR

In the case of hantaviruses, rodents and insectivores act as carrier, humans and non-rodent mammals (cats, dogs, foxes, deer, boar, etc.) act as dead-end hosts. So far, only infected humans can become ill although no sufficient evidence exists to exclude other mammals as competent hosts (Zeier et al., 2005). In most cases the virus is transmitted from animal to human by inhalation of an aerosol of infected rodent excreta (Heyman et al., 2009c). In order to make this possible three main conditions must be met.

[1] http://www.iucnredlist.org/

[2] http://www.enivd.de/FS/fs_encdiseases.htm

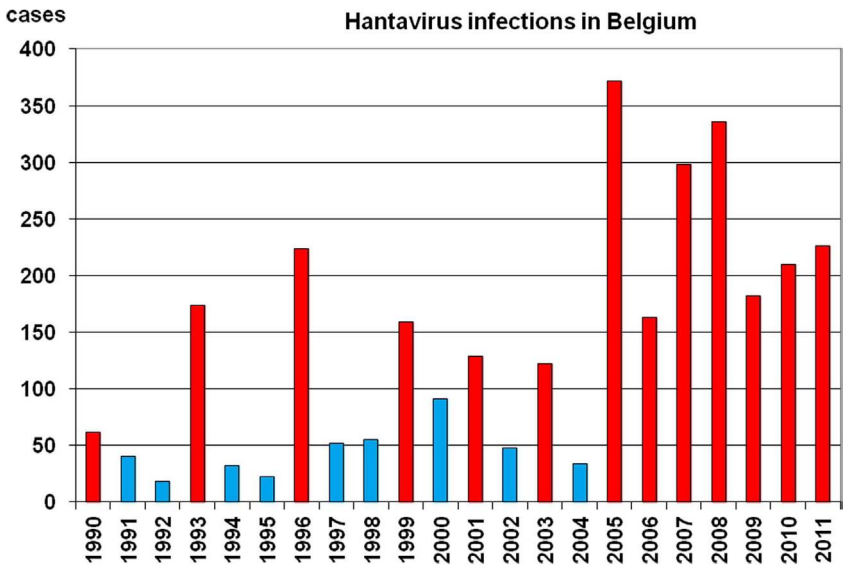

FIGURE 1 | Yearly number of hantavirus cases in Belgium as diagnosed by the Reference Laboratory for Vector-Borne disease, Brussels. The epidemic years are depicted in red, the non-epidemic years in blue.

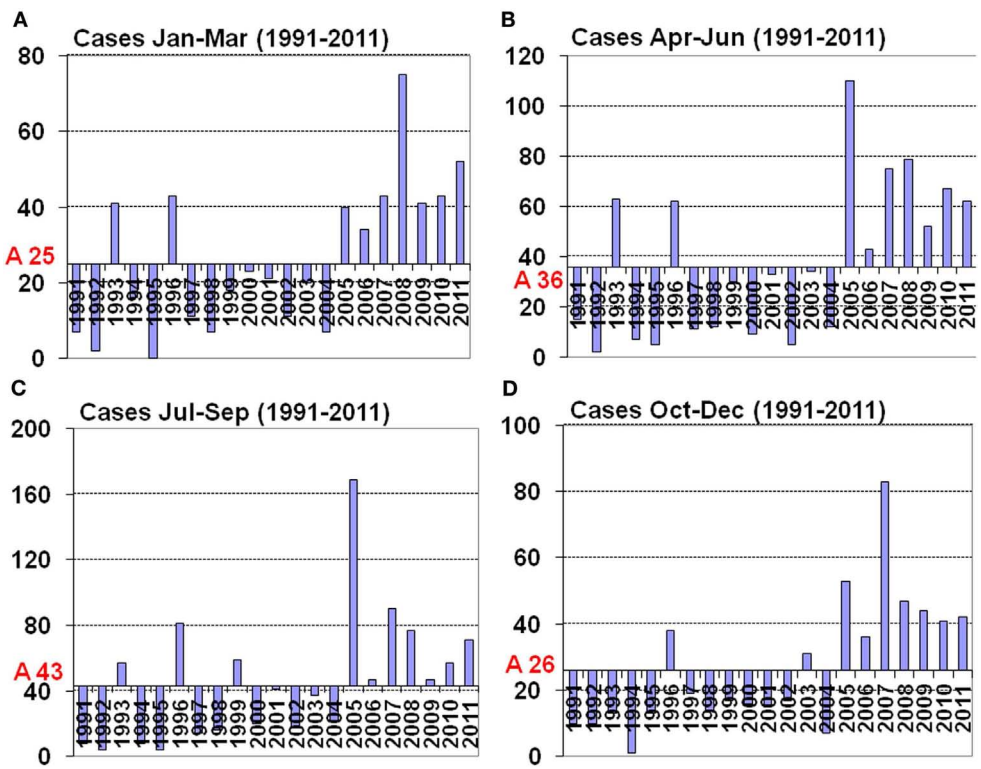

FIGURE 2 | Average of seasonal hantavirus cases and seasonal distribution (calculated as deviation from the average) in Belgium for the years 1991–2011. (A) Months January to March with an average of 25 cases, **(B)** months April to June with an average of 36 cases, **(C)** months July to September with an average of 40 cases, **(D)** months October to December with an average of 26 cases.

a. The carrier must be present and be sufficiently numerous.

There is sufficient evidence that the rodent population density in Belgium is directly related to the percentage of infected carriers in the population and to the number of infected hosts (Heyman et al., 2002b). Some hantaviruses can also spill-over to other habitat sharing rodent species (*M. glareolus* to *Apodemus*

sylvaticus in the case of PUUV; Klingström et al., 2002; Heyman et al., 2009b). In some cases the secondary carrier species can be heavily infected and – although there is no proof provided – chances are that both primary and secondary carrier species could infect humans (Klingström et al., 2002; Heyman et al., 2009b; Schlegel et al., 2009).

b. The host must have access to the habitat of the carrier or vice-versa.

In order to infect the host, he must remain in the carrier habitat for a sufficiently long period of time and also be involved in activities that put the host in direct contact with the virus particles. Entering and cleaning long abandoned places (cabins, attics, cellars, etc.) or performing work that disturbs carrier nests (renovation, cleaning, etc.) or other activities that put the host in prolonged contact with the carrier's habitat (camping, sleeping on the ground, military exercises, etc.; Linard et al., 2007a; Tersago et al., 2011a). This rule is also valid for non-human mammals although the involved species usually are predating on the carrier species (Dobly et al., 2012). The observation that about 85% of the hantavirus cases occur in the forested South of the country, where wood is commonly used for heating and building, may support this.

c. The contact between aerosol and host must take place within a limited time frame, i.e., the virus must still be viable. In a minority of the cases direct contact with rodents through handling dead rodents or rodent's nesting material of live rodents can play a role.

Although hantaviruses are RNA viruses that are considered to be vulnerable for various biotic and abiotic parameters once they are outside the carrier, studies have revealed that the virus can remain viable for an extended period of time (days to weeks) outside the carrier if conditions are favorable (Kallio et al., 2006a). This implies that it is by no means necessary to actually see or encounter rodents in order to become infected. Again, the more common manipulation and use of wood in south Belgium may support this (Campioli et al., 2009). The observed change of the climatic conditions in Belgium with warmer winters and wetter summers (Tricot et al., 2009) might favor the survival of the virus in the environment and contribute to the higher number of cases.

RODENT ECOLOGY

During the glacial events of the Quaternary in Europe, deciduous forests were generally confined to the Mediterranean peninsulas (Deffontaine et al., 2005). Temperate forest mammal species such as bank voles shifted their range according to their habitat, thus surviving the glacial maxima in the Mediterranean peninsulas (Taberlet et al., 1998; Deffontaine et al., 2005). Interglacial and postglacial recolonizations of central and northern Europe, by plants and tree accompanied by – amongst others – rodents, therefore originated from these refuges (Taberlet et al., 1998; Michaux et al., 2005).

Depending on food availability, predation and environmental characteristics, and stress, various endemic rodent species display more or less regular cyclic population changes. Whether population numbers and cycles of endemic rodents in Belgium have changed is not known but with the cutting of forests and the human population increase since Medieval times it seems reasonable to assume that nowadays rodent populations are a fraction of what once was. Rodents often were a serious economic and public health problem; one of the most striking examples were the consecutive plague events in Europe between the fourteenth century and the last occurrence in Marseille in 1720 (Duchene and Contrucci, 1998) that killed in certain regions up to 30% of the population. A curious side effect of the significant human population decrease was that from about 1,350 on CO_2 levels in the atmosphere dropped due to reforestation as less wood was needed for building and heating for the decimated population (Ruddiman, 2003).

Bank vole populations in Belgium indeed show cyclic population density changes (Heyman and Saegerman, 2009). A 10-year (1999–2008) rodent surveillance project organized by the Belgian Ministry of Defense with rodent trapping on 15 different sites demonstrated that between 1999 and 2005 fluctuations in trapping success (and thus rodent population density) correlated with hantavirus prevalence (measured by detection of IgG antibodies) and with the relative number of human cases. From 2006 on, however, this relation is much less clear, i.e., rodent population densities remain high in the absence of a mast event – in this case beech and/or oak – and virus prevalence remains higher than expected (**Figure 3**).

Although allegedly not critical for rodent cycles in the temperate deciduous forest zone, predation, or behavioral changes due to the presence of predators could also play a role in Belgium. Population densities of the various animals (mammalian and avian) that predate on *M. glareolus* are not well known, but – apart from population density increases of birds of prey species (Robinson and Sutherland, 2002), Population densities of *Mustelidae* (weasels) and foxes (*Vulpes vulpes*) are on the rise since the 1980s in accordance with bank vole population fluctuations (Vervaeke et al., 2003).

BIODIVERSITY

Due to intensive agriculture, pesticide, and insecticide use and urbanization, the mammal biodiversity in Belgium is in almost all habitats changed. Although 21 rodent and 6 insectivore species are endemic in Belgium (Wilson and Reeder, 2005), trapping data – as obtained in the forenamed 1999–2008 study of the Belgian Ministry of Defense – reveals that only five rodent (*Microtus arvalis*, *M. glareolus*, *A. sylvaticus*, *Apodemus flavicollis*, *Rattus norvegicus*) and two insectivore species (*Sorex araneus*, *Crocidura leucodon*) are readily captured in live traps. In the study Ugglan Special No2 traps (Grahnab, Gnosjö, Sweden) that are suitable for trapping the vast majority of *Apodemus*, *Myodes*, *Microtus*, *Sorex,* and *Crocidura* species were used. *Talpa europaea* is also common but requires a specific trapping strategy due to its subterranean life. In Belgium, *M. arvalis* is the carrier for Tula virus (TULV; Heyman et al., 2002a), *M. glareolus* carries PUUV, and *A. sylvaticus* is a spill-over host for PUUV (Heyman et al., 2009b), *A. flavicollis* carries Dobrava virus (DOBV) but this virus is so far not found in Belgium, *R. norvegicus* carries Seoul virus (SEOV) which is readily present in Belgian rural brown rats (Heyman et al., 2009a) but to this point no human cases have been detected. The remaining 22 species are rare (Pucek, 1989). It is generally regarded that high

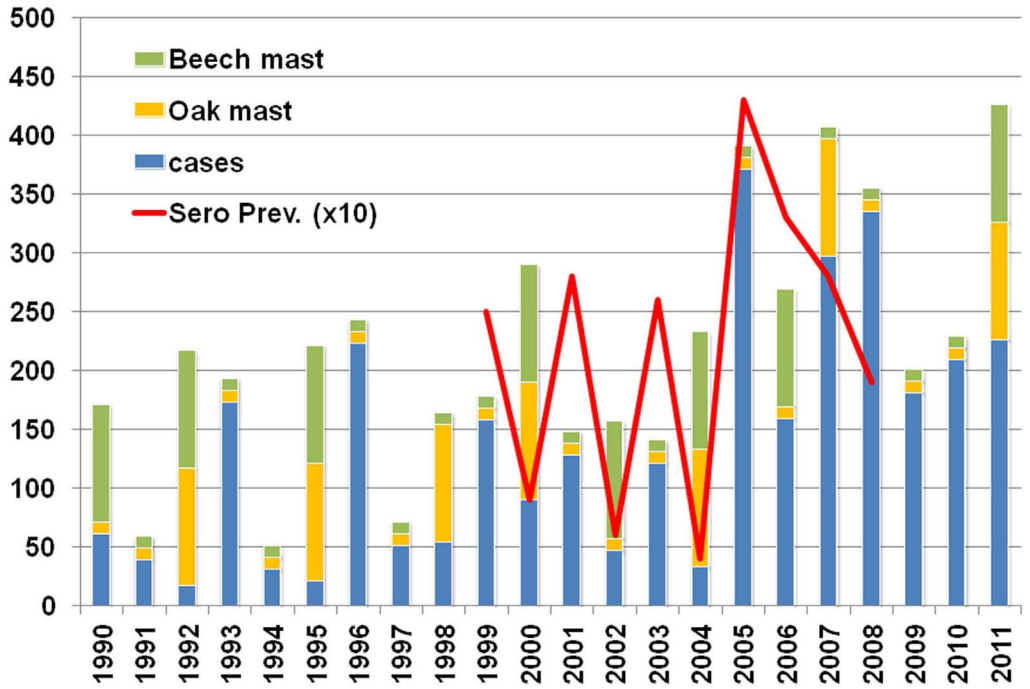

FIGURE 3 | Oak (*Quercus robur*) and Beech (*Fagus sylvatica*) mast years, hantavirus cases, and seroprevalence in *M. glareolus* during the period 1999–2008.

biodiversity has a diluting effect for pathogen transfer to a single host (Mills, 2006; Peixoto and Abramson, 2006; Keesing et al., 2010), deteriorating landscape and subsequent decrease of biodiversity could thus favor hantavirus infections. The disturbance of trophic interactions is considerable for virtually all species (Olsson et al., 2010).

LANDSCAPE

Endemic species are amongst the most affected if an original forest is converted into an anthropogenic habitat. The native vegetation is in most cases partially replaced by non-native or even invasive plant species by gardening activities. A matrix of altered habitats enclosing forest remnants in human dominated landscapes may be inhospitable for wildlife in general. Endemic species are in this case under increasing pressure from non-native or invasive species and both invasive species and generalist species tend to survive the changes (Umetsu and Pardini, 2007).

Habitat fragmentation is also regarded as a possible triggering factor for biodiversity loss (Fahrig, 2003). The landscape fragmentation and absence of corridors can also be responsible for creating so-called islands where rodent populations display the typical Island syndrome features (differences in demography, reproduction, behavior, and morphology when compared to normal populations; Adler and Levins, 1994; Eccard et al., 2011). Landscape changes may thus partially explain the success of the bank vole in virtually all habitats in Belgium and the increasing risk for hantavirus infections. Unfortunately, the intensive agriculture techniques applied in Belgium turned the landscape into a green desert, where wildlife or wild plant species have no place (Peeters et al., 2006, 2007). A significant disturbance of trophic interactions can occur and only generalist and invasive species may profit (Erhard, 2009; Jonsson et al., 2010).

CLIMATE

Climate change is often referred to as one of the main causes of the increase in magnitude and amplitude of the hantavirus infection curve in Belgium, the problem however is that climatic events are often locally, vaguely, and qualitatively defined (Krebs and Berteaux, 2006). The average temperature in Belgium is defined by two parameters: the distance from the sea and the altitude of a certain location.

The fourth IPCC report (The Core Writing Team, 2007) stipulates that "warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice and rising global average sea level," albeit that changes in temperature and rainfall are predicted to be more pronounced at higher latitudes and along the equator than in Western Europe.

The Belgian Royal Meteorological Institute is monitoring the climatic parameters in Belgium since 1833 and is currently relying on a network of more than 250 daily reporting stations throughout the country. In its 2009 climate report, detailed information is made available concerning the tendencies of the Belgian climate in the past century (Tricot et al., 2009). The average temperature has risen with about 2°C since 1833, but this happened in two rather abrupt stages (**Figure 4**); the first significant increase took place around 1910 with primarily a rise in maximum temperature, the second around 1990 with a rise of both maximum and minimum temperatures. Both times the increase was about 1°C. The first frost day happened around the 30th of October

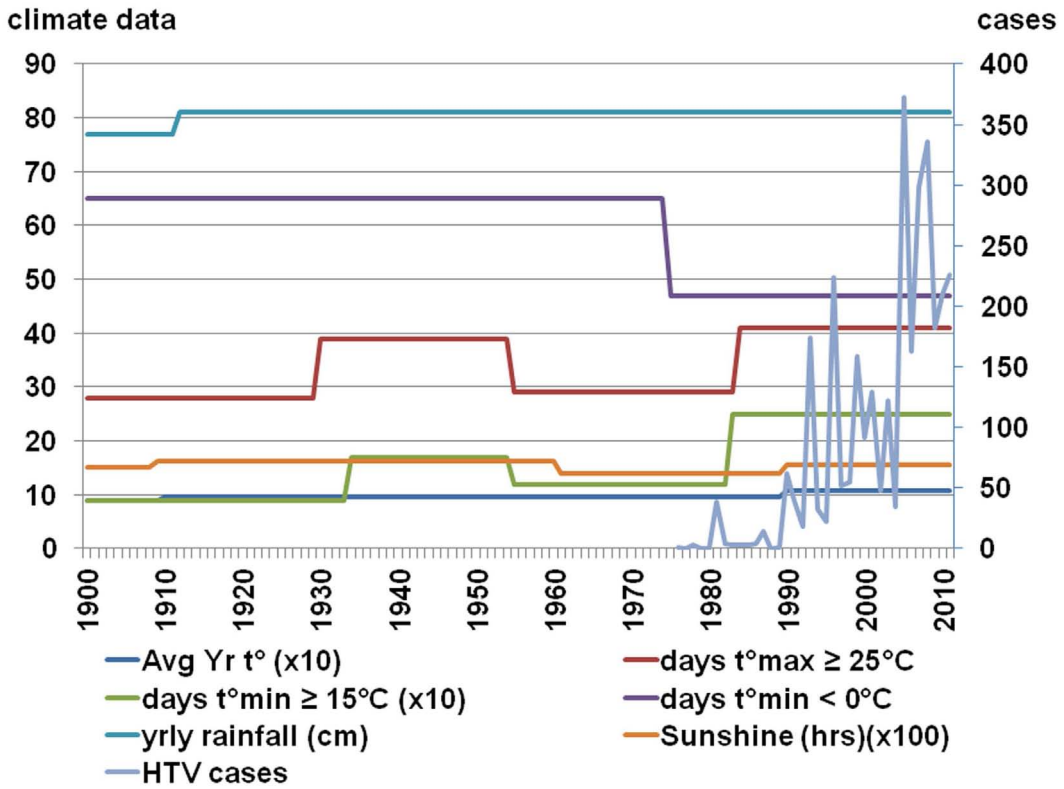

FIGURE 4 | Climatic conditions in Belgium according to Tricot et al. (2009).

from 1900 up until 1955, than an abrupt delay of – on average – 10 days occurred. The last frost day shifted from around the 22nd of April to 06th of April with – again – two abrupt changes in 1930 and 1980 (**Figure 4**). The number of days with at least 25°C and the number of days with temperatures of at least 15°C showed two warmer periods, i.e., from 1930 to 1950 and from 1983 until present. Although the number of days with >25°C has increased, there is no evidence that the number of sunshine hours (related to UV index) has increased as this is associated with cloud cover which is difficult to measure. The UV index increase – important for inactivation of RNA viruses – is thus not established. Finally, the yearly rainfall of on average 770 mm increased around 1910 to 810 mm. The Belgian climate has changed during the twentieth century. Two sudden temperature increases – one in the first half of the century, the other during the 1980s – were noted, the heat wave frequency has increased in the 1990s, the frequency of cold waves decreased in the 1970s.

