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Current Opinion in Virology

Animal virus discovery: improving animal health, understanding zoonoses, and opportunities for vaccine development

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The characterization of viral genomes has accelerated due to improvement in DNA sequencing technology. Sources of animal samples and molecular methods for the identification of novel viral pathogens and steps to determine their pathogenicity are listed. The difficulties for predicting future cross-species transmissions are highlighted by the wide diversity of known viral zoonoses. Recent surveys of viruses in wild and domesticated animals have characterized numerous viruses including some closely related to those infecting humans. The detection of multiple genetic lineages within viral families infecting a single host species, phylogenetically interspersed with viruses found in other host species, reflects past cross-species transmissions. Numerous opportunities for the generation of novel vaccines will arise from a better understanding of animal viromes.

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Current Opinion in Virology 2012, **2**:344–352

This review comes from a themed issue on
Vaccines
Edited by Connie Schmaljohn and Christian Mandl

Available online 15th March 2012

1879-6257/$ – see front matter
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DOI 10.1016/j.coviro.2012.02.012

Introduction

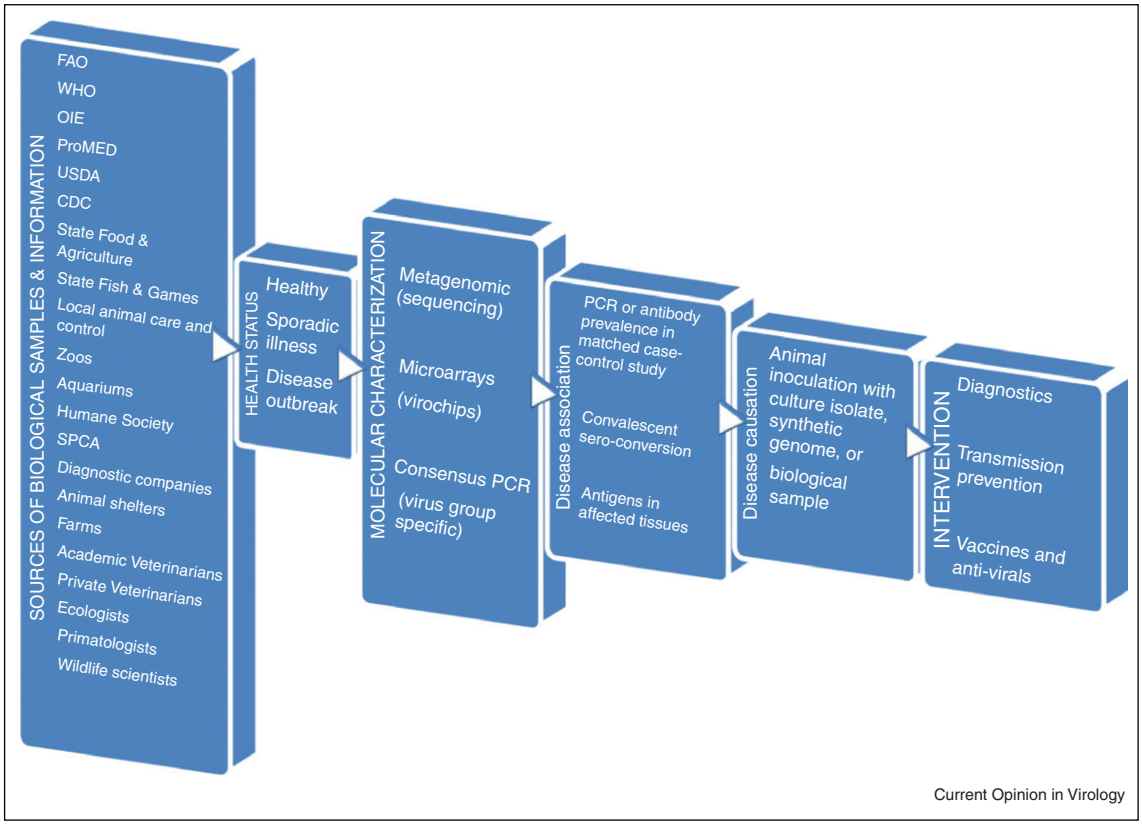
The rate of viral discovery has recently increased due to the introduction of next generation sequencing technologies and the analyses of biological samples of diverse geographic origins from multiple host species. By 2006 the number of known human viral species was estimated at approximately 180 [1]. In 2009 the number of all ICTV defined viral species, including both eukaryotic viruses and bacteriophages, stood at approximately 2200 (http://www.ictvonline.org/virusTaxInfo.asp). Compared to the sustained efforts in human virus discovery, viruses infecting other species, including >4200 species of mammals [2], have been greatly under-sampled. While the number of known, globally prevalent human viruses (excluding geographically restricted and emerging viruses), may eventually reach a plateau, the rate of discovery of animal viruses is expected to rapidly increase. The generation of more fully characterized animal viral genomes, from more host species, will improve our understanding of viral evolution, cross species transmissions, and will provide new opportunities for animal vaccine development particularly for domesticated and endangered wild species.

