Vector Hazard Report: Mosquitoes and Ticks of Djibouti

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Introduction

The nation of Djibouti It is located in the Horn of Africa, bordering the Gulf of Aden and the Red Sea, between Eritrea and Somalia. Slightly smaller than the state of New Jersey, the area of Djibouti is approximately 23,200 km^2 (CIA, 2022). The country has 195 miles of coast line and there are eight mountain ranges, some with peaks over 2,000 meters tall (Sun & Zoubir, 2016). Djibouti is a highly significant location for international shipping and is adjacent to the world's most trafficked shipping lanes . As of 2021, Djibouti is also host to over 5,000 refugees from Yemen and over 10,000 refugees from Somalia (Navy, 2022). Djibouti is home to the only permanent U.S. military base on the continent of Africa, Naval Expeditionary Base: Camp Lemonnier. The Base supports approximately 4,000 U.S., joint and allied forces military and civilian personnel and U.S. Department of Defense contractors. Additionally, the base provides employment for approximately 1,000 local and third country nation workers (Navy, 2022). The People's Republic of China (PRC) continues to rival U.S. influence throughout in Africa, and maintains a military base in Djibouti with approximately 2,000 permanent personnel (Bath, 2022; Sun & Zoubir, 2021). Djibouti is considered one of the hottest inhabited places in the world with average day-time temperatures ranging from 29 °C (84 °F) during the winter months (December to February) and 40 °C (104 °F) during peak summer (September). The climate of Djibouti is described as extremely dry, mostly desert with an annual average rainfall of just over 12 inches (NOAA, 2022). Although extremely rare, tropical storms and cyclones that form in the Arabian sea can make landfall in Djibouti (NOAA, 2022). Camp Lemonnaire appears to be one of the hottest places in Djibouti. A recent study of historical heatwave data revealed that a monitoring station at Camp Lemonnier consistently recorded longer and hotter heatwaves than any other monitoring station in the country (Liu, 2019). The biodiversity of the Horn of Africa remains understudied and has experienced several invasive species introductions in recent years. Arthropods that spread disease are an obvious concern, but other organisms such as birds, reptiles and mammals play a significant role in disease cycles as well.

Mosquitoes of Djibouti

Although malaria is one of the most the most significant threats to force health protection (FHP) in Djibouti, the threat of arboviruses may be growing because of climate change (Rogers and Randolph, 2006; Altizer, et al. 2013). Changes to mosquito distributions and human population movement are expected to have major impacts to arbovirus transmission in Africa over the coming decades (Mordecai et al. 2020). Arboviruses also remain a significant threat as there are currently very few prophylaxis or vaccine protections available. There are at least 12 species of mosquitoes reported from Djibouti that can transmit arboviruses to humans (Wilkerson, Linton & Stickman, 2021). The most significant of these pathogens include, Chikungunya (CHIKV), dengue (DENV), Japanese encephalitis (JBEV), Rift Valley fever (RVFV), sindbis (SINV), West Nile (WNV) and yellow fever (YFV) viruses (Rodhain, et al. 1977). WNV is considered endemic to Djibouti with *Culex* spp. playing the most significant roles in human transmission (Faulde, Spiesberger, & Abbas, 2012). Previous studies have found that *Cx*. *pipiens* (As ssp. *torridus*) is present in most urban areas where WNV transmission occurs, while *Cx*. *quinquefasciatus* is mostly associated with peri-urban and rural environments (Faulde & Ahmed, 2010; Harbach, 2012). A seroprevalence study conducted in Djibouti in 2014 found that 20% of study participants had antibodies to dengue virus (Andayi et al. 2014). Andayi (2014) also found low levels of antibodies for CHIKV and RVFV in serum samples.

