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Detection of Emerging Zoonotic Pathogens: An Integrated One Health Approach

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Abstract

The emergence of novel zoonotic pathogens is one of the greatest challenges to global health security. The advent of increasingly sophisticated diagnostics tools has revolutionized our capacity to detect and respond to these health threats more rapidly than ever before. Yet, no matter how sophisticated these tools become, the initial identification of emerging infectious diseases begins at the local community level. It is here that the initial human or animal case resides, and it is here that early pathogen detection would have maximum benefit. Unfortunately, many areas at highest risk of zoonotic disease emergence lack sufficient infrastructure capacity to support robust laboratory diagnostic systems. Multiple factors are essential for pathogen detection networks, including an understanding of the complex sociological and ecological factors influencing disease transmission risk, community engagement, surveillance along high-risk human-animal interfaces, and a skilled laboratory workforce. Here we discuss factors relevant to the emerging disease paradigm, recent technical advances in diagnostic methods, and strategies for comprehensive and sustainable approaches to rapid zoonotic disease detection.

THE EMERGING ZOONOSES PATHOGEN CONTEXT

Few threats to human health have the potential to cause more impact than emerging zoonoses. More than half of all known human pathogens have their origins in animals (1–7), and all but one of the 17 US National Institutes of Health Category A pathogens capable of causing severe human illness and mortality are zoonoses (8). However, the entire list of all known pathogens represents what is likely to be only a small fraction of the total potential pathogens that exist in nature (9, 10). It is the threat posed by these unknown, yet-to-emerge pathogens that requires a reinvigorated and sustainable pathogen detection network based on One Health principles to prevent a global widespread pandemic resulting from a zoonotic agent.

A striking example of the need for broad-based and robust detection networks is the recent emergence of a relatively well-known pathogen, Ebola virus (*Zaire ebolavirus)*, in 2013 in Guinea, Sierra Leone, and Liberia (11–13). Although Ebola virus disease has been recognized for over 40 years, this devastating outbreak rapidly intensified and was eventually over 60 times larger than any previous Ebola outbreak (14–16). Over nearly three years, more than 11,000 lives were lost, and the virus affected individuals in 7 additional countries, as it stretched local and international pathogen detection and disease control capacities to their utmost limits (17, 18). Despite tremendous advances in diagnostic technology since the virus's discovery in 1976, this outbreak demonstrated that technology alone is not sufficient if resilient and integrated human and animal disease surveillance and laboratory systems are not prepared. A key lesson is that human capital and technical expertise are needed to rapidly identify potential health threats, no matter where they may occur, which requires consistent and sustainable funding, training programs, and engagement from national and international partners.

Numerous authors (2, 4, 19–21) have defined the modern concept of an emerging or reemerging pathogen. Engering et al. (19) elegantly summarized the pathogen-host-environment interplay in their 2013 work, and their concepts can, at their core, be most simply thought of as an unexpected disease in an unexpected place. It is the concept of time and place that provides the context of emergence. This scenario can vary dramatically, from newly recognized and never before detected threats, such as Lassa fever, Lujo hemorrhagic fever, SARS, and Heartland virus disease (22–25); to macrolevel shifts of known pathogens to new geographic locations, illustrated by migration of West Nile virus to the United States (26, 27) and Ebola virus to West Africa (11); to species jumps into new hosts, including H5N1 avian influenza virus to humans (28) or Nipah virus from bats to pigs to people (29). The concept of place further extends down to the microscopic level, as changes in a pathogen's tropism in tissues and disease pathogenesis result in novel clinical presentations, such as Zika virus neurological sequelae in human newborns (30, 31), or to the molecular scale of genomic mutations resulting in enhanced antimicrobial drug resistance (32). Thus, the drivers of emergence can occur at any level in our broader environment, from massive global-scale events, such as intense climactic weather events resulting in Rift Valley fever virus outbreaks in East Africa (33) to changes in pathogenesis or transmission dynamics that can give rise to a wholly new disease entity, like variant Creutzfeldt-Jakob disease (34). The massive breadth over which emergence can occur defines a primary challenge in detecting newly emerging zoonoses.

Beyond biological factors, the ecology, sociology, and behaviors of humans and animals greatly influence the transmission interface and enable the spillover of a new pathogen from an animal reservoir host into the first human case and can greatly influence further dissemination and transmission through the human population (6, 35–38). Here we seek to explore the complex and dynamic factors leading to zoonotic pathogen emergence, review diagnostic methods to detect these new health foes, and discuss approaches to enhance disease surveillance through local community engagement to ensure rapid identification of new human and animal health threats.

HUMANS, ANIMALS, AND PATHOGENS: AT THE INTERFACE OF BIOLOGY AND BEHAVIOR

Humans have been interacting with animals and their associated pathogens since the beginning of our species (6, 35, 39). As human society developed, certain animal species were found to be beneficial as sources of food, fiber, and companionship, as well as for use as beasts of burden, and were domesticated and habituated to human contact. Other animals remained wild and were either too elusive to farm, like the African duiker antelope (*Sylvicapra grimmia*), or simply too dangerous for regular close contact and possible domestication by humans, such as the North American brown bear (*Ursus arctos*). In modern times, as a result of explosive human growth and changes in animal and land use patterns, we have dramatically increased our contact with previously wild and otherwise unknown animal species (40–42).

