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Article

Wildlife Pathology Studies and How They Can Inform Public Health

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Abstract

Emerging zoonoses have had a serious impact on human and animal health in recent decades. More often than not, these disease outbreaks have taken public health by surprise because we have failed to shift the epidemiological curve to the far left and detect zoonoses in animal populations prior to spillover to people. Not only can animals serve as valuable sentinels for emerging zoonoses but also much can be gained by the study of the animals themselves.

Key words: animal studies; bat viruses; emerging infectious zoonoses; West Nile virus; wildlife pathology; wildlife surveillance

Introduction

Rudolf Virchow said, "Between human and animal medicine there is no dividing line—nor should there be" (Klauder 1958). This has never been more valid than today. Emerging zoonoses have increased in recent years with serious consequences for public health. Severe acute respiratory syndrome (SARS), Nipah virus (NiV), Hendra virus (HeV), West Nile virus (WNV), monkeypox, and, most recently, Ebola virus and Middle Eastern respiratory syndrome (MERS-CoV) are all animal-related diseases. These zoonoses have served as wake-up calls to the public health, wildlife, and veterinary communities and heralded the need for closer collaboration (Chomel et al. 2007).

Historically, public health, veterinary, and wildlife agencies have not had close working relationships. More often than not, we have not been able to shift the epidemiology curve to the far left and identify zoonotic diseases in animal populations before spillover into humans. Instead public health has used taxpayers as sentinels. Such was the case with WNV when early warning came in the form of dead crows 2.5 months before any human infections took place. Veterinary pathology studies on wildlife were ultimately the key to the recognition and understanding of WNV. Wildlife studies have been critical to the understanding of other recent emerging zoonoses, and their importance cannot be underestimated.

Revenge of the Rainforest—Emerging Infectious Diseases and Wildlife

Most emerging infectious diseases (EIDs) are zoonoses (60.3% of EIDs), and the majority of these (71.8%) have originated in wildlife (Jones et al. 2008). A recent retrospective study of 335 emerging infectious episodes over a 64-year period (1940–2004) also emphasized the role of wildlife as a source of emerging infections (Cutler et al. 2010). Rodents and bats, in particular, have been associated with many serious disease outbreaks such as hantavirus pulmonary syndrome, Lassa fever, and NiV encephalitis (Morens and Fauci 2013). SARS was linked to colonies of horseshoe bats (*Rhinolophus sp.*) infected with a coronavirus in Malaysia that subsequently spread to Hong Kong and Toronto, Canada. NiV and HeV have also both been linked to bats (Artois et al. 2011).

In spite of the role that wild animals have played in recent zoonotic disease outbreaks, research efforts have typically been focused

toward either humans or domestic animals (Cutler et al. 2010), and few surveillance programs are specifically aimed at wildlife (Chomel et al. 2007). There are few diseases of wildlife that must be reported to regulatory agencies (Daszak et al. 2000), and wildlife agencies tasked with doing surveillance are critically underfunded both in the United States and overseas. Wider surveillance in wildlife has been called for, but few coordinated efforts have been implemented to preempt zoonotic disease emergence with wildlife surveillance (Morse et al. 2012).

Don't Blame Wildlife—Human Activities and Emerging Zoonoses

There has been a tendency to blame wildlife for emerging zoonoses. The public can be quick to call for eradication of wildlife when the fact is human activities are the driving force for where and how zoonoses occur (Karesh et al. 2012).

The emergence of these pathogens as significant health issues is associated with a range of causal factors, most of them linked to human activity. Changing agricultural practices, movement of domestic animals, and a range of environmental factors result in the emergence of zoonoses (Bengis et al. 2004). There has, however, been little emphasis on the roles of human-induced habitat destruction or wildlife population stress on EID spread or on the negative impacts of disease on wildlife (Buttke et al. 2015).

Kyasanur Forest disease in 1957 occurred in previously undisturbed forest where land was clearcut for a cashew tree plantation. Workers who cleared the forest became ill. In the 1950s, the emergence of Argentine hemorrhagic fever was directly linked to a switch to corn production that supported an explosion of the virus's main reservoir, the corn mouse (*Calomys musculinus*). NiV in Malaysia in 1998 and 1999 was the result of deforestation and expansion of pig farmers into bat habitat in association with the production of fruit-bearing trees that led to indirect exposure to bats that shed the virus (Chomel et al. 2007). It was the destruction of bat habitat that triggered encroachment of bats into pig farming/fruit tree growing areas (Wynne and Wang 2013). NiV infection caused 265 human cases of encephalitis with a 38% mortality rate.

