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INDENTIFYING AND MODELING THE DISTRIBUTION OF CRYPTIC RESERVOIRS OF EBOLA VIRUS USING ARTIFICIAL INTELLIGENCE

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Running title: Distribution of Cryptic Reservoirs of Ebola Virus

ABSTRACT
Fruit bats (Megachiroptera) have been found to be the principal reservoirs of Ebola virus (EBOV) to humans. However, bats do not appear to be the primary reservoir in the environment and between outbreaks. The cryptic reservoir species of EBOV and its distribution have not been identified. The purpose of the study was to identify the most likely cryptic reservoir species of EBOV and the probable distribution of cryptic reservoir species where EBOV could be maintained in Sierra Leone. The Bioagent Transport and Environmental Modeling System (BioTEMS) was used to analyze mammals, arthropods, plants and protists in order to identify the most likely species to be the cryptic reservoir for EBOV. ArcGIS and BioTEMS were used to determine the probable distribution of cryptic reservoir species. BioTEMS identified free-living pathogenic amoebae (FLPA) as the probable cryptic reservoir species (Test Performance = 93.3). Diptera in the order Chrysops were also identified as possible secondary reservoirs and mechanical vectors of EBOV. Distribution of likely hot spots for FLPA and phytotelmata/tree-holes were identified in several regions of Sierra Leone, primarily in the southeast and are similar to those predicted by other authors, but at a much higher resolution (15 m for BioTEMS verses up to 5 km in other studies). Water-filled cavities (phytotelmata), specifically tree-holes, were identified as the most likely sites for the cycle of transmission to occur among FLPA and susceptible secondary reservoirs. Free-living pathogenic amoebae are not only pathogenic to humans and animals but they serve as reservoirs and Trojan horses of infection as well. Identifying what and where cryptic reservoirs of EBOV persist between outbreaks provides an opportunity for the first time to conduct environmental epidemiologic surveillance to mitigate outbreaks and to test anti-microbial delivery systems such as the ProVector® to reduce EBOV and FLPA.

Keywords: Filovirus, Amoeba, Epidemiology, Machine Learning, Vector, Disaster Management

IDENTIFICATION ET MODÉLISATION DE LA DISTRIBUTION DES RÉSERVOIRS DU VIRUS EBOLA CRYPTIQUE EN UTILISANT L'INTELLIGENCE ARTIFICIELLE

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Runimg Email Titre : Distribution de réservoirs cryptique du virus Ebola

Résumé
Des chauves souris (Megachiroptera) ont été trouvés à être les principaux réservoirs du virus Ebola (EBOV) à l'homme. Cependant, les chauves-souris ne semblent pas être le réservoir principal de l'environnement et entre les poussées. Les espèces réservoirs cryptique du virus Ebola et de sa distribution n'ont pas été identifiés. Le but de l'étude était d'identifier les plus susceptibles d'espèces réservoirs cryptique EBOV et la distribution probable des espèces réservoirs cryptique où EBOV pourrait être maintenu en Sierra Leone. La modélisation des transports et de l'environnement Bioagent Système (BioTEMS) a été utilisé pour analyser les mammifères, les arthropodes, les plantes et les protistes afin d'identifier les espèces les plus susceptibles d'être le réservoir pour EBOV cryptique. ArcGIS et BioTEMS ont été utilisés pour déterminer la distribution de déterminer l'évolution probable des espèces réservoirs.

