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Anticipating the Species Jump: Surveillance for Emerging Viral Threats

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The mission of the Defense Threat Reduction Agency (DTRA) is to safeguard America and its allies from weapons of mass destruction (chemical, biological, radiological, nuclear, and high explosives) by providing capabilities to reduce, eliminate, and counter the threat, and mitigate its effects.

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Executive Summary

Emerging infectious diseases (EID) pose international security threats because of their potential to inflict harm upon humans, crops, livestock, health infrastructure, and economies. Some zoonotic (animal) viruses pose unique challenges because of their ability to infect new host species. For example, influenza and human immunodeficiency viruses originally infected animals, but subsequent mutations enabled these viruses to “jump” to new human hosts. Zoonotic disease surveillance is typically triggered after animal pathogens have infected humans. Yet, what might be achieved by surveillance that *precedes* human infection? Can it be done? How? Where? By whom?

On 3-4 December 2009, the Advanced Systems and Concepts Office of the Defense Threat Reduction Agency (DTRA-ASCO) convened a workshop of experts to conceptualize the future of predictive surveillance for viruses that jump from animals to infect humans. Virologists, ecologists, and computational biologists from academia, US Government and nongovernmental organizations discussed opportunities as well as obstacles to prediction of species jumps using genetic and ecological determinants from virus, host, vector and reservoir.

A majority of emerging pathogens are zoonoses. If one assumes that some instances of zoonotic infections in humans are preceded by infections in animal reservoirs and/or intermediate hosts, then early detection of virus in these animals or vectors may preclude imminent human infection. In the short term, such early detection may enable human avoidance of high-risk areas, prophylaxis, or timely mobilization of medical resources to cope with imminent or emergent disease. In the future, it is conceivable that sustained virus surveillance in animals could detect the genetic and ecological changes that likely precede a species jump. As our understanding of virus-reservoir-host ecology becomes sufficiently robust, we may be able to recognize patterns, processes and mechanisms entrained in species jumps, and these signals could enable prediction of a species jump before it occurs, allowing us to prevent outbreaks of human infections. Development of this future predictive capability was the focus of the workshop.

Conclusions and recommendations from workshop participants are summarized as follows:

1. **Leverage current advances in genomic technologies until whole-genome sequencing becomes more cost effective.**

Recommendation 1: Support expanded whole genome sequencing of viruses, sentinel human and animal hosts (including insect vectors) to enable elucidation of whole-genome influences on virus-host ecology. In the short term, increase use of standard and advanced PCR techniques for diagnostic surveillance purposes.

2. **Deriving predictive value from genetic sequences will require elucidation of the complex relationship between genotype and phenotype.**

Recommendation 2: Research funding agencies and professional societies should work together with APHIS and CDC to synergize those research and policy efforts needed to elucidate genotype phenotype relationships.

3. **The ability to predict species jumps is presently limited by organizational obstacles that hamper needed scientific progress.**

Recommendation 3: Form a trans-disciplinary permanent working group that can seek orthogonal approaches to key research questions, including: Can laboratory viral adaptation to animals or cell cultures be used to model species jumps? What evolutionary drivers underlie species jumps in wild-type viruses? What human host factors and polymorphisms ameliorate or exacerbate viral pathology?

4. **Future prediction capability must rest on a foundation of basic science that currently exists only in fragmented parts. ‘Gap-filling’ research will further progress in diverse fields ranging from vaccine development to microbial forensics to biosecurity policy.**

Recommendation 4.1: Support systemic study of a simple virus-host ecological model at the molecular, genetic, organismal, population and ecosystem levels, such as canine parvovirus.

Recommendation 4.2: Improve repositories and archival materials from retrospective studies relevant to species jumping research. The working group should organize and

facilitate an open source, interagency repository resource(s) to facilitate species jumping research.

Recommendation 4.3: Support the discovery, development, and testing of computational models incorporating essential biological, ecological, and evolutionary phenomena to enable prediction.

- 5. Before species jumps can be predicted, sustained animal surveillance systems must be in place in potential regions of emergence. In order to conduct predictive surveillance in the long term, stakeholders must lay the groundwork for optimizing surveillance efforts in the short term.**

Recommendation 5.1: Plans should be made to 1) efficiently and effectively collect and process samples; 2) format and share data to maximize usefulness and minimize access to sensitive information; and 3) develop cross-border standards and cooperative agreements to enable equitable data and sample sharing.

Recommendation 5.2: Practitioners should develop data formats and confidence measures that decision-makers would require before taking actions based on surveillance data and model predictions. Short-term and long-term surveillance goals can produce measurable societal benefits if those outputs are accepted among decision-makers internationally.

