



High-Throughput Sequencing for Understanding the Ecology of Emerging Infectious Diseases at the Wildlife-Human Interface

Georgia C. Titcomb^{1*}, Christopher L. Jerde² and Hillary S. Young¹

¹ Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, Santa Barbara, CA, United States, ² Marine Science Institute, University of California, Santa Barbara, Santa Barbara, CA, United States

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*Correspondence:

Georgia C. Titcomb
georgiatitcomb@gmail.com

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Rising rates of emerging infectious diseases (EIDs) demand creative, efficient, and integrative investigations to understand their transmission, ecological contingencies, and dynamics at wildlife-human interfaces. High-throughput sequencing (HTS) methodologies provide enormous potential to unravel these contingencies to improve our understanding, but their potential is only just starting to be realized. While recent work has largely focused on novel pathogen discovery at likely interfaces, high-throughput methods can also allow disease ecologists to better explore the critical effects of climate, seasonality, and land-use changes on EIDs. HTS can facilitate the creation of entire host-pathogen networks, integrate important microbiome and co-infection data, and even pinpoint important exposure routes at interfaces through environmental media. Here we highlight studies at the frontier of HTS and disease ecology research, identify current limitations, and outline promising future applications for EIDs.

Keywords: high-throughput (HT) approaches, emerging infectious disease (EID), wildlife-human interaction, metabarcoding analyses, metagenomic analyses, eDNA, pathogen diversity

INTRODUCTION

Increasing globalization, agricultural intensification, urbanization, and climatic changes have resulted in a marked recent increase in emerging infectious diseases (EIDs) (Jones et al., 2008, 2013; Hassell et al., 2016). While EIDs are often zoonotic, viral, and triggered by shifts in host behavior and ecology (Jones et al., 2008; Morse et al., 2012), they are also incredibly diverse and often display complex transmission dynamics, rendering prediction challenging (Holmes, 2013). Fortunately, despite this complexity, pressing questions in emerging disease ecology – including the effect of environmental conditions on infection risk, the role of host microbiome in altering disease dynamics, and multi-host multi-pathogen relationships (Lively et al., 2014) – may be effectively broached using molecular advances in the form of high-throughput sequencing (HTS). HTS already enables EID surveillance at interfaces where humans, wildlife, and domestic animals overlap, by screening for pathogens in likely reservoir hosts (Carroll et al., 2018). Yet, we have made relatively little use of the extraordinary potential of HTS approaches to understand how climate, season, behavior and other ecological factors affect pathogen diversity, in both the community of pathogens and the within-pathogen heterogeneity that allow for increases in virulence and infectivity.

While traditional PCR methods target a small slice of pathogen diversity in a sample, HTS allows researchers to identify a wide range of DNA sequences simultaneously using a specified genetic region (metabarcoding/amplicon-based methods) or all genetic material (metagenomics, **Box 1**). To date, health-oriented researchers have harnessed HTS extensively for novel pathogen discovery and surveillance (Didelot et al., 2012), and ecologists have used HTS to understand energy flows in food-web ecology (e.g., Pompanon et al., 2012), microbial functions in soil sciences (e.g., Fierer et al., 2012), and the movement of invasive, rare, or endangered species using environmental DNA (eDNA) (e.g., Rees et al., 2014). However, HTS has only recently been used to reveal the complex ecology of host-pathogen-environmental interactions, although interest in the topic is rapidly expanding (**Figure 1**). With dropping costs and development of field-stable preservation methods (e.g., Wegleitner et al., 2015), HTS is increasingly feasible worldwide. This will certainly allow for more novel pathogen discovery and expansion of effort in remote and under-served locations where EID risk is highest (Jones et al., 2008) and many pathogens are undescribed. It should also facilitate more robust investigations across broad geographic regions over time, which is ultimately needed to help identify important locations for continued surveillance.

Identifying Important Interfaces

Given cost and data handling constraints, it is critical to identify locations and contexts that are likely EID interfaces (Morse et al., 2012). In general, interfaces may be locations of increased human-wildlife contact, such as forest edges (Wilcox and Ellis, 2006), locations with substantial land-use change, such as increased agricultural intensification (Jones et al., 2013), or sites where humans have high contact rates with animals, such as rodents, that thrive in urban environments (Williams et al., 2018). EID transmission at dynamic interfaces of wildlife, livestock, and humans occurs via media, such as air, water, food, and bodily fluids (including vector bites), and many interactions

are likely influenced by seasonal (Altizer et al., 2006), climatic (Harvell et al., 2002), or ecological context (Patz et al., 2004).