Sources of biological samples for animal virus discovery

Specimens to analyze for new viruses include those from animals with diseases affecting farm productivity or the survival of critically endangered or threatened species. Farmers, with their extensive knowledge and experience of animals, can readily identify new health problems. The health of animals in zoos and aquariums is also of interest given their high level of care, the diverse viral exposures they may experience, and their close proximity to human handlers. Companion animals, due to their extensive health care and close contact with owners may also be a readily accessible source of animal samples for pathogen discovery. Shelters for abandoned or feral animals, because of their crowded conditions and the high susceptibility to infections of their often undernourished and weakened residents also provide a fertile breeding ground for viral epidemics and pathogen discovery (Figure 1).

Outbreaks of acute disease on farms and in animal shelters greatly facilitate virus-disease association studies if appropriate data and sample collection occurs. The affected animals should all exhibit pathogen-specific markers of infection, such as sero-conversion or the presence of a newly characterized virus. The pathogenicity of a new virus can also be tested in animals following direct inoculation with viral isolates (minimally passaged to prevent attenuation), the original biological samples (if shown to contain no other virus by metagenomics), or by synthesizing the genome and transfecting it *in vitro* to generate infectious particles. Because of their protected status, such inoculations are not feasible in endangered species where more indirect means of testing disease causation, akin to the situation for novel human viruses, are required [3,4]. By identifying unusual symptoms or disease outbreaks, both academic and private veterinarians and scientists also contribute to the identification of previously unknown or emerging animal pathogens. Federal departments such as the USDA and CDC, state organizations concerned with fish and game or food and agriculture, and local government groups involved

Figure 1



Flow chart of animal virus discovery, pathogenicity determination, and interventions.

in animal care and control can also identify disease outbreaks in wild, farm or companion animals and collect samples for further studies. The Humane Society and the Society for the Prevention of Cruelty to Animals, by closely monitoring and promoting animal health, may also detect and report early signs of emerging infections. International organizations such as the Food and Agriculture and World Health Organizations of the United Nations, World Organization for Animal Health (i.e. OIE), and ProMED can also assist in the recognition of emerging animal health problems, dissemination of information, and in coordinating international collaborations (Figure 1). A growing realization of animals as the source of most emerging human and animal infections has led to the One Health Initiative to foster collaborations between physicians, veterinarians, and scientists to monitor the exchange of infectious agents between species [5–12].