Over time, changes in human population density, mobility, lifestyle, behaviors, and food choices have all influenced the dynamics of zoonotic disease emergence and have served as drivers of pathogen transmission (43–46). As the human population has grown, the influence of increased urbanization, coupled with higher incomes, has also led to greater demands for domesticated and wild animal meat products. This development has greatly expanded both our consumption of animal-source protein and our need for land to grow these animals and their feedstuffs, leading to tremendous modification of previously remote and seldom-visited regions into ever more intense livestock and agricultural production areas (47). This expansion of animal production into areas with greater abundances of wildlife has resulted in numerous examples of zoonotic disease emergence directly related to agricultural practices, including henipaviruses (pigs and horses); Middle East respiratory syndrome-coronavirus (CoV) (camels); Crimean-Congo hemorrhagic fever virus (ostriches); and other tick-borne bunyaviruses, such as severe fever with thrombocytopenia syndrome virus (livestock) (48–51).

However, despite the importance of domesticated and wild animal interactions in zoonotic disease emergence, a key driver of emerging zoonoses remains direct human contact with wildlife, their by-products (e.g., guano), and their meat for consumption (19, 52, 53). The intensification of wild animal meat hunting in the so-called bushmeat trade is driven by human taste preferences and allows for the direct contact of susceptible humans with animal reservoirs of a tremendous diversity of potential pathogens (53–56). In 2005, Wolfe et al. (46) reported that an estimated 4.5 million tons of bushmeat are harvested in the Congo basin alone. Human contact with wild animals during hunting, slaughtering, and consumption has been directly linked to the emergence of several known high-consequence zoonoses, including monkeypox, SARS-CoV, and ebolaviruses, as well as simian retroviruses such as Simian foamy virus and T-lymphotrophic viruses 3 and 4 (57–61).

Understanding and appreciating human behaviors related to wild animal use and the importance of local belief systems and customs regarding the concepts of disease, death, fear, and funeral practices are an often-neglected part of emerging disease surveillance activities (62, 63). The recent West Africa Ebola outbreak exemplifies the need for greater understanding of these human behaviors and how they may further interactions with animal reservoirs and their pathogens. Greater appreciation of these human factors can lead to insights into the magnitude of high-risk behaviors undertaken by community members (64–66). Local traditional health workers are often a societal pillar in many communities and can play a very important role in the recognition and eventual control of emerging pathogens (62, 67). The influence of these traditional healers as key sources of information in local communities can greatly help or hinder efforts to reduce the intensity of disease outbreaks (68). It is essential that they be recruited and encouraged early in the design of any disease surveillance system to become partners in the activities. If properly informed, these individuals can be tremendous facilitators, encouraging ill individuals to seek treatment, influencing

changes to burial customs if needed, and helping to mitigate the impacts of fear on a population (69, 70).

During the West African outbreak, lack of initial engagement with traditional healers by health professionals, coupled with a pervasive mistrust of outsiders or government workers using non-traditional methods, led to an early reluctance of communities to engage in disease surveillance efforts (71, 72). This misstep likely compounded early efforts to slow the spread of the virus, as reactions to international efforts during the outbreak ranged from reluctance to seek medical help at Ebola treatment centers to, most strikingly, the attacking and killing of international health responders in eastern Guinea (73). To help overcome these tremendous anticipatable challenges in the future, it is imperative to promote early and intensive community-level engagement by medical anthropologists and disease surveillance professionals working in combination with local traditional healers and local village-level governance structures so that these community leaders become a part of any integrated and comprehensive zoonotic disease surveillance system.

THE EMERGING PATHOGEN-DETECTION PATHWAY

The increasingly intense and complex interactions of people, animals, and pathogens across the local and global landscapes all point to an ever-increasing risk of the emergence of a zoonotic pathogen with true pandemic potential that could threaten the survival of millions of animals and people (37, 74, 75). The initial detection of this threat, by necessity, begins at the local level with the observation of sick individuals (persons or animals) by someone familiar with the common diseases of the area. Oftentimes, this initial observation is never reported beyond the local area, as the disease is not recognized as unusual or does not spread, but in some instances the recognition of initial cases or the resulting chain of multiple events may lead to the eventual engagement of regional or national government authorities and possible involvement of international health responders. In many countries, centralized systems have been developed in which epidemiologic and laboratory diagnostic technologies and capacities reside in referral national-level centers distant from many of the high-risk human-animal interfaces at the forefront of disease emergence (76). In these instances, patient or animal clinical information, reports of die-offs or other unusual illnesses, and eventually diagnostic specimens for testing are pulled up from the local level to these referral centers.

Despite successes, this centralized pull pathway can fail at a variety of levels, as the barriers for the flow of information and diagnostic specimens up from the household to the national level can be difficult to overcome. Issues, such as poor transportation or communication networks, lack of trained health workers, limited laboratory systems, poor interagency or ministerial communications between the animal and public health sectors, mistrust of government officials, and sometimes suboptimal national reporting systems, all contribute to delays in the recognition, diagnosis, and eventual control of emerging health threats.