Host-Specific Surveillance for EIDs

Many HTS surveillance efforts directly screen wildlife hosts for pathogens that are either the causative agent or closely related to current EIDs, and research efforts have thus concentrated on viruses in domestic animals, bats, rodents, and non-human primates (e.g., Temmam et al., 2014; Carroll et al., 2018), which are primary reservoirs of EIDs. In bats alone, HTS studies have revealed viruses closely related to and known to cause Ebola, Nipah, Marburg, and other coronaviruses (Han et al., 2015). In particular, a prominent multinational research program, PREDICT, uses a combination of simple molecular and HTS techniques for viral surveillance in wildlife, livestock and humans at likely interfaces (Morse et al., 2012). These efforts have revealed more than 800 novel viruses and have contributed to an extensive database of zoonotic pathogens (Epstein and Anthony, 2017). Other studies have focused on diverse parasite types across hosts, including Chagas-like trypanosomes in bats (Dario et al., 2017), Babesia and Rickettsial pathogens in non-human primates (Nakayima et al., 2014), and pathogenic bacteria in urban mice (Williams et al., 2018).

While these studies collect valuable pathogen detection and identification data, most do not assess prevalence, ecological risk factors, or infection dynamics using multi-pathogen methods (Bodewes, 2018). Collecting concurrent data on host movements at interfaces across seasons could allow researchers to understand spatial, temporal, or host-specific attributes that contribute to pathogen diversity, especially in investigations of viral evolution and transmission pathways following outbreaks (e.g., Ramey et al., 2017). For example, one recent study used metabarcoding to determine feeding patterns and population structure of vampire bats that could be used to inform projections of rabies transmission across interfaces (Bohmann et al., 2018). Other studies have connected host microbial communities to parasite infection, revealing potential important evolutionary links (Zaiss and Harris, 2016; Rosa et al., 2018). Other studies that focus on ecological risk factors for pathogen infection (e.g., Oakgrove et al., 2014; Titcomb et al., 2017; Young et al., 2017) have been constrained to one or a few focal pathogens, but their scope can be expanded via HTS, promising to reveal insights for a wide range of diseases (**Table 1**).

Environmental Surveillance for EIDs

Despite swelling databases of host-parasite associations (e.g., Nunn and Altizer, 2005; Bensch et al., 2009; Urban et al., 2015), we still know relatively little of mechanistic sources of infection risk. Extending HTS use to explore hypotheses of transmission mechanisms and environmental sampling should enable researchers to pinpoint infection sources or dangerous interfaces. Although there is a clear need for elucidating mechanisms (Littlefair and Clare, 2016), progress had been slow for EIDs without insect vectors, given that indirect pathways are often complex and dynamic. For example, increasing evidence suggests that bat viruses are predominantly transmitted via secondary reservoirs that consume fruits contaminated with bat

Box 1 | Key definitions

Bioinformatics: a method of "conceptualizing biology in terms of molecules and applying information techniques (derived from disciplines such as applied math, computer science and statistics) to understand and organize the information associated with these molecules, on a large scale" (Luscombe et al., 2001).

Environmental DNA (eDNA): "genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material." (Thomsen and Willerslev, 2015).

High Throughput Sequencing (HTS); also Next Generation Sequencing (NGS): "... [a] method used to determine a portion of the nucleotide sequence of an individual's genome. This technique utilizes DNA sequencing technologies that are capable of processing multiple DNA sequences in parallel." (National Cancer Institute Dictionary of Genetic Terms)

Metabarcoding: "...[T]he automated identification of multiple species from a single bulk sample containing entire organisms or from a single environmental sample containing degraded DNA" (Taberlet et al., 2012).