- 6. In order to implement any of the above recommendations, an adequately trained workforce is essential. Practitioners with training in mathematical modeling, informatics, veterinary science, virology, immunology, ecology, and evolutionary and vector biology are required.**

Recommendation 6: Funding agencies should create surveillance-specific grants that require and reward interdisciplinary teams and include training funds for students. Further, US Government research entities within Departments of Energy, Health and Human Services, and Defense should increase field and laboratory research positions where practitioners can develop and perform sustained surveillance activities using dedicated funds.

Background

Disease surveillance has primarily been an epidemiologic endeavor, capturing information from symptomatic humans or animals that were shown to be infected using diagnostic tests. Zoonotic disease surveillance is typically triggered after animal pathogens have infected humans. Are there ways to identify high risk pathogens before they emerge in humans? If so, then how and where can identifications be made, and by what methods? These were the fundamental questions driving a workshop to examine the future of predictive surveillance for viruses that might jump from animals to infect humans. Virologists, ecologists, and computational biologists from academia, US government and nongovernmental organizations discussed opportunities as well as obstacles to prediction of species jumps using genetic and ecological determinants from virus, host, vector and reservoir. This workshop marked an important first step toward envisioning both scientific and organizational frameworks for this future capability. Canine parvoviruses, seasonal H3N2 and pandemic H1N1 influenza viruses are discussed as exemplars that suggest *what to look for* in anticipating species jumps. To answer the question of *where to look*, prospects for discovering emerging viruses among wildlife, bats, rodents, insect vectors and the occupationally exposed are discussed. Finally, obstacles and opportunities are identified and accompanied by recommendations for *how to look* for species jumps. Taken together, these suggestions constitute the beginnings of a conceptual framework for achieving a predictive virus surveillance capability in the future.

Introduction

The majority of emerging pathogens in humans are zoonoses. Consequently, most zoonotic infections in humans are preceded by increased infection rates in animal reservoirs (which potentially include previously recognized intermediate hosts), suggesting that timely detection of virus in vectors or reservoirs may provide an early warning for imminent human infection. In the short term, protective mechanisms made possible by early detection could include human avoidance of high-risk areas, prophylaxis, or timely mobilization of surveillance and medical resources to cope with imminent or emergent disease.

In the future, persistent surveillance in sentinel animals could detect changes in pathogens that precede a species jump. As our understanding of virus-reservoir-host

ecology becomes sufficiently robust, we may be able to recognize patterns, processes and mechanisms entrained in species jumps, and these signals could enable prediction of a species jump before it occurs, allowing us to prevent outbreaks of human infections.

Defining Predictive Virus Surveillance. There are many current initiatives to improve disease surveillance (see Hitchcock et al. [1] for a review of these). The term *surveillance* is broadly applied, and the different modes of surveillance overlap or complement one another. *Public health surveillance* entails the ongoing collection of clinical incidence data for specific diseases within human populations. In the United States, epidemiologists working at local, state and national levels collect incidence data on reportable diseases and disseminate these data regularly to the public. *Syndromic surveillance* is the monitoring of generalized clinical phenomena that are associated with disease outbreaks (for example, collection of hospital reports of influenza-like illness (ILI)). *Infectious disease surveillance* typically refers to humans and entails collection of incidence data for diseases with infectious etiologies (versus incidence of noninfectious diseases like type II diabetes). *Emerging infectious disease surveillance* pertains to diseases that are new (e.g., HIV, SARS) or recurring after a period of low incidence (e.g., tuberculosis). *Wildlife surveillance* entails monitoring of pathogens within wildlife populations (e.g. H5N1 influenza in migratory birds), while *animal health surveillance* generally refers to monitoring of livestock, pet or captive animal populations.

The prospects for predicting infectious disease outbreaks have been reviewed and discussed by a relatively small number of scientific experts [2,3,4,5,6,7,8,9]. These articles suggest risk-based prioritization of viral surveillance efforts and represent promising first steps to that end. However, the complexity of the problem of predicting viral host jumps, particularly those involving changes in zoonotic viruses that make them more infectious to and transmissible among humans, will necessitate broad-based collaborations among scientists, public health experts of all types and funding agencies.

Spillover Events and Species Jumps. Zoonotic viruses cause infections in animals that can in some cases be transmitted to humans. Broadly speaking, humans may become infected with zoonotic viruses in one of two ways. In the first case, humans become infected with zoonotic viruses to which they are susceptible but are rarely exposed. These infections are called *spillover events*, and tend not to spread sustainably from human to

human. In the second case, zoonotic viruses undergo genetic changes that render them newly able to spread efficiently among humans. In this case the viruses previously may (or may not) have been able to cause sporadic human infections, but once these zoonotic viruses cause widespread and transmissible human infections, this shift from animal to human hosts is known as a *species jump* (see Table 1 for historical examples of each).

TABLE 1. HISTORICAL EXAMPLES OF SPILLOVER EVENTS [A] AND SPECIES JUMPS [B].