The central challenge then in the rapid detection of emerging zoonoses at a systems level is how best to create robust and resilient surveillance networks capable of detecting the rare and isolated health event at the local level and to link those observations with a highly skilled public health laboratory workforce (77). Is a highly centralized and concentrated network in national- or regional-level reference institutions or government ministries, or instead in a distributed network of local partner-driven surveillance teams with basic laboratory capacity for point-of-care rule-in/out diagnosis, or perhaps even a combination of both most likely to succeed? Key questions that influence the determination as to which of these approaches is most appropriate for a particular country relate to the sustainability of funding, training needs, and sophistication of laboratory techniques needed for pathogen detection. Examples of successes from a combination of

national- and local-level surveillance networks with support from international organizations have been developed in several countries (78, 79).

Disease surveillance systems that seek to integrate the human and animal health sectors as closely as feasible are likely to be best positioned to rapidly detect emerging zoonotic threats. In these combined surveillance activities, human and animal health workers and field ecology teams would work together with the common scientific and public health goal of detecting novel emerging pathogens from animal reservoirs and the human population as quickly as possible. Combining this integrated approach with efforts to push down as close to the local levels as possible the technical training and laboratory infrastructure needed for zoonotic disease detection may be ideal. Systems that use a locally driven and distributed component may be potentially more expensive and more difficult to manage than exclusively centralized systems, but by being closer and more integrated into the local community, their ability to rapidly detect rare health events for follow-up is likely to be enhanced over completely centralized systems.

Since 2009, the US Agency for International Development (USAID) Emerging Pandemic Threats Program has funded the PREDICT project, providing one such example of this type of integrated program for zoonotic disease detection. Since its conception, the PREDICT program has endeavored to engage with communities and reinforce zoonotic disease surveillance activities by providing in-depth capacity building for human and animal health field and laboratory workers while simultaneously conducting active surveillance and laboratory detection for emerging novel zoonotic viruses in people and animals. The goal of the PREDICT project is to rapidly identify emerging novel viral threats to human and animal health using techniques that are pushed to the local level as far as possible to rapidly detect new and unknown pathogens while building capacity in national systems to characterize and mitigate epidemic risk. The PREDICT team is also active in the global health community, working to assess the potential of these newly discovered viruses to cause large-scale outbreaks or pandemics. At its core, PREDICT seeks to build capacity throughout the world by providing training to scientific staff in over 30 countries, developing networks of institutions, public health and veterinary staff, and technical experts to enable in-country detection of emerging disease threats. PREDICT teams concurrently conduct wildlife, domestic animal, and human surveillance in disease emergence hotspots by safely collecting biological specimens and ecological risk data and conducting behavioral risk assessments from ill human patients at high-risk transmission interfaces (see sidebar titled Note from the Field: The USAID Emerging Pandemic Threats PREDICT Program).

BUILDING EFFECTIVE AND RESILIENT SURVEILLANCE NETWORKS

To be functional, integrated approaches require long-term, sustainable funding and investment in human capital, infrastructure, and laboratory and communications technology to build local or regional surveillance hubs. One impetus that has sped the development of integrated animal and human health surveillance systems for zoonoses is the adoption in 2005 of International Health Regulations (IHR) by 196 World Health Organization (WHO) member countries in the wake of the SARS pandemic in 2001 (80). These regulations, coupled with the increased recognition that disease emergence in one country could easily spread to another through movement of animals or people, has provided an opportunity to more closely link the human and animal disease surveillance sectors. IHR requires the timely notification (<24 h) of outbreaks "of disease with the ability to cause serious public health impact and to spread internationally" and may constitute a "Public Health Emergency of International Concern" (81). The regulations do not stipulate the source of the infection (human or animal) and are meant to be applied as broadly as possible by all member nations.

NOTE FROM THE FIELD: THE USAID EMERGING PANDEMIC THREATS PREDICT PROGRAM

PREDICT, a project of USAID's Emerging Pandemic Threats Program, was initiated in 2009 to strengthen global capacity for detection and discovery of viruses with pandemic potential that can move between animals and people. Currently, in its second five-year phase, the project is focused on the detection of emerging zoonotic pathogens such as coronaviruses, the family to which SARS and MERS belong; paramyxoviruses, like Nipah virus; influenza viruses; and filoviruses, like the Ebola virus. The PREDICT consortium led by the University of California, Davis, One Health Institute strives to build upon One Health partnerships. These cross-disciplinary collaborations are critical for gaining a fuller understanding of the integral links among human, animal, and environmental health that can provide opportunities for prevention or early detection and control of disease threats. Working across sectors and including a diverse range of stakeholders and expertise help to operationalize efforts that promote public health, effective natural resource management, and development. Toward this overarching goal, the program has worked closely with a wide range of government ministries, scientific and educational institutions, local organizations, and other stakeholders to further One Health initiatives.