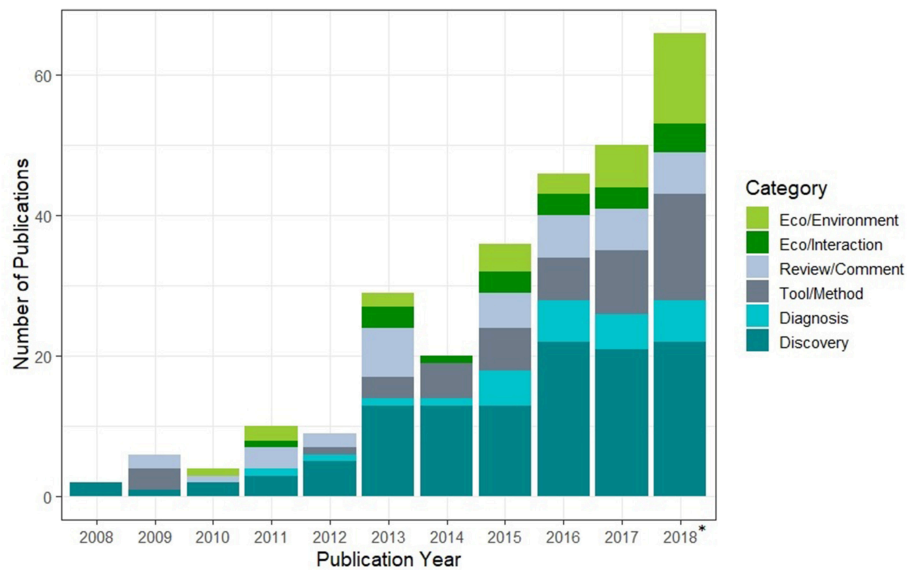


FIGURE 1 | A Web of Science search for combinations of the words metabarcod* or metagenom* or eDNA and emerging pathogen, parasite, or emerging disease revealed 278 relevant publications, demonstrating the accelerating interest in using HTS approaches to understand the ecology of emerging diseases across a range of applications. Articles for 2018 do not include December publications. Categories were defined as: Eco/Environment—HTS used to detect changes across pathogen communities in different environments, Eco/Interaction—interactions of microbiota or other organisms with pathogen communities, Review/Comment—reviews or commentaries, Tool/Method—new protocols, tools, or comparisons of methods, Diagnosis—HTS used to diagnose disease or identify aetiological agents, Discovery—HTS used to discover or describe new pathogens.

saliva (Han et al., 2015; Mann et al., 2015). Application of HTS to these suspected transmission sources could pinpoint problematic interfaces. Given that bats are frequently persecuted for their zoonotic connections (Schneeberger and Voigt, 2016), surveillance via intermediate hosts or likely transmission sources to humans (i.e., fruits) may even decrease persecution by identifying feasible interventions.

Just as individual hosts may act as pathogen superspreaders, specific interfaces may act as hotspots of pathogen transmission (Paull et al., 2012). HTS now allows researchers to interrogate environmental media (e.g., water, soil, food) for a wide variety of pathogens simultaneously, a feat that was impossible until very recently. Application of HTS to eDNA has numerous potential benefits for advancing understanding of diseases at interfaces and in environmental media (soil, water, air) (Bass et al., 2015). Monitoring water sources using metagenomics has become increasingly popular (Nieuwenhuijse and Koopmans, 2017), with applications revealing pathogen community shifts across land-use types (Gu et al., 2018), pathogen movement via commercial ships (Lohan et al., 2016), and mapping of high-risk sites from wastewater sampling (Cantalupo et al., 2011). Further useful applications might enable researchers to explore the effect of environmental policy measures, land management changes (e.g., dam creation), or species (re)introductions on pathogen prevalence and diversity at important interfaces.

Vector Surveillance for EIDs

Screening vectors for pathogens is a clear application of HTS to understanding EID transmission at human-wildlife interfaces.

This practice has expanded in the past decade, with particular focus on mosquitoes and ticks (Temmam et al., 2014). For example, recent work detected an emerging rickettsial pathogen in ticks in California (Bouquet et al., 2017), novel Bunyaviruses (a viral family that includes Crimean-Congo Hemorrhagic Fever and others) (Sakamoto et al., 2016; Bouquet et al., 2017), and numerous potentially-pathogenic bacterial genera in saliva across tick species (Qiu et al., 2014). While novel pathogen discovery is critical, understanding the ecology of vector-borne pathogens requires consideration of host communities, environmental and seasonal effects, and vector-pathogen interactions, among others (Table 1).