[A] Spillover Events					
Virus (species name)	Animal Hosts	Date	Location	Ref. #	
Marburgvirus (<i>Lake Victoria marburgvirus</i>)	Unknown*	1967	Marburg and Frankfurt, Germany**	[10, 11]	
Hantavirus (<i>Sin Nombre virus</i>)	Deer mouse (<i>Peromyscus maniculatus</i>)	1993	Four Corners area, US	[12]	
Monkeypox (<i>Monkeypox virus</i>)	Monkey, prairie dog, African rodents, et al.	1970	Liberia, Sierra Leone, Democratic Republic of Congo	[13]	
[B] Species Jumps					
Human-adapted Virus	Animal-derived Virus	Animal Host	Date of First Detected Human Outbreak/Case	Location	Ref. #
SARS coronavirus	SARS-like coronavirus	Civet (<i>Paguma larvata</i>), raccoon dog (<i>Nyctereutes procyonoides</i>)†	2003	Multi-country (Viet Nam, China, Singapore, Thailand, Canada)	[14,15,16]
HIV-1	SIVcpz (simian immunodeficiency virus chimpanzee)	Chimpanzee	before 1959‡	Leopoldville, Belgian Congo (now Kinshasa, Democratic Rep of Congo)	[17,18,19]
Influenza A subtype pdmH1N1	Influenza A subtype H1N1	Pig	2009	Northern Mexico	[20]
<p># We must take care when drawing a line of distinction between spillover events and species jumps. Spillover events are defined as occurring in humans already susceptible to infection. However, there are insufficient data to rule out the possibility that spillover viruses had undergone genetic changes that increased likelihood of human infection and fueled the spillover. In such a case, one might argue that a species jump occurred before spillover, blurring the distinction between the two concepts.</p> <p>* Recently, Marburg viral RNA and antiviral serum antibodies were detected in Egyptian fruit bats (<i>Rousettus aegyptiacus</i>) in Uganda (Towner et al., 2009).</p> <p>** While these outbreaks occurred in Germany, both were caused by exposure to the same lot of green monkeys (<i>Chlorocebus</i> sp, formerly genus <i>Cercopithecus</i>) imported from Uganda.</p> <p>† While infected animals have been detected in markets, they have not yet been detected in the wild.</p> <p>‡ Two more recent studies have narrowed this estimate to 1915-1941 (Korber et al. 2000) and 1884-1923 (Worobey et al. 2008) using phylogenetic analyses.</p>					

Most zoonotic surveillance efforts are **reactive** – that is, surveillance entails collection of incidence data from people who are already sick and seeks the sources of viruses that have already spread to humans. By contrast, **predictive** surveillance efforts aim to identify conditions that precede animal disease outbreaks using knowledge of host/pathogen biology and correlating that knowledge with climate and ecological data in order to predict outbreaks and provide timely warning to human populations [21,22]. In similar fashion, species jumps have historically been revealed by public health surveillance, but only once people were already sick. A limited number of surveillance programs, such as those undertaken by the Global Viral Forecasting Initiative and the EcoHealth Alliance, attempt predictive surveillance for species jumps. As this report will demonstrate, there are numerous obstacles, both technical and organizational, that challenge the development of predictive surveillance for species jumps – not least of which is the fact that such surveillance efforts, like the viruses they target, are emergent.

What to Look For: Virus-Host Ecology

Viral disease results from complex interactions between a **virus** and its **host**, and these interactions are shaped by the **environment** in which they take place (Box 1). In addition, there may be intermediate host reservoirs and/or insect vectors involved in the viral life cycle that can affect viral access to the host.

Box 1: Examples of factors that influence virus-host ecology

Viral factors: infectivity (proportion infected), pathogenicity (proportion ill), virulence (proportion severely ill), transmissibility, immunogenicity, mutation rates, environmental stability, genotype, phenotype, etc.

Host factors: genotype and phenotype, age, socio-cultural practices, gender, nutritional status, health status (co-infections or chronic illness), immune status, income, occupation (rural or urban), etc.

Environmental factors: climate (temperature, rainfall, seasonality), geography, land use, vegetation index, air quality, human/animal migration, global travel, introduction of exotic species (trade or smuggling), regional/global livestock trade, etc.

Levels of Virus-Host Interactions. There are multiple barriers to productive infection that a virus must surmount to infect and spread among individuals and populations. Virus-host interactions take place at molecular, cellular, organismal, population and ecosystem levels (Figure 1). Among these, the molecular and organismal levels are the best characterized – the result of decades of classical virological,

immunological and genetic studies. Obstacles to completion of the viral life cycle at the molecular level are outlined in Box 2.