It should also be taken into account that – even in a country like Belgium where the greatest distance between its borders is no more than 300 km – climatic conditions sometimes significantly vary between the coastal region and the inland regions. There exists an average temperature difference of 2.5°C between the coast region and the Ardennes region, a temperature decrease of approximately 0.6°C per 100 m of altitude increase is observed[3].

[3]http://www.meteo.be

Thus, explaining the increase in the number of NE cases in the last decade (2001–2011) in Belgium just by climate change is difficult because the key years for changes in hantavirus activity were 1999 and 2005; if milder climatic conditions caused the increase in cases, these events should have been visible much earlier as the key years for climatic changes in Belgium occurred long before (**Figure 4**). If – even in the absence of the necessary diagnostic tools – hantavirus epidemics had taken place before the first reliably recorded outbreak of 1995–1996 (Heyman et al., 1999) they would not have gone unnoticed given the sometimes severe clinical course of hantavirus disease, i.e., acute renal failure (ARF) and/or acute respiratory distress syndrome (ARDS). In Russia for instance, hantavirus infections were already diagnosed based on clinical features as early as 1935 (Sirotin and Keiser, 2001).

MAST

"Mast" is a term used to describe the seeds of shrubs and trees that are eaten by wildlife. "Hard mast" refers to nuts of beech and oak, whereas "soft mast" refers to leaves, flowers, and berries of a variety of species. A number of different levels of mast are used such as full, half, dispersed, or deficient but these criteria are not always quantitatively measured. The mast theory – although recently claimed by some in Belgium – is by no means new. Charles Elton (1900–1991) was the first to systematically study animals in their natural habitats and their interactions with their surroundings (Elton, 1933).

Hundreds of animal species use mast as staple food. No less than 171 species (16 amphibian, 9 reptile, 102 bird, and 44 mammal species) are known to depend on it in beech or oak stand habitats (DeGraaf et al., 1992). The interspecies competition already suggests the use of alternative food sources for mammals as they can also feed on soft fruits, plant, or insect species (Bozinovic, 1997; Elkinton et al., 1998).

The connection between mast and hantavirus infections was – in Belgium – already suggested in 2001 (Heyman et al., 2001) and re-examined recently (Tersago et al., 2009; **Figure 5**). The scarcity of detailed mast data, regional mast data, the short time frame for which detailed data are available and the complete lack of data on other than oak and beech species that have a cyclic or yearly masting event limits the value of the observations. Only oak and beech mast were involved in the various studies but closer examination shows that – in the habitat of *M. glareolus* – between 11 and 33 (November to February) and 95–1,292 (March to October) flowering plant species[4] are available as food source (Kollmann et al., 1998). As beechnuts and acorns are only available between October and January and are also the staple food for large mammals like *Sus scrofa* (wild boar) or *Capreolus capreolus* (roe deer) and various bird species, it seems obvious that rodents need to rely on other food sources for most – if not the whole – of the year. Besides evidence for an animal-based food preference in spring (Eccard and Ylönen, 2006), it was also established that hard fruits only account for about 40% of the bank vole's diet (Wereszcynska and Nowakowski, 2004). Bank voles are generalists and omnivorous, they eat insects, leaves, and soft fruits as well as hard fruits (Kollmann et al., 1998).

As far as oak and beech mast is concerned, Belgian data show that hantavirus epidemics are to some extent related, but only for the period between 1995 and 2007 (Tersago et al., 2011b). Not in favor of the role of beech and oak in hantavirus epidemics and probably the proverbial straw that breaks the camel's back is the fact that both species only account for 9 and 17%, respectively, Norway spruce (*Picea abies*) represents more than 36% of the total tree stand composition (Campioli et al., 2009). Before 1995 mast did – to our best knowledge as reliable data are lacking – not correlate with hantavirus disease epidemics. After 2007, i.e., the last 3 years (2008, 2009, 2010) were no mast years but hantavirus activity was nevertheless high (**Figure 5**). In fact, only the 1993, 1996, and 2005 epidemics correlate with a previous oak-beech mast event, 2001 should have been an epidemic year, after the oak-beech mast event of the year 2000, but was not. And finally, all years after 2005 – six in total – were epidemic years in the absence of oak-beech masting events (**Figure 5**). From **Figure 5** it can be concluded that any mast event can provide sufficient food for rodent populations to peak and induce a hantavirus epidemic in the next year. Thus, not only beech or oak trigger these events. Another observation that does not support the Belgian "mast theory" (Clement et al., 2011) is the fact that mast is considered to take place on a large scale, even on sub-continental level (Kelly et al., 2008; Olsson et al., 2010), the variation of epidemic years in neighboring countries however does not support this (Heyman et al., 2011). Even locally, i.e., in

[4]www.wilde-planten.nl

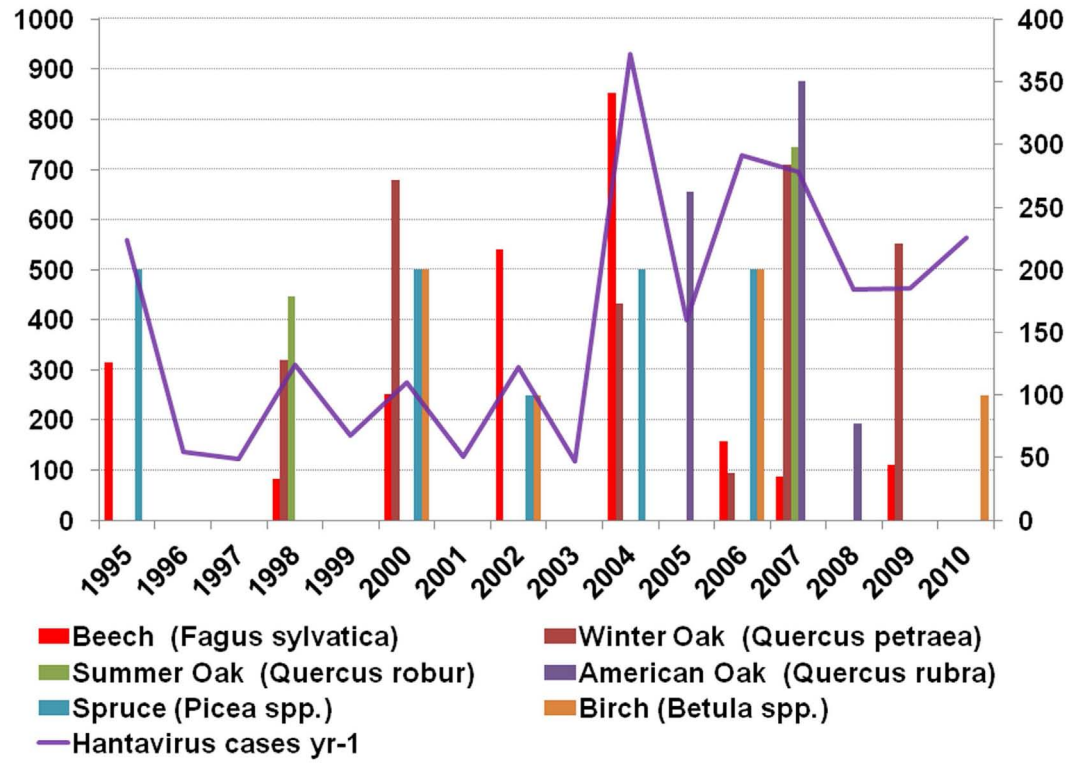

FIGURE 5 | Masting events and masting species (Source: Comptoir forestier de Wallonie).

Belgium, masting is not present at the same time in all regions; the 2011 mast of oak-beech is present in the Flanders region, the North and South – but not in the middle part – of the Wallonia region, this does not mean however that other plant species did not display mast. One other intriguing fact is that although hantaviruses are supposed to co-evolve with their carriers for millions of years – clusters of hantavirus disease (NE, ARF) were not reported before the 1980s (van Ypersele de Strihou et al., 1983). Even in the absence of diagnostic tools the syndrome caused by hantavirus infection should have been noticed and periodically, i.e., the year after a mast event, reported by clinicians.

EPIDEMIOLOGICAL FEATURES IN FRANCE

HANTAVIRUS SPECIES IN FRANCE

In France, PUUV is the most prevalent hantavirus species (Artois et al., 2007). As already explained in the Belgium chapter, this virus is closely associated with its natural reservoir host, the bank vole *M. glareolus*, but the transmission to other dead-end hosts (including humans) remains possible (Deter et al., 2008a). During a survey in France, PUUV seropositive wood mice (*A. sylvaticus*) were detected during the peak of prevalence in bank voles (Sauvage et al., 2002). PUUV was also serologically found in 0.3% (9/263) of the montane water voles (*Arvicola scherman*) captured in the Jura region during 2002, 2003, and 2004 (Charbonnel et al., 2008). Genetic analysis of S and M segments of French PUUV strains revealed their proximity with strains circulating in Belgium and Germany and also in Slovakia (Plyusnina et al., 2007).

Seoul virus, another pathogenic hantavirus, was detected in 2003 in rats captured near Lyon (Heyman et al., 2004). Using reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing, two wild *R. norvegicus* were found infected with a SEOV strain, most closely related to an Indonesian and a Cambodian wild-type strain (Heyman et al., 2004). Human sera positive for SEOV antibodies have been reported in Europe using highly cross-reactive immunofluorescence assays, but have never been confirmed by reliable methods such as neutralization. Nevertheless, as opposed to Belgium, SEOV-neutralizing antibodies have been detected in the convalescent serum of a French patient suffering from HFRS (Lundkvist, personal communication).

More recently, TULV has been identified using RT-PCR in one vole (*M. arvalis*) trapped in the Department of Jura, close to the Swiss border (Artois et al., 2007). This report was confirmed later, with 6.5% (11/170) of *M. arvalis* found seropositive for TULV (Deter et al., 2008b). Based on partial sequence of the M segment, TULV strains circulating in France were found to form a distinct lineage, close to strains isolated in central and Eastern Europe (Plyusnina et al., 2007). TULV has not definitely been linked to human disease (Heyman et al., 2011).

To date, DOBV, responsible for severe cases of HFRS, has not been reported in France, although one of its carriers, *A. flavicollis* is present (Mitchell-Jones et al., 1999).

GEOGRAPHICAL DISTRIBUTION

In France, most of the HFRS cases are reported in the northeastern quarter of the country (Augot et al., 2006; **Figure 6**). In this endemic area, foci of hot spots are well known, more particularly in the Ardennes department, near the Belgian border, which historically accounts for 30–40% of the total number of human cases in France (Sauvage et al., 2002).

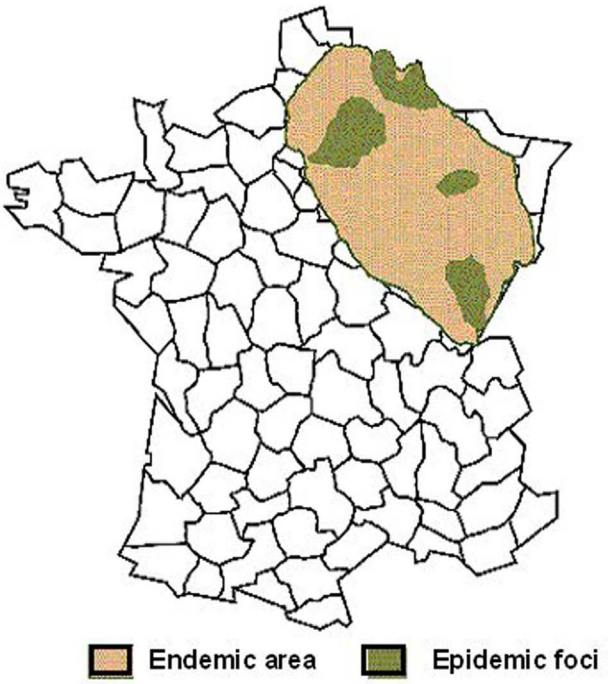

FIGURE 6 | Geographic distribution of human Puumala hantavirus infections in France (Source: French Ministry of health).

As illustrated in **Figure 7**, endemic foci stay rather stable over years, without a real trend for extension. In addition to the historical department of the Ardennes, a new hot spot appeared in the department of Jura, bordering Switzerland, since the epidemic of 2005 (**Figure 7**).

In fact, *M. glareolus* is distributed in the whole country, excepted along the Mediterranean coast (Mitchell-Jones et al., 1999), in a much larger area than the endemic area for HFRS.

INFECTION IN RODENTS

Several studies have been carried out, in the endemic area, in order to assess the prevalence of PUUV in *M. glareolus*, and to elucidate the dynamics of circulation of the virus in its wild reservoir and thus estimate the risk for human beings. To date, no results have been published for surveys outside the endemic area.

As shown in **Table 1**, the seroprevalence levels are highly variable, between areas and in the same area, according to the year and the season (Augot et al., 2008).

Transmission among the rodent populations occurs via excreta, i.e., saliva, urine, and feces, directly or indirectly, probably through aerosols. The voles may become infected through the sniffing of contaminated excreta marks, even if they avoid direct encounters. The virus remains active outside the host, allowing indirect transmission, without physical contacts with infectious rodents. PUUV can survive and remain infectious for 15 days at room temperature, and probably much longer in cold and moist environments

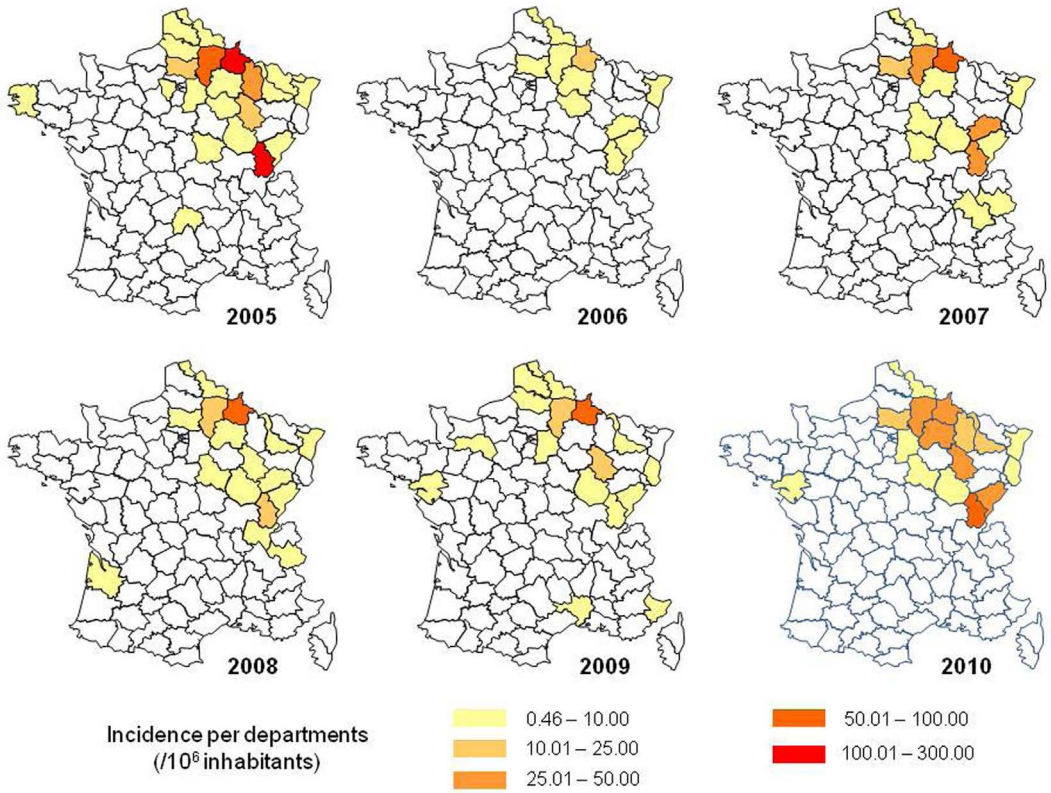

FIGURE 7 | Incidence rate of hantavirus infections in France, according to the department of residence of the patients (Source: Institut de Veille sanitaire, IVS).

Table 1 | Seroprevalence study of Puumala virus infection in bank vole (*Myodes glareolus*) populations in France (95% confidence interval in brackets).

Location	Period	Seroprevalence (PUUV positive/investigated rodents)	Confidence interval (%)	Reference
Jura	2004 and 2005	9.0% (12/133)	4.2–13.9	Deter et al. (2008a,b)
Ardennes	2000–2002	22.4% (204/912)	19.7–25.1	Augot et al. (2008)
Ardennes	2004 and 2005	37.6% (291/773)	34.2–41.1	Augot et al. (2006)
Ardennes	2008 (autumn)	13.5% (37/274)	9.5–17.6	Guivier et al. (2011)
Ardennes	2008 (September–October)	11.8% (37/313)	8.2–15.4	Salvador et al. (2011)

(Kallio et al., 2006a). Vertical infection seems improbable (Deter et al., 2008b) as well as the contamination from mother to progeny. Indeed, juveniles are protected during 2 or 3 months because of the transfer of maternal antibodies (Bernshtein et al., 1999) and the dispersal from the natal site starts before. However, in case of high densities, the dispersal of bank voles is delayed until next spring and juveniles can get infected from their contaminated mother, after the disappearance of the maternal antibodies (reviewed in Deter et al., 2008a).

Usually, the adult males are more often infected than females and juveniles or sub-adults for behavioral and/or physiological reasons (Bernshtein et al., 1999). In the Ardennes, the antibody prevalence rate also increased with age (weight) of the bank voles, suggesting that horizontal infection could be important (Augot et al., 2006). Later, the same author concluded that a strong positive correlation is usually found between seroprevalence and age (estimated with the weight) of the rodents (Augot et al., 2008).

The effect of sex on the prevalence of infection remains unclear, as in some French studies males are more often infected (Deter et al., 2008a) but in others, no significant difference is found (Augot et al., 2008). Nevertheless, the behavior between males and females differs. Female territories are smaller than those of males, especially during the reproductive season (Mazurkiewicz, 1994), and males are much more tolerant for territorial overlap (Augot et al., 2008). Compared to females, when males leave the nest, they traverse greater distances and could introduce the virus in virus-free rodent populations (Augot et al., 2006). Behaviors and social features are critical for understanding the persistence and the spread of hantavirus among rodents populations.

The fluctuations of PUUV prevalence in the bank vole populations have generated multiple hypotheses. Some authors consider prevalence is linked, sometimes with a delay, to the density of the rodent reservoir (Davis et al., 2005). Other authors found that the seroprevalence is not always linked to the density of rodents but usually, higher numbers of seropositive bank voles are captured when the populations decrease from a peak year (Kuenzi et al., 1999; Augot et al., 2008). In other surveys, PUUV seroprevalence and abundance of rodents are weakly linked (Augot et al., 2008).