Recent molecular studies have considered how vector microbiomes may affect pathogens in different contexts. For example, one study used metabarcoding to determine that mosquito microbiota were more diverse in rural areas compared to urban areas, and varied substantially by species (Thongsripong et al., 2018). Other investigations have sought to identify microbial differences in infected mosquitoes (Villegas et al., 2018), ticks (Narasimhan et al., 2014), and triatomines (Gumiel et al., 2015). Furthermore, harnessing HTS to reveal feeding information with that of standard vector surveillance can allow the development of transmission networks, increasing our knowledge of disease dynamics, and ultimately informing public health approaches by identifying key species or pathogen superspreaders. One study detected decreasing diversity of hosts for sandflies and mosquitoes across a disturbance gradient (Kocher et al., 2017), demonstrating a potential system for investigation of pathogen prevalence and vector

TABLE 1 | Questions in disease ecology that have been addressed with HTS approaches.

Question	Media/taxa	Target DNA	Findings	References
How do anthropogenic changes affect pathogen diversity and prevalence?	Mosquitoes	Microbiota	Mosquito microbiota that affect pathogen prevalence were less diverse in urban areas.	Thongsripong et al., 2018
	Sandflies and Mosquitoes	Host and vector	Diversity of hosts used by mosquitos and sandflies declines along a disturbance gradient.	Kocher et al., 2017
	Water	Waterborne viruses	Land use and type of water source drive viral community structure.	Gu et al., 2018
	Ballast water	Protist parasites	A broad range of protistan parasites can be transported across oceans via cargo vessels.	Lohan et al., 2016
What are the likely transmission pathways for prominent diseases? Can infection sources be identified for management purposes?	Bats	Rabies	Metabarcoding revealed bat feeding preferences and population structure to inform local prevention of rabies transmission.	Bohmann et al., 2018
	Humans and water sources	Typhoid strains	Genome sequencing revealed spatial clustering of typhoid strains, importance of indirect transmission at water sources.	Baker et al., 2011
	Bait water	Broad range of pathogens	Bait water has substantially greater abundance and diversity of pathogens than natural lakes, revealing a potential pathway of fish pathogen spread.	Mahon et al., 2018
Can host-parasite sharing networks be mapped and compared across contexts?	Triatomines (kissing bugs)	Triatome microbiome, hosts, trypanosome parasites	Triatomines switch hosts with unexpected frequency: the majority of hosts are humans, domestics, and synanthropes.	Dumonteil et al., 2018
	Sandflies and mosquitoes	Vertebrate host DNA	Associations between important vectors and hosts were revealed across habitat types.	Kocher et al., 2017
How do host demography, location, behavior, group structure, movement, and microbiome affect entire pathogen communities? Do community level results correspond to single host-parasite findings?	Cattle	Intestinal nematodes	Parasite diversity was higher in cattle in warmer climates; anti-helminthic treatment modified parasite communities but did not eradicate them.	Avramenko et al., 2017
	Lemurs	Intestinal parasites and microbiota	Infection with certain parasites substantially altered gut biota and diversity.	Avelo and Norberg, 2017
	Humans	Intestinal parasites and microbiota	Certain bacteria were associated with helminth infection. Bacterial functions were linked to helminth biology and survival in the host.	Rosa et al., 2018
	Ticks	Microbiota	Tick gut microbiota facilitate colonization by <i>Borrelia burgdorferi</i> .	Narasimhan et al., 2014

feeding networks in response to anthropogenic change. Another recent study simultaneously characterized diverse host species and prevalence of *Trypanosoma cruzi*, the causative agent of Chagas disease, by sequencing the guts of Triatomines to create a potential transmission network (Dumonteil et al., 2018). By comparing host feeding and transmission networks across ecological gradients or following changes to host communities (e.g., wildlife loss, increased livestock, invasive species) researchers could model likely future disease outcomes in changing ecosystems.