Bats, rodents, and primates are notorious sources of zoonotic infections, possibly a result of their very large colony sizes facilitating maintenance of viral transmission chains, frequent association with humans, and their close genetic relatedness to humans respectively. The consumption of wild animals as bush meat, particularly of non-human primates, also provides a portal of entry of animal viruses into human populations [8,10,11]. Large unbiased or viral family specific surveys of these and other mammalian groups to characterize their viruses will enhance our understanding of the original animal reservoirs of many current human viruses. Viral infections may be mostly asymptomatic in their long-term hosts, but pathogenic in a new host species. Viral metagenomics and more virus family specific surveys have therefore been used to characterize viral populations in both sick and healthy animals [13,14,15•,16–28]. The buildup of known animal viral genome sequences will also allow their inclusion in updated high-throughput virus detection assays, such as micro-array 'virochips,' able to very sensitively detect known viruses and their close genetic relatives [29,30,31•]. Including probes from the growing number of viral genomes on micro-arrays also allows simultaneous disease association studies for multiple viruses using animal (and human) cohorts. The availability of biological samples from large numbers of epidemiologically matched unexplained disease cases and healthy controls is likely to be a major limiting factor for determining which of the rapidly growing number of animal viruses are likely pathogens and therefore targets for transmission control measures or vaccine development.

Molecular methods for viral discovery

Many classical methods of viral discovery such as cell inoculation and monitoring for cytopathic effects can yield pure viral cultures, but are subject to the availability of susceptible cell lines and infectious inoculums. The introduction of molecular methods has greatly simplified the genome characterization of both known and emerging or previously unrecognized viruses. Consensus PCR, targeting conserved viral genome regions, [32,33] can be used to rapidly screen large numbers of samples for any group of related viruses such as herpes viruses [34], astroviruses [35,36], and enteroviruses [37]. The downside of this sensitive method is the requirement for a priori knowledge of which viral family is likely to be present in order to avoid the need for numerous PCR primer sets targeting a large number of different viral families. Rolling circle amplification preferentially amplifies circular DNA viral genomes and has greatly enhanced their discovery but is less efficient for linear DNA or RNA genomes [38–40]. Microarrays spotted with oligonucleotides of the most conserved viral regions have also been highly successful but are limited by the amount of mismatch they can tolerate such that highly divergent species (relative to those previously known and spotted on arrays) may not hybridize [29,30,31•,41–44]. Random nucleic acid amplification with or without prior enrichment for viral particles [45], followed by DNA sequencing (including next generation sequencing) and in silico similarity searches for sequence related to those of known viruses has been highly productive [46–49,50•,51,52,53•,54,55]. This metagenomic approach is limited by the need for novel viral sequences to show detectable protein or nucleic acid sequence similarity to those of the many already sequenced viruses.