In France, as more generally in Atlantic and continental Western Europe, mast events seem to be linked to hantavirus epidemics (Heyman et al., 2011). Here in comparison with Belgium several studies have shown that masting in the previous autumn increases food resources and improve the winter survival of rodents which start to breed earlier and can have a second litter, bringing high densities of rodents early in summer (Sauvage et al., 2002; Vapalahti et al., 2003). It appears that animals from the first litter, born in late winter or early spring develop rapidly and become fertile the same year whereas animals from the second litter, born late spring or mid-summer, become fertile only next year. The seroprevalence was significantly higher in animals from the first litter (48%) when compared to the second litter (8%; Augot et al., 2006). The high proportion of susceptible animals could favor a high level of PUUV circulation and a massive shedding of virus in the environment, source of rodent, and human infections (Bernshtein et al., 1999; Escutenaire et al., 2002). In fact, the amount of virus shed during the first month of infection is far higher than during the consecutive chronic phase (Bernshtein et al., 1999; Sauvage et al., 2007). More than the global density, the number of newly infected voles, still in the acute phase of the infection, appears critical to lead to exceptionally high virus concentrations in the environment and thus human exposure and epidemics. In more stable populations, the low incidence of newly infected individuals could explain a lower contamination of the environment. This assumption could explain the presence of only sporadic cases outside the endemic area for hantavirus in France, whereas *M. glareolus* is distributed nearly in all the territory. In brief, epidemics could occur in areas where multi-annual fluctuations of bank vole populations induce at the same time a high number of infected rodents and high proportions of those rodents in the acute phase of the excretion.

As is the case for Belgium, hantavirus epidemics in France can probably not solely be related to mast cycles as outbreaks appear to be localized in foci when mast events are supposed to occur over large areas (Heyman et al., 2009c). The prevalence of the infection in rodents seems also linked to the habitat (Escutenaire et al., 2002; Olsson et al., 2005; Zeier et al., 2005). Forest environments seem more favorable for rodent infection than fragmented, heterogeneous landscapes as hedge networks (Guivier et al., 2011). Different factors can explain this difference. First, hedges are usually considered of a lower quality for food resources than adjacent forests (Guivier et al., 2011). Besides, hedge networks are more exposed to important variations in temperature, humidity, and UV radiations, altering the survival of the virus in the environment and the efficiency of indirect transmission (Guivier et al., 2011). In addition, the helminth community of bank voles is linked to the environment and was shown to be associated with PUUV infection. In particular, bank voles infested by *Heligmosomum mixtum* were more often PUUV positive (Salvador et al., 2011).

The risk for human infection seems to be strongly correlated with the prevalence of PUUV in populations of its reservoir host species (Salvador et al., 2011). But additional factors intervene as the survival of the virus in the environment may relate to temperature, humidity, and UV as well as human behavior, necessary to come into contact with the virus.

HUMAN INFECTIONS

In France, the first clinical case of hantavirus infection was reported in 1977, in the forest of the Ardennes, near the Belgian border (Cousin, 1979). Nevertheless, the epidemics of "trench nephritis" described during world war one (Atenstaedt, 2006) were probably caused by hantavirus.

The national reference laboratory for hemorrhagic fevers (CNR) is in charge of hantavirus infection surveillance in France. The serological diagnosis relies on indirect immunofluorescence since 1982. In 1993, a confirmation with the research of specific IgM antibodies using an enzyme-linked immunosorbent assay (ELISA) has been added (Le Guenno et al., 1994). When a positive or a doubtful serological diagnosis is made by another laboratory, which is possible since 2002, a confirmation is systematically performed by the CNR, making the centralization of the results and the surveillance possible. In case of a positive laboratory result, a questionnaire is sent to the clinician of the patient in order to identify the risk factors. Later the Institut de Veille Sanitaire (IVS) analyses these data and provides public health recommendations.

The annual number of hantavirus infections reported in France from 1987 to July 2010 is shown in **Figure 8**. If 60 cases in average are diagnosed every year, with an increase during the last decade, the incidence varies greatly and epidemic years (in red in **Figure 8**) occur every 2–4 years. The increased number of cases reported in epidemic years is not a consequence of a better physician awareness leading to a greater number of analyses. In **Figure 9**, the curve shows that the proportion of positive results among all the analyses is not diminished during epidemic years. Neither, the improvement in the diagnostic tools can explain the increase in the reported cases.

As PUUV, the most prevalent hantavirus in France, is responsible for a mild form of HFRS, the number of human infections could be under-estimated. Surveys of seroprevalence in endemic areas show that 0.45 of the population has anti-Puumala antibodies. Among people working in the countryside, the overall seroprevalence rate was 1% and even reaching 5% in some areas (Le Guenno, 1997). Most human infections occur during late spring and summer (Sauvage et al., 2002; Vapalahti et al., 2003). For the period 2001–2009, June was the month with the maximum of cases reported, followed by July and May (**Figure 10**).

Most of the cases are diagnosed in the endemic area (**Figure 7**) but sporadic cases are reported elsewhere (**Figure 7**). An infection has been proven using RT-PCR on a Belgian citizen who had been camping near Perpignan, in the Pyrenean mountains, in southern France. After investigations, this patient had no other risk factors than this stay in France (Keyaerts et al., 2004). In the area of Paris, a

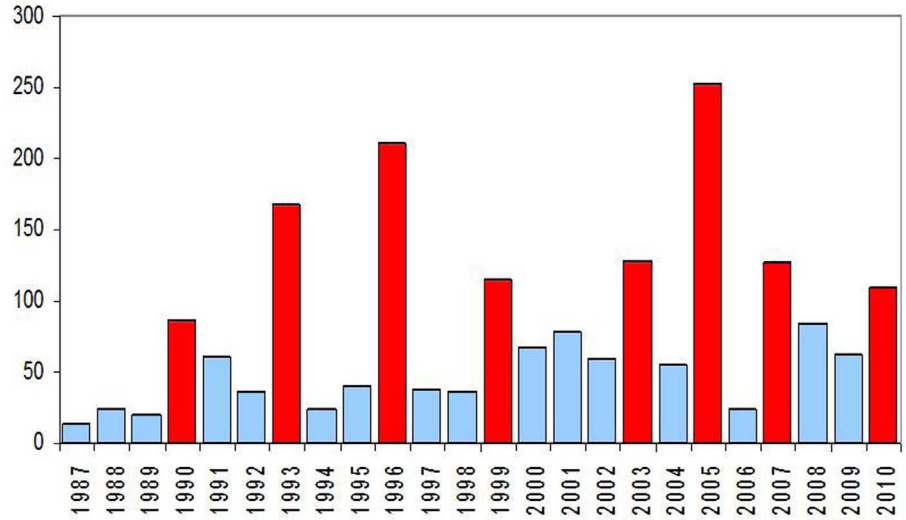

FIGURE 8 | Number of hantavirus infections in France during the period 1987 – July 2010 (data: Institut de Veille Sanitaire, IVS).

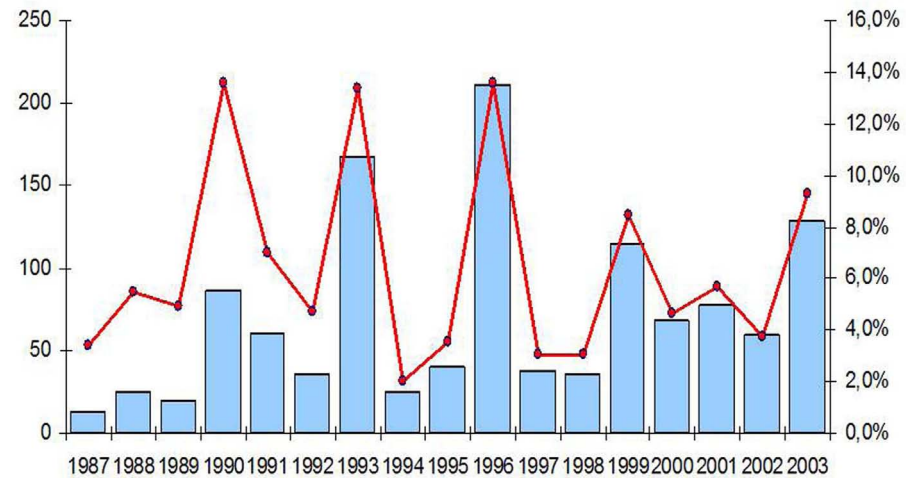

FIGURE 9 | Number (graph in blue) and proportion of positive results (curve in red) of hantavirus infections in France, period 1987–2003 (data: Institut de Veille Sanitaire, IVS).

retrospective study identified 14 cases in 1999 and 2000 (Lautrette et al., 2003).

As previously seen, the risk for human infection is correlated with the prevalence of PUUV in the populations of *M. glareolus* (Salvador et al., 2011). In the Ardennes, the prevalence was 39% in the epidemic year of 2003 while it was 26.5% in 2002 and 29.9% in 2001. In addition, the number of *M. glareolus* trapped was between twice and three times more in 2003 when compared with 2002 and 2001 (Mailles et al., 2005a). As for the contamination within rodent populations, the amount of virus in the environment, linked with the number of recently infected rodents, is critical.

The presence of infected rodents is not the only condition leading to human infections. In an epidemiological survey in the department of the Ardennes, high seroprevalence rates were found in *M. glareolus*, in sites where no human cases were reported (Augot et al., 2008).

Transmission to humans occurs mainly indirectly by inhalation of virus-contaminated aerosols from excreta of infected rodents (McCaughey and Hart, 2000). Human behavior must make such a contact possible. In a Franco-Belgian case-control study, the main risk factors identified were a significant exposure in forests, entry in buildings where rodents are present and living less than 50 m from a forest (Crowcroft et al., 1999).

EPIDEMIOLOGY OF HANTAVIRUSES IN GERMANY

BRIEF HISTORY ON HANTAVIRUS INFECTIONS IN GERMANY

In Germany human hantavirus infections are characterized by a mild HFRS and were first published in 1985 (Zeier et al.,

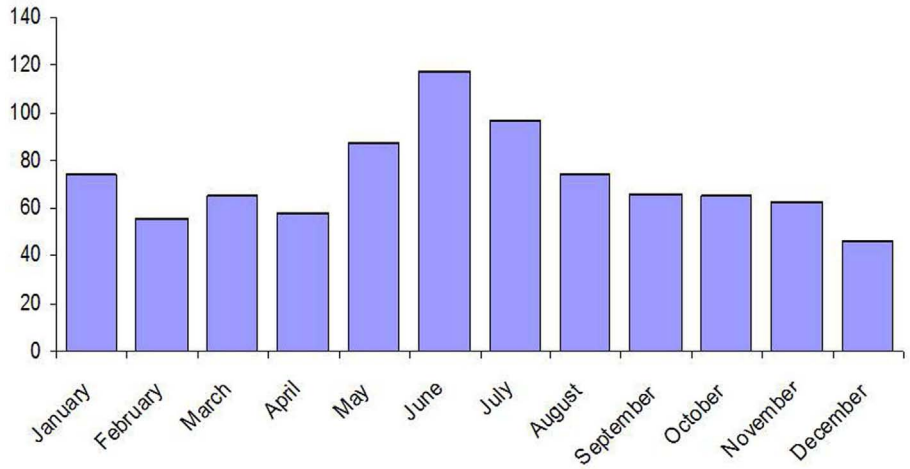

FIGURE 10 | Distribution per month of the hantavirus cases in France during the period 2001–2009 (data: Institut de Veille Sanitaire, IVS).

1986) followed by sporadic descriptions in the early nineties of the last century (Gärtner et al., 1988; Zeier et al., 1990; Mettang et al., 1991; Pilaski et al., 1991, 1994). An accumulation of hantavirus infections speculating to local hantavirus outbreaks were already postulated for 1983, 1990, and 1993 in different regions in Germany, e.g., in the Swabian Alb, Baden-Württemberg, including also sites for military maneuvers around Ulm (Clement et al., 1996). These human cases were defined by clinical signs of the disease and/or serological evidence for hantavirus antibodies. However, detailed knowledge on the etiological agent such as virus isolates or sequence data on German hantaviruses were not available (Kulzer et al., 1992, 1993; Clement et al., 1994). It still took 10 years until hantaviruses were characterized on the molecular level in Germany, i.e., the PUUV strain Berkel from an HFRS-patient (Pilaski et al., 1994) and strain Erft from a bank vole collected ∼70 km away from the area in North-Rhine Westphalia where this patient was infected (Heiske et al., 1999). Finally, in the year 2001 in Germany clinically apparent hantavirus infections became notifiable to official health authorities due to the German Infection Protection Act (Infektionsschutzgesetz, IfSG). By definition, a combination of any of the following symptoms is indicative for HFRS and considered as a case-definition according to the Robert-Koch-Institute (RKI): acute onset of disease with temperatures >38.5°C, back- and/or abdominal pains, headache, proteinuria, and/or hematuria, elevation of serum creatinine levels, thrombocytopenia, and oliguria or polyuria, respectively. The suspected disease is confirmed either serologically or directly through molecular-biological methods. However, the infectious agent can only be detected within the first few days of the disease during the viremic phase using RT-PCR. Thus, a negative RT-PCR result does not necessarily exclude an infection with hantavirus[5] (accessed January 9, 2012).

[5]www. rki.de

2001–2011: CHANGES IN THE HANTAVIRUS EPIDEMIOLOGY IN GERMANY?

Since the beginning of the mandatory notification, approximately 200 HFRS cases are counted in non-epidemic years with an average incidence of 0.25 per 100,000 inhabitants. Local outbreaks have been reported in 2004 in the federal state of Bavaria (Essbauer et al., 2006; Schilling et al., 2007) and in 2005 (Mailles et al., 2005b; Heyman et al., 2007) affecting regions in Lower Saxony (Ulrich et al., 2008), North-Rhine Westphalia (Essbauer et al., 2007), and Bavaria (Mertens et al., 2009, 2011c). In 2007 a large outbreak occurred with 1,688 registered cases affecting the federal states Baden-Württemberg, Bavaria, Lower Saxony, and North-Rhine Westphalia (Heyman et al., 2007; Koch et al., 2007; Hofmann et al., 2008; RKI, 2012). Further, in the year 2010 a record number of 2,017 cases was reached with outbreaks in the same federal states as in 2007 including also parts of Hesse (Faber et al., 2010; Heyman et al., 2011; Ettinger et al., 2012; RKI, 2012). The summary of annual cases is shown in **Figure 11**. As described for France there exist some hot spot regions in Germany, i.e., several parts of Baden-Württemberg including the Swabian Alb, in Bavaria the Main-Spessart region and Lower Bavaria, in Lower Saxony the administrative district of Osnabrück (see **Figure 12**).

Changes in the epidemiology of clinically apparent hantavirus infections in Germany therefore are characterized by a tremendous rise of cases in years 2005, and most notably in 2007 and 2010 but also by an appearance in so far unrecognized regions or in new urban areas. As already discussed in general a changed risk for hantavirus infections for humans may also be inferred from a change in behavior as the working population tends to shift recreational activities to natural settings. In 2005 an unusual outbreak in the cities of Cologne, Aachen, and Osnabrück were recognized (Abu Sin et al., 2007; Essbauer et al., 2007). In Cologne human cases were found close to a wooded city recreation area, an adjacent tennis park, and football stadium. In the forest city park, PUUV was found in high prevalence in the bank vole population and therefore some risk for human infections persists in this area

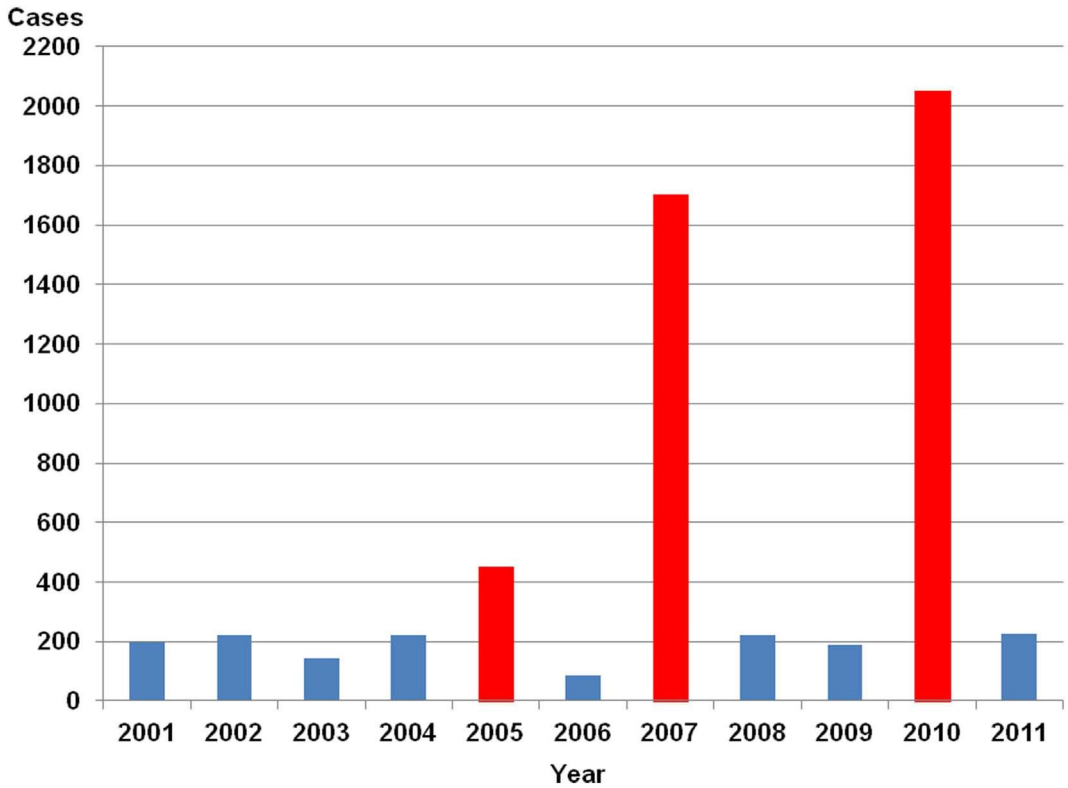

FIGURE 11 | Yearly number of hantavirus cases in Germany. Source of data is the SurvStat of the Robert Koch-Institut Data available from http://www3.rki.de/SurvStat, data as of January 25th, 2012, RKI (2012).

(Essbauer et al., 2007; Ulrich et al., 2008). In 2010 in the urban district of Stuttgart even 166 cases were registered (RKI, 2012).

What do we know about seasonal patterns of hantavirus infections in Middle Europe? In general, Germany and other western European countries usually (2001, 2002, 2004, 2005, 2007, 2010) see a summer peak in human PUUV cases. **Figure 13** shows the seasonal oscillations of hantavirus infections in Belgium and Germany illustrating the high variability of seasonal peaks for these two neighboring countries. This is in contrast to Fennoscandia (Finland and Scandinavia) where autumn and winter peaks occur (Piechotowski et al., 2008; Evander and Ahlm, 2009). However in Germany in 2006 (very few cases), 2008, and 2009 winter peaks were obtained. Further, in the years 2003 (roughly, very few cases) and 2011 winter and summer peak were observed. In 2010 health authorities were aware of the steep rise of human infections in the winter months and early spring in the year and it was hypothesized that bank voles might move closer to human housings in winters with extreme cold conditions (Faber et al., 2010). In summary, these data indicate a quite complex seasonal pattern of clinically apparent hantavirus infections for Germany that also might be regionally variable (see **Figure 14**).