Holistic Approaches

Integrating data on pathogen sequences found in hosts, vectors and the environment will enable researchers to reveal seasonal, climatic, or ecological patterns affecting EID transmission. However, many HTS applications focus on pathogen detection in either hosts, vector, or the environment, and studies that do focus on these in tandem are usually constrained to single pathogens. For example, one study investigated prevalence of *Cryptosporidium parvum* in cattle hosts, humans, and water sources simultaneously to show potential pathways of environmental transmission (Peng et al., 2003). Another study allowed researchers to confirm that the majority of typhoid infections in Nepal were indirectly transmitted, and likely to

be spread via public water spouts, where sequences of two different typhoid-causing pathogens were found (Baker et al., 2011). Future studies using HTS could allow researchers to draw similar conclusions for an entire suite of parasites. An integrated research approach that reveals pathogen diversity within hosts and across interfaces may allow researchers to better understand why certain pathogens are successfully transmitted while others are not. When armed with data on pathogen diversity in wild hosts, their behavior at interfaces, and pathogen prevalence in environmental media that humans and their livestock encounter, we may be able to develop successful barriers to transmission that promote wildlife conservation.

Limitations

There are several important limitations to HTS-based inferences about pathogens. For example, the metagenomic process tends to compound errors, particularly in environmental samples (Darling and Mahon, 2011), obscuring inferences about pathogen diversity. While noninvasive genetic approaches, such as eDNA with HTS, have a well-documented road map for methodological development to account for errors (Beja-Pereira et al., 2009), pathogens pose additional challenges beyond those encountered in the search for macrofaunal biodiversity from eDNA. In particular, pathogens and parasites are enormously diverse

(Poulin and Morand, 2000), making development of primers to target a range of pathogens for metabarcoding challenging. Furthermore, database coverage of pathogens is substantially less-developed than in other organisms (Ryan et al., 2017), resulting in identification uncertainty and inaccurate estimates of pathogen richness. The solution is increased storage and management of pathogen genome and metagenome databases (McNeil et al., 2007; Carroll et al., 2018; Zhang et al., 2018) coupled with additional field and laboratory expertise to document the breadth of global pathogen diversity. Yet, costs are still high, especially in regions where pathogen diversity is highest and likely to have the strongest impacts on human and animal health (Jones et al., 2008; Ryan et al., 2017).

Technology is rapidly improving our ability to identify sequences to species or strain (Luo et al., 2012). Single marker studies with short base pair read lengths can provide uncertain results with sequences identified only to pathogen groups (i.e., *Vibrio*, *Legionella*, *Mycobacterium*) (Mahon et al., 2018). While multiple marker inferences (Olds et al., 2016) and growing bioinformatics programs will improve our ability to identify specific pathogens, technology, and primer development for longer and reliable sequence reads will be beneficial. Similarly, improved methods to detect rare and elusive species (Jerde et al., 2011), and to evaluate sampling adequacy (Grey et al., 2018) have been developed, but the broader experimental design framework to guide future work is still an area of active research. Increased application of HTS across a wide range of scientific fields has also increased demand for innovation in multivariate statistics to compare pathogen communities over space, time, and environmental gradients (Paliy and Shankar, 2016).

Finally, a critical challenge lies in determining which pathogens are, or will be, virulent in humans or their domestic animals and in what contexts (Temmam et al., 2014; Epstein and Anthony, 2017). While continued surveillance of both healthy and diseased animals will help to identify harmful pathogens (Levinson et al., 2013), HTS methods will often need to be used as a complement to traditional methods in diagnosing infectious diseases (Miller et al., 2013; Simner et al., 2018). While infectious disease outbreaks remain challenging to predict, HTS methods offer substantial advantages in improving

much-needed surveillance and broader understanding of EIDs (Holmes et al., 2018).

CONCLUSIONS AND FUTURE DIRECTIONS

In the past decade, the number of articles that combine HTS and disease ecology has increased more than 10-fold, revealing the potential for enormous gains in understanding the underlying ecology of EIDs. However, many studies have focused on novel pathogen discovery, and vital contextual information is missing. While substantial, current technological limitations are being addressed, expanding the breadth and reliability of HTS studies. By pairing ecological concepts with HTS and epidemiological expertise, researchers will better address outstanding knowledge gaps: the effect of anthropogenic changes on pathogen diversity, exposure, and infectivity; the primary and secondary routes of EID transmission through environmental media; multi-host-multi-pathogen dynamics; and development of complete host-parasite networks that further our understanding of these critically-important diseases.

AUTHOR CONTRIBUTIONS

GT conceived the article and wrote the initial draft. CJ contributed sections and provided feedback. HY helped conceive the article and provided feedback.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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