Now working with partners in over 30 countries and in over 60 in-country laboratories, PREDICT is continuing to build platforms for viral surveillance and for identifying and monitoring zoonotic pathogens, or those that can be shared between animals and people. Using the One Health approach, the project is investigating the behaviors, practices, and ecological and biological factors driving disease emergence, transmission, and spread. Through these efforts, PREDICT is improving global disease recognition and beginning to develop strategies and policy recommendations to minimize pandemic risk. PREDICT's surveillance for emerging pathogens focuses on areas of the world at the highest risk for zoonotic disease emergence. The goal is to move countries away from a reactive post-outbreak response to a proactive approach in which pathogens of pandemic potential are discovered at their source before large-scale epidemics occur in people.

PREDICT has made significant contributions to strengthening global surveillance and laboratory diagnostic capabilities for new and known viruses. During the first five-year period (2009 to 2014) the project has successfully detected over 800 novel viruses in more than 20 countries. The program's viral detection success lies in the use of broadly reactive consensus (genus/family level) PCR supplemented with high-throughput sequencing (95). These powerful tools produce specific, high-resolution data, allowing for rapid detection of known and new potential pathogens. PREDICT has developed and optimized detection protocols and built capabilities in laboratories serving the countries in which the program is engaged to improve regional capacity to detect pandemic threats (**http://www.predict.global**).

The costs of developing strong disease detection systems that would enable the full enactment of the IHR are relatively modest and attainable (82). The World Bank estimated in 2008 the total costs of implementing a robust One World–One Health work force to be approximately US$852 million in the 49 lowest-income and most at-risk countries (1). This figure, when compared with the estimated costs of the single 2001 SARS pandemic (US$40–50 billion), is cost effective by any measure (83). The comprehensive World Bank approach to building this workforce would leverage outside international aid funding (e.g., World Bank, WHO, UN Food and Agriculture Organization, USAID, or other national governments) to collaborate with and augment existing academic centers, private institutions, and local government partners to ensure that initial start-up costs and training burdens are managed to facilitate rapid training and enhancement of local surveillance and diagnostic capacities (82). Ideally, over time, as technical expertise is established, the role of international aid agencies can be reduced, leaving behind a core staff capable of responding to emerging health threats within each country or region.

A key first step in this process is the identification of local, national, and regional partners to collaborate and develop structures in concert with these international agencies and to identify talented and motivated individuals. These persons ideally should come from diverse scientific backgrounds and across all sectors, including human, animal, and wildlife health specialists; epidemiologists; laboratory and behavioral scientists; and other junior and senior personnel, to build an experienced and integrated zoonotic disease-detection network of individuals and institutions.

Training needs may vary from country to country and are highly dependent on the preexisting academic and governmental training programs and infrastructure. For example, the PREDICT program (**http://www.predict.global**) has been engaged with multiple countries spanning the spectrum from those with highly advanced tertiary medical care centers and strong internationally focused academic centers to those lacking basic infrastructure and preexisting individuals who can readily conduct disease surveillance or laboratory testing. At a minimum, close peer-to-peer mentoring by experts in the field and hands-on in-person training are necessary to prepare any integrated One Health surveillance team. To sustain these efforts, other key skills, such as scientific writing, design of hypothesis-driven experimental approaches, and grant and proposal preparation, must be included among training activities. By encouraging an academic and entrepreneurial spirit of collaboration and engagement, these teams are more likely to be able to identify sources of funding from the governmental or private sectors to maintain their research efforts well beyond the initial phases of the integrated surveillance project.

INTEGRATED LABORATORY TESTING TO SPAN THE DIAGNOSTIC SPECTRUM

It is important to note the differences in the dynamics of pathogen infection between potential reservoir and nonreservoir hosts (**Figure 1**). In true reservoirs, infection is generally not overtly deleterious to the host in a healthy state and often leads to the establishment of a nonsymptomatic persistent infection with intermittent reactivation of pathogen replication and environmental shedding in response to environmental or hormonal queues, such as those observed for hantaviruses and arenaviruses in rodents or marburgviruses in Egyptian fruit bats (*Rousettus aegyptiacus*) (84–86). This healthy carrier pattern in reservoirs is markedly different from the classical infection paradigm in nonreservoir, susceptible hosts, where infection leads to pathogen replication and either pathogen clearance via activation of immune response or, if immune control is unsuccessful, eventual disease and either a chronic persistent infection, a potential convalescent carrier state, or lethality. The initial design of a comprehensive pathogen-discovery program must consider these differences so that the appropriate diagnostic technologies [i.e., molecular nucleic acid amplification techniques (NAAT) versus serological antibody detection–based assays] are used to afford the best opportunity for pathogen/infection detection. To be most effective, laboratory scientists and experts in diagnostic technologies should be involved at the conception and program design stages, as well as be invited to contribute extensively in the implementation of zoonotic pathogen surveillance activities. This integration will help to ensure that relevant specimens are collected, transported, and stored appropriately to allow for the best chance for later identification of etiologic agents and surveillance successes.