HANTAVIRUS SPECIES AND RODENT HOSTS

The picture of hantavirus species present in Germany seems to be more complex in comparison to Belgium and France or also Scandinavian countries. As indicated until the beginning of the century only few data on circulating strains were available (Pilaski et al., 1994; Heiske et al., 1999). Several species have been reported so far, yet PUUV found in human patients and bank vole populations in South and West Germany is the predominant virus. Since 2004 intense studies on hantaviruses in their natural reservoir the small mammals have been started in outbreak regions of human infections. Comparable to the French data shown in **Table 1**, in Germany PUUV-prevalences in affected rodent populations might be quite high reaching 30 up to 60% prevalence in outbreaks (Essbauer et al., 2006; Ulrich et al., 2008; Mertens et al., 2011a). Presently, at least eight geographic and phylogenetic distinct clusters predominate in Germany (Essbauer et al., 2007; Hofmann et al., 2008; Ulrich et al., 2008; Mertens et al., 2011c; Ettinger et al., 2012). Meanwhile, in order to understand the oscillation of human cases intense longitudinal investigations of rodents in several areas have been initiated (Ulrich et al., 2008; Mertens et al., 2011c). Secondly, DOBV is present in Germany (Meisel et al., 1998, 2006; Klempa et al., 2004; Schlegel et al., 2009). Since 2001 approximately 30 human infections with DOBV were diagnosed in Northeast Germany (RKI, 2012). The usually *Apodemus agrarius*-associated strain DOBV-Aa ("Saarema") was found in *A. flavicollis* in the Federal States of Mecklenburg-Western Pomerania, Lower Saxony, Brandenburg, and also in Schleswig-Holstein (Schlegel et al., 2009; RKI, 2012).

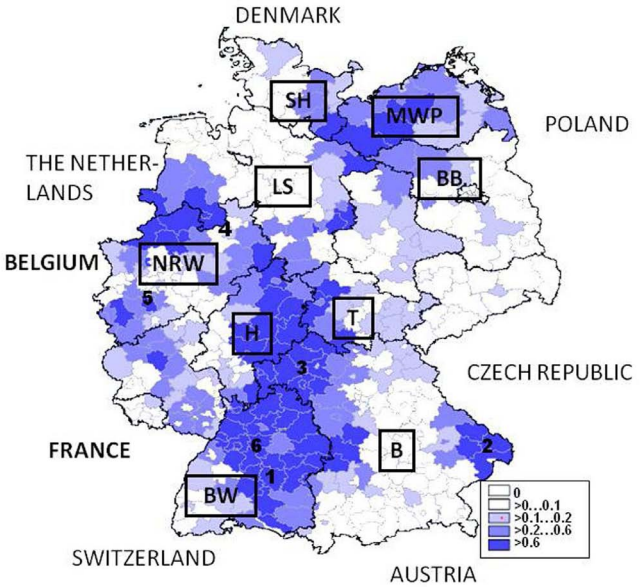

FIGURE 12 | Germany: map with hantavirus cases/100.000 inhabitants January 1st 2001–January 1st 2012. Some hot spot regions for PUUV infections are numbered: 1 –Swabian Alb, 2- Lower Bavaria, 3-Main-Spessart, 4-Osnabrück, 5-Cologne, 6-Stuttgart. Source of data is the SurvStat of the Robert Koch-Institut Data available from http://www3.rki.de/SurvStat, data as of January 25th, 2012, RKI (2012). MWP, Mecklenburg-Western Pomerania; BB, Brandenburg; LS, Lower Saxony; SH, Schleswig-Holstein; NRW, North-Rhine Westphalia; H, Hesse; T, Thuringia; BW, Baden-Wuerttemberg; B, Bavaria.

Thirdly, mild human TULV infections were discussed but the definite role of this virus for human infections is quite unclear (Schultze et al., 2002; Klempa et al., 2003). As already mentioned for France multiple vole hosts such as *Microtus agrestis*, *M. arvalis*, and *Arvicola amphibius* were shown to be reservoirs (Schmidt-Chanasit et al., 2010; Schlegel et al., 2012). TULV was found to be present in several geographic regions in Germany, i.e., Baden-Württemberg, Bavaria, Lower Saxony, and Brandenburg (Mertens et al., 2011b). Its evolution seems to be not host-related but several different genetic geographic clusters were shown (Schmidt-Chanasit et al., 2010; Schlegel et al., 2012). Concisely, intense studies on hantaviruses in insectivores have been initiated without knowing the impact of these viruses on human health (Schlegel and Ulrich, personal communication).

RISK GROUPS AND FACTORS FOR HUMAN POPULATION

The data as hitherto describe clinically apparent hantavirus infections in humans and some aspects of viruses in rodents. However, the officially reported human cases only present the tip of an iceberg as many infections progress with unspecific symptoms and therefore might not be detected. Seroprevalence studies in the human population and different risk groups were started in the early 1980s in order to understand the "true" impact of hantavirus infections on humans. During these studies serological diagnostics started with classical immune fluorescence assays and were in recent years improved by implementing ELISAs and Western Blots with homologous antigens (Zöller et al., 1993, 1995; Razanskiene et al., 2004; Meisel et al., 2006; Mertens et al., 2009, 2011b). The first detailed seroepidemiological investigation was performed on 13,358 human sera (Zöller et al., 1995), and followed by further studies that showed a general seroprevalence in Germany of approximately 1–2% with higher prevalences (2%) found in South-West Germany (Martens, 2000; Kimmig et al., 2001; Mertens et al., 2009). In endemic regions the hantavirus seroprevalence may locally be quite variable and reach up to 5–10% as it was, e.g., shown in Baden-Württemberg (Zöller et al., 1995; Kimmig et al., 2001) and some communities in Lower Bavaria (Mertens et al., 2009, 2011c; Essbauer, personal communication). Due to their occupational exposure to small mammals and their excreta, several professions are at a higher risk for hantavirus infections, i.e., soldiers (Antoniadis et al., 1985; Clement et al., 1996; Mertens et al., 2009), construction workers (Abu Sin et al., 2007), muskrat hunters (Zöller et al., 1995), workers at horse breeding farms (Zöller et al., 1995), farmers (Rieger et al., 2005), lumberjacks, and woodsman (Zöller et al., 1995; Rieger et al., 2005; Mertens et al., 2011b). The most recent seroepidemiological study in Brandenburg showed that forest workers there have higher TULV and DOBV seroprevalences concluding that both virus types might be predominant and possibly underdiagnosed in this region (Mertens et al., 2011b).

In order to define further risk factors for the human populations as in Belgium and France some further epidemiological studies were performed in Germany. After the 2005 outbreak two case-control studies were initiated. In the area of Osnabruck for 30 eligible hantavirus patients registered between August 2004 and 2005 and a control-group of 43 persons it was shown that staying or living close to a forest, leisure activities in a forest such as camping, jogging, going on a mushroom foray are significant risk factors (Siffczyk et al., 2006). In comparison, a German-wide case-control study was performed between May and August 2005 with 215 appropriate patients and 150 matched controls participating. Living close to forested areas, and noticing mice, but not leisure activities in a forest was found to be a risk for human PUUV infections (Abu Sin et al., 2007). A further study after the 2007 epidemics showed with 191 matched case-control pairs (April to June 2007) in Baden-Württemberg that participants were more likely to have visited a forest and to have seen small rodents or their droppings. Besides, cleaning utility rooms such as sheds, attics, cellars, and garages, or visiting forest shelters in order to make a barbecue or to shelter from the rain were additional risk factors (Winter et al., 2009). In summary, risk factors for Belgium, France, and Germany are defined by contact to nature and rodents but might to some degree depend on the study sites, the annual and local epidemic situation, and the questions asked in the survey.

FACTORS DRIVING HANTAVIRUSES (OR NOT) IN GERMANY

So far the epidemiology and some risk settings for humans acquiring a hantavirus infection have been summarized for Germany. However, investigations of factors, which drive human hantavirus infections' oscillations such as the prevalences of the virus in

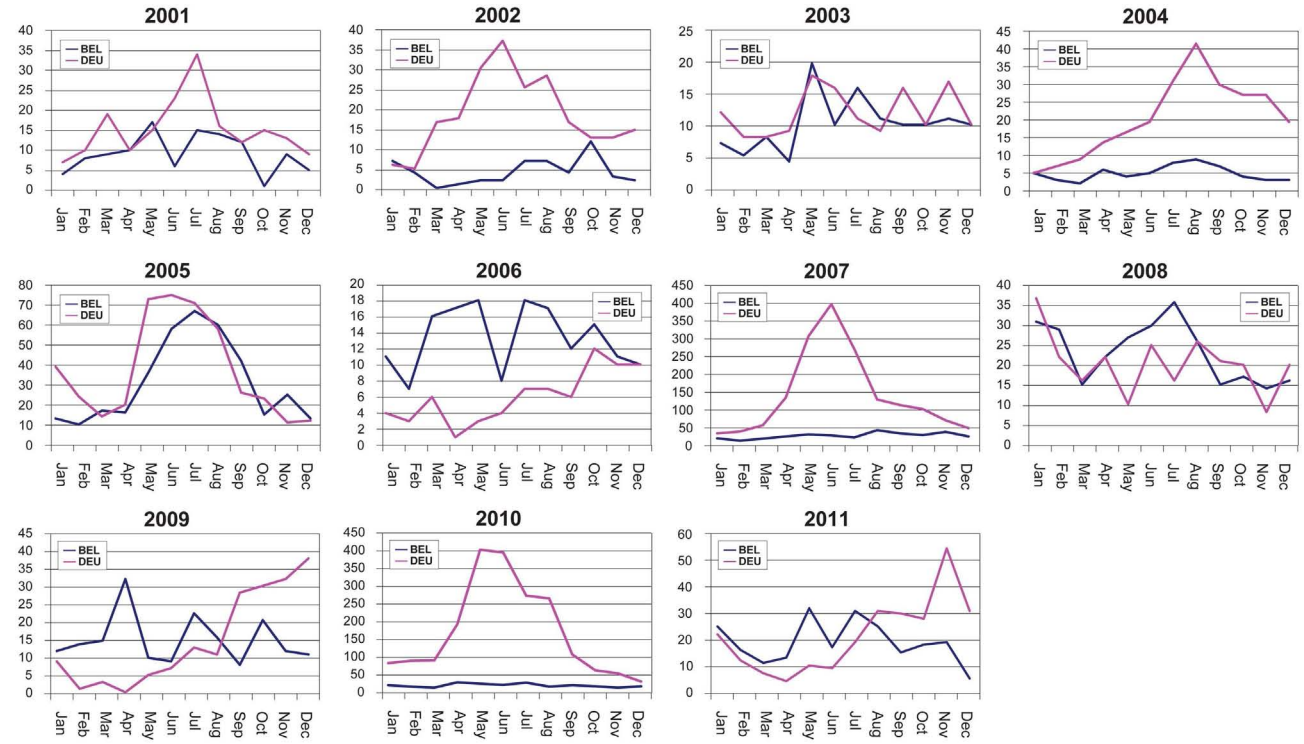

FIGURE 13 | Monthly oscillations of clinically apparent hantavirus cases in Belgium and Germany, 2001–2011.

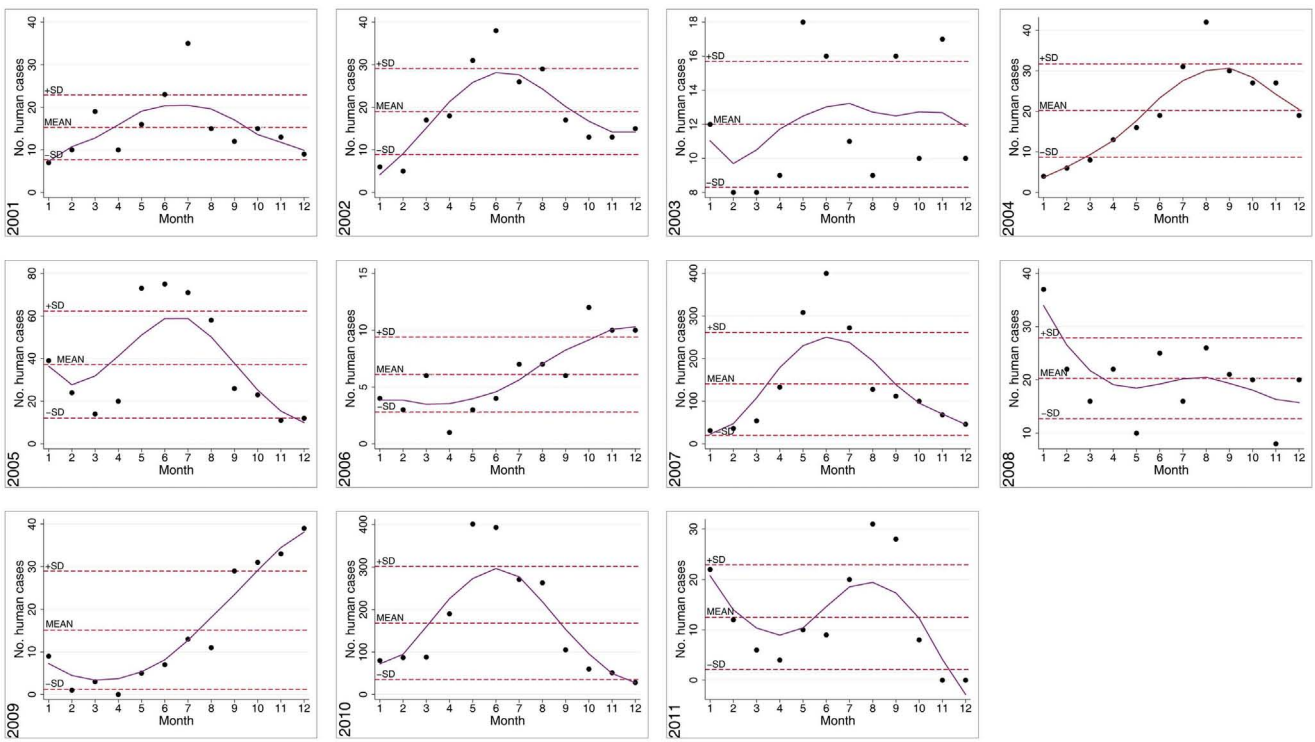

FIGURE 14 | Seasonal/Monthly oscillations of clinically apparent hantaviruses in Germany, 2001–2011. Notified human PUUV cases by year were charted to month of notification (1 = January, 2 = February, etc.). Mean monthly cases per year ± SD and yearly smoothed trend lines are shown.

rodent populations, the environmental, faunistic, floristic, weather (climatic), and other parameters are still at an early stage. This also includes factors that might have a direct impact on the virus stability in the environment. In general, Germany consists of a broad variety of landscapes formed by former glaciers. In the North these include the Islands of the North- and Baltic Sea, the North German Plain. In Central Germany uplands and numerous river valleys, e.g., Rhine are present. Southern Germany is characterized by several linear hills and high mountain ranges such as Swabian and Franconian Alb, Bohemian Forest, and the Alps. There are three main biogeographic regions, the Atlantic, the continental-middle European, and the alpine (Glaser et al., 2007). Hence, there exist a broad variety of habitats and biotopes for rodents and makes the situation for these animals and therefore associated diseases quite complex. In 2004 a network on rodent-associated pathogens with an intense focus on hantaviruses was established in order to bring scientist with different expertise together to investigate aforesaid issues (Ulrich et al., 2008). Several studies are presently performed, e.g., financed by the Robert-Koch-Institute, the Federal German Environmental Agency, the Bavarian State Ministry of Environment and Public Health[6], or in parts by the EEC (EDENnext[7]). Details of this network and actual projects have been actually summarized recently by (Rosenfeld, personal communication).

In the following subchapter actual data on factors driving hantaviruses in Germany available from the literature are reviewed. In part, so far unpublished results from a 3-year-study in the German hantavirus hot spot region Bavarian Forest National Park in Lower Bavaria are presented (see marked in **Figure 12**). Here from 2008 to 2010 small mammals and their associated pathogens (e.g., PUUV and *Rickettsiae*; Schex et al., 2011; Silaghi et al., 2012) were analyzed along an altitude gradient together with environmental, i.e., vegetation and climatic data generated in the BIOKLIM project (Biodiversity and Climate Change Project; Bässler et al., 2009). In brief, data from 661 small mammals including 275 bank voles trapped at 23 sites were used in order to analyze if rodent-associated factors, i.e., biometric, diversity, environmental, and climatic or weather conditions influence the prevalence of PUUV in lower Bavaria in order to reflect the risk of infections for the human population (Thoma, personal communication).

BIODIVERSITY

As mentioned earlier in this review biodiversity may influence host-pathogen interactions and thus the prevalence of infectious agents leading to the emergence/re-emergence of diseases. However, the role of biodiversity on pathogen pattern changes is actually controversially and extensively discussed (Maillard and Gonzalez, 2006; Aguirre and Tabor, 2008; Maillard and Sparagano, 2008). While the hypothesis that high species diversity decreases pathogen prevalence through mechanisms such as decreased host density, reduced encounters between hosts, alterations in host behavior or reduced host survival ("Dilution Effect") commonly exists another theory is postulated ("Amplification" or "Rescue Effect") where increased pathogen prevalence is associated with greater species diversity through increased encounters between hosts, or through the presence of secondary hosts (Keesing et al., 2006). The "Dilution Effect" has been demonstrated for pathogens other than hantaviruses, i.e., for Leptospirae it was shown that a reduced mammal species biodiversity increased its incidence (Derne et al., 2011). Similar observations have been made for other rodent-borne viruses (Mills, 2006) including also several New World hantaviruses. In example, for Sin Nombre, Choclo, and Laguna Negra virus a negative relationship between species diversity and hantavirus prevalence has been emphasized (Yahnke et al., 2001; Ruedas et al., 2004; Dizney and Ruedas, 2009). In Belgium the PUUV prevalence in *M. glareolus* was negatively affected by the relative proportion of the non-host *A. sylvaticus* (Tersago et al., 2008). Further, for Sin Nombre Virus there is also experimental evidence for the "Dilution Effect," which has been demonstrated through manipulation of small mammal biodiversity in wild reservoir populations in Panama (Suzán et al., 2009). In contrast to the above studies, for PUUV in Lower Bavaria, where *M. glareolus*, *A. flavicollis*, *A. sylvaticus*, *M. agrestis*, and *Sorex* spp. are predominant, the association of reduced rodent diversity and higher prevalence of PUUV could not be confirmed (Thoma, personal communication). Here, increased species diversity was correlated with higher PUUV estimates in the host rodent, which is in line with the "Amplification Effect." With regard to this phenomenon several hypotheses may be formulated: (i) species other than the primary host could have the ability of functioning as a further reservoir for PUUV and thus lead to an increased transmission of the virus and overall prevalence; (ii) the presence of a species with a low reservoir capability could lead to higher contact rates between the primary hosts and subsequent higher transmission of the virus (Clay et al., 2009). Nevertheless, whereas biodiversity may play a key role in pathogen transmission further factors which drive regional differences must exist.

LANDSCAPE AND HABITAT FACTORS

Landscape attributes might have a beneficiary effect on rodent host population dynamics. There exists excellent data on the influence of topography and soil properties on dynamics of common voles from almost 40 years collected in eastern Germany. The analyses showed for example that mean elevation, area-related percentage of Chernozem soils, and soil air capacity are variables that have an influence on the common vole outbreak risks at different sites (Blank et al., 2011). The link to forest environments has been discussed in earlier chapters. More precisely, deadwood and denser ground vegetation, which are found both in forest reserves and managed forests to varying degrees, may serve as a microhabitat and deadwood is used by many species as a food resource, breeding habitat, and for shelter. Further, species richness correlates high with deadwood volume (Siitonen, 2001; Maguire, 2002). Deadwood thus represents an important feature and may therefore relate to higher bank vole population densities, which may increase the overall hantavirus prevalence in the rodent host, and provoke hantavirus epidemics (Rooney and Hayden, 2002). Analogous to deadwood, dense ground vegetation may also serve as an ideal habitat for bank voles and has

[6]http://www.lgl.bayern.de/das_lgl/forschung/forschung_interdisziplinaer/fp_vicci_project5_en.htm
[7]www.eden-fp6project.net

been associated with PUUV foci in other countries (Escutenaire et al., 2002; Tersago et al., 2008). In Lower Bavaria, increasing percent coverage of deadwood and dense ground vegetation significantly increases the probability of PUUV prevalence in *M. glareolus* (Thoma, personal communication). In conclusion, despite the important aspects of deadwood for a healthy ecosystem and the tendency to launch such traditional and untouched forests enhanced deadwood may be one factor to increase a risk for PUUV infections.