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BioTEMS a identifié des amibes pathogènes (FLPA) comme le probable espèces réservoirs cryptiques (Test Performance = 93,3). Dans l'ordre des Diptères Chrysops ont également été identifiées comme vecteurs mécaniques secondaires possibles de EBOV. La distribution des points chauds susceptibles de FLPA et phytotelmata/arbre-trous ont été identifiés dans plusieurs régions de la Sierra Leone, principalement dans le sud-est et sont similaires à ceux prévus par d'autres auteurs, mais à une beaucoup plus haute résolution (15 m pour BioTEMS les versets jusqu'à 5 km dans d'autres études). Cavités remplies d'eau (phytotelmata), en particulier les trous d'arbres, ont été identifiés comme sites les plus probables pour le cycle de transmission de se produire entre FLPA et sensible des réservoirs secondaires. Des amibes pathogènes ne sont pas seulement des pathogènes pour l'homme et les animaux, mais ils servent de réservoirs et les chevaux de Troie de l'infection. Identifier ce qui et où les réservoirs de EBOV cryptique persistent entre les poussées est l'occasion pour la première fois d'effectuer la surveillance épidémiologique de l'environnement pour atténuer les épidémies et de tester les systèmes de prestation anti-microbiens tels que le ProVector® pour réduire EBOV et FLPA.

Mots-clés: Filovirus, amibes, l'épidémiologie, de l'apprentissage machine, scénario, la gestion des catastrophes

INTRODUCTION

The first cases of Ebola virus (EBOV), family Filoviridae, appeared in 1976, causing acute and often fatal illness. There have been five species described, with the Zaire EBOV having the highest rate of fatality, 88% (1). Since its discovery, there have been over 31,000 cases and nearly 13,000 deaths attributed to EBOV, most of which occurred in the latest outbreak in 2014-2016 (2). The index case for the 2014-2016 outbreak was traced to a boy living in Meliandou, Guinea who was most likely playing near a large harboring bat (2,3). From Guinea it spread to Sierra Leone, Nigeria, Liberia, Nigeria, Senegal, Spain, the United States and Mali, with Sierra Leone experiencing the most cases and the second highest number of fatalities. In a retrospective epidemiologic study, EBOV was introduced into Sierra Leone by a woman who had visited the home of the index case in Meliandou (4). Sierra Leone is within the area modeled where the mammalian reservoir species, e.g. fruit bats, and environmental conditions are suitable to sustain EBOV transmission but no zoonotic transmission has been recorded.[5] However, the identity of the cryptic reservoirs of EBOV and where these infected reservoirs are located have not been modeled in Sierra Leone or in other areas where EBOV may persist.

Great progress has been made in understanding the pathogenesis and molecular biology of EBOV; however, the maintenance and transmission of EBOV in the environment remains unclear (6,7). In a review of animal sampling, suggested four strategies which should be undertaken for the purposes of identifying reservoir sources and to isolate suspected virus in an animal outbreak; 1) surveillance of free-ranging non-human primate mortality and morbidity,2) investigation of every wildlife morbidity or mortality event as this may hold the most promise for locating virus or viral genome sequences, 3) surveillance of suspected bat species to detect evidence of exposure, and 4) prioritization of dogs and pigs for testing, including morbidity, mortality, and serology to detect virus or previous exposure (8). Pourrut noted that human outbreaks and animal mortality do not appear to be reliable indicators of the presence of the virus in a region, and suggested antibody detection may serve as a better epidemiological indicator of the presence of EBOV in an area (9).

The three bat species suspected of serving as the primary reservoirs of EBOV are in the suborder (Megachiroptera); *Hypsignathus monstrosus, Epomops franqueti*, and *Myonycteris torquata;* other mammals may become infected when eating fallen fruit contaminated with saliva or feces from infected bats (10). Insectivorus bats (Microchiroptera) may also serve as reservoirs of EBOV in West Africa (1). The source of EBOV infection in bats has not been determined. The conditions of EBOV transmission from bats to great apes or even to humans are also unknown; environmental factors, animal demography and viral factors may all contribute to the outbreak of EBOV, e.g. during dry seasons when great ape mortality increases from EBOV, two of the three primary bat species give birth, and when apes and bats compete for scarce fruit.[9] Species positive for EBOV and/or EBOV antibodies, polymerase chain reaction (PCR) and isolation have been identified in several mammal orders: Primates, Chiroptera, Artiodactyla, Carnivora and Rodentia (5,8,11).