Anticipating zoonoses

The sources of many emerging viral diseases are animals in contact with the new viral host or with an intermediate bridge species [5–12]. Initially, cross-species transmissions are thought to result in weakly adapted viruses that through mutations may evolve to increase their pathogenicity and transmissibility in the new host species. A well understood example of cross-species transmission is of a feline parvovirus adapting to dogs in the late 1970s followed by its global spread and increase in pathogenicity [10,56]. Mutations in the feline parvovirus surface glycoprotein allowed infection and transmission in dogs [57,58••]. Further adaptation of the original canine parvovirus may have occurred through intermediate species such as raccoons [59]. The emergence of HIV1 groups M and N from chimpanzees, HIV group P from gorillas and HIV2 from sooty mangabeys, most likely through bush meat hunting, butchering, and consumption is also generally accepted [60,61••,62,63]. SIVcpz, the presumed progenitor of HIV, may itself be a recombinant of two retroviruses from monkeys preyed upon on by chimpanzees [64]. Influenza viruses are especially notorious for their ability to transfer from birds to mammals such as pigs, that can act as intermediate hosts, facilitating recombination with porcine influenza viruses before transmission to humans [56,65,66]. Bats and rodents appear to be frequent sources of viral zoonosis but the very high number of these animal species and their global distribution makes a systematic determination of their viromes difficult. The genetic characterization of viruses per se in these frequent virus donor species does not a priori provide information regarding the likelihood of successful transmissions to human. Zoonoses are dependent on complex interactions between viral phenotypes and host genetics (particularly surface receptors and innate immune responses), cross-neutralization by antibodies to related viruses, and epidemiological factors influencing viral exposures. The high mutability of viral genomes indicates that, provided some chronic low-level replication occurs in a new host species, these viruses have the potential to further adapt increasing both their viral load and the possibility of transmission in that species. The diversity of emerging or re-emerging human viruses such as HIV (*Retroviridae*) and the SARS virus (*Coronaviridae*) that are transmissible between humans, zoonotically acquired viruses capable of only limited transfer between humans such as the Ebola virus (*Filoviridae*) and Lassa virus (*Arenaviridae*), 'dead end' zoonoses without the necessary adaptation to facilitate ongoing transmission between humans such as rabies virus (*Rhabdoviridae*), Hendra virus (*Paramyxoviridae*), and monkeypox virus (*Poxviridae*), as well as the arthropod vectored West Nile and Japanese encephalitis viruses (*Flaviviridae*), Crimean-Congo hemorrhagic fever virus (*Bunyaviridae*), and Chickungunya virus (*Togaviridae*), indicate that sequence members of any of the known viral families infecting animals could potentially become epidemic in humans. The recent demonstration of an adenovirus (*Adenoviridae*) from a titi monkey outbreak of respiratory symptoms infecting a scientist at a primate center and this person transmitting the virus to a human contact, further illustrates the wide range of viral families that can be considered capable of at least some level of replication in multiple host species [67••]. This study exemplified the speed with which an adenovirus could be transmitted from an unknown host to titi monkey, between titi monkeys, from a titi monkey to a human and between at least two humans. Some genotypes of hepatitis E virus (*Hepiviridae*) are capable of oral-fecal transmission between human while other genotypes are acquired by consuming infected animal meat, but are inefficiently transmitted between human [68,69]. Simian foamy and T-lymphotropic viruses (*Retroviridae*) that have infected persons exposed to non-human primates can also be considered as viruses constantly 'probing' human populations but that, unlike HIV1 and HIV2, have not adapted sufficiently to be transmitted between humans [70,71,72••,73–75]. Certain human viruses such as influenza (*Orthomyxoviridae*) are periodically acquired directly from avian or mammalian hosts. The highly lethal

H5N1 influenza circulating in birds is currentlyy poorly transmissible between humans or between other mammals although rapid passage experiments in ferrets or direct mutagenesis have exposed its latent capacity to rapidly increase its pathogenicity and transmissibility [76,77,78•].

The wide diversity of viruses capable of switching host species therefore highlights the difficulty in predicting from which viral family will emerge the next human viral pandemic. Because increasing genetic distances between hosts is a significant block to cross species transmission [79••,80,81], there has been a focus on identifying viruses and immune response shared between non-human primates and people exposed to them [70,71,72••,73,74,82]. Since the frequency and intensity of viral exposure can also be expected to increase the likelihood of cross-species transmission, the study of viruses in farm or companion animals with extensive contact with both humans and wildlife should also uncover viral species of concern for future zoonoses. Sero-surveys for antibodies to these viruses would reveal the extent of their replication in highly exposed humans. Arboviruses also present a growing threat as seen with resurgent West Nile, Dengue, Japanese encephalitis and Chikungunya viruses following introductions in new locales or extension of the range of their insect vectors [83•]. Monitoring for new arboviruses in anthropophilic arthropod vectors may provide novel viral genomes whose capacity to infect humans or other mammals can then be tested serologically. Vaccinating animal reservoirs for some arboviruses could warrant considerations to reduce spill-over infections into humans.

The recent characterization of the closest known genetic relative of the human HCV [84••] and enteric Aichi viruses in canine samples [14,85] point to dogs as a potential zoonotic origin of these now common human infections. The direction of transmission (dogs to human or human to dogs) cannot be revealed by genetic similarities alone and future viral discoveries may reveal yet closer relatives of these and other human viruses [16]. As sampling of animal viruses increases, a complex network of past cross-species transmission will likely emerge. For example until recently only a single species of astrovirus (HAstV) and of parvovirus (B19) were known to infect humans. Viral survey in human have now shown that multiple genera and species within the *Parvoviridae* [46,86–89] and *Astroviridae* [90–92] can infect humans. Multiple lineages of these viral families can also be found in pigs and other animals [15•,17] (Figure 2). Distinct phylogenetic clades within a viral family also include viruses found in different mammal hosts, likely reflecting cross-species transmission of parvoviruses [93–96] (as was recently documented for the feline to canine CPV2 transfer in the 1970s) and of astroviruses (Figure 2) [90–92].