In 2014, the Asian malaria vector *Anopheles stephensi* Liston, 1901 was found in Djibouti, the first record of this species in Africa (Faulde, Rueda, & Khaireh, 2014). News of this discovery raised alarm bells across the continent as *An*. *stephensi* is a notoriously efficient vector of *Plasmodium falciparum* and *P*. *vivax* in urban environments across its native range of Asia and the middle East (Dev, & Sharma, 2013; Sinka, et al. 2011). *Anopheles stephensi* specimens discovered in Ethiopia in 2018, provided molecular evidence that the introduction was likely from Pakistan. When compared to molecular evidence from samples collected in Djibouti they were found to be related to a separate population also originating in Pakistan (Carter, et al. 2018). This species has since been found in Somalia, Eritrea and Sudan (WRBU, 2022). A recent study modeled the potential distribution of *An*. *stephensi* across Africa and predicted its range could extend to include all densely populated areas on the continent, putting millions of people at risk and setting back years of malaria eradication efforts (de Santi, 2021). Djibouti had largely been declared malaria free in the years leading up to the discovery of *An*. *stephensi* (Faulde, 2014). The extremely hot, dry climate did not support many of the native Anophelines who prefer, natural water sources in rural/ peri-urban areas as oviposition sites. However, only a few years after the initial discovery, Djibouti has seen its first reported autochthonously acquired case of *P*. *vivax* malaria with case numbers steadily rising year over year (Seyfarth, 2019). *Anopheles stephensi* is now driving additional malaria outbreaks across the Horn of Africa (Takken, & Lindsay, 2019; Tadesse, et al. 2021).

Ticks of Djibouti

Ticks transmit a variety of infectious diseases among humans and animals in Northeast Africa. Native and invasive tick species ranges are also changing, further complicating the tick-borne disease (TBD) risk landscape in the region. Despite being a significant threat within the Horn of Africa, the tick fauna and tick-borne diseases found within this area are largely uncharacterized. Such gaps in knowledge can impede attempts to prevent future outbreaks of TBD, which can have dramatic impacts on local economies and military operations. This is especially the case within Djibouti, which regularly experiences large human movements between the African continent and Arabian peninsula. In 2015 alone, around 100,000 people had migrated through or into Djibouti. Crimean-Congo Hemorrhagic Fever virus (CCHFV) and Alkumra (AHFV) viruses were first detected from ticks collected in Djibouti relatively recently (Horton, et al. 2016a). Originally described from Saudi Arabia in 1995, AHFV has since been detected in multiple Africa countries likely spread by a combination of mammalian and avian host species (Hoffman, et al. 2018). In the last 20 years, knowledge of what Rickettsial pathogens are circulating on the African continent and Djibouti specifically, has increased (Parola et al. 2001). *Rickettsia parkeri* (Spotted fever group rickettsiae (SFGR)) was first detected from ticks collected in Djibouti in 2007 (Socolovschi, et al. 2007). Since then, SFGR infected ticks have been detected in neighboring Chad and Ethiopia as well (Mura, et al. 2008). A 2016 study found slaughterhouse workers in Djibouti testing positive for *Rickettsia parkeri*, *R*. *africae* (African tick-bite fever)) as well as *Orientia tsutsugamushi* (Scrub typhus) infections (Horton, et al. 2016b). Of particular concern is the ability of some tick species for transovarial and trans-stadial transmission of *Rickettsia* spp. (Socolovschi, et al. 2009). Seroprevelance studies have also detected Tick-Borne Encephalitis virus (TBEV) in human patients throughout the Horn of Africa including Djibouti (Andayi, et al. 2014; Im, et al. 2020). Finally, transmission of *Coxiella burnetii* (Q-Fever) has also been detected in serological studies of humans in Djibouti, however transmission appears to be primarily occurring via contaminated goat, sheep and camel meat (Devaux, et al. 2020). How significant the role ticks are playing in the transmission of Q-Fever directly to humans remains unclear.