Species other than mammals have been hypothesized as possible reservoirs for EBOV. Monath suggested possible non-mammalian reservoirs of EBOV could include plants, blood-feeding and other arthropods, and that step wise mutation may be an alternative to transmission through a cryptic reservoir (12). Despite a relatively long search for the origin of Ebola viruses, their reservoirs remain elusive; including origin and ecology of Ebola viruses. In a study of Marburg virus, over 2,000 insects were tested by PCR using a Filovirus specific primer set; all were negative (13). Leendertz recommended biologically plausible hypotheses of persistence and transmission be developed that may not align with current dogma; suggesting EBOV emerges in mammals when the precursor virus jumps from mayflies or other riverine insects to insectivorous bats, made possible by the thermodynamics of the digestive system of insectivorous bats (14). In a thermodynamic model, to support the possibility emphemeral insects may serve as reservoirs Gale proposed the following; 1)

the evidence that arthropods are refractory is not definitive, 2) a combination of filovirus filament length and the high temperature of the insect virus once ingested by a flying bat, in combination with the large number of insects eaten by bats (e.g. during an ephemeral mass emergence of mayflies), facilitated jumping the species barrier, 3) phospholipid phosphatidylserine in the virus envelope promotes filament formation through fusion of single glycoprotein (Gc) particles within the ingested insect within the high temperature of the bat, increasing their infectivity to bats, and 4) increasing the temperature from 27°C to 42°C could increase the affinity of the filaments for bats, while having no effect on the binding affinity of the single Gc virions (15).

Despite decades of research and thousands of animals tested and over 40 years of research, monitoring of EBOV is limited or impossible due to the paucity of knowledge about its ecology e.g. when, where, and how EBOV circulates in the environment **(1,16).** The exact reservoir species that harbors the virus is not determined, although most wildlife epidemiologic efforts have been focused on fruit bats**(17).** Future sampling campaigns, in-depth serological studies, and modeling efforts should take into account the possibility that fruit bats may not always be the ultimate source of EBOV outbreaks.[1] Where EBOV survives between outbreaks and replicates is unknown; this unknown species is defined as the cryptic reservoir of EBOV. There are two opposing hypotheses for the emergence of EBOV, the first is long-term local persistence in a cryptic and infrequently contacted reservoir; the second is a recent introduction of EBOV with spreading through susceptible populations **(6).** Here we present a hypothesis that free-living pathogenic amoeba (FLPA) serve as the cryptic reservoir of EBOV, with susceptible mammalian species and possibly invertebrate reservoirs becoming infected through contact with FLPA in the environment, most likely via phytotelmata. Also presented is the probable distribution cryptic reservoirs where EBOV could be introduced and maintained in Sierra Leone.

MATERIAL AND METHODS

Biotic and abiotic factors were analyzed in order to determine the location of the probable cryptic reservoir species. Niche analysis of protists, mammals, arthropods and plants was conducted to identify the most likely cryptic reservoirs of EBOV. ArcGIS geospatial analysis software, Statistica statistical software, and the Bioagent Transport and Environmental Modeling System (BioTEMS) were used to analyze geographic information and conduct data analysis. BioTEMS utilizes up to several hundred abiotic and biotic factors to produce risk and vulnerability assessments for biological agents and infectious diseases. Examples of biotic and abiotic factors include pathogen strain, nucleic acid sequences, vector/host relationship, vectorial capacity, host/vector physiology, colonization ability, population dynamics of hosts and vectors, plants species, soil, and weather conditions, such as wind, temperature, precipitation, and shade. Analytical methods within BioTEMS include artificial intelligence, fuzzy logic, niche analysis, Baysian and general additive regression. The BioTEMS consists of a set of algorithms and models that have been used as a stand-alone system for risk assessments of bioagents, infectious diseases, vectors, and to supplement HPAC. BioTEMS has been used to analyze risk of selected bioagents and/or to optimize placement of Biological Integrated Detection Systems (BIDS) units at military installations within the US and overseas, during national and international training exercises, national political conventions and to assist the Defense Threat Reduction Agency during a presidential inauguration. The BioTEMS risk assessment maps (RAMS) have also been developed for several infectious and zoonotic diseases and distribution of vectors e.g., Zika, West Nile and Eastern Equine Encephalitis viruses, Lyme disease, Rocky Mountain spotted fever, avian influenza, plague, *Shigella*, tularemia, mosquito, tick and mite species. The BioTEMS RAMS have been produced for several countries, including Bangladesh, Brazil, Cameroon, China, India, Iran, Libya, Georgia, Turkey, United Arab Emirates, Sierra Leone, and several cities in the US (Kollars in press). BioTEMS was used in the present study to analyze abiotic and biotic factors to identify the location of sites of hot spots for cryptic reservoirs of EBOV and to conduct niche analysis to determine likely reservoir species of mammals, arthropods, plants and microbes. BioTEMS and ArcGIS were used to identify locations of hot spots and validate the model using geographic coordinates of published EBOV cases, isolations and other published models of EBOV.