Fortunately, the variety and quality of diagnostic technology and specimen collection materials have expanded tremendously over the past 40 years (87). The evolution of diagnostics and the use of these technologies has led to a vast increase in our understanding of the microbial world and the potential zoonotic pathogens capable of impacting human and animal health (**Table 1**). Ideally, the methods employed will be selected or designed both to allow for the detection of emerging

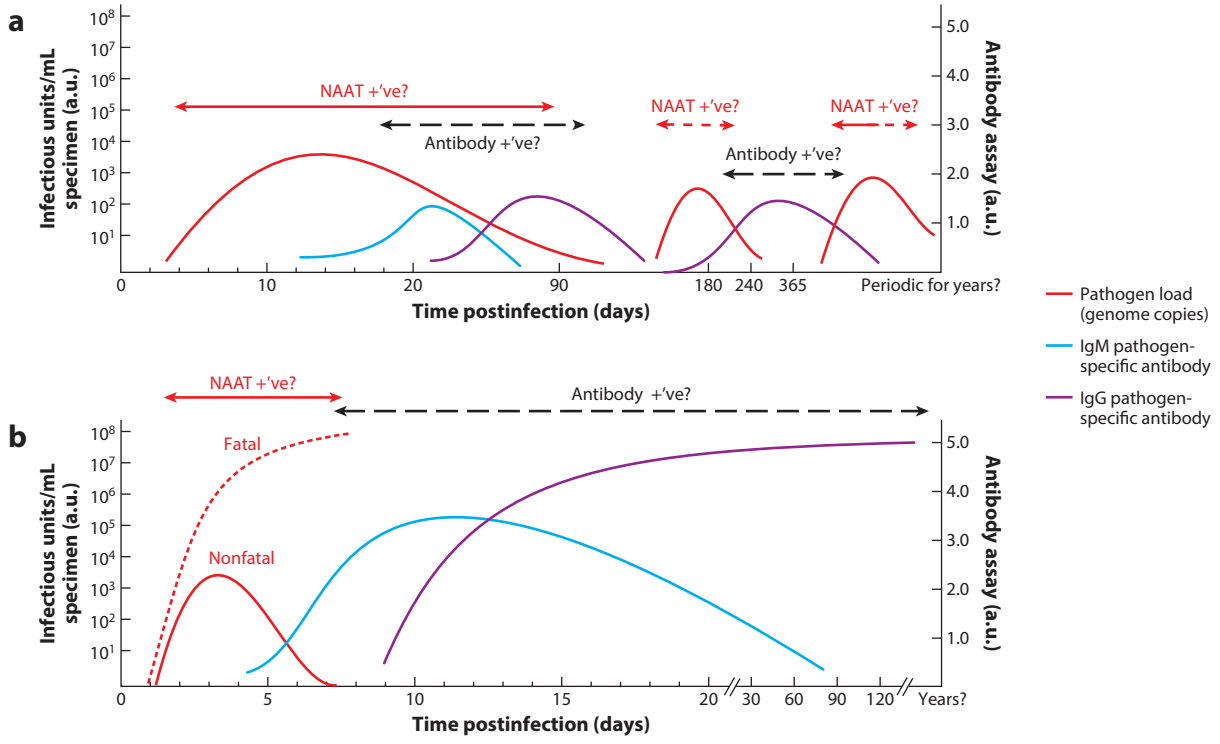

Figure 1

Hypothetical schematic comparing the kinetics of pathogen replication and host immune responses between a potential reservoir and a nonreservoir host that influence detection assay performance and utility. (*a*) Reservoir dynamics: reinfection or persistent infection with intermittent pathogen shedding and detection. (*b*) Nonreservoir dynamics: acute infection, immune response, and pathogen clearance. Comprehensive disease diagnostic systems require both nucleic acid amplification techniques (NAAT) for acute-phase disease detection and serologic techniques to detect convalescent humans or animals, including persistently infected reservoirs. Note that typically classical techniques, such as pathogen culture or isolation, are possible only during the acute-phase (NAAT +'ve) time window. Red line indicates pathogen load (genome copies), and blue and purple lines indicate immunoglobulin M (IgM) or IgG pathogen-specific antibody, respectively.

zoonoses during the acute phase of infection, while the patient or animal remains acutely infected with active pathogen replication or shedding, and to monitor for antibody development and persistence after recovery, when the potential pathogen may no longer be present and available to cause disease in the monitored patient or other individuals (**Figure 1**). For many known high-consequence viral pathogens, the acute phase of infection is only a few days long and typically ranges from 2 to 21 days postinfection (88, 89), thus limiting the time that molecular diagnostic techniques targeting the pathogen genomic material, such as polymerase chain reaction (PCR) and its derivatives, may be diagnostically useful.