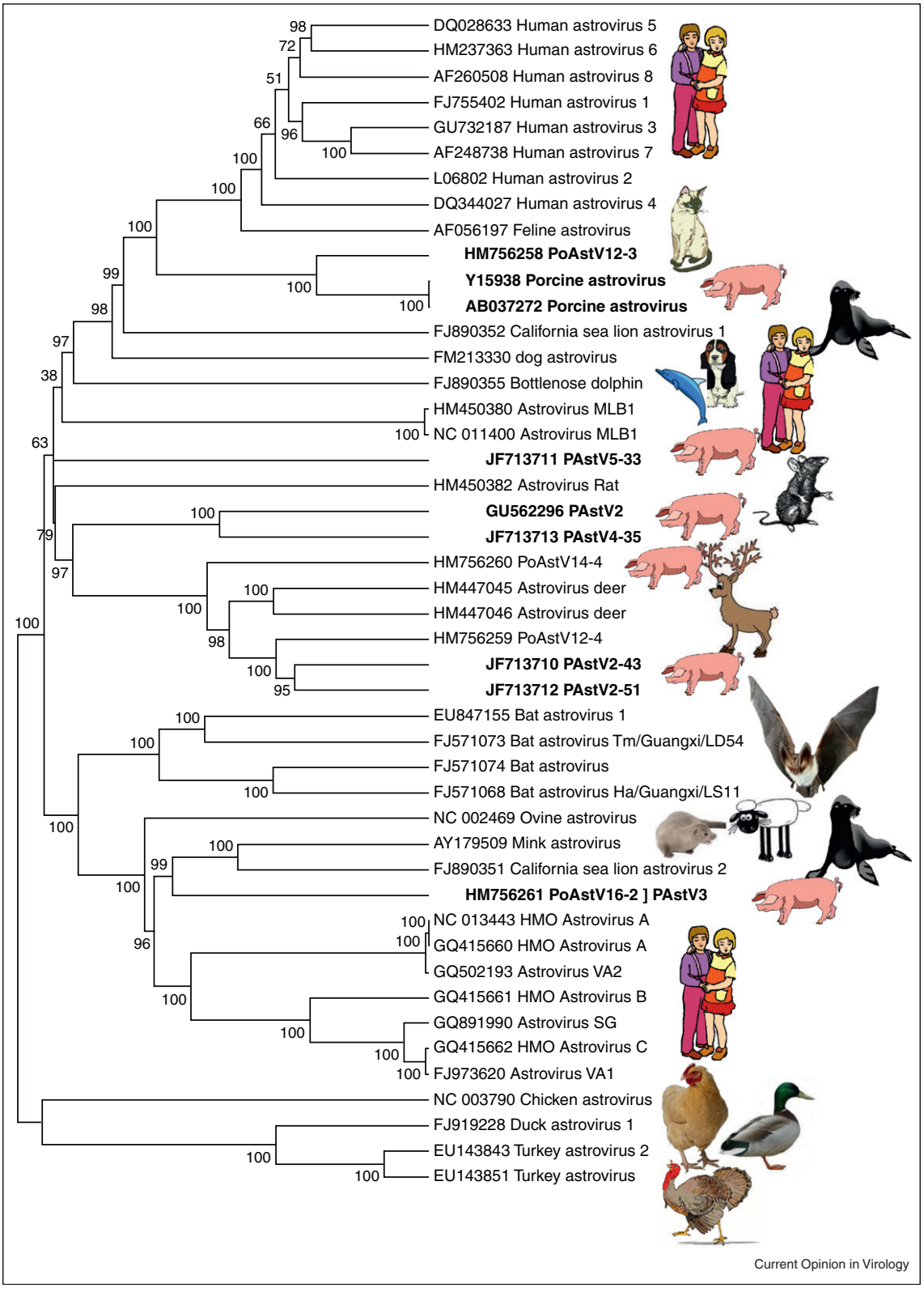
While the occurrence of cross-species transmissions is well established the overall frequency of such events is harder to estimate using molecular clocks calibrated based on short-term observations of viral evolution. Estimates of the time to last common ancestor of existing lineages of related viral RNA species yields date of thousands of years which differ greatly from dates derived using molecular clocks based on ancient viral genomes recently found on host chromosomes whose ages since integration are in the millions of years [97,98,99••]. In the absence of longitudinally collected epidemiological data confirming the recent emergence of a virus in a new host species, as was possible for HIV1/2, SARS-CoV, and canine parvovirus CPV2, estimating the age and therefore the frequency for other cross-species jumps based on molecular clocks derived from short term viral evolution data, is therefore problematic [100–102].