RESULTS AND DISCUSSION

BioTEMS identified phytotelmata/ tree holes as the principal microhabitat for cryptic reservoir species of EBOV, and where long term survival of EBOV between outbreaks and the cycle of EBOV transmission occurs. BioTEMS identified free-living amoeba, specifically free-living pathogenic amoeba (FLPA) species of *Acanthamoeba*, *Naegleria* and *Balmuthia*, as the most likely cryptic species of EBOV; other FLA such as *Hartmanella* and *Dictyostelium* may also serve as reservoirs. Transmission in tree holes is predicted to occur among FLA and secondary reservoirs, principally fruit bats, secondarily in primates, and possibly arthropods (Test Performance = 93.3). Distribution of likely hot spots for FLPA and phytotelmata/tree-holes are shown in Figure 1.

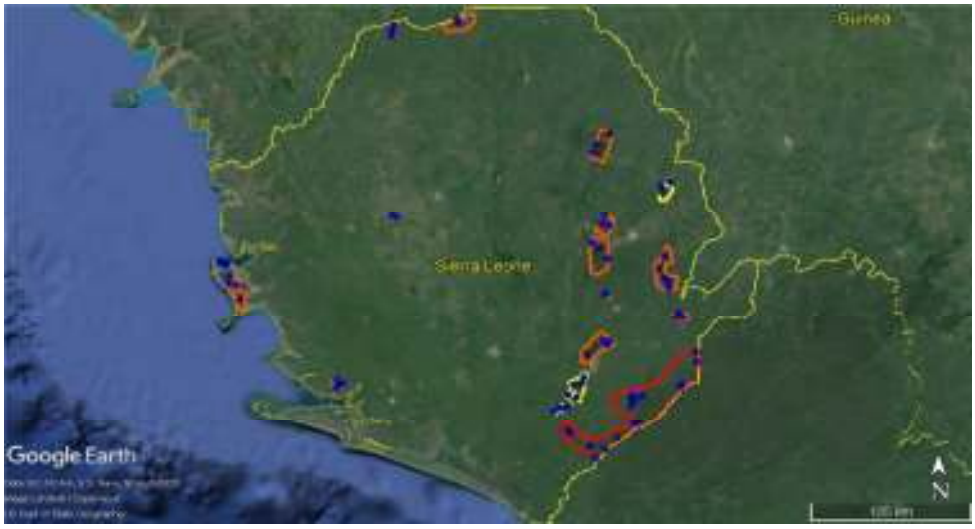

FIGURE 1 BIOTEMS RISK ASSESSMENT MAP OF EBOV AND FLPA IN SIERRA LEONE (RED POLYGON- HIGHEST RISK OF ENVIRONMENTAL SURVIVAL OF EBOV
Orange polygon- moderate risk of EBOV, Yellow polygon-lower risk of EBOV; blue circles identify recommended sites for EBOV surveillance and environmental sampling.