MAST

Mast already was defined in detail and critically discussed above in the overview on PUUV in Belgium. As described in detail for Belgium criteria for mast may be very versatile. The comparison of the seasonality of human hantavirus infections in Belgium and Germany (**Figure 13**) also demonstrates that oscillations in these two countries are quite variable and therefore a general conclusion on mast, rodent populations, and hantavirus infections should be avoided. In Germany an annual report on the conditions of German forests ("Waldzustandsbericht") is published from the Federal Governmental Ministry of Consumer Protection, Food, and Agriculture (e.g., for 2007[8]). The report includes an overview of the status and also damages on different trees but does not include concrete data on mast. Additionally, in Germany certain officers responsible for forest and agriculture from each federal state meet once a year in order to exchange data on mast, rodent damages, and actual prognostic trappings. Despite the importance of this work in recent years, manpower, temporal, and financial possibilities have been reduced. In general, differences on results of mast of different trees, rodent populations, and also at the investigated sites are tremendous and therefore cannot be compared with the conditions in Scandinavia. The results of these meetings are embedded in the network rodent-borne diseases in order to combine the different expertise and to communicate the data also to health offices (Ulrich et al., 2008).

As already mentioned in the chapters above, in principal food availability (as, e.g., beechnut mast) has an influence on population dynamics of bank voles. In contrast to other rodent species (e.g., *M. agrestis* and *M. arvalis*) bank voles may be more dependent on tree seeds such as beechnut. Data from Lower Saxony from 16 years on beech fructification and the abundance of bank voles showed that the fruits have a positive effect on the population in the next year. For negative trends the context between fruit and vole was true in 75% of the cases (Kühn et al., 2011). As beech forest might be beneficial habitats for this species and populations may reach high densities some investigations were performed in Baden-Württemberg. There highest beech forest cover was found in the Swabian Alb (17.7%) and lowest cover in the eastern and middle regions and major cities of Baden-Wuerttemberg (1.6%). Data on annual mast production of beechnuts was obtained between 2000 and 2006. Cumulative incidence (2001–2007) of PUUV infections in Baden-Wuerttemberg is reported highest in the Swabian Alp region. Highest maximum incidence rates for human PUUV cases ranged from 2.28 per 100,000 population in 2007 to 1.16 per 100,000 population in 2006, which is considered a year with beech crop failure (Schwarz et al., 2009). For the year 2010 a growth of the bank vole rodent population could be expected as at two study sites in Swabian Alb and around Münster there was an enhanced beechnut mast in 2009 (Jacob and Ulrich, personal communication). However, the 2010 PUUV outbreak in Germany again showed significant local differences. In conclusion, there exists no enough valid, longitudinal collected and critically summarized data on mast, rodent populations, and associated diseases in Germany as it was also discussed for Belgium and France.

CLIMATE

Germany is characterized by a warm temperate humid midlatitude climate with predominating westerly winds carrying humid air masses from the Atlantic Ocean. This influence weakens from the northwest to the southeast. In the northwest and the north the climate is extremely oceanic and rain falls all the year round. Winters there are relatively mild and summers comparatively cool. In the east the climate shows clear continental features; winters can be very cold for long periods, and summers can become very warm. Dry periods are often recorded. Special effects occur in the eastern parts of South Germany especially bringing summery weather conditions during the winter half year, occasionally[9] (accessed January 4, 2012). Only few analyses on the influence of weather data on rodent populations and rodent-transmitted agents are available in Germany.

Mild winters and springs may positively influence rodent survival rates and early food supply and thus affect rodent population dynamics. The density of the rodent population may well drive the distribution and prevalence of disease/associated agents in the rodent host. For *M. arvalis* populations in the Eastern Federal States of Germany the impact of weather parameters in winter and early spring was revealed to be of some importance for outbreaks in the autumn. Data was collected over almost four decades but also indicated that there may exist regional differences of the impact of weather and that winter and early spring records only may manipulate extremely high and low outbreak risks (Imholt et al., 2011). These results again indicate the complexity of the system and that not only one group of parameters might stimulate rodent populations and therefore associated pathogens. Besides for PUUV carried by bank voles some efforts have been started in order to understand the impact of weather conditions or climate on the oscillations in Germany. Data from 2001 to 2007 from Baden-Wuerttemberg showed that in 2001, 2003, 2004, and 2007 winter temperatures were above long-term averages and in 2001, 2002, 2003, 2004, and 2007 spring temperatures exceeded long-term averages. The maximum winter temperature deviation was recorded in 2007 when reported human hantavirus cases in Germany rose to over 1,500 cases. In marked contrast, in 2006 winter temperatures were lowest as were cases of NE reported in Baden-Wuerttemberg ($n = 17$). Interestingly, as compared to other years between 2001 and 2007, in 2005 there also seemed to be a beech crop failure (Piechotowski et al., 2008; Schwarz et al., 2009). Human hantavirus prevalences may further be driven by the fact

[8] http://www.bmelv.de/cae/servlet/contentblob/383616/publicationFile/22185/ErgebnisseWaldzustandserhebung2007.pdf

[9] www.dwd.de

that human contact with rodent host abundance is increased when humans shift their recreational activities to bank voles' environments during mild weather conditions, preferably in early summer (Schwarz et al., 2009). Fluctuations in common vole population densities and thus prevalence of hantavirus in the rodent host may also be correlated with December snowfall, January sunshine duration, and snow fall in April (Ulrich et al., 2008). Interestingly, as opposed to findings in Baden-Wuerttemberg in Lower Bavaria no significant link between winter and spring temperatures and elevated hantavirus prevalences could be corroborated, which again highlights the complex nature of hantavirus disease transmission (Thoma, personal communication).

As described earlier the viability of a hantavirus outside the host may be dependent on climatic factors such as temperature and moisture. Yet, additional factors such as UV radiation may also have an impact on virus tenacity. In experimental studies for appropriate inactivation of Hantaan virus the virus was effectively inactivated through UV irradiation in cell cultures and supernatants (Kraus et al., 2005). Photochemical inactivation of alpha- and poxviruses was demonstrated in another study. Effective inactivation of the viruses was dependent on the type of photochemical used (e.g., 5-iodonaphthyl 1 azide or amotosalen) in combination with UVA (Sagripanti et al., 2011). In the environment, it could be postulated therefore that high values of solar radiation may have an influence on the stability or occurrence of PUUV. In Germany, in the Bavarian Forest National Park in Lower Bavaria, it was shown that after adjusting for confounding factors such as elevation high values of mean annual solar radiation were associated with a decreasing PUUV prevalence in bank voles (Thoma, personal communication). This finding has yet to be shown in other hantavirus hot spots in Germany.

RODENT SPECIFIC FACTORS

Age

There is good evidence that in the wild PUUV-prevalences in bank voles are age-dependent (Bernshtein et al., 1999; Escutenaire et al., 2002). The age of bank voles is commonly estimated using bodyweight as a proxy as the basis for weight class categories, e.g., juvenile, subadult, and adult (Escutenaire et al., 2002; Kallio et al., 2007). Another method applied for age determination of voles is by assessing the development of the molars (Bernshtein et al., 1999; Olsson et al., 2002). As described for France, age-dependency of virus prevalence in the rodents may be indicative for a horizontal transmission of PUUV in the rodent host (Tersago et al., 2011b). An explanation for this might be that adult rodents will have been exposed to viruses longer than young ones and on more occasions. Another theory discusses that older male rodents may suffer from immunosuppression due to high levels of stress hormones, which are associated with breeding, making them more vulnerable to infections (Deter et al., 2008a). That sexually mature rodents may be at higher risk for infection due to fighting rituals, mating, and generally close social behavior was already mentioned (Escutenaire et al., 2002). Moreover, juvenile rodents will have benefited from maternal antibodies during the first 3 months of their live and therefore less often been infected during suckling (Kallio et al., 2006b, 2010). Findings in Lower Bavaria affirm these observations. In the large part older animals are infected with PUUV. The odds of the tested bank voles being PUUV seropositive constantly increased with each unit increase of the rodents' bodyweights (Thoma, personal communication). By implication, given the horizontal transmission and as indicated by the respective chapters in this paper climatic and environmental factors may have a benefit on the survival and aging of rodent populations and on population density. Together, increasing rodent population densities may lead to increased rodent contacts, which may foster the transmissibility of the virus, resulting in higher prevalences, and eventually driving and influencing hantavirus epidemics.

Male bank voles are believed to be of greater risk of acquiring and spreading PUUV and thus play an important role in the dynamics of PUUV epidemics (Bernshtein et al., 1999; Olsson et al., 2002; Deter et al., 2008a). *R. norvegicus* and *Calomys musculinus* show a similar pattern regarding transmission of SEOV and Junin virus, respectively (Mills and Childs, 1998). Especially in the reproductive season male animals are very mobile and have close contacts with other members of their species. The effects of aggression and wounding due to sexual activity of male animals during mating season on the dissemination of disease have been discussed, which may be considered as a driving force for hantavirus epidemics (Bernshtein et al., 1999; Olsson et al., 2002; Deter et al., 2008a; Tersago et al., 2011b). In contrast to this observation, in rodents investigated in the Bavarian Forest National Park in Lower Bavaria the PUUV prevalence levels in males were not significantly higher than in females (Bernshtein et al., 1999; Olsson et al., 2002; Deter et al., 2008a). These findings suggest that there is at least in that region no gender specific affinity to the virus. However, local genetic differences in the bank vole population in this region may produce subpopulations, which render differently and gender-independent with regard to susceptibility to the virus. Various patterns of PUUV infection and resistance (tolerance) depending on different genetic variants of the TNF-α locus were for example discussed for bank voles in France and Germany (Guivier et al., 2010). With regard to other hot spot regions in Germany no reliable data on gender exist and has yet to be shown in further longitudinal studies. Again, these findings highlight the complex nature of driving factors for hantavirus transmission and host ecology.

CONCLUSION

Our findings indicate that the occurrence of clinically apparent human hantavirus infections in Belgium, France, and Germany are highly variable in time and space. Comparing the epidemiological features in the three forenamed countries also indicated that hantavirus outbreaks in our three countries are also not always triggered by the same factors.

We defined six major groups that could act as triggers or regulators of hantavirus outbreaks in Western Europe; behavioral factors of reservoir and host, landscape and changes in land-use, climate, food availability, biodiversity, and physiological features of the reservoir population. Care should however be taken not to generalize the relationships as some have already been proven wrong in the past (Brown and Ernest, 2002).

The ever-present pitfall however, is relating the occurrence of infectious diseases in general and hantavirus infections in particular to one particular trigger, e.g., food availability. Climate

change, for instance, is a likely facilitator but climate change is an ancient and ongoing process (Ruddiman, 2003) that we experience through a narrow window in time. The environment seems a vital intermediary factor when it concerns pathogen transmission between carrier and host; which points to the possibility that the transmission rate is not only determined by carrier abundance or pathogen prevalence, but also by rodent ecology, virus survival, local climatic conditions, and human behavior (Lambin et al., 2010). We have for most of the regulating factors – unfortunately – hardly an idea where we come from and where we are going to in this matter. Relationships between abiotic and biotic parameters, rodents, humans, and hantavirus infections are first and foremost highly complex and highly intertwined but – should we be able to evaluate them accurately – can be valuable for predicting hantavirus epidemics.

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Malaria in East African highlands during the past 30 years: impact of environmental changes

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East African highlands are one of the most populated regions in Africa. The population densities in the highlands ranged between 158 persons/km^2 in Ethiopia and 410 persons/km^2 in Rwanda. According to the United Nations Population Fund, the region has the world's highest population growth rate. These factors are likely behind the high rates of poverty among the populations. As there were no employment opportunities other than agricultural, this demographic pressure of poor populations have included in an extensive unprecedented land use and land cover changes such as modification of bushland, woodland, and grassland on hillsides to farmland and transformation of papyrus swamps in valley bottoms to dairy pastures and cropland and changing of fallows on hillsides from short or seasonal to longer or perennial. Areas harvested for food crops were therefore increased by more than 100% in most of the highlands. The lost of forest areas, mainly due to subsistence agriculture, between 1990 and 2010 ranged between 8000 ha in Rwanda and 2,838,000 ha in Ethiopia. These unmitigated environmental changes in the highlands led to rise temperature and optimizing the spread and survival of malaria vectors and development of malaria parasites. Malaria in highlands was initially governed by low ambient temperature, trend of malaria transmission was therefore increased and several epidemics were observed in late 1980s and early 2000s. Although, malaria is decreasing through intensified interventions since mid 2000s onwards, these environmental changes might expose population in the highlands of east Africa to an increase risk of malaria and its epidemic particularly if the current interventions are not sustained.

Keywords: malaria, East African highlands, environmental changes

GEOGRAPHY AND CLIMATE OF EAST AFRICAN HIGHLANDS

In Africa, the highlands are defined to be altitude higher than 1500 m elevation above sea level or with daily mean temperatures of below 20°C. This area covers about one million km^2 equivalent to 4% of the total land area of sub-Saharan region. Ethiopia, Kenya, Tanzania, Rwanda, Burundi, Uganda, and Madagascar constitute about 82.4% of all African highlands. Ethiopian highlands make up 60% of all highlands in East Africa, while other countries constitutes of variable proportions of the remainder (**Figure 1**) (Hurni, 1990). The highlands of East Africa have been endowed with a combination of moderate temperatures, adequate rainfall (falling in two distinct seasons for much of the highlands), and productive soils that make the region one of the best suitable regions for agricultural development in Africa.

POPULATIONS OF EAST AFRICAN HIGHLANDS

Although the highland zone is a small fraction of the total land area of sub-Saharan Africa, it accounts for large populations of human and livestock due to its high agricultural productivity potential (Getahun, 1978). In general, East African highlands are considered as the most populated parts of the region (Bloom and Sachs, 1998). Despite the fact that the highland constitutes 23% of the total landmass in the region, their human dwellings represent more than 64% of its population with a population densities ranged between 158 persons/km^2 in Ethiopia and 410 persons/km^2 in Rwanda (**Table 1**). Densities above 500 persons/km^2 is common in some parts of the highlands i.e., the population densities in Kigezi District, the southwestern highlands of Uganda, is 518 persons/km^2 (Bolwing, 2006) and it is exceed to 1200 persons/km^2 in Vihiga district, western highlands of Kenya (Delve and Ramisch, 2006). The United Nations Population Fund (Formerly- United Nations Fund for Population Activities or UNFPA) data showed that Eastern Africa region already in 2010 having advanced into the most populated region in Africa and still has the world's highest population growth rate (UNFPA, 2010).

Agriculture is the main livelihood for the populations of East African countries i.e., more than 85, 80, and 75% of the total labor force is engaged in agriculture in the highlands of Ethiopia, Uganda, and Kenya, respectively. Although the highlands include the most favorable agricultural production areas, the populations are characterized by disappointingly high rates of poverty and one of the reasons behind this is the extreme population density (Place et al., 2006c). In Kenya, for example, about 53–56% of the population lives below the Kenyan poverty line

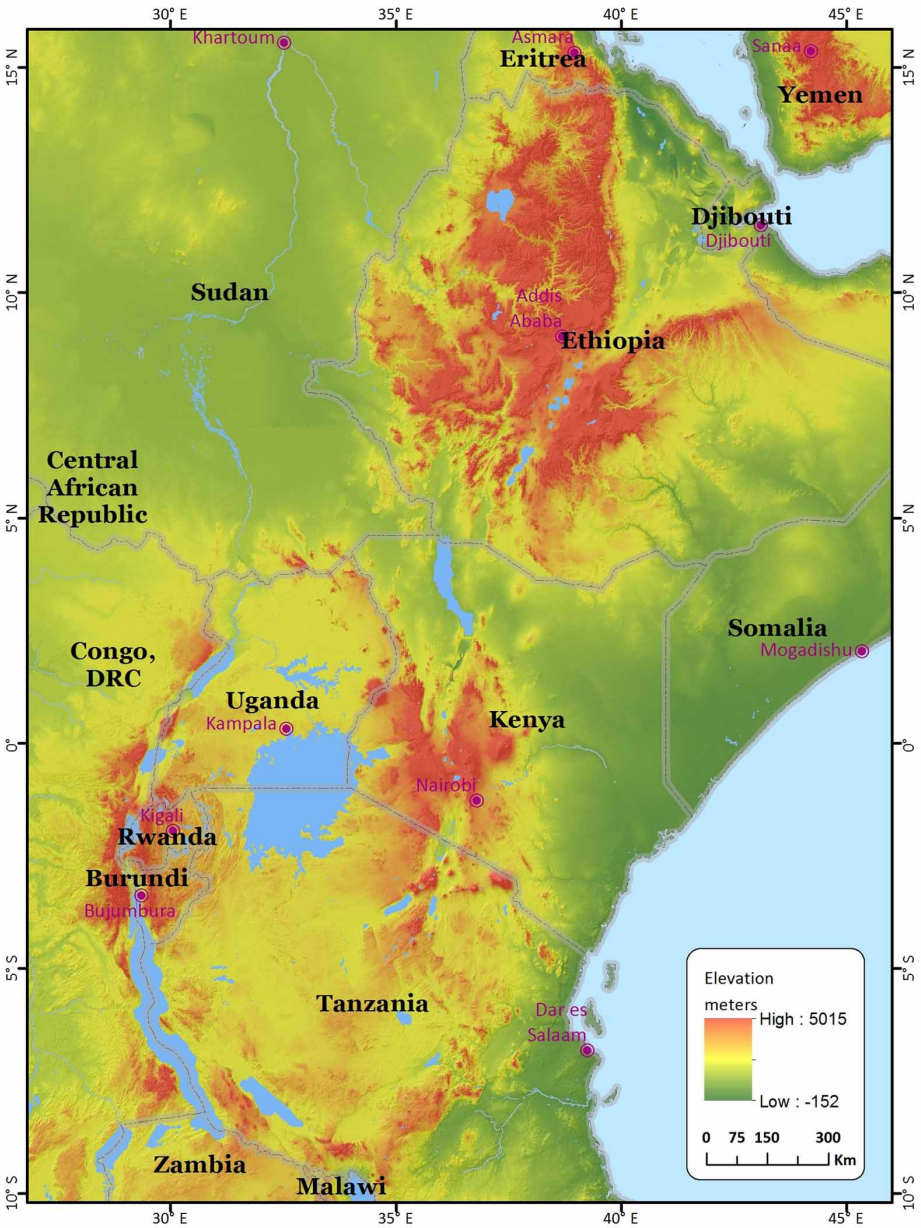

FIGURE 1 | Highlands areas in East African countries.

of $0.55/day in central and western Kenya highlands (Pell et al., 2004). Unfortunately, non-agricultural employment opportunities are not growing rapidly enough to provide the engine for a viable poverty reduction strategy for the short to medium term (Place et al., 2006b).

HISTORY AND CURRENT SITUATION OF MALARIA IN EAST AFRICAN HIGHLANDS

During nineteenth century, highland areas have been considered to be free or negligible malaria incidence (Matson, 1957; Lindsay and Martens, 1998). Movement of people to lowlands associated with the opening of civil and military posts, increased agricultural activities probably introduced malaria into the highlands (Malakooti et al., 1998; Shanks et al., 2005). The first malaria epidemic was documented following the influenza pandemic during troop demobilization and resettlement after World War I in 1918 and 1919 in western Kenya highlands (Matson, 1957). Between the 1920s and the 1950s infrequent malaria epidemics were reported in eastern Africa highlands (Fontaine et al., 1961). Malaria epidemics were not reported between the 1960s and the early 1980s after a malaria eradication campaign (Roberts, 1964a,b,c). Records from tea estates in the Kericho district of western Kenya highlands showed that malaria re-emerged in the 1980s (Shanks et al., 2005) (**Figure 2**). Thereafter,

Table 1 | Population density in East African highlands.