Newly characterized animal viruses and disease association

While the rate of viral discovery has greatly accelerated, the epidemiological studies required to associate infections with symptoms has lagged behind due to difficulties in obtaining large numbers of the most appropriate biological samples. In order for human or animal viral vaccine development to proceed, convincing evidence of pathogenicity is required [4,103]. Genetically characterizing novel viral genomes in diseased animals provides the information required to design high throughput PCR assays with which to compare viral prevalence in epidemiologically matched disease cases versus healthy controls (Figure 1). Matching between cases and controls should optimally include age, sex, location, and type of environment (e.g. high intensity farming or free range). Disease association studies as well as temporal association of symptoms with IgM detection, rising IgG can both provide evidence in support of pathogenicity. Disease induction following animal challenge with the purified virus amplified in cell culture, or other pure virus inoculum can directly demonstrate disease causation (Figure 1). Complicating factors to be considered which may influence clinical outcome include variable host genetics, passive immunity due to maternally acquired antibodies, cross-protection by prior infections with related but less pathogenic viruses, and co-infections with other agents. Repeatability in independent studies can also validate prior conclusions. The severity of symptoms and their frequency must also be onerous enough to justify the cost of vaccine development, efficacy testing under realistic conditions, and ultimately large-scale vaccination.

Recent successes in identifying animal viruses and associating them with disease include the piscine reovirus (PRV) associated with heart and skeletal muscle inflammation in farmed salmon, where viral prevalence and viral loads were higher in affected than in healthy fish and viral

Figure 2



Maximum likelihood phylogenetic analysis of capsid proteins of astroviruses showing that diverse astroviruses infect some mammalian hosts species likely reflecting past cross-species transmissions. Bootstrap values of $\geq$70% are indicated at each branching point.

expression was detected in affected tissues [50•]. A new bornavirus was also detected and associated with proventricular dilatation disease in psittacine birds [44,49,104].

Animal viral vaccines

Once pathogenicity has been established, the efficacy of vaccination must be shown to provide cross-protection against genetically diverse viral 'field' strains. In situations where the challenge viruses are highly diverse, the use of multiple viral strains as vaccine antigens may be considered to widen the breadth of cross-protection.

Given the rapid rate of animal virus genome characterization using deep sequencing and other molecular approaches and the ever wider surveys of domesticated and exotic animal populations, it can be anticipated that a subset of the newly identified viruses will be shown to be pathogenic [15•,17,18,36,54,55,93,96,105–119]. The decision to develop animal viral vaccines will depend largely on economic calculations and/or the need to protect animal and/or human health. The ease of developing vaccines for animals, relative to human vaccines, including direct viral challenges and the requirement for only short term protection, will facilitate the rapid manufacture and testing of novel attenuated, inactivated, or subunit animal viral vaccines. Opportunities for vaccine development protecting farm, companion, and endangered animals should therefore rapidly expand in the near future.

Acknowledgements

Funding was received from NHLBI R01 HLO83254 and BSRI for this study.

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