The predicted sites for the cryptic species are similar to those predicted by other authors, but at much higher resolution than most (15 m for BioTEMS verses up to 5 km in previously published models.[5, 18,19] BioTEMS identified species within the genus *Chrysops* as a secondary reservoir and mechanical vector of EBOV. It is unclear whether EBOV can replicate within arthropods. One laboratory study demonstrated replication of Marburg virus, another Filovirus, in the mosquito *Aedes aegypti*; other studies failed to demonstrate EBOV replication in *Aedes aegypti* (20,21). A lesion from a fly or spider bite coincided with a patient with Marburg virus (22). Whether arthropods can mechanically transport EBOV has also not been demonstrated. Laboratory trials indicated low potential for mechanical transmission of EBOV by house flies under the conditions tested, but repeated expsosure from EBOV/fly exposures could reach the 100 plaque forming units/ml threshold necessary to result in mucosal transmission (23).

In addition to vertebrates (proven) and invertebrates (hypothesized) as reservoirs or mechanical vectors for EBOV, we suggest free-living pathogenic amoeba (FLPA) are the most likely cryptic and maintenance reservoirs of EBOV. That BioTEMS accurately predicted FLPA as the cryptic reservoir is supported by biological and environmental evidence; 1) FLPA act as suitable hosts for invasion and replication for several pathogens (bacteria and viruses), 2) FLPA can vector pathogens into hosts, 3) FLPA infect vertebrates and invertebrates 4) FLPA protect pathogens from harsh environmental conditions, 5) FLPA are effective air-borne colonizers of micro-habitats, 6) FLPA can survive harsh environmental conditions for decades, 7) FLPA undergo blooms when environmental conditions are right, and 8) some species of pathogens become more pathogenic when passaged with FLPA. Figure 2 demonstrates the life cycle during wet and dry periods. During suitable environmental conditions, some FLPA species such as *Acanthamoeba* are in the amoeba form, other species, e.g. Naegleria can exist as an amoeba or a flagellate form. During harsh environmental conditions the FLPA can encyst in order to survive the inhospitable conditions of their microhabitat.

Ebola viruses (EBOV) are enveloped RNA viruses that infect cells through a pH-dependent process mediated by viral glycoproteins involving endocytosis of virions and their routing into acidic endosomes (24,25). Host cell factors used by EBOV to invade macrophages include Mer, integrin, and NPC1 are required for efficient GP-mediated transduction and EBOV infection of macrophages (26). Perhaps these same mechanisms enable EBOV to infect free-living amoebae (FLA). Free-living amoebae are virtually free-living macrophages living in the environment. FLPA may be more suitable reservoirs than closely related non-pathogenic species/strains due to cell membrane composition. For example, *Naegleria fowleri* demonstrated a higher concentration of integrin-like proteins than *N. lovaniensis* (27). Perhaps, the integrin-like protein in *N. fowleri* enables binding and entry of EBOV. Several bacteria and virus pathogens are able to invade and replicate within FLPA and during replication they are protected from harsh environmental conditions and even anti-microbials when the FLPA encyst The(28,29). cyst of FLPA can stay viable for at least 20 years, (30) and we suggest, serve as a maintenance reservoir for EBOV. Although often temporal, tree holes can serve as

exceptional aquatic micro-ecosystems for several microbial species and are hotspots for microfungi (31). Protists feed upon micro-fungi and bacteria and have been found in phytotelmata ecosystems, on tree limbs and llianas (32,33). Larval mosquito species decrease several forms of protists in tree holes, such as ciliates; however, cyst stages become more abundant (34). Cyst forming amoebae appear to be very effective colonizers of aquatic habitats, and have even been isolated in the atmosphere.[35] *Naegleria fowleri*, the etiologic agent of primary amoebic meningo-encephalitis is found in soil, water, wastewater, and biofilms, even on rough top tanks (36). Temperature and bacterial density are positively correlated with the population density of *N. fowleri* biofilms (37). In addition to air-current transport of infected FLA cysts, mechanical transport of infected cysts or secretions from infected reservoirs, could deposit material in tree-holes, thereby infecting FLPA inhabiting the tree hole and secondary hosts utilizing resources in the treehole.