The only publication focused exclusively on documenting tick fauna in Djibouti was completed over 50 years ago by Hoogstraal (1953). At that time, he reported the results of a brief survey from 5 locations in and around Djibouti city reporting a total of 13 species (Hoogstraal, 1953). However, these collections were conducted during July when rainfall is especially low, which may have limited the numbers of ticks. Others have made contributions to the species list for Djibouti, most notable Walker (2003) who compiled a list of 28 tick species known to occur in the Northwest region of Africa including Djibouti, Ethiopia, Eritrea and Somalia. The most comprehensive list of global tick species identifies which tick species have an Afrotropical distribution, but does not provide a comprehensive checklist for each country (Guglielmone, et al. 2014). Specimens deposited in the United States National Tick Collection (USNTC) and collected in Djibouti were used to redescribe all life stages of the CCHFV vector *Hyalomma impeltatum* Schulze & Schlottke, 1930 and *Hyalomma somalicum* Tonelli Rondelli, 1935 (Apanaskevich & Horak, 2009). However, a full accounting of all USNTC specimens from Djibouti is not publicly available.

Historically, more research attention has been focused on hard ticks rather than soft ticks and Djibouti is no exception. This is likely due to the difficulty in collecting soft ticks and a lack of expertise to accurately determine species. To date, *Ornithodoros savignyi* Audouin, 1827 is the only soft tick reported from Djibouti, this is almost certainly an incomplete picture of all soft tick fauna (Mouchet, 1971). A survey conducted in neighboring Ethiopia, found 33 species of ticks associated with domestic livestock, with *Amblyomma variegatum*, found to be the most common species detected

(Pegram, Hoogstraal, & Wassef, 1981). *Amblyomma variegatum* is known to frequently parasitize humans and is capable of transmitting CCHFV and *Rickettsia africae* (Horton, et al. 2016; Guglielmone and Robbins, 2018). In addition, there is evidence that slaughterhouse workers in Djibouti have been exposed to *Rickettsia* and *Orientia* infections while processing meat (Horton, et al. 2016). Of particular concern regarding ticks and domestic livestock, is the widespread trade of these animals across the continent providing a mechanism for introducing ticks to new environments (Socolovschi, et al. 2007).

Finally, it is important to note the taxonomic status of *Rhipicephallus sanguineus* s.s., commonly called the 'brown dog tick,' is not fixed (Nava, et al. 2015). As a result, this ticks widely reported geographic range is likely obscured by repeated misidentifications between at least 12 possible species comprising the *Rhipicephalus sanguineus* group (Sánchez-Montes, et al. 2021). Nevertheless, *Rh*. *sanguineus* is reported from multiple sources as occurring in Djibouti and given its status as a vector of CCHFV and Boutonneuse fever, it is important to include in this checklist. However, due to the high likelihood that at least some *Rh*. *sanguineus* records will ultimately be overturned as misidentifications, we reported all instances of this species from Djibouti as *Rh*. *sangunieus* s.l.

Materials and Methods

We relied on recently published global compendiums of tick and mosquito distributions as well as a systematic literature review to compile checklists of mosquito and tick species known to occur in Djibouti. We also developed a simple ranking system to prioritize entomological collections of vector species capable of spreading diseases that have the highest impact on FHP. Finally, we present a comprehensive list of tick and host associations compiled from peer-reviewed literature.

In 2021, the first compendium of all described mosquito species was published in over 30 years. This resource presents the current taxonomy, medical importance and bionomic profiles for all implicated vector species as well as their documented, country-level distributions (Wilkerson, Linton & Stickman, 2021). This publication was preceded in 2014, by a catalog of the hard ticks of the world, also detailing the current taxonomy, medical importance and global distribution for all described ticks (Guglielmone, et al. 2014).

As part of a larger study that included 6 East African countries (Chad, Djibouti, Ethiopia, Kenya, Tanzania and Uganda), a systematic literature review was conducted using PubMed, Scopus, Web of Science, and CABI online databases. We targeted peer-reviewed articles published between 1901-2020. Search terms were standardized and included terms [Ixodidae] or [Ticks] and [Djibouti]. A total of 83 unique articles were initially identified from Djibouti during the search but 68 were removed during the title and abstract review. The remaining 15 articles underwent a full review to identify high-quality, mappable distribution data. Of these, 8 articles met our inclusion criteria. Tick surveillance data were extracted to a MS excel template from articles capturing information related to tick species, location, collection method, associated hosts and pathogen testing results. Collection sites were georeferenced using coordinates reported by authors while named places were assigned coordinates from an online gazetteer (GeoNames, 2021; Wieczorek & Wieczorek, 2021).