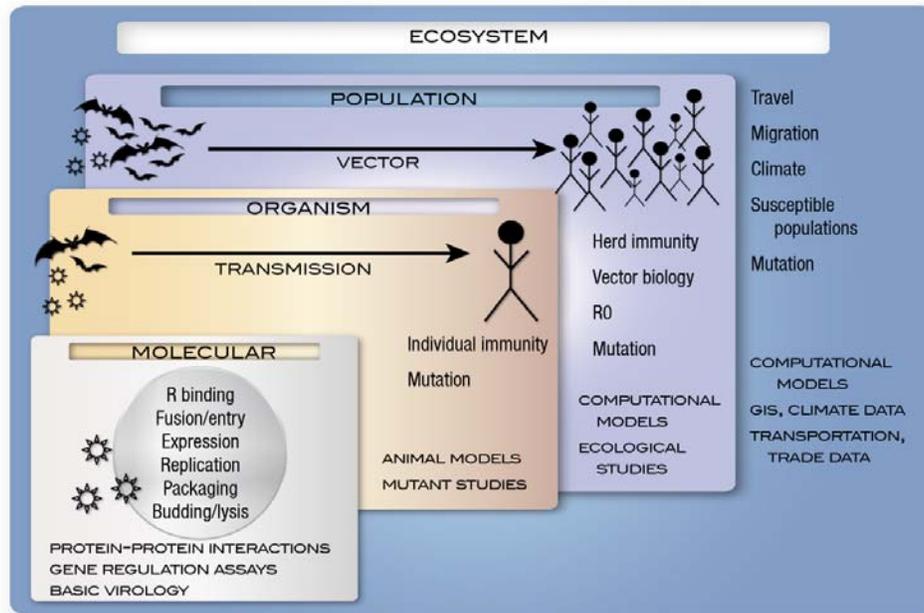


FIGURE 1. LEVELS OF VIRUS-HOST INTERACTION. VIRUSES INTERACT WITH THEIR HOSTS ON MULTIPLE ECOLOGICAL LEVELS; EACH LEVEL CAN BE CHARACTERIZED BY DIFFERENT BARRIERS (OR OPPORTUNITIES) TO PRODUCTIVE INFECTION AND TRANSMISSION, AS WELL AS TOOLS THAT MAY BE USED TO BETTER UNDERSTAND THESE MECHANISMS AND PROCESSES.

At the organismal level, animal models have provided significant insights into host susceptibility and immune responses to viral infection. Less understood is the role of natural population variation (versus the homogeneous populations used in animal studies), which is magnified at the metapopulation level. For example, influenza infection models in mice or other hosts yield different results depending upon which strain of host is used. At the population level, a virus must achieve a threshold reproduction rate in order to spread to susceptible new hosts; otherwise, the virus will become extinct within this population. Remaining unclear are the relative contributions of the numerous steps in transmission associated with failure of most viruses to achieve this threshold, or what genotypes, viral properties or host qualities make transmission possible. (See Woolhouse, Haydon and Antia [23] for a brief explanation of reproduction rate within a larger discussion of species jumps.)

Box 2: Molecular-level obstacles to host infection

Access/Receptor binding. Virus particles (virions) must gain physical access to susceptible host cell types, i.e., those that express surface receptors to which virions can bind. Routes of host entry include respiratory, gastro-intestinal, and reproductive mucosa (via inhalation, ingestion, and sexual exposure, respectively) as well as blood or lymph (via broken skin or injection). *Tropism* is the affinity that a given virus has for particular host cell receptors, cells or tissues.

Fusion/Entry. Binding of virions to receptors on host cells is necessary, but may still be insufficient to initiate infection. Thereafter, virions must effectively enter the cell and release their genetic material (RNA or DNA) into the cytoplasm.

Expression. Some virus types already contain proteins that orchestrate replication of viral genetic material (e.g., poxviruses). For other types, viral RNA or DNA must first be transcribed and translated by host systems to produce the viral proteins necessary for virus replication. This may be a host-specific process.

Replication and Packaging. Viral RNA or DNA must be copied and packaged into a coat of viral proteins and in some cases membrane envelopes to produce progeny (offspring).

Budding. Progeny viruses must fuse with and bud out of the host cell membrane before infecting adjacent cells.

Transmission. Viruses must spread from one host organism to the next to perpetuate their life cycle.

Influenza Viruses. To predict which influenza viruses may jump from avian, swine or other animal species to humans, it is essential to understand both how influenza viruses switch *tropism* (affinity for one species over another) as well as how *virulence* is manifested within a host species to cause disease. Despite extensive investigation of these mechanisms in influenza viruses, a holistic picture of the prerequisite adaptations for species jumps remains elusive. Furthermore, without a comprehensive understanding of host-specific replication, transmission or virulence mechanisms, scientists are unable to predict tropism shifts nor anticipate how new human hosts will be affected.