Among the FLA, only species within four genera, *Acanthamoeba, Naegleria, Balamuthia and Sappinia,* are responsible for opportunistic and non-opportunistic infections in mammals (38,39). Infected animals can potentially contribute in spreading pathogenic *Acanthamoeba* in homes by contaminating the domestic environment; bedding, fur, cornea and tissue may be infected with FLPA (40). Free-living pathogenic amoebae are also able to infect insects, such as locusts, mosquitoes, and biting flies. Larvae of syrphid fly larvae, *Eristalis tenax*, may have been infected with Marseillevirus either by direct ingestion or through the ingestion of an infected free-living pathogenic amoeba (41). *Acanthamoeba* can invade and cause disseminated infection within insects likely providing a vehicle for bacterial and viral pathogens to be disseminated as well (42). *Mycobacterium* species, a pathogen that replicates in several free-living amoeba species, can be mechanically transmitted by Syrphid flies to pigs (43). *Mycobacterium ulcerans*, the etiologic agent of Burndi ulcer, is acquired from the environment, but the exact mode of transmission remains a mystery. *Mycobacterium ulcerans* presents an example of a pathogen that may be transmitted by free-living pathogenic amoeba or through an insect vector. Laboratory trials demonstrated ICR mice were infected by M. ulcerans through passive infection through skin abrasions, and co-culturing of M. ulcerans–with AP enhances pathogenesis (44).

In Australia, *M. ulcerans* was detected by PCR in several species of mosquitoes during peak transmission time, and the hypothesis was proffered that transmission by mosquitoes offers a partial explanation for outbreaks in southeastern Australia (45). Viruses and bacteria are often isolated from the same soil and water environments as are amoebae, and experimental models using *Acanthamoeba spp.* and *Dictyostelium discoideum* and other FLA have demonstrated these organisms are resistant to digestion by free living amoeba, are able to replicate and the FLA are a training ground for pathogens (46,47).

Several studies have demonstrated the ability of FLPA to infect mammals. Contact with *Naegleria* species by wild mammals was demonstrated for the first time with the discovery of antibodies to FLA in several mammalian species with variation among species and age groups (48). Several species of primates have been infected by *Balmuthia*; the mandrill (*Papio sphinx*), white-cheeked gibbon (*Hylobates concolor*), a western lowland gorilla (G*orilla gorilla*), Kikuyu colobus monkey (*Colobus guereza*) (49). In zoos in America, infection with *B. mandrillaris* has been reported to account for 2.8% of captive gorilla deaths in North America over the past 19 years (50). In Africa, *Balmuthia* and *Acanthamoeba* have been found in humans living in the Tai Forest area of Côte d'Ivoire, near the Liberian border; interestingly the prevalence of *Balmuthia* was correlated with hunting activity and age in this area where hunters may have become exposed to *Balmuthia* through handling bloody meat or exposure to the environment through cuts and abrassions (51). *Balmuthia* and *Acanthamoeba* are capable of infecting through abrasions in the skin (39). *Acanthamoeba, N. fowleri*, and *B. mandrillaris* have been isolated from water bodies from Guinea-Bissau, and this is the first report of the isolation of *B.mandrillaris* from environmental sources in Africa (52). *Naegleria* and *Acanthamoeba* species have also been isolated from Benin (53). *Acanthamoeba* and *Naegleria* species have been isolated from the Queen Elizabeth Park in the same area of western Uganda, where the first outbreak of Bundibugyo EBOV occurred in western Uganda.[54,55] This does not mean that there is causation, however, it does demonstrate the wide dispersion of FLPA in areas where EBOV is present at least temporarily. There would seem to be an opportunity for EBOV to interact with FLPA in the environment and within infected mammals (Figure 2).