The taxonomic status of reported species names was reviewed to update synonyms and invalid names (Guglielmone, Petney and Robbins, 2019; Wilkerson, Linton & Stickman, 2021). Collection data were formatted for inclusion in the VectorMap database under the map service TickMap (VectorMap, 2022). Additional tick collection data from the Horn of Africa were downloaded from the VectorMap website and combined with data generated during this literature review before analyses were conducted. Tables were constructed to identify unique mosquito and tick taxa reported from Djibouti and host associations were derived from collections made in Djibouti, Eritrea, Ethiopia and Somalia.

To prioritize entomological surveillance efforts in Djibouti, we consulted a study that developed an algorithm for evaluating investment decisions by ranking infectious disease threats to the US military (Burnette, et al. 2008). This system ranks infectious diseases by assigning each a Global Risk-Severity Index (GRSI), considering the expected incidence and severity of disease. GRSI values were presented as maximum, minimum and median scores that range from the highest priority (Malaria, Maximum GRSI= 8,188) to the lowest priority (St. Louis encephalitis, Maximum GRSI=

5). To produce a conservative estimate of risk, we chose to use only the maximum GRSI scores presented. Due to the wide range of GRSI values we chose to divide the scores into tiers scoring them 1-3 based on severity:

- Max GRSI 0-100= 1
- Max GRSI 100-1000= 2
- Max GRSI 1000+= 3)

Each pathogen is scored for each species of mosquito and tick reported from Djibouti, and scores are added together for a total pathogen score.

Tick surveillance targets are additionally ranked based on the frequency at which they parasitize humans. Another recent global compendium compiled a list of all known reports of ticks parasitizing humans, ranking this behavior as very rare to very frequent, including which life stages have been documented (Guglielmone & Robbins, 2018). We adapted this ranking system to provide additional depth to our prioritized list of tick species by scoring each species based on the frequency of parasitization and number of life stages known to feed on humans. The ranking system is as follows:

- Not collected on humans = 0
- Very rare = 1 (+1 for each life stage)
- Rare = 2 (+1 for each life stage)
- Sporadic = 3 (+1 for each life stage)
- Frequent = 4 (+1 for each life stage)
- Very frequent = 5 (+1 for each life stage)

Final parasitization scores are added to the total pathogen score for a final score that is used to rank each species. **Figure 1** summarizes this ranking process for *Am*. *variegatum*.

Figure 1:
Example of surveillance prioritization work-flow. Parasitism and pathogen scores are added for a final score.

Scientific Name	Associated VBD	Parasitism Frequency
Amblyomma variegatum Fabricius 1794	CCHFV, R. africae	Frequent, M, F, L

Parasitism Frequency	Parasitism Score	Prioritized Pathogen Association	Prioritized Pathogen Score	Final Score
Frequent, M, F, L **(4+1+1+1)**	**7**	CCHFV (Rank: 10, Max GRSI: 783); Rickettsioses (Rank: 22, Max GRSI: 282) **2+2**	**4**	**11**