Country	Population in 1985	Population in 2005	Population in 2010	Population growth 2005–2010	Population density	Total area in highlands (km^2)	Population in highlands (%)	Population density in highlands
Burundi	4922	8162	9863	3.90	298	23,182	82.1	349
Ethiopia	42,227	74,980	88,013	2.51	72	489,500	88.0	158
Kenya	19,761	34,912	40,047	2.65	69	128,300	82.5	257
Madagascar	10,029	18,312	21,282	2.66	28	16,825	26.2	331
Rwanda	5987	9611	11,056	2.76	380	25,918	96.2	410
Tanzania	21,618	37,771	41,893	2.47	46	119,600	61.4	215
Uganda	14,392	28,199	33,399	3.24	136	20,000	16.0	268

Source: The population and population growth rate is based on the estimates taken from the 2006 edition of the United Nations World Population Prospects report. Population density figures are sourced from year 2005 data in United Nations World Population Prospects (2004 revision).

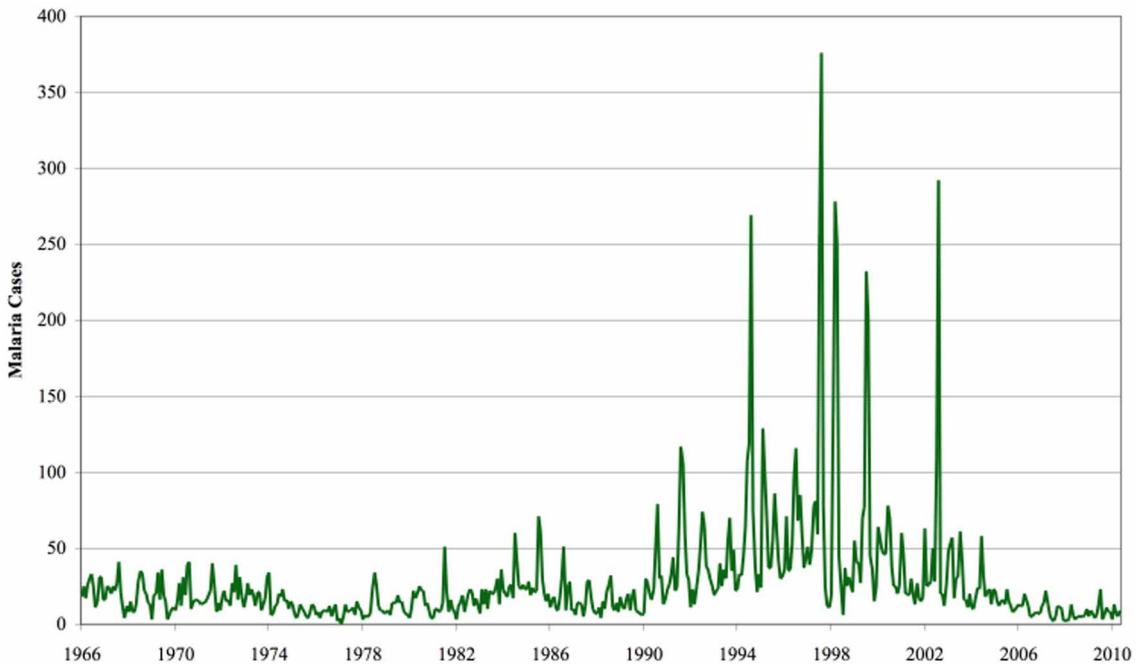

FIGURE 2 | Monthly malaria cases at Kericho Unilever Tea Kenya Ltd. Hospital. Source: Stern et al. (2011), with permission from Dr. David I. Stern, Crawford School of Economics and Government, Australian National University.

several malaria epidemics were reported during the decades of 1980s and 1990s in most countries of eastern Africa highlands. These include Uganda (Mouchet, 1998), Kenya (Some, 1994; Malakooti et al., 1998; Lindblade et al., 2000a), Ethiopia, Tanzania (Matola et al., 1987; Fowler et al., 1993; Mboera and Kitua, 2001), Rwanda (Loevinsohn, 1994), and Madagascar (Lepers et al., 1988; Mouchet, 1998). In western Kenya highlands alone between late 1980s and early 2000s, malaria outbreaks were reported at more than 20 highland sites causing serious mortality and morbidity (Lepers et al., 1988; Loevinsohn, 1994; Some, 1994; Lindsay and Martens, 1998; Mouchet, 1998; Shanks et al., 2000; Hay et al., 2002a,b).

The patterns of malaria epidemic during the 1990s were characterized by expanded geographic areas, increased frequencies and case-fatality rates (Githeko and Ndegwa, 2001; Zhou et al., 2004; Shanks et al., 2005; Pascual et al., 2008). Both the frequency of malaria epidemics and the number of malaria outpatients have dramatically increased compared to those in the 1980s such as in Kericho (**Figure 2**) (Snow et al., 1999; Shanks et al., 2005). Compared to the 1960s, case fatality rate increased from 1.3% to 6% in the 1990s (Shanks et al., 2000). A significant human mortality in both children and adults was reported during the last malaria epidemics in 2002 and 2003 in Kericho and Nandi districts of western Kenya highlands (Hay et al., 2002b, 2003; John et al., 2004; Shanks et al., 2005). The estimates of WHO in 2003 showed that malaria epidemics kill 110,000 people each year and 110 million people were considered at risk in the highlands (WHO, 2003).

A complex set of environmental, biological, and socioeconomic factors including climate change (Loevinsohn, 1994; Lindsay and Martens, 1998; Afrane et al., 2006), land use changes (Matola et al., 1987; Lindsay and Martens, 1998; Malakooti et al., 1998; Mouchet, 1998), drug resistance (Malakooti et al., 1998), cessation of malaria control activities (Mouchet, 1998), and demographic changes (Lindsay and Martens, 1998; Mouchet, 1998) were hypothesized to influence malaria in the highlands. Apparently, a significant environmental change occurred. This was attributed mainly to dramatic increases in the human population i.e., populations of each of the East African countries increased by more than 100% since 1985 (**Table 1**), and this included an extensive unprecedented land use and land cover changes (Whitmore, 1997).

LAND COVER CHANGE AND ITS IMPACT ON MALARIA TRANSMISSION

There is no doubt that population pressure in eastern African highlands profoundly changed land cover over the course of the last one or two centuries, and even earlier than that (Bolwing, 2006). Data presented in **Table 2** shows that lost of forest areas during 1990–2010 in the countries mentioned of east Africa due to human activities was ranged between 8000 ha in Rwanda and 2,838,000 ha in Ethiopia. Recent study from Madagascar indicated that about 88.91 km^2 was shrunk only between 2003 and 2004 of forest near Ranomafana National Park (Brooks et al., 2009). This occurred mainly due to increased human demand for forest products and land for agricultural purposes. In Rwanda, deforestation of the entire country is almost completely due to overpopulation, with a mere 230 square miles (600 km^2) remaining and an ongoing loss of about 9% per annum (McMichael, 2003). In highlands of Kenya, reduction in tree cover was found in areas with more densely populated (Pell et al., 2004). The rapid loss of primary forest due to subsistence agriculture (Myers, 1979, 1984; Brooks et al., 2009) represents one of the greatest environmental changes that create disequilibrium to local natural balance and global biodiversity. It was concluded that unmitigated deforestation under increasing demographic pressure make the East African highlands one of the most fragile ecologies in the world (McMichael, 2003).

The implication of this change in land cover on malaria transmission is that deforestation can lead to changes in microclimate of both adult and larval habitats, hence increase larvae survival, population density, and gametocytes development in adult mosquitoes (Afrane et al., 2006; Munga et al., 2006, 2007; Kweka et al., 2011). For example, the mean indoor temperatures of houses located in the deforested area of western Kenya highland were found to range between 0.7°C and 1.2°C higher than in houses located in the forested area, which resulted in a significant increase in net reproductive rate and intrinsic growth rate for adult mosquitoes (Afrane et al., 2006). This enhanced mosquito reproductive fitness and mosquito population growth, shortened the duration of the gonotrophic cycles by 1.4–1.5 days. Deforestation could augment the vectorial capacity of *Anopheles gambiae* with 29–106% increase compared with forestation areas (Afrane et al., 2005, 2006). Deforestation was found to increase water temperature of larval habitat, hence increase immature stages survivorship of malaria vectors by shortening larval development period and reducing the chance of larvae encountering predators (Tuno et al., 2005; Munga et al., 2007). Not only, changing microclimate of both larval and adult stages of malaria vectors, and development of malaria parasite, but also deforestation was shown to create more suitable breeding sites (Minakawa et al., 2005; Munga et al., 2005; Mushinzimana et al., 2006). Forest reclamation for agriculture, was found to change water chemistry which is suitable for larval development and survival (Munga et al., 2005; Tuno et al., 2005). Therefore, the overall consequences of deforestation, particularly due to expansion of agricultural activities can increase malaria cases and transmission e.g., in western Usambara forest (Bodker et al., 2003, 2006).

LAND USE CHANGE AND ITS IMPACT ON MALARIA TRANSMISSION

In more humid highlands area having bimodal rainfall patterns and sufficiently good soils, production of perennial cash crops such as coffee is common e.g., highlands of central Kenya (Place et al., 2006c), Eastern highlands of Uganda (Pender et al., 2006), and much of southwestern Ethiopia. Perennial food crops are also common in such areas, but annual food crops (especially maize) are become more important in many areas, particularly in western Kenya highlands, where maize–livestock production is the dominant farming system (Place et al., 2006b).

There is no doubt that population pressure in the region led to expanded and intensified agricultural activities and pastoral

Table 2 | Trends in natural forest cover (1000 ha) (Deforestation) in East African, 1990–2010.

Country	Natural forest cover (1000 ha)				Loss (1000 ha)	Coverage %	Annual deforestation rate (%)		
	1990	2000	2005	2010	1990–2010	2000	1990–2000	2000–2005	2005–2010
Burundi	289	112	103	103	186	3.7	−6.1	−6.12	−0.8
Ethiopia	14,623	13,214	12,509	11,785	2838	4.2	−1.0	−0.96	−1.08
Kenya	3470	3370	3320	3270	200	30.0	−0.3	−0.29	−0.30
Madagascar	13,461	12,850	12,548	12,138	1323	20.2	−0.5	−0.45	−0.55
Rwanda	70	62	62	62	8	n.a	−1.1	−1.14	0
Tanzania	41,345	37,262	35,215	33,188	8157	43.9	−1.0	−0.99	−1.09
Uganda	4717	3837	3398	2937	1780	21.0	−1.9	−1.87	−2.35

Data source: Rhett A. Butler/mongabay.com. San Francisco, USA. Available at: http://rainforests.mongabay.com/deforestation/Retrieved on January 20, 2012.

land use systems as a response to increasing population densities and market opportunities (Bolwing, 2006). For example, the increase in area harvested for only one single crop of maize ranged between 4.7% in Burundi and 225.7% in Rwanda (**Table 3**). In the southwestern "Kigezi" highlands of Uganda, for example, remotely sensed land cover data from the early 1990s show that small scale farmland covers 57% and 68% of the land area in Kabale and Kisoro districts respectively, while natural forests

Table 3 | Increase in harvested area of maize in East Africa.

Country	Total area in highlands (km^2)	% of African highlands[a]	Area harvested (ha) 1980	Area harvested (ha) 1995	Area harvested (ha) 2010	% increase in area harvested
Burundi	23,182	2.6	1,300,00	1,200,000	1,256,000	4.7
Ethiopia	489,500	49.2	n.a.	1,464,080	1,772,250	21.0
Kenya	128,300	12.9	1,350,000	1,438,740	2,008,350	45.8
Madagascar	16,825	1.2	1,278,900	1,838,400	3,712,000	132.3
Rwanda	25,000	2.5	718,000	500,000	1,846,580	225.7
Tanzania	119,600	12.0	1,400,000	1,368,000	3,100,000	124.3
Uganda	20,000	2.0	258,000	571,000	890,000	110.7

[a] *Other non eastern African highlands represent 17.6%.*

Source: Food and Agriculture Organization (FAO), FAOSTAT, Available at: http://faostat.fao.org/DesktopDefault.aspx?PageID=567#ancor. Retrieved on 23 January 2012.

Data for total area in highlands (km^2) were obtained from Jahnke, H.: "Dairy development in the highlands of tropical Africa: an overview of planning considerations"; paper presented at the International Livestock Centre for Africa, Workshop on Smallholder Dairy Development in the East African Highlands, August 1980. Report is available at: http://www.ilri.org/InfoServ/Webpub/fulldocs/Bulletin11/Smallholder.htm#TopOfPage Retrieved on 20 January 2012.

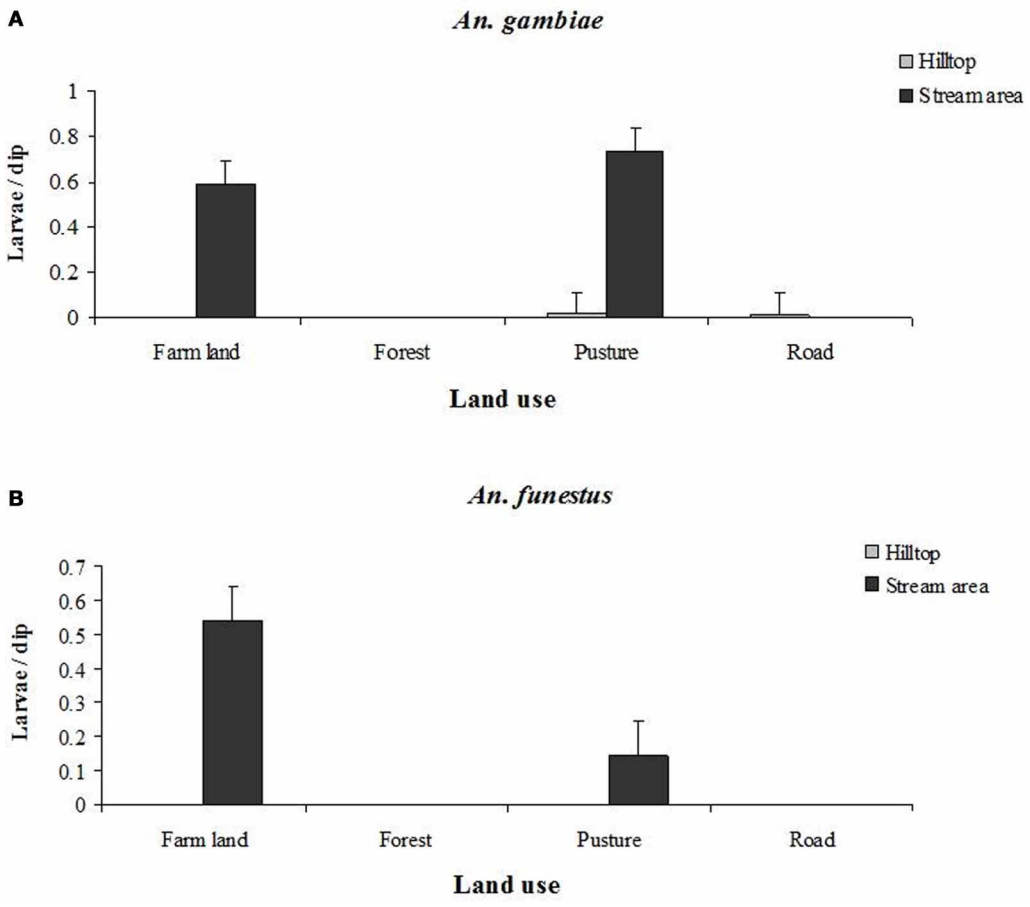

FIGURE 3 | Larvae density (larvae/dip) of *A. gambiae* (A) and *A. funestus* (B) among the different land use types found in Iguhu area in western Kenya highlands. Error bars show standard errors. Source: Himeidan (2009).

cover only 2.0% of Kabale and 16.3% of Kisoro (Bolwing, 2006). During the last four decades, in the Bale Mountains in south-east Ethiopia the area changed from a quite natural to a more cultural landscape. Within a representative subset of the study area (7957.5 km^2), agricultural fields increased from 1.71% to 9.34% of the total study area since 1973 (Kidane et al., 2012). Cultivation of valley papyrus swamps around Lake Bunyonyi produced major changes in land uses (Lindblade et al., 2000b). This activities of claiming natural swamps for cultivation started more than 70 years ago when the British administration encouraged the drainage of swamps containing *Cyperus papyrus* (papyrus) and other swamp grasses to increase the land available for crop cultivation (Carswell, 1996). Of an estimated 69.7 km^2 of papyrus swamp surveyed in 1954–1955 in Kabale District, south-western highlands of Uganda, only about 15% remains today in its natural state (Lindblade et al., 2000b). Bolwing (2006) summarized the changes in land use between the 1950s and 1990s in the highlands of East Africa where; bushland, woodland, and grassland on hillsides was changed to farmland; papyrus swamps in valley bottoms was changed to dairy pastures and cropland; grazing land on steep slopes have been changed to fallow areas and short (seasonal) fallows on hillsides have been changed to longer fallows on hillsides.

These changes in land use were reported to create and spread habitats for malaria vectors breeding as well as changing microclimate by altering temperature to that more suitable for larval development and adult survival (Lindblade et al., 2000b). Some earlier reports noted that anophelines would not breed in papyrus swamps but could be found in the ditches formed during cultivation (Goma, 1958). Fifty years later, it was confirmed that *A. gambiae* and *Anopheles funestus* were the dominated farmland areas of western Kenya highlands (**Figure 3**) (Minakawa et al., 2005; Munga et al., 2006; Himeidan, 2009). Entomological surveys revealed that at least more than third of malaria vectors larval habitats observed in farmlands. These habitats constituted 40% of positive anopheline larval habitats (Minakawa et al., 2005), but almost 100% of habitat produce the vectors with rate of 1.82 *A. gambiae* s.l. emerged/m^2/week compared to zeros in forests and swamps of western Kenya highlands (Munga et al., 2006). The successful of these habitats for producing the vectors was associated with the increase in maximum temperature of water of the breeding sites which was 6.6°C and 2.4°C higher in farmlands compared to forest and natural swamps, respectively (Munga et al., 2006). Lindblade and others (Lindblade et al., 2000b) compared mosquito density, biting, sporozoite, and entomological inoculation rates between eight villages located along natural papyrus swamps and eight villages located along swamps that drained and cultivated in Kabale District, south-western highlands of Uganda. They observed that both mean maximum and minimum temperatures were approximately 0.9°C higher in villages located along cultivated swamps than in villages located along natural swamps and that all malaria indices investigated were higher near cultivated swamps. The authors concluded that replacement of natural swamp vegetation with agricultural crops led to increase temperatures, which might be responsible for elevated malaria transmission risk in cultivated areas. This supported by the fact that several malaria outbreaks occurred near cultivated swamps and that was attributed to availability of potential breeding habitats for *Anopheles* vectors in highlands (Steyn, 1946; Goma, 1958). Overall, external environmental influences of household associated by land use changes such as living on flat land, in close proximity to maize fields, and on land lacking nearby trees were shown to increase malaria risk in East African highlands (Ernst et al., 2009).