The advent of highly sensitive and specific molecular techniques that can directly detect a pathogen's genome based on NAAT technologies, such as PCR amplification of RNA (after reverse transcription) and DNA, has revolutionized pathogen discovery. NAATs such as consensus PCR, real-time quantitative PCR, and Sanger whole-genome sequencing have forever changed the diagnostic landscape, especially for viral pathogens (90). However, the exquisite sensitivity and specificity of these techniques limit their use in the detection of widely divergent and unknown novel pathogens, or even variation among strains of known pathogens, owing to genomic sequence

Table 1 Diagnostic methods and zoonotic pathogens

Method	Technical requirements	Costs	Advantages	Disadvantages	Key examples	Reference
Acute phase of disease						
Electron microscopy	+++++	++++	Visualization of pathogen in situ	High technical expertise required; expensive	Ebola virus, SARS-CoV	16, 24
Pathogen isolation or culture	+++	+++	Sensitive, pathogen available for further assessment, suitable for unknown pathogens	Lab biosafety requirements; choice of cell line/media may limit sensitivity and unknown pathogen detection	Sosuga virus, SARS-CoV, Nipah virus, Ebola virus	16, 24, 105, 106
Histology/ immunohistochemistry	+++++	++++	Assessment of tissue pathology	Requires cross-reactive antisera and high technical expertise	Zika virus (in neonatal CNS tissue)	30
Rapid diagnostic tests or lateral flow assays	+	+	No electricity needed, generally thermostable, low skill required to operate	Limited sensitivity; interpretation of weak results can be difficult; not suitable for unknown pathogen discovery	Influenza; Ebola virus Multiple commercial examples	107, 108
Antigen capture and IgM ELISA	+++	+++	Adaptable to high-throughput screening, excellent counterpart to molecular testing for known pathogens during outbreak responses	Requires cross-reactive antisera, moderate technical and lab requirements; some humans/ animals have broadly reactive sticky serum yielding false positives	Multiple commercial/ experimental examples	98, 109
Conventional PCR/RT-PCR and sequencing	++ to +++	++ to ++++	High sensitivity and specificity depending on primer design; can be broadly reactive; PCR amplicons can be sequenced for further confirmation and pathogen characterization	Moderate technical and lab requirements; cold-chain requirements for reagents; easily contaminated techniques can result in inaccurate or difficult-to-interpret results	Sin Nombre virus, Ngari virus coronaviruses, Bundibugyo virus, MERS-like CoV	95, 109–112
Quantitative real-time PCR/RT-PCR	+ to +++	+++ to +++++	Highest sensitivity and specificity available for known pathogens; some systems approaching push-button ease of use; reduced potential for lab contamination and false negative/positive results	Not suitable for unknown pathogen discovery; PCR amplicons not suitable for subsequent genome sequencing and confirmation	Detection of Marburg virus in bat reservoir	109

(*Continued*)

Table 1 (*Continued*)

Method	Technical requirements	Costs	Advantages	Disadvantages	Key examples	Reference
Whole genome Sequencing, VirCap (HTS/NGS etc.)	+++++	+++++	Unbiased genomic detection, highly adaptable to unknown pathogen discovery; bioinformatics risk assessment possible; can be used to track pathogen dispersal in a population (reintroduction versus ongoing transmission)	High cost; high technical expertise required especially for bioinformatics analyses; overall reduced sensitivity compared with PCR techniques	Heartland virus, Sosuga virus, Bas-Congo virus, SFTS virus	25, 105, 113–115
Convalescent phase of disease						
IgG ELISA techniques	++	++	Broad detection of past infections based on specific or cross-reactive antibodies; primary tool for postexposure serosurveys	Requires specific antisera and antigens; validation and establishment of positive/negative cutoff OD values required; antibody cross-reactivity can confound results; host species limitations	Multiple commercial/experimental assays	
Phage display antibody detection	+++++	+++++	Potential to detect wide range of antibodies to diverse pathogens and overall exposure history	Currently in experimental proof-of-concept stage; expensive and technically demanding	Experimental	99
Western Blot (antipeptides)	++++	++++	Highly specific; can be targeted to widely conserved protein peptides	Technically demanding; requires moderate laboratory infrastructure	Multiple commercial/experimental examples	

Abbreviations: CNS, central nervous system CoV, coronavirus; ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M.

variation. In the past 10 years, strategies to overcome this limitation have been developed and successfully employed in pathogen detection, such as unbiased next-generation sequencing (NGS) or high-throughput sequencing (91–94) and refinements to traditional consensus PCR strategies to use highly degenerate primers to detect and amplify genomic material across entire virus families (95–97).

However, despite the ever-increasing sensitivity and throughput capacities of molecular diagnostics, the amount of pathogen genome in a given specimen type will begin to decrease and can rapidly fall below detectable levels as the infected person or animal ceases to actively shed infectious pathogens. In these circumstances, further pathogen monitoring becomes reliant on specific antibodies that are circulated following activation of the immune response during the acute phase of infection. These form an individual's pathogen exposure record (**Figure 1**). Evidence of previous infection in these individuals may be demonstrated by assays designed to detect pathogen-specific antibodies, such as the commonly employed enzyme-linked immunosorbent assay (ELISA). These techniques and other related antibody-detection modalities are essential to determining the overall scope of disease activity in a given location and species distribution and should ideally be part of any comprehensive laboratory plan (**Table 1**).