H1N1 Jump from Swine to Humans. Shortly after its detection in humans, the 2009 H1N1 pandemic influenza A virus (A/H1N1pan) was determined by phylogenetic analysis to have arisen from combinations of viruses that were previously infecting human, swine and avian hosts [24]. Subsequent animal studies revealed host-specific differences in virulence among A/H1N1pan strains – that is, animal species (mice, ferrets, macaques) were affected differently depending upon the strain with which they were infected [25].

These pathotype variations suggest that for a given A/H1N1pan strain, different species are more or less susceptible to infection, and/or develop different immune responses that diminish or worsen outcomes of infection, leading to species-specific differences in morbidity and mortality. Despite fears, A/H1N1pan has exhibited a global mortality rate of far less than 1%, compared to an estimated 2.5% for 1918 pandemic influenza A virus [26,27]. The data collected from the 2009 pandemic may provide new insights into the tropism and virulence mechanisms utilized by the strains that caused it. For example, Smith et al. [28], utilizing a Bayesian molecular clock analysis of swine-origin influenza virus (S-OIV) outbreak strains, estimated that the A/H1N1pan common ancestor emerged between August 2008 and January 2009. Additional retrospective analyses may help reveal why sentinel cases during this timeframe went unnoticed. The possibility that A/H1N1pan emerged up to eight months before detection illuminates the uncertainties, opportunities and risks accompanying the current zoonotic viral surveillance vacuum.

H3N2 Epochal Evolution. Seasonal H3N2 influenza viruses are capable of evading immune recognition through continual antigenic drift of their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), complicating long-term control of the disease through vaccination. Despite high mutation rates, HA/NA genetic diversity is constrained. This limited diversity is evident in its phylogeny, which shows high extinction rates that result partly from some degree of cross-immunity between similar strains. That is, many HA/NA mutants go extinct because they fail to spread efficiently from host to host due to the presence of previously infected individuals. Several hypotheses have been suggested to explain how competition between closely related strains interacts with other factors to limit the observed diversity of HA and NA. One hypothesis suggests that short-term, strain-transcending immunity may limit the growth and mutation of influenza strains [29]. Another hypothesis is that punctuated antigenic changes in HA may precipitate selective sweeps, allowing sufficiently novel mutants to outcompete related strains of the same subtype [30]. This process has been termed “epochal evolution,” as the discovery of new antigenic phenotypes depends on periods of extensive genotypic change with generally minor but occasionally dramatic effects on phenotype.

How are the patterns of seasonal influenza in humans useful to predicting species jumps? Understanding the dynamics of influenza in human hosts sheds light on the

potential of the human population to be infected by new strains, the probability that a spillover virus can acquire evolutionary adaptations to facilitate spread in humans, and the abilities of intermediate hosts (such as chickens and pigs) to generate pandemic viruses. Seasonal influenza creates cycles of relatively higher and lower immunity in humans: epidemics deplete susceptibles, leaving a higher fraction of the population with protective immunity. Some of this immunity has been shown to be partially protective against viruses of other subtypes (e.g., infection with seasonal influenza can confer partial protection to infection with H5N1). In addition, the diversity of viruses circulating in humans should in theory correlate with the potential for an emerging virus to swap gene segments with an adapted resident, which could increase the emerging virus's rate of transmission. Reassortment events are commonly associated with seasonal influenza and appear to be an integral evolutionary step in pandemics. The generation of pandemic viruses through reassortment depends sensitively on parallel dynamics in the intermediate host population, including the amount of herd immunity in non-human hosts and the dynamics of viral diversity in that host population. For example, it has been observed that pig populations can contain a much greater diversity of H3N2 viruses, including antigenic variants of H3 HA that have long been extinct in the human population [31], but the rate of viral antigenic evolution in pigs is slower than in humans. As with humans, an important question is how host immunity interacts with geography, birth and death processes, and viral mutation to generate the observed patterns of influenza diversity. Understanding these basic processes should allow the long-term effects of interventions on viral evolution to be predicted and shed light on which steps (such as key mutations or rates of contact between hosts [32]) limit emergence.

Arguably, the infectious disease field is at a disadvantage when it comes to elucidating influenza viruses, because those viruses are segmented and prone to reassortment in addition to mutation within segments. Seasonal, epidemic and pandemic influenza viruses remain significant and persistent public health threats. Experimental work with simpler zoonotic viruses would also yield additional insights into tropism and virulence changes that accompany species jumps, and may in turn help to elucidate how influenza viruses make these jumps.

Parvovirus Jump between Cats, Dogs and Raccoons. Parvoviruses infect several carnivorous species, including domestic dogs and cats as well as wild foxes, mink and raccoons. While those viruses are not infectious to humans, these viruses are known to have made a species jump from cat to dog, and also to raccoons. Their small, single-stranded DNA genomes (comprised of two genes that encode four proteins) and widespread occurrence among domestic and wild carnivores make the parvoviruses particularly useful as model viruses for understanding how species jumps occur.