Results

A total of 29 mosquito species are reported from Djibouti, of which 14 are identified as vectors of pathogens to humans. A prioritized list of mosquito taxa is presented in **Table 1**. The highest priority mosquito surveillance targets are arbovirus vectors *Culex bitaeniorhynchus* (vector of DENV, RVFV, SINV), *Cx*. *pipiens* (vector of RVFV, SINV, WNV), *Culex quinquefasciatus* (vector of RVFV, SINV, WNV), *Cx*. *tritaeniorhynchus* (vector of DENV, RVFV, WNV), and *Aedes vittatus* (vector of CHIKV, DENV, JBEV, WNV, YFV, ZIKV). A total of 21 tick species are reported from Djibouti, with *Rhipicephalus* spp. being the most reported, followed by *Hyalomma* spp. A single record for one *Dermacentor* spp. was identified but a species determination was not made available. We found no other reference to *Dermacentor* spp. in our reviews, however species of this genera are present throughout the rest of Africa, so it is very likely additional *Dermacentor* spp. are present in Djibouti. *Rhipicephalus camicasi* and *R*. *sanguineus* s.l. were the most reported species from Djibouti in the literature. See **Table 2** for a full checklist of species reported from Djibouti ranked by surveillance priority. The highest priority tick surveillance targets are *Amblyomma variegatum* (vector of CCHV and *R. africae*), *Rhipicephallus sanguineus* s.l. (vector of CCHFV and Boutonneuse fever) and *Rhipicephalus pulchellus* (vector of *Coxiella burnetii*). These species are prioritized based on multiple life stages that parasitize humans and their frequency, and the high GRSI values associated with the pathogens they transmit. Additionally, our literature search documented a total of 76 host animals that are associated with the 21 tick species reported from Djibouti. S**upplement Table 1** displays these host associations for all ticks reported from Djibouti. The host animals with the highest diversity of associated tick species are cattle (*Bos* spp.), sheep (*Ovis* spp.), goat (*Capra* spp.), and camels (*Camelus* spp.).

Figures

Table 1: Prioritized Checklist to the Mosquitoes of Djibouti

ScientificName	Feeds on Humans?	Associated VBD	Final Score
Culex bitaeniorhynchus Giles, 1901	Yes	DENV, RVFV, SINV	9
Culex tritaeniorhynchus Giles, 1901	Yes	DENV, RVFV, WNV	9
Aedes vittatus (Bigot, 1861)	Yes	CHIKV, DENV, JBEV, WNV, YFV, ZIKV	8
Culex pipiens Linnaeus, 1758	Yes	RVFV, SINV, WNV	8
Culex quinquefasciatus Say, 1823	Yes	RVFV, SINV, WNV	8
Aedes aegypti (Linnaeus, 1762)	Yes	CHIKV, DENV, ILHV, YFV, ZIKAV	7
Anopheles sergentii (Theobald, 1907)	Yes	*P. falciparum*, *P. ovale*, *P. vivax*, RVFV	7
Anopheles stephensi Liston, 1901	Yes	P. *falciparum*, *P. vivax*, *CHIKV*	7
Aedes caspius (Pallas, 1771)	Yes	RVFV, WNV	6
Anopheles gambiae Giles, 1902	Yes	P. falciparum, *ONNV*, *ILEV*	6
Culex univittatus Theobald, 1901	Yes	SINV, WNV	5
Anopheles arabiensis Patton 1905	Yes	*P. falciparum*, *P. vivax*	4
Culex sitiens Wiedemann, 1828	Yes	MOSV	1
Anopheles dthali Patton, 1905	Yes	None	1
Anopheles azaniae Bailly-Choumara, 1960	No	None	0
Anopheles dancalicus Corradetti, 1939	No	None	0
Anopheles harperi Evans, 1936	No	None	0
Anopheles rhodesiensis Theobald, 1901	No	None	0
Anopheles salbaii Maffi & Coluzzi, 1958	No	None	0
Anopheles turkhudi Liston, 1901	No	None	0
Culex laticinctus Edwards, 1913	No	None	0
Culex simpsoni Theobald, 1905	No	None	0
Culex tenagius van Someren, 1945	No	None	0
Culex thalassius Theobald, 1903	No	None	0
Culiseta longiareolata (Macquart, 1838)	No	None	0
Lutzia tigripes (de Grandpre & de Charmoy, 1900)	No	None	0
Mimomyia mediolineata (Theobald, 1904)	No	None	0
Mimomyia mimomyiaformis (Newstead, 1907)	No	None	0
Uranotaenia balfouri Theobald, 1904	No	None	0