An exciting area of diagnostics development in the past five years has been the growth of so-called rapid diagnostics tests, or RDTs, for use in resource-poor settings. These lateral-flow assays are typically self-contained devices that, after addition of clinical specimen, use surface tension to flow specimens and reagents across an absorbent membrane precoated to detect the presence of either specific pathogen antibodies or even pathogen antigens present in the clinical specimen. Results generated from the specimen and relevant controls are observed visually or with a detection reader that quantifies the change in color on the device, indicating the presence or absence of the pathogen in question. The primary advantages of these devices are that they are often thermostable, require no electricity, and have minimal training requirements. Sensitivity and specificity of RDTs are typically lower than other, more sophisticated molecular and antibody techniques, such as PCR and ELISA testing, but their extremely low technological and infrastructure requirements make them highly useful in certain circumstances and represent the ultimate thus far in the ability to push diagnostic technology as far down locally as possible. RDTs for malaria detection and Ebola virus infection rule-out during corpse surveillance in the recent 2013–2016 Ebola virus outbreak in West Africa demonstrated this technology's potential to radically alter the diagnostic possibilities available after further refinement and development of this promising technology (98).

At the other extreme of the technology spectrum is the coupling of high-throughput next-generation sequencing and the power of bacteriophage-display technology to generate multivalent antibody-detection assays (99). This approach could allow for simultaneous detection of the entire repertoire of an individual's viral pathogen or virome exposure history, rather than screening one or a few closely related pathogens using ELISA-based technology. Although still highly experimental, this technological advance may revolutionize pathogen detection in a manner similar to what PCR technology did in the 1980s and 1990s (100).

Despite the revolution and success of modern molecular and antibody detection techniques to identify acute zoonotic pathogen infections, other more classical techniques (i.e., indirect fluorescent antibody assays, virus isolation and bacterial culture, histology, immunohistochemistry, and electron microscopy) remain highly valuable and should not be neglected (**Table 1**). If a novel zoonotic pathogen is discovered by NAAT or serological techniques, follow-up activities, including pathogen characterization and virulence determination, even fulfilling Koch's postulates if possible, and therapeutics and vaccine development require the isolation of the etiologic agent in question, and these classical techniques are critical. However, certain pathogens are difficult to culture under laboratory settings owing to challenges in determining the selection of appropriate

cell lines and growth conditions. These difficulties are exemplified by the nearly 16-year period between the discovery of hepatitis C virus in 1989 and the first successful in vitro cell culture of the virus in 2005 (101, 102).

Beyond the technical issues, the growth and characterization of zoonotic pathogens require well-equipped laboratories, higher biosafety and biosecurity infrastructure to protect investigators, specialized reagents, and highly trained laboratory staff that may be difficult to sustain in resource-poor settings, but the value of isolation of the etiologic agent in question cannot be overstated. Because of the high technical demands and the importance of biosafety and biosecurity, the use of these types of classical techniques may be best suited in some circumstances in a comprehensive push-pull detection strategy that employs collaboration between local/national laboratories and an international referral laboratory, as is available through the WHO Global Outbreak Alert and Response Network in an integrated system of laboratories (**http://www.who.int/ihr/alert_and_response/outbreak-network/en**). These latter facilities can assist local and national laboratories in the classical work-up, as well as provide further confirmation of putative PCR or serological assays using molecular NAAT assays that were pushed down to regional or locally based laboratories (103).

THE TANZANIA VISHA (VIRUS-SHARING) PROJECT: AN EXAMPLE OF CAPACITY BUILDING THROUGH INTEGRATED FIELD SURVEILLANCE AND PUSH-PULL LABORATORY SYSTEMS FOR NOVEL ZOONOTIC VIRUS DETECTION

In 2007, the University of California, Davis, One Health Institute and the Sokoine University of Agriculture in Morogoro, Tanzania, collaborated on a model One Health project to foster technical capacity building to improve in-country disease detection to promote the health of animals and livestock keepers throughout central Tanzania. Now in its tenth year, this collaboration, referred to as Healthy Animals and Livelihood Improvement (HALI, **http://haliproject.org/**) (104), has strengthened field ecology and laboratory systems; trained staff and students; and conducted wildlife, livestock, and human surveillance and laboratory detection of a variety of zoonotic pathogens, considering environmental influences and ecological drivers on population and pathogen dynamics. Recently, the collaboration was expanded to include the Ifakara Health Institute, another local counterpart specializing in human health activities. Together, these three research partners are conducting broad cross-sectional disease surveillance in wildlife and people in central Tanzania (**Figure 2**). At four sites in the region with high-risk human–wildlife interfaces, patients presenting at local clinics with nonmalarial undifferentiated febrile illnesses are offered participation in a pathogen discovery project. After informed consent is obtained, a detailed case investigation form is completed for each patient, and whole blood, serum, and oral and fecal swabs are collected. Later, a case investigator administers a survey instrument to determine the individual's exposure and interactions with wildlife and livestock to determine what risk factors may be present at the household level. Concurrently, field ecology teams sample wildlife (nonhuman primates and bats) in the catchment areas surrounding the study clinic sites. From these animals, oral and fecal swabs and blood specimens are obtained via nondestructive sampling techniques. Human patient enrollment forms and specimens are referred to the Ifakara Health Institute for consensus reverse transcriptase (RT)-PCR-based assays capable of detecting five priority virus families (coronaviruses, filoviruses, influenza viruses, paramyxoviruses, and flaviviruses). Identical NAAT assays are completed on all animal specimens at Sokoine University of Agriculture. Presumptive positives obtained via these techniques are then forwarded to the University of California, Davis, for further molecular confirmation (repeat RT-PCR, NGS, and other bioinformatics techniques)