In the late 1970s, canine parvovirus (strain 2, CPV-2) emerged as a new pathogen infecting dogs and spread globally within the year [33]. That virus was clearly shown to be a descendant of a cat virus (feline panleukopenia virus, FPV) that jumped from cats to dogs within five years prior to its emergence. Since that time, CPV-2 has continued to evolve, and in one of those steps it re-acquired the ability to infect cats while continuing to evolve within its canine host. Phylogenetic analyses reveal that changes of residues on the surface of the viral capsid proteins. Although those are single-stranded DNA viruses, they show high levels of variation, similar to that seen for RNA viruses. Parrish and colleagues have shown that many of the genetic differences between CPV and FPV associated with host range variation occur in these capsid protein genes, resulting in a tropism shift that enabled the species jump from cats to dogs. They further characterized the viruses structurally, and showed that they differ in their antigenicity and exhibit species-specific differences in attachment to the host cell receptor (transferrin receptor type 1, TfR) [34]. In the case of the FPV-to-CPV jump, genotypic changes (likely about 5 mutations) gave rise to the changes of the viral capsid that enabled the new virus (CPV-2) to bind to the transferrin receptor in the canine host.

However, further research to elucidate the genotype-phenotype relationships for other viruses must be undertaken in order to determine how to identify viruses with altered host range properties. Since many zoonotic viruses bind to animal host receptors for which orthologous receptors exist in humans, laboratory studies using pseudotyped zoonotic viruses in human cell systems may reveal genotypic and phenotypic changes that enable tropism shifts.

Where to Look: Discovering Spatial Patterns

The preceding section outlined levels of virus-host interactions and, using specific examples of species-jumping viruses, suggested some means by which viruses adapt to new host species. However, in these and other examples, the sources of any viral samples collected for analysis are crucial to detecting informative changes. To develop a predictive capability for species jumps, it is important to consider not only what to look for, but also where to look.

Wildlife Reservoirs of Viruses. Historical reviews [7,35,36] of emerging infectious disease (EID) events have shown that 1) most are of zoonotic origin; 2) among zoonotic EID events, most originated in wildlife; and 3) an estimated 10-40 new human viruses are expected to emerge by 2020. In 2008, Jones and colleagues [24] found that “Wildlife host species richness [a measure of the geographic distribution of 4219 terrestrial mammalian species] is a significant predictor for the emergence of zoonotic EIDs with a wildlife origin.” When plotted on a global map, the areas at greatest risk for zoonotic pathogen emergence (“hotspots”) were equatorial developing nations. (By contrast, the most intensive EID research and surveillance efforts were concentrated in developed countries.) These investigators and others [37] suggest that surveillance efforts can be rationally focused both geographically and based on income. These data were compiled before the emergence of A/H1N1pan in 2009 in Mexico, but as more geolocated virus sample information becomes available, biogeographic relationships may be revealed and predictors identified.

Zoonotic surveillance efforts focused in hotspots, such as those undertaken by investigators from the U.S. Centers for Disease Control (discussed below) and the Global Virus Forecasting Initiative (GVFI), offer evidence that such efforts provide information that makes predictive surveillance possible [4], including discovery of a novel retrovirus in monkey and human populations [38,39]. The ability to make correlations between homologous viruses transferred between proximal species will be fundamental to predicting species jumps.

Bats and Rodents. Of the more than four thousand known mammalian species, ~50% are rodents and ~25% are bats. This rich species diversity, plus other ecological traits (high population densities and reproductive rates), suggest that some surveillance efforts would be well focused on rodents and bats. Rodents are typically small and can be

trapped in large numbers for surveillance, and they are easier to handle and less expensive to keep in laboratory settings than large animals. The ability to study viral infections in animal hosts under controlled laboratory conditions is central to understanding virus-host ecology at molecular and organismal levels, including the duration and severity of infection, immune response, tissue tropism and pathology. Laboratory-induced infections can also clarify the species that are true reservoirs among the various susceptible host species.

As with other wildlife, importation of exotic rodents can drive viral emergence. In 2003, a multi-state U.S. monkeypox outbreak was driven by exposure to prairie dogs (*Cynomys* sp.), which were infected by exposure to Gambian giant rats (*Cricetomys* sp.) [40]. Also, one human case was acquired from a rabbit that became infected when exposed to a prairie dog in a veterinary setting. In this case, rodents commercially captured in forested areas of southern Ghana were the sources of the U.S. outbreak, and a 2010 study by the U.S. CDC found that 53% of nearby human residents had been previously exposed to monkeypox [41]. While the 2003 outbreak was likely a spillover event, surveillance efforts focused on the international rodent pet trade may detect such events and enable genotypic/phenotypic characterization of viruses that jump among rodent species and to humans and pets.