IMPACT OF LAND USE AND LAND COVER CHANGES ON MALARIA VECTORS DISTRIBUTION

Malaria vectors for many years were found in lowlands and restricted to highlands because of climatic conditions with low temperatures and high forest canopy (Githeko et al., 2000). Deforestation and land use changes created both temporal and permanent potential breeding habitats exposed to sunlit hence increase larvae survivorship and growth rate (Minakawa et al., 2004, 2005; Munga et al., 2005, 2006). The high rate of deforestation leads to rise of temperatures in highlands area (Githeko and Ndegwa, 2001; Afrane et al., 2005). Therefore, due to these changes, malaria vector mosquitoes (*A. gambiae* s.s and *A. funestus*) invaded the highland areas in western Kenya (Zhou et al., 2004; Ndenga et al., 2006). *Anopheles arabiensis* is highest vector ever reported and continue to re-colonize new areas in highlands of Kenya (Chen et al., 2006; Imbahale et al., 2011; Kweka et al., 2011). The high proportions of new species in East African highlands might increase the risk in malaria foci expansion and subsequently epidemics due to low immunity against malaria parasites among highland populations (Lindsay and Martens, 1998).

REGIONAL CLIMATE CHANGE AND MALARIA EPIDEMIC

Both land use/land cover changes and global warming may contribute to regional change in climate of East African highlands. The presence of greenhouse gases in the atmosphere is the best known impact of human activity on climate change, variations in land use/land cover, and surface cover may be of equal importance (Pielke, 2005). In a transient climate simulation, agricultural expansion results in significant additional warming over the Amazon (Feddema et al., 2005). Though, as malaria in highlands is limited by low temperature (Hay et al., 2002b; Balls et al., 2004), regional climate changes have been proposed as a major factor accountable for the recent epidemics in African highlands (Martens, 1995; Lindblade et al., 2000b). However, assessing the impact of climate in malaria resurgence is difficult due to high spatial and temporal climate variability and the lack of long-term data series on malaria cases. Therefore, association between climate change and the re-emergence of malaria epidemic in the East African highlands is subject of debates during the last two decades. However, different observations were reported on climate change and their association with malaria incidence in African highlands. Despite an increase of 0.74°C, in the global average temperature between the years 1906 and 2005 (IPCC, 2007), Hay et al. (2002a) claimed that mean temperature and rainfall did not change significantly in the past century at four locations in the East African highlands, where malaria incidence increased. Patz et al. (2002) argued this conclusion to the use of spatially interpolated climate data that criticized for its

inappropriateness for trend analysis in areas known to have a high spatial heterogeneity in temperature. The primary argument here was that climate was the main driver behind higher malaria incidence, but that its role could not be ruled out on the basis of lack of evidence for temperature warming in the region (Pascual et al., 2006). Based on this assumption, Pascual et al. (2006) revisited result of Hay et al. (2002b) using the same temperature data, with updated from 1950 to 2002 and found evidence for a significant warming trend at all four studied sites. Chaves and Koenraadt's (2010) assessed the conclusions from both sides of the argument that supporting and rebutting the role of climatic change as a driving force for highland invasion by malaria and concluded that evidence for the role of climate in these dynamics was robust. This conclusion was supported by Omumbo et al. (Myers, 1979) who analyzed quality controlled daily observations (>97% complete) of maximum, minimum, and mean temperature at Kericho meteorological station, sited in a tea growing area of Kenya's western highlands for 30 years (1 January 1979–31 December 2009). Evidence of a warming trend was also confirmed in this analysis and an upward trend of ≈0.2°C/decade was observed in maximum, minimum, and mean temperatures at Kericho in western Kenya highland (Chaves and Koenraadt, 2010; Omumbo et al., 2011; Chaves et al., 2012). Stern et al. (2011) compared a new robust trend test to the original monthly time series from the Climate Research Unit Time Series (CRU TS) 1.0 data set used by Hay et al. (Fowler et al., 1993) for the four locations in highland East Africa to the more recently published CRU TS 2.1 data used by Chaves and Koenraadt (2010) and Pascual et al. (2008) and to a newest data-set, CRU TS 3.0 as well as the data from the Kericho meteorological station used by Omumbo et al. (Myers, 1979). The authors confirmed the significant trends observed in the data extracted from newer editions of the database used by Chaves and Koenraadt (2010) and Pascual et al. (2008). The trend was also significant in the newest data-set, CRU TS 3.0 and in the data of the Kericho meteorological station but not in the older version (CRU TS) 1.0 for periods ending in 1996 used by Hay et al. (2002a) (**Figures 4**, **5**). The role of this unambiguous warming trend observed on malaria transmission may need to be assessed again but indications provided in this review suggest that in this region, change of 0.7–1.2°C in temperature can have a significant effect on transmission (Afrane et al., 2005, 2006). Overall, it has been projected that climate changes will significantly affect the spread of malaria in tropical Africa well before 2050 and that the geographic distribution of areas where malaria is epidemic e.g., highlands might have to be significantly altered in the coming decades (Ermert et al., 2012).

COMPLEXITY OF HIGHLANDS MALARIA TRANSMISSION

The fact is that the occurrence of increased malaria transmission trends observed during 1980s and 1990s was not homogeneous across the East African highlands (Chaves et al., 2012). Topography synergize with increase rainfall associated with El Niño (Myers, 1984; Mboera and Kitua, 2001; Bodker et al., 2003; Kweka et al., 2011; Chaves et al., 2012), interaction between

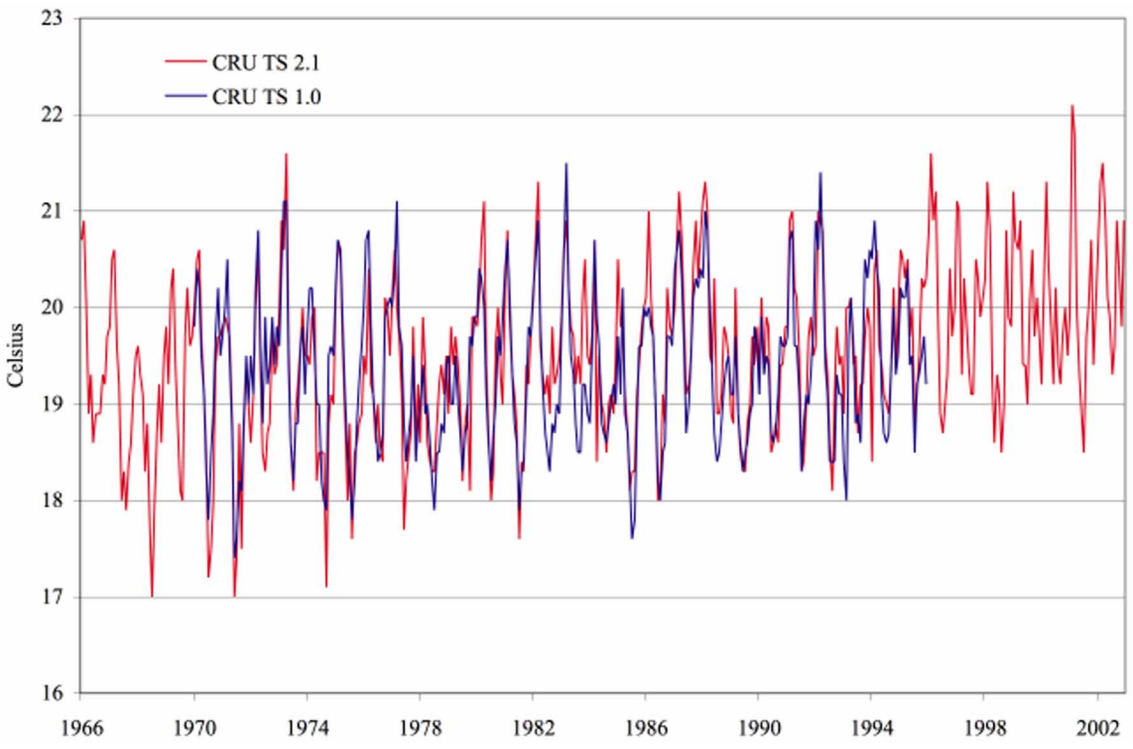

FIGURE 4 | Temperature series for Kericho: CRU TS 1.0 vs. CRU TS 2.1. Source: Stern et al. (2011), with permission from Dr. David I. Stern, Crawford School of Economics and Government, Australian National University.

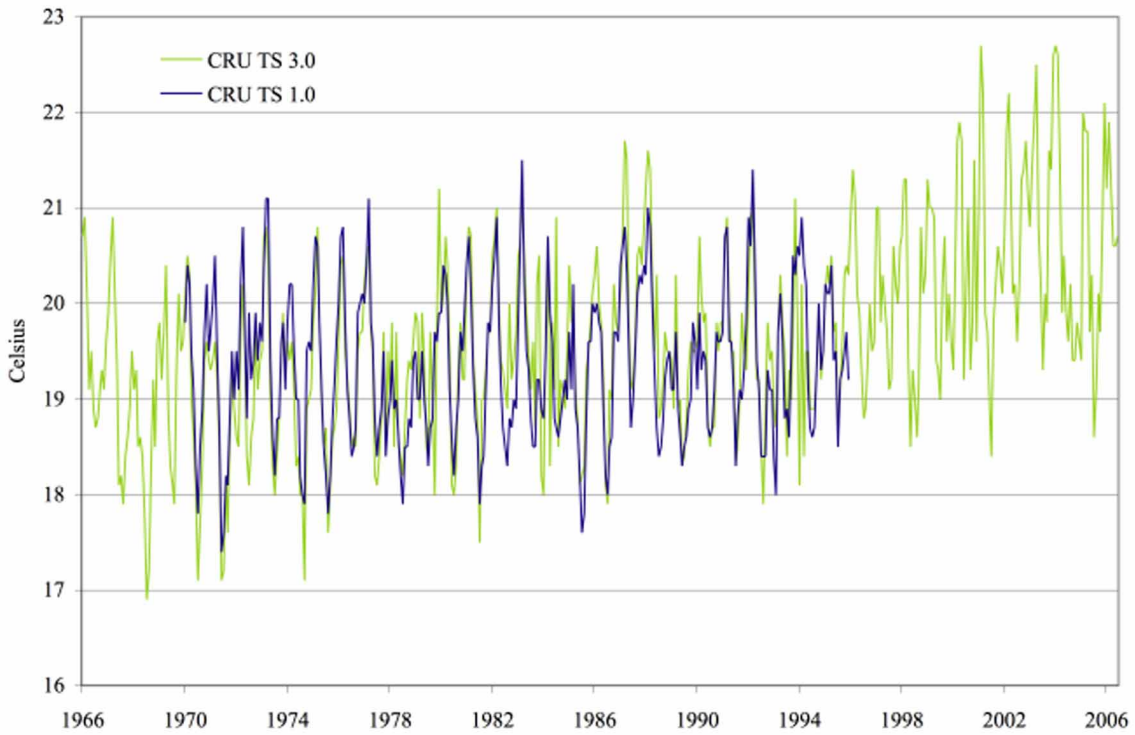

FIGURE 5 | Temperature series for Kericho: CRU TS 1.0 vs. CRU TS 3.0. Source: Stern et al. (2011), with permission from Dr. David I. Stern, Crawford School of Economics and Government, Australian National University.

rainfall and temperature (Zhou et al., 2005), were major drivers of epidemics confirmed above 1600 m (Chaves et al., 2012). Although, shared biophysical environments can produce clusters of higher transmission, other factors at the individual and household levels can mediate this risk (Ernst et al., 2009). In this context, migration of human derived earlier by colonies administration; for settlement the highlands that favored by rich soil, maximum water resources and lower incidence of human diseases, or occurring later to the region associated with rapid population growth rate is probably playing role during both earlier emergence and the resurgence of malaria epidemics in the East African highlands (McCallum et al., 2001). This is probably indirect role occurred through a large proportion of land use changes that have been observed on a nearly continuous basis for many decades if not centuries (Place et al., 2006a). Forests and natural swamps of the highlands have mostly disappeared, and locals are still clearing the last patches of forest and drained the remaining natural swamps (Lindblade et al., 2000b; Verschuren et al., 2002), just for their self interest of increasing land available for agriculture (Ernst et al., 2009). It is believed that even there are no additional productive lands to be brought under cultivation and therefore, to reduce the poverty, agricultural growth must be through intensification of production (Place et al., 2006a).

However, the concentration of the residences around valley bottoms, where these activities of claiming swamps and forest for agriculture is running, is just exposed them to a large number of vectors and high level of malaria transmission (Ndenga et al., 2006). Accordingly, closer proximity to valley bottoms has been associated with increased malaria risk as these habitats enhanced *Anopheles* breeding sites and their microclimates being more suitable for prolong adult vector survival and parasite development; hence a 500-m threshold for these relationships has been demonstrated (Minakawa et al., 2004; Zhou et al., 2004; Ernst et al., 2009). Although these results indicated that malaria risk may cluster near specific land covers and topography, still some efforts are needed to identify such high risk areas, as even in these apparent hotspots, malaria-free households has been observed suggesting that environmental, socio-demographic, biological, and behavioral variables are important (Ernst et al., 2009).

The current malaria interventions (mainly ITNs) in the highlands have greatly reduced malaria prevalence in 2000s onwards (Stern et al., 2011; Chaves et al., 2012). Transmission has been interrupted in some areas (Zhou et al., 2011), which might lead to decrease in immunity of the residences (Githeko et al., 2012). With the reported environmental changes that alter malaria transmission, peoples in the highlands would be at high risk never like before if the interventions is not sustainable. Such present factors have already contributed to this scenario include reduced efficacy of ITNs, insecticide resistance and lack of proper use of ITNs (Protopopoff et al., 2008; Atieli et al., 2011; Zhou et al., 2011).

AUTHORS' CONTRIBUTIONS

Yousif E. Himeidan identified the idea, framed, drafted, and wrote up the manuscript, Eliningaya J. Kweka wrote up and reviewed the content. All authors read and approved the final version.

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Intertwined arbovirus transmission activity: reassessing the transmission cycle paradigm

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Arboviruses are emerging/reemerging infectious agents worldwide. The factors within this scenario include vector and host population fluctuations, climatic changes, anthropogenic activities that disturb ecosystems, an increase in international flights, human mobility, and genetic mutations that allow spill-over phenomenon. Arboviruses are maintained by biologic transmission among vectors and hosts. Sometimes this biological transmission is specific and includes one vector and host species such as Chikungunya (CHIKV), Dengue (DENV), and urban Yellow Fever (YFV). However, most of the arboviruses are generalist and they use many vectors and hosts species. From this perspective, arboviruses are maintained through a transmission network rather than a transmission cycle. This allows us to understand the complexity and dynamics of the transmission and maintenance of arboviruses in the ecosystems. The old perspective that arboviruses are maintained in close and stable transmission cycles should be modified by a new more integrative and dynamic idea, representing the real scenario where biological interactions have a much broader representation, indicating the constant adaptability of the biological entities.

Keywords: arbovirus, St. Louis encephalitis virus, transmission cycles, West Nile virus, transmission network

Arboviruses are emerging/reemerging infectious agents worldwide; Chikungunya (CHIKV), Dengue (DENV), Yellow Fever (YFV), St. Louis encephalitis (SLEV), and West Nile (WNV) are some examples of this phenomenon. Although not fully understood, several factors are thought to promote reemergence. For instance, environmental disturbs from anthropogenic activities (Vasconcelos et al., 2001), climatic changes affecting vector and host population fluctuations (Weaver and Reisen, 2010), human movements through airplanes, animal trade and migration (Pfeffer and Dobler, 2010), and genetic mutations that cause spill-overs (Weaver and Barrett, 2004; Kuno and Chang, 2005).

Basically, arboviruses (arthropod-borne viruses) are maintained by biological transmission through an arthropod vector to a vertebrate host, hence representing an ecological rather than a taxonomic grouping. For most arboviruses (SLEV, Usutu virus—USUV, WNV, Japanese encephalitis virus—JEV, Eastern, Venezuelan, and Western equines encephalitis virus—EEEV, VEEV, WEEV) human beings are dead-end hosts, which means that viremias are not high enough to infect the arthropod vector. Therefore, humans are not necessary for virus maintenance and they represent just an accident during the biological transmission among vectors and hosts. However, CHIKV, DENV, and YFV are exceptions, given that these viruses can replicate and generate viremia titers in the human host high enough to infect vector mosquitoes (Morris, 1988; Scott, 1988; Reisen and Monath, 1989).

Based on ecological terms, infectious agents can be classified as generalist or specialist according to the number of host/vector they can infect. Specialist arboviruses are those transmitted by specific species of host/vector. Thus, as a result of centuries of coadaptation CHIKV, DENV, and YFV are particularly efficient in being transmitted by *Aedes aegypti/Ae. albopictus* mosquitoes and amplified by humans in urban environments (Weaver and Reisen, 2010). In certain cases, some viruses make a change of species and have the ability of being transmitted by another species of host/vector. For example, due to a special mutation, CHIKV is transmitted by an alternative mosquito species: *Aedes albopictus* (Tsetsarkin et al., 2007).

On the other hand, those viruses maintained in nature by more than one host/vector species are considered as generalists, such as: SLEV, WNV, JEV, EEV, WEEV, and VEEV. The analysis of the dynamics maintenance of these viruses is more related to a transmission network than to a transmission cycle.

Several intrinsic and extrinsic requirements (physiological/behavioral—ecological/environmental) must be fulfilled for species to be considered a vector or a host (**Table 1**). Ecosystems are inherently variable across time and space. The intrinsic characteristics are not modified by time, however, there might be exceptions such as certain selective processes that affect the population, determining susceptibilities to the differential infection among hosts and vectors, e.g., environmental stress and detrimental nutrition affect both vector and host competence (Kramer and Ebel, 2003; Reisen et al., 2003). In contrast, the extrinsic factors such as species availability, density, and abundance are modified in time and space. If we analyze certain ecosystem where the arbovirus is maintained by the network of interactions between its hosts and vectors, we are likely to see that the cycles, part of that network, change through time (**Figure 1**).

Table 1 | Intrinsic and extrinsic characteristics fulfilled by a vector/host of an arbovirus.

Characteristics	Vectors	Hosts
Intrinsic	Viral replication	
	Susceptibility to viral infection	
	Host feeding preference	–
	Behavior	
Extrinsic	Abundance and dispersal	
	Seasonal breeding patterns	
	–	Attractiveness to mosquito vector
	Host feeding selection	–
	Distribution	

In systems like the arbovirus, characterized by multi-host-vector interactions, the ecological dynamics may alter the epidemiological patterns and scenarios (Allan et al., 2009). Many studies have recently focused on the effects of biodiversity over arbovirus activity (Ezenwa et al., 2006, 2007; Swaddle and Calos, 2008; Allan et al., 2009; Loss et al., 2009; McKenzie and Goulet, 2010). It has been hypothesized that high diversity of host would result in a reduced viral activity. This could be caused by two different mechanisms, firstly, due to a decrease in the probability that the vector comes into contact with the host of higher competence; resulting this out of a decrease in the relative abundance of the host with the higher competence or an increase in the relative abundance of hosts of low competence. Secondly, higher host diversity could increase interspecific interactions, such as predation and competition, thus potentially regulating the abundance of the most competent host (Allan et al., 2009).