FIGURE 2 PHYTOTELMATA (SUCH AS TREE HOLES) SERVE AS SITES FOR INTERACTION AMONG CRYPTIC RESERVOIRS (FREE-LIVING PAGHOGENIC AMOEBAE), SECONDARY RESERVOIRS (SUCH AS BATS, PRIMATES, RODENTS) AND POSSIBLY MECHANICAL VECTORS (*CHRYSOPS* SPECIES), ALLOWING EBOLA VIRUS TO SURVIVE BETWEEN OUTBREAKS, REPLICATE AND INFECT SECONDARY RESERVOIR
(Illustrated by Kathryn E. Kollars)

Reperant, suggested the application of the theory of island biogeography, developed by MacArthur and Wilson, to emerging pathogen epidemiology to identify: 1) interactions among recipient host species, reservoirs and vectors, 2) intraspecific interactions of reservoir interactions, and 3) mechanism driving disease emergence within host species (56,57). Application of the theory of island biogeography to emerging pathogens may assist in predicting from which animal species future zoonotic and vector-borne pathogens are most likely to emerge and to identify arthropod/parasite/host interactions and distribution (56,58). Habitat islands, created by human activity, have a major impact on biodiversity (59). Forest fragmentation creates habitat islands associated with EBOV. Spillover from wildlife reservoirs of EBOV to humans occurred mainly in hotspots of fragmented forest and reduction of closed forests (60,61).
Tree holes can be described as islands in the forest; the diversity of microbial communities utilizing tree holes can vary depending on size, and tree holes in fact serve as island reservoirs for species, protecting them from a hostile environment (62,63). Tree holes are often created when trees are damaged, and logging often damages adjacent trees. Free-living amoebae thrive in islands of fertility, were water penetrates and remains in gaps (64). Fruit bats and other bat species will use tree holes as roosting places in West Africa (65,66). Monkeys and apes use tree holes as a source for drinking water, during habitat-specific and seasonal water shortages (.67)

Host and spatial heterogeneity are linked and management strategies can be developed to quantitatively assess the contribution of habitat patches, pathogens, and reservoirs to cycles of transmission (68). Niche models are also used to identify patterns of pathogen distribution. Peterson stated that limitation using GARP niche models may occur due to current small sample sizes and ecologic dimensions of the model that do not capture other dimensions that may be more important, such as climate variability.[18] There is host specificity in the filovirus host-parasite system with patterns of cophylogeny and codistribution and they survey for EBOV; 1) African EBOV reservoirs would be primarily be found in evergreen broadleaf forest, 2) the main focus of the geographic distribution of the reservoir(s) would be in the Congo Basin; 3) a disjunct distributional area would be present in West Africa; 4) a related taxon in eastern Africa would range in more arid habitats; 5) the reservoir would belong to a clade more broadly distributed across Africa and Southeast Asia(18). BioTEMS identified the probable cryptic reservoirs of EBOV and was used to map its potential distribution between and during outbreaks.

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Conclusion

BioTEMS has been used to accurately predict the presence and distribution of infectious diseases, vectors and to develop risk assessment maps for bioagents at military installations (69). Several members of the FLPA fit the characteristics of the prioritized EBOV reservoir; 1) protists are found in high density in phytotelmata, 2) Chiroptera may play a critical role in distribution of infected FLPA as well as infecting FLPA in the Congo Basin, when roosting or drinking from tree-holes, 3) phytotelmata are microhabitat islands within the fragmented forest islands that would serve as abundant and optimal habitats for the cycle of transmission between FLPA and secondary reservoirs, and 4) FLPA are found at higher densities in habitat islands in arid habitats, and 5) there is increased evidence of the broad distribution of FLPA in tropical environments. There is evidence that FLPA serve as the cryptic reservoir for EBOV; however this needs to be tested across western Africa by field sampling and in laboratory trials. Antimicrobial technologies, auto-disseminated through devices such as the ProVector®, may be utilized to reduce EBOV in FLPA in microhabitats where hotspots have been identified and to keep EBOV below the epidemiologic threshold, thereby reducing the risk of an EBOV outbreak. Additional studies should be conducted to determine the environmental epidmiologic threshold required for an EBOV outbreak in order to assist medical and public health officials in developing medical prevention and emergency response plans to reduce morbidity and mortality due to EBOV

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