Insect Vectors. Many viruses are transmitted to animals and humans from insect vectors. West Nile, Chikungunya and Yellow Fever viruses are examples of insect-borne viruses that have jumped to new mosquito host species. Insects are themselves members of the animal kingdom and flying insects can greatly expand viral access to bird, wildlife and human hosts. While collecting samples from wildlife is a resource-intensive endeavor, large numbers of known insect vectors can be collected at much lower cost, making virus surveillance in insects an attractive goal. Furthermore, geographic information systems (GIS)-based maps that layer environmental measurements (temperature, precipitation, land use) and vector/host distribution data can be used to inform rational decisions about when and where surveillance samples should be collected. This approach has been used to correlate environmental factors with competent West Nile Virus vectors trapped in urban areas of the northeastern United States [23,42]. Assembling such risk-based maps would concentrate surveillance efforts to maximize impact and minimize cost. While detecting genetic precursors to species jumps in sampled viruses is a long-term goal for which

underlying knowledge is lacking, in the short-term, characterizing endemic viruses harbored by local insect vector populations would provide baseline information required for future prediction. Such knowledge can be used to assess risk to human populations and drive mitigation strategies (e.g., vector control strategies).

Occupational Infections. There are occupations whose members are frequently (and in some cases continually) exposed to zoonotic viruses, including veterinarians, farmers, ranchers, tanners, and food processors. Immunity acquired among members of this "front line" group, whether through symptomatic or asymptomatic infection, would alter the dynamics of infection and the spread of zoonotic pathogens. Yet there are surprisingly few serological surveys in the literature reporting the patterns and mechanisms of exposure, including the consequences for immunity, among the occupationally exposed.

For example, exposure to swine influenza has caused elevated levels of anti-swine influenza antibody among animal workers. Olsen et al. [43] found higher seropositivity to swine-adapted influenza viruses among swine farm employees and their families than in people with no swine contact. Myers et al. [44] found that farm workers, veterinarians, and meat-processing workers all had greatly elevated serum antibody levels for swine isolates of H1N1 and H1N2, compared with controls. Extension of such serological surveys to other at-risk occupational groups would help define a baseline for the frequency of cross-species transfer of zoonotic viruses.

How to Look: Needs and Recommendations

The following is a discussion of recurrent issues that present both obstacles and opportunities to achieving a predictive virus surveillance capability, accompanied by recommendations for achieving progress.

Leverage Advances in Genomic Technologies. While whole-genome sequence data may be ideal in the long term for maximizing information about emerging or re-emerging viruses, deep sequencing remains in the short term a relatively expensive and time-consuming method. This is especially true when considering the large number of samples that sustained surveillance efforts would require. Standardized PCR assays are a

quicker, less expensive alternative, but primer sets may fail to capture mutant strains or new viruses. MassTag PCR is a relatively quick and inexpensive tool that has successfully identified novel pathogens, including members of the parvovirus [45], rhinovirus [46], and arenavirus [47] families. The TIGER broadband pathogen detection system was also extremely useful in identifying the A/H1N1pan index case in the United States, which was “untypeable” human influenza A by standard methods [48].

Recommendation: Support expanded whole genome sequencing of viruses, sentinel human and animal hosts (including insect vectors) to enable elucidation of whole-genome influences on virus-host ecology. In the short term, increase use of standard and advanced PCR techniques (including MassTag and TIGER) for diagnostic surveillance purposes.

Elucidate Genotype-Phenotype Relationships. Deriving predictive value from genetic sequences will require elucidation of the complex relationship between genotype phenotype, pathotype and ecotype [2]. Over-reliance on genomic (versus phenotypic) studies will not enable prediction of which viruses will jump to new host species. Even for well-characterized viruses like HIV and influenza, it is currently challenging to determine from sequence information whether a given viral strain will be more or less virulent (or able to replicate) in a given host. Understanding the relationship between genotype and phenotype is one of biology’s “holy grails,” the elucidation of which will require the combination of many different and diverse approaches [2].

Recommendation: The US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and the CDC are tasked with populating the Select Agent List, comprised of those organisms whose possession and use are regulated by the U.S. Government. There is impetus to develop a system of oversight that makes use of DNA sequence-based screening to make determinations about which microbial agents belong on the Select Agent List (and therefore, which agents will be regulated). A committee was formed by the National Research Council to address the monumental challenges to determining virulence (phenotype) from sequence (genotype) data [49]. Research funding agencies and professional societies should work together with APHIS and CDC to synergize the research and policy efforts needed to elucidate genotype/phenotype relationships.

Overcome Organizational Obstacles. The ability to predict species jumps is presently limited by organizational obstacles that hamper needed scientific progress. First, prediction requires inputs derived from many disparate bioscience fields (virology, ecology, evolutionary and computational biology, immunology, veterinary science, wildlife biology, etc.) that have little history of collaboration or current impetus to do so. No single field can accomplish the required research, obtain the desired knowledge or develop actionable models, but such trans-disciplinary collaboration can push experts and funding agencies outside their comfort zones, creating barriers to progress.