Empirical studies aimed to test the hypothesis that higher host diversity reduces transmission rate and viral concentration in the ecosystem had produce inconclusive results. While some studies have found support for this hypothesis (Ezenwa et al., 2006; Allan et al., 2009), others have not found a relation between host diversity and virus transmission (local spatial scale vs. regional/national) (Loss et al., 2009). Moreover, diversity effects on transmission dynamics vary through time. For instance, by using a country scale analysis, Allan et al. (2009) found that the fluctuation of WNV incidence in the American population

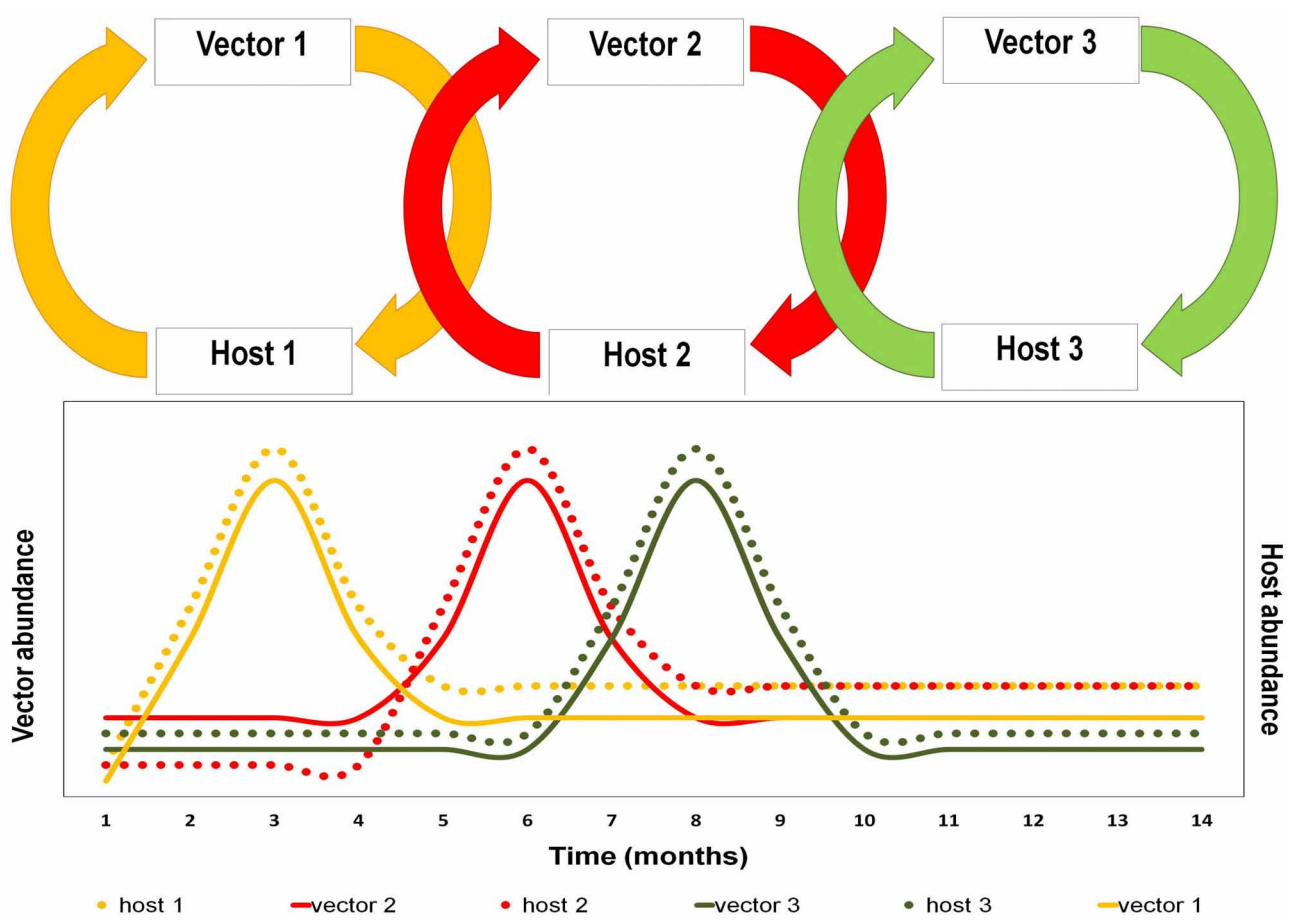

FIGURE 1 | Schematic representation of the sequencing changes as function in time in arbovirus transmission cycles. Foot figure: this figure represents the network for a hypothetical arbovirus in which transmission network made by three vector-host cycles. We would like to mention how population fluctuations in vectors and hosts (temporal dynamics) force the virus to adapt itself in relation to availability of vectors and hosts.

between 2002 and 2004 is explained through bird diversity, while other factors such as human density and the community competence index vary in their relevance during the years. These studies emphasize the need to consider the diversity of host and vector within an ecosystem when analyzing virus dynamics (Kilpatrick, 2011). Some authors pointed out the hypothesis that host competence could be associated to evolutive relatedness (Ezenwa et al., 2006). Kilpatrick et al. (2007) showed that host competition varies more among families than within members of the same family. Therefore, when assessing the suitability of an ecosystem to an arbovirus it is important to know and consider not only the diversity (abundance, richness) but also the species composition of potential vectors and hosts.

Besides its geographical distributions, the virus adaptability for its maintenance also occurs at a seasonal level, being the viral flow driven by the feeding preference of the vectors (Kilpatrick et al., 2006b). Thus, the strength level of a certain host/vector association can be quantified by measuring the vector-feeding preference. Nowadays, thanks to the incorporation of molecular techniques (e.g., gene sequencing for Cytochrome Oxidase I), the vectors blood-feeding patterns can be identify (Apperson et al., 2002; Goldstein and DeSalle, 2011). Based on both blood-feeding patterns and host population densities, a feeding selection index can be determined and later translated into a host-vector association measurement (Manly et al., 2002; Hamer et al., 2011). This feeding preference is modified by extrinsic factors such as host abundance, vector densities, and/or avian defensive behavior (Kilpatrick et al., 2006b; Molaei et al., 2006; Thiemann et al., 2011). For instance, in late summers at a countrywide scale in the USA, WNV vectors (*Cx. pipiens, Cx. nigripalpus, Cx. tarsalis, Cx. salinarius*) showed a shift in their feeding preference from birds to mammals (Kilpatrick et al., 2006b). Although the impact of genetic it not yet explored, physiological or other intrinsic changes within the mosquito population may contribute to this host shift.

As empirical examples we here reconsider the transmission pattern of WNV and SLEV in the American continent, mainly based on the data gathered in USA and Argentina (**Figures 2A,B**).

Since its introduction in 1999 in the USA, WNV has become one of the arboviruses of most medical concern in the American continent. Thanks to a decade of ecological and epidemiological research carried out in the USA, most aspects of its transmission dynamics have been analyzed. WNV is maintained through biological transmissions in which *Culex* spp. mosquitoes are involved as vectors and Passeriformes birds as hosts (Komar et al., 2003; Hayes et al., 2005). The main species of vector are *Cx. pipiens*, *Cx. restuans* (Kilpatrick et al., 2005), *Cx. quinquefasciatus* (Turell et al., 2005), *Cx. nigripalpus* and *Cx. tarsalis* (Turell et al., 2002; Blackmore et al., 2003); while only a few non-*Culex* species have been considered as possible vectors, such as *Aedes albopictus* and *Ae. vexans* (Turell et al., 2005). Regarding hosts, the main participants could be listed as: the American Robin (*Turdus migratorius*), the Northern Cardinal (*Cardinalis cardinalis*), the House Sparrow (*Passer domesticus*), the Blue Jay (*Cyanocita cristata*), the Northern Mockingbird (*Mimus polyglottos*), the Western Scrub-Jay (*Aphelocoma californica*), the American Crow (*Corvus brachyrhynchos*), and the Black-billed Magpie (*Pica hudsonia*) (Kilpatrick et al., 2007). Geographical and seasonal variation in host and vectors were observed across the USA. In the northeastern region of USA, the suggested main vectors are *Cx. pipiens* and *Cx. restuans* (Kilpatrick et al., 2005), while *Cx. salinarius* might have local significance as a bridge vector (Molaei et al., 2006). The American Robin has been suggested to be the main host in this region (Apperson et al., 2002, 2004), as well as in Tennessee (Savage et al., 2007) and in the mid-eastern region of the country (Kilpatrick et al., 2006a; Griffing et al., 2007). Additionally, the Northern Cardinal, the House Finch, and the House Sparrow are suggested to be important hosts (Molaei et al., 2006) for the region. The most important vector in Florida are *Cx. nigripalpus* and *Cx. quinquefasciatus* (Sardelis et al., 2001; Goddard et al., 2002; Rutledge et al., 2003); and in terms of hosts, the Northern Cardinal, the House Sparrow, the Blue Jay, and the Northern Mockingbird were mentioned for this state (Komar et al., 2005). In the western area of USA the principal vector species are *Cx. tarsalis, Cx. quinquefasciatus* and *Cx. stigmatosoma* (Goddard et al., 2002; Reisen et al., 2005). Birds species suggested as host are the House Finch, the House Sparrow, the Western Scrub Jay (*Aphelocoma californica*), the Mourning Dove (*Zenaida macroura*), and the Common Ground Dove (*Columbina passerina*) (Reisen et al., 2005).

Cx. erraticus, Coq. perturbans, and *Cx. salinarius* may play a more significant role in the transmission of WNV in the USA mid-southern region (Cupp et al., 2007). Other potential host competences are the Western Scrub-Jay, the American Crow, the Black-billed Magpie, the Common Grackle (*Quiscalus quiscula),* the House Finch, and the Ring-billed Gull (*Larus delawarensis*) (Kilpatrick et al., 2007). WNV mosquito vectors show seasonal variation across their geographical distribution. *Cx. restuans* is generally found in spring and early summer, while other *Culex* species are present later in the season (O'Meara et al., 1989; Andreadis et al., 2001; Ebel et al., 2005). The *Cx. pipiens/restuans* complex (not differentiated due to morphological similarity) responded differently to weather variables in western New York (USA) than another potential WNV vector, *Ae. vexans* (Trawinski and Mackay, 2008). Conversely, *Cx. pipiens* and *Cx. quinquefasciatus* showed similar seasonal distributions in Tennessee (USA), though *Cx. quinquefasciatus* had a broader seasonal distribution and there was variation between sites (Savage et al., 2008). In some locations, since *Cx. salinarius* frequently feeds on both birds and mammals (Kilpatrick et al., 2005) it could also be an important epidemic or bridge vector (Andreadis et al., 2004).

Alternative transmissions mechanisms contribute to the maintenance of WNV in nature. For instance, in vertical vector transmission the virus is transmitted to its descendant through an infected female mosquito. This way tends to have low transmission rates, but gains importance in mild areas as an overwinter mechanism. Laboratory and field studies have confirmed that WNV can be vertically transmitted in at least *Culex* and *Aedes* mosquitoes (Anderson and Main, 2006; Unlu et al., 2010). Other alternative mechanism is the direct transmission among hosts. This mechanism has been observed in several species of birds maintained under laboratory conditions, where viruses transmissions occurs both, due to the intake of WNV-infected food

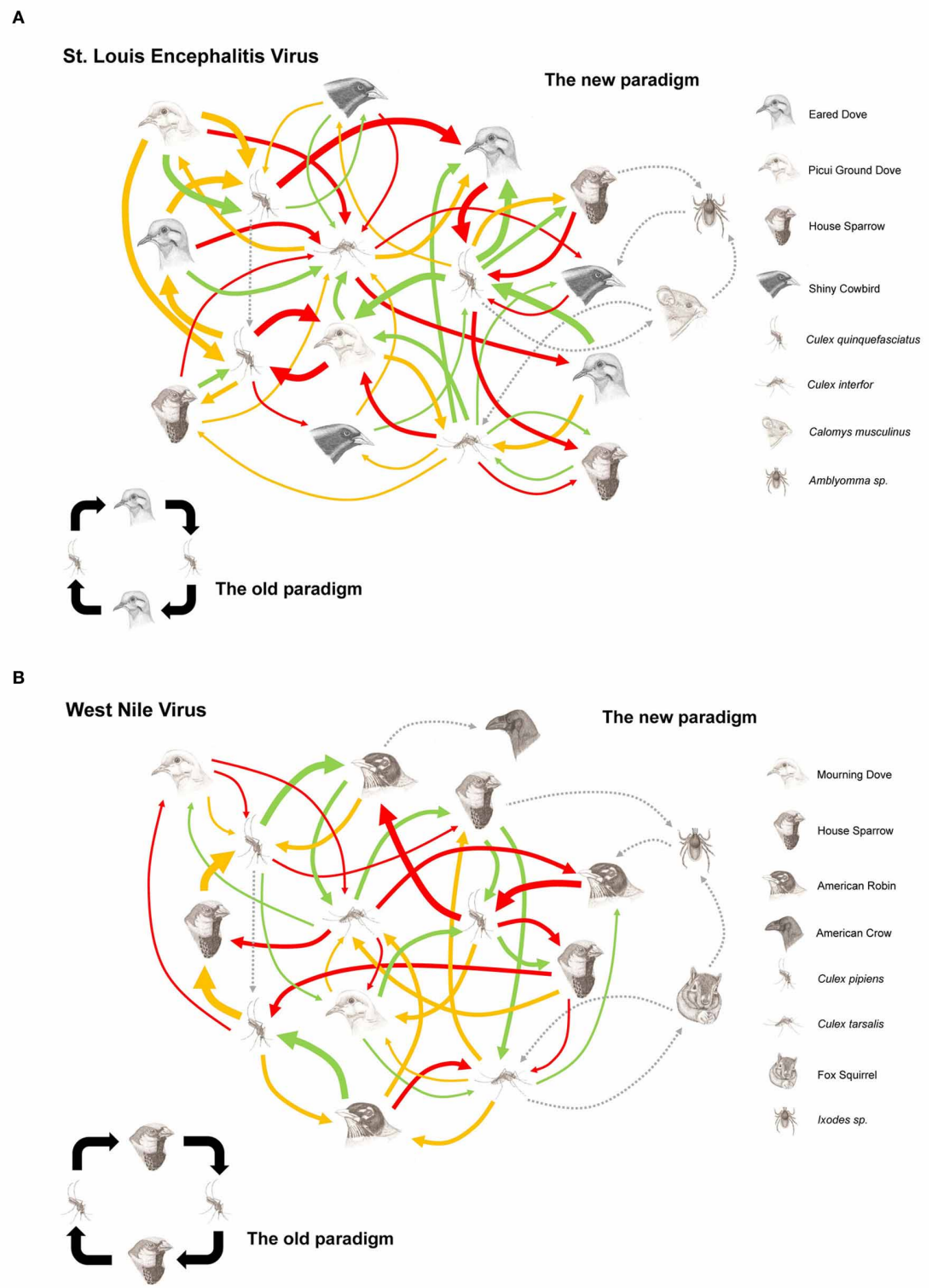

FIGURE 2 | Hypothetical transmission networks for St. Louis encephalitis virus in central area Argentina (A) and West Nile virus in USA (B). Foot figure: The arrows represent the viral flow between the vectors and hosts involved in the arbovirus maintenance network. The thickness of the arrow represents the amount of existing virus between the particular connection of host and vector (which is determined by the vector host preference, vector-host population density, vector and host competence). The spotted line arrows represent alternative transmission way (venereal and/or transovarial transmission), hosts (mammals) and vectors (ticks). The colored arrows represent the season in which the vector-host relation takes place (green: Spring, red: Summer, orange: Fall).

[e.g., Great Horned Owl (*Bubo virginianus*), American Crow, Common Grackle, House Finch and House Sparrow, or direct transmission between partners (Ring-billed Gull, Blue Jay, Black-billed Magpie, and American Crow) (Komar et al., 2003)]. Apart from mosquitoes and birds being the key participants in the maintenance of the virus in nature, other animals such as fox squirrels (*Sciurus niger*) (Root et al., 2006) and ticks (*Dermacentor andersoni, D. variabilis, Ixodes scapularis*) (Anderson et al., 2003) might play a fundamental role.

A second example where the maintenance of an arbovirus in nature exceeds the vector-host cycle model of transmission is the SLEV flavivirus. This multi-host-vector virus is a reemerging close relative of WNV and JEV, and it is exclusively distributed in the American continent. SLEV is maintained through the biological transmission among *Culex* spp. mosquitoes and Passeriformes and Columbiformes hosts (Reisen, 2003). Since many avian host and *Culex* mosquito vector species can transmit this virus, it can be consider as a generalist. For example, in California, at least three species of *Culex* mosquitoes (*Cx. tarsalis*, *Cx. stigmatosoma,* and *Cx. quinquefasciatus*) can transmit the virus to different passerines birds (House Sparrow, House Finch). However, in most part of the USA (center and eastern) it is mainly maintained by the House Sparrow and *Culex quinquefasciatus*. In contrast, in Argentina the House Sparrow has an insignificant role in the maintenance of the virus in nature. Host competence studies have shown that in Argentina the Eared Dove (*Zenaida auriculata*) and the Picui Ground Dove (*Columbina picuí*) are the principal amplifying hosts. However, despite their lower viremia, other species (the House Sparrow, the Spotted Winged Pigeon—*Patagioenas maculosa,* the Shiny Cowbird—*Molothrus bonariensis*, the Bay Winged Cowbird—*Agelaioides badius*), replicate the virus with a titer high enough to infect the *Cx. quinquefasciatus* vector (Diaz, 2009). With regards to vectors, SLEV has been found in the mosquitoes *Ae. aegypti*, *Ae. albifasciatus*, *Ae. scapularis*, *An. albitarsis*, *Cx. apicinus*, *Cx. interfor*, *Cx. quinquefasciatus*, *Psorophora ferox* (Díaz et al., 2012). However, certain field and laboratory assays strongly suggest that *Cx. quinquefasciatus* and *Cx. interfor* are the main SLEV vectors (Diaz et al., 2006; Diaz, 2009).

As seen for WNV, vertical transmission has been observed for SLEV. This mechanism could explain the permanence of the virus in nature (Flores et al., 2010; Díaz et al., 2012). A hypothetical alternative transmission cycle for SLEV can be thought to exist between *Calomys musculinus* and *Mus musculus* rodents and mosquitoes such as *Ae. albifasciatus, Ae. scapularis,* and *Cx. quinquefasciatus* (Sabattini et al., 1998; Diaz, 2009).

Resuming, the scheme of biological interaction between vectors, host, and viruses is complex. These intricate networks of interactions would favor a given virus with a greater stability against biological and adverse environmental conditions (e.g., population declaims of one of host and vector). The existent strength of viral-flow cycles between the vectors and host conforming the network (or degree of association) are affected by environmental and climatic factors that vary in time and space (**Figures 1** and **2**). Given the inherent complexity of the vectorial transmission among hosts, it is necessary to carry out ecological and epidemiological research for each epidemical event.

The mechanisms for the maintenance of generalist arboviruses are inherently complex, and should be taken into account, and incorporated, when designing and constructing mathematical models that allow us to predict its enzootic/epidemic activity in a given ecosystem (wild, urban, agricultural, etc.). By facing these new designs from an integral, networkable perspective we can improve our predictive ability. The ultimate goal will be to improve our understanding of viruses' dynamics and thus improve preventive measures in vector-control and public health policies.

IN CONCLUSION

Arbovirus maintenance in nature depends on host and vector coexistence in time and space. From an ecosystemic approach of transmission cycles, it is unlikely that the maintenance of a virus is restricted to determined vectors and hosts, particularly when considering a multi-host-vector virus, like certain arboviruses (e.g., SLEV, WNV). Therefore, the determining factors for the maintenance of an arbovirus in nature are the intertwined biological links that integrate a transmission network rather than a transmission cycle. Consequently, to better understand the activity pattern and transmission networks of arboviruses, it is fundamental to understand the species assembly of hosts and vectors, their interactions, and fluctuation through time and space.

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