Table 2: Prioritized Checklist to the Ticks of Djibouti

ScientificName	Associated VBD	Parasitism Frequency	Final Score
Amblyomma variegatum Fabricius 1794	CCHFV, *R. africae*	Frequent, M, F, L	11
Rhipicephalus sanguineus sensu lato	CCHFV, Boutonneuse Fever	Very frequent, M, F, N, L	11
Rhipicephalus pulchellus (Gerstäcker, 1873)	Q-fever	Sporatic, M, F, N, L	10
Hyalomma anatolicum Koch, 1844	CCHFV	Frequent, M, F	8
Hyalomma marginatum Koch, 1844		Very frequent, M, F, N	8
Hyalomma rufipes Koch, 1844	CCHFV, Tick typhus	Sporatic, M, F, L	8
Rhipicephalus turanicus sensu lato		Very frequent, M, F, N	8
Amblyomma marmoreum Koch, 1844		Sporatic, M, F, N, L	7
Rhipicephalus annulatus (Say, 1821)	CCHFV	Sporatic, M, F	7
Rhipicephalus evertsi Neumann, 1897		Sporatic, M, F, N, L	7
Hyalomma dromedarii Koch, 1844	CCHFV	Rare, M	5
Hyalomma excavatum Koch, 1844		Frequent, M, F	5
Hyalomma impeltatum Schulze and Schlottke, 1929	CCHFV	Rare, M, N	5
Hyalomma truncatum Koch, 1844		Sporatic, M, F	5
Rhipicephalus decoloratus Koch, 1844		Rare, M, F, L	5
Rhipicephalus pravus Dönitz, 1910		Sporatic, M, F	5
Amblyomma gemma Dönitz, 1909	Q-fever	Very rare, M	4
Amblyomma lepidum Dönitz, 1909		Rare, M, F	3
Rhipicephalus praetextatus Gerstäcker, 1873		Rare, F	3
Rhipicephalus guilhoni Morel & Vassiliades, 1963		Very rare, M	2
Amblyomma cohaerens Dönitz 1909		Very rare, unknown life stage	1

Supplement 1: Updates to Tick/ Host Associations

See supplemental Excel table. Note, records marked with * indicate the host association was derived from outside of Djibouti.

Discussion

Given the complex nature of the vector-borne disease threats facing U.S forces in Djibouti, a One Health approach is needed to fully characterize the risks and more effectively inform FHP. There are currently information products designed to inform military personnel of vector-borne disease threats of Djibouti such as Disease Vector Ecology Profiles (DVEP) produced by the Armed Forces Pest Management Board (AFPMB) or the Entomological and Zoonotic Operational Risk Assessments (EZORA) produced by the Army Public Health Center (APHC). However, these products lack sufficient guidance for prioritizing entomological surveillance targets or identifying potential disease reservoirs and spillover risks. We aim to present prioritized mosquito and tick species checklists ranked by their association with diseases of high importance to FHP and frequency of biting humans. We highly recommend additional surveillance be carried out in

Djibouti to include screening of host/ reservoir animals as well as environmental sampling. Birds remain a particularly challenging biosurveillance target in Djibouti given their role in transporting ticks around the world or serving as reservoirs for diseases such as WNV (Hasle, 2013; Flaisz, 2018; Rocklöv, & Dubrow, 2020;). For instance, The Smithsonian Institution, in collaboration with the U.S. Navy, Natural Resources Program, recently completed their first biodiversity study of the birds of Djibouti in over 100 years (Dove et al. 2020). In addition to documenting 143 total species, 96 species were also preserved as museum specimens. These surveys also resulted in new records for Djibouti including the semi-collared Flycatcher *Ficedula semitorquata* and the first record of River Warbler *Locustella fluviatilis* in Djibouti (Dove, et al. 2017; Buechley, et al. 2019).

There are many challenges associated with conducting entomological surveillance in Djibouti. The landscape transitions from coastline to mountainous within a relatively small geographic area. The climate is generally arid with a brief rainy season resulting in long-periods of drought (Razack, Jalludin & Houmen-Gaba, 2019). Security issues related to international terrorism are also a concern for field work in Djibouti (CIA, 2022). However, the regular movement of people and domestic livestock connects Djibouti to not only Africa, but the Arabian Peninsula and Asia as well. Future surveillance efforts should certainly target the prioritized vector species identified here, but also expand to include disease reservoirs and/or species responsible for the introduction of invasive vector species.

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