Second, there exists within the U.S. Government no single funding agency with the mission to achieve a future capability to predict and prevent species jumps. At the same time, there are several stakeholder agencies that house scientific expertise and/or manage funding streams, many of which are accustomed to working together. New biosurveillance supply/demand architectures would be required to achieve real progress in this area.

Recommendation: Form a trans-disciplinary permanent working group that can seek orthogonal approaches to key research questions, including: Can laboratory viral adaptation to animals or cell cultures be used to model species jumps? What evolutionary drivers underlie species jumps in wild-type viruses? What human host factors and polymorphisms ameliorate or exacerbate viral pathology?

Fund Basic Research. Future prediction capability relies on a foundation of basic science that currently exists only in fragmented programs. ‘Gap-filling’ research will yield synergistic benefits and further progress in diverse fields ranging from vaccine and drug development to microbial forensics to biosafety and biosecurity policy. The aforementioned working group should advocate for advancing, among others, three program areas:

Recommendation 1: Support systemic study of a simple virus-host ecological model at the molecular, genetic, organismal, population and ecosystem levels, such as canine parvovirus and other models of host switching where fundamental mechanistic details can be revealed.

Recommendation 2: Improve repositories and archival materials from retrospective studies relevant to species jumping research. Carefully curated collections

allow for “hypothesis-generating” (vs. hypothesis-testing) research; these community resources should go beyond commercial and boutique collections in their depth, range, accession quality/specificity, and validation testing. The working group should organize and facilitate an open source, interagency repository resource(s) to facilitate species jumping research.

Recommendation 3: Support the discovery, development, and testing of computational models incorporating essential biological, ecological, and evolutionary phenomena to enable prediction. Multidisciplinary teams should be organized and funded to curate data sets, build and validate new models, improve extant models, and most importantly, define data requirements and quality objectives for future predictive models that could be used to drive new sample collection and algorithm improvements.

Devise a Global Surveillance Strategy. Before species jumps can be predicted, sustained animal surveillance systems must be in place in potential regions of emergence. This is a challenge in a world where developing countries lack resources, and developed countries lack the mandate and the infrastructure for livestock or in some cases human EID surveillance. In order to conduct predictive surveillance in the long term, stakeholders must lay the groundwork for optimizing surveillance efforts in the short term, and for understanding the properties of potentially emerging viruses.

Recommendation 1: Plans should be made to 1) efficiently and effectively collect and process samples; 2) format and share data to maximize usefulness and minimize access to sensitive information; and 3) develop cross-border standards and cooperative agreements to enable equitable data and sample sharing.

Recommendation 2: Practitioners should develop data formats and confidence measures that decision-makers would require before taking actions based on surveillance data and model predictions. Short-term and long-term surveillance goals can produce measurable societal benefits if those outputs are accepted among decision-makers internationally.

Train and Sustain the Workforce. In order to implement any of the above recommendations, an adequately trained workforce is essential. Practitioners with training

in mathematical modeling, informatics, veterinary science, virology, immunology, ecology, and evolutionary and vector biology are clearly required. Furthermore, these workers need be accustomed to working together, across disciplinary boundaries, and toward common goals. Anecdotal evidence suggests that practitioners in required fields like entomology have steadily declined, while newer fields like modeling and informatics still have too few trainees. While increasing the number of trainees is frequently recommended for advancing science and technology, a corollary need for job and career opportunities is often overlooked: Passionate trainees become seasoned practitioners only when they have a viable and rewarding career path.

Recommendation: Funding agencies should create surveillance-specific programs that require and reward interdisciplinary teams and include training funds for students. Further, US Government research entities within Departments of Energy, Health and Human Services, and Defense should increase field and laboratory research positions where practitioners can develop and perform sustained surveillance activities using dedicated funds.

Conclusions

Pepin et al. [2] and Childs [50] have published comprehensive reviews of the mechanisms, processes and dynamics that shape host/species jump risks. Their analysis and the considerations discussed here suggest that the problem space is vast, and distinguishing causal predictive signatures for host/species jump risk is challenging. They further suggest that viral convergent evolution could be a driver for adaptation to new hosts, and that biosurveillance systems tailored to recognize salient changes in viral fitness for alternative hosts could cue early warning of species jumps such as the evolution of SARS. The emergence of A/H1N1pan in North America highlights the uncertainties and challenges in differentiating convergent viral evolution from true human-to-human transmission chains – yet understanding the sources of new viruses is critical to understanding how they emerged. The extant zoonotic viral surveillance vacuum [51] relegates the power of sequence and phylogeny-based analytics to the reactive realm of outbreak reconstruction. There is an urgent need for pervasive surveillance capability at nodes of disease emergence. This surveillance could proactively direct tools for disease characterization, response, and mitigation to flash points while localized outbreak control is still possible.

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