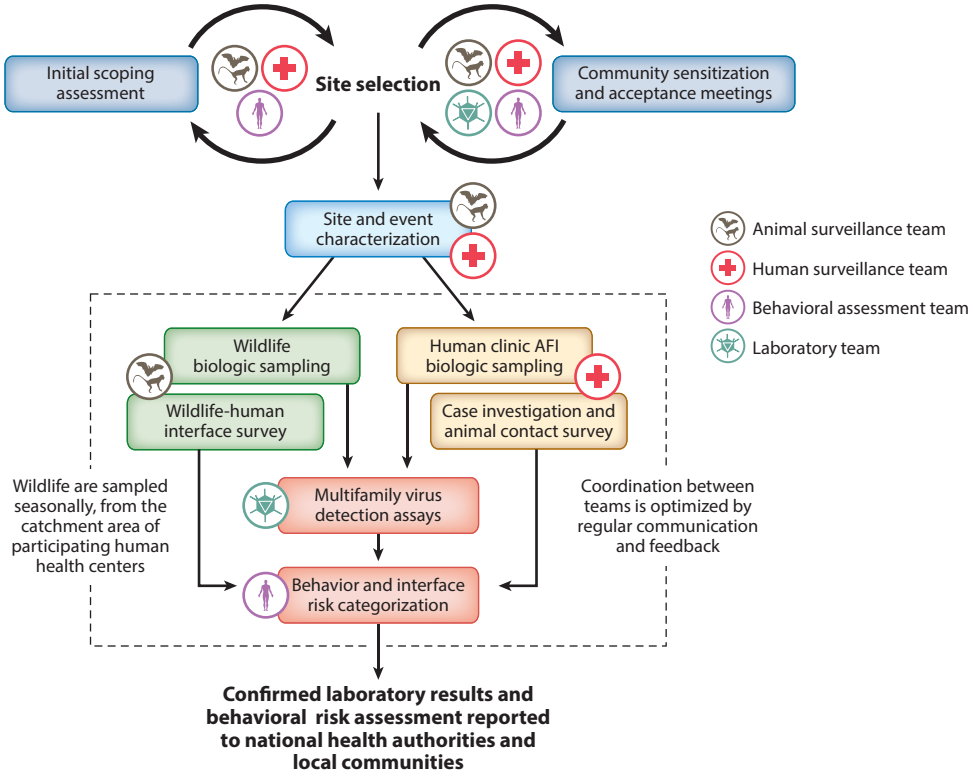

Figure 2

Schematic of the Health for Animals and Livelihood Improvement (HALI) virus sharing project (VISHA) implemented in central Tanzania. Active surveillance in both human health clinics for acute febrile illnesses (AFI) and adjacent wildlife populations affords the opportunity to monitor virus spillover and disease emergence at high-risk disease transmission interfaces. This strategy benefited from intellectual developments from the PREDICT Project and its leadership. The authors thank Dr. Leilani Francisco for her assistance with this graphic.

and to provide baseline assessments of the zoonotic and possible pandemic potential of any novel virus detected. Confirmed positives are then pulled to the appropriate international diagnostic reference center specializing in the isolation and characterization of the potential pathogen discovered. Through these combined efforts in active surveillance of animals and humans, novel virus discoveries and the demonstration of contemporaneous virus sharing among these populations are possible. Combining novel virus detection with the risk-behavior data from the patient and household surveys will enable the study team to craft public health messaging to reduce the potential exposure of further individuals to these health threats. Over the course of this US Defense Threat Reduction Agency–funded project, further training and capacity development have been provided for more than 25 individuals ranging from senior investigators to university interns and government field staff in the areas of laboratory diagnostics, field ecology, and human study bioethics and procedures, as well as to clinic staff in the four rural Tanzanian health clinics. It is hoped that the capacities built during this project will provide the basis for continued sustainable growth in Tanzania of a scientific workforce capable of zoonotic disease surveillance to improve the health of animals and the Tanzanian people.

SUMMARY

Despite tremendous technological advances in the past 25 years, there remain massive challenges to developing robust systems capable of rapidly detecting emerging zoonotic disease threats. Establishing long-term, sustainable, and diagnostic modalities as close to the local level as feasible is a key step to rapidly identifying and alerting public health authorities and avoiding the next global pandemic. Regardless of the technology used and the scientific capacities developed, failure to engage and build trust with local political and thought leaders, traditional health workers, and community groups in disease detection and control will delay diagnosis and response, with potentially disastrous consequences. The rapid expansion of the West Africa Ebola crisis of 2013–2016 is a stark warning that zoonotic threats (novel or known) can emerge and quickly threaten global health security. Avoiding a repeat of this scenario and halting whatever the next global pandemic may be begins at the animal-human interface and with the initial spillover event in local communities. We must be prepared to recognize the signs, identify the threat, and rapidly work together to reduce the spread of infections and health consequences before they harm the health of animals and people throughout the world.

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