NiV also continues to be a serious problem in many rural areas of Bangladesh and India but for a different reason. Bats and people share a predilection for consuming raw date palm sap that is collected from trees in hanging containers. Bats come to feast on the sap and in doing so urinate into the collection pots. The sap is later consumed raw by humans (Cutler et al. 2010). Something as simple as placing barriers on the pots may decrease transmission of NiV to people.

Human activities have also resulted in human infections with Ebola virus. The bush meat trade has been implicated in recent Ebola outbreaks. In the May to November 2007 outbreak of Ebola in the Democratic Republic of Congo, researchers found that fruit bats were massively hunted and eaten by villagers during their annual migration. Bats represent a major source of protein for the human population (Leroy et al. 2009) but are also a source of infection.

Tracking, capturing, and butchering of wildlife in the field and transporting of bushmeat all involve risks and have also resulted in human cases of Ebola virus. Particularly high risks are associated with the hunting of nonhuman primates (Cutler et al. 2010). Human transmission has resulted from the handling of gorilla, chimpanzee, or duiker carcasses (Leroy et al. 2004).

Of note, the wild animal outbreaks of Ebola occurred before each of the five human outbreaks between 2001 and 2003. Monitoring information was used to alert health authorities of an imminent risk of exposure weeks before a human outbreak occurred on two occasions (Balmer 2014).

"Bomb the Bats"

When HeV was first discovered in Australia, there was a populist call for bat eradication. A politician was quoted as saying "Bomb the bats" (Degeling and Kerridge 2013). This is an example of an uninformed and reactive response to a wildlife-related emerging infectious disease. In fact, although it is true that more than 15 virus families have been identified in 200 species of 12 bat families and that they are more likely to be infected with more zoonotic viruses than rodents (O'Shea et al. 2014), studies have shown no evidence that a decrease in bat density would reduce viral prevalence (Plowright et al. 2015). Instead, they found that disrupting bat colonies and increasing bat stress may increase the amplitude of viral shedding. (Halpin et al. 2011). In one study, Pteropus bats from Australia and Malaysia were inoculated with NiV and HeV by natural routes of infection. In spite of intensive sampling, researchers found no NiV in Malaysian bats, and HeV was reisolated from only one Australian bat. These results suggest opportunities for henipavirus transmission may be limited and that the probability of spillover is low (Halpin et al. 2011).

A more balanced and reasoned approach to bat-associated EIDs would be to restore bat feeding habitat to discourage urbanization of bat populations due to nutritional stress. Simple things like vaccinating and fencing horses away from fruit-bearing trees where bats may roost and feed could decrease exposure to HeV (Plowright et al. 2015). Changing agricultural practices by the creation of buffer zones between fruiting trees and domestic animals would decrease the incidence of NiV and HeV (Smith and Wang 2013). In Bangladesh, the installation of barriers on the date palms that prevent the bats from accessing the collection vessels is a simple strategy currently being investigated to control transmission of NiV (Smith and Wang 2013). Not only is wanton destruction of bat populations unfeasible, it is also ill advised. Bats have remarkable immune systems, and we need to study them. "The bat immune system is astonishingly tolerant of most pathogens—a trait that could pose risks to people, but that also offers clues to preventing human disease of aging, including cancer" (Angier 2015; Baker 2014). Bats have evolved mechanisms to control viral replication more effectively than most other mammals (O'Shea et al. 2014).

What sets bats apart from other mammals is their ability to fly. Flight results in a 15-fold to 16-fold increase in bat metabolic rate in comparison with the sevenfold increase in rodents running to exhaustion or the twofold increase in metabolic rate of most flying birds (O'Shea et al. 2014). Bats use 20 times more energy than other mammals of the same size during an average day (Slezak 2012). The body temperature is consistently above normal in a fever-like state. This persistent fever state keeps viral replication at a low level, and the virus never overruns the bat immune system (Grant 2014). But all this energy production generates free radicals that damage DNA. In spite of that bats, are extremely long lived, some living up to 40 years of age. They live 3 to 10 times longer than other mammals of similar size (Grant 2014) and demonstrate low levels of cancer (Wynne and Wang 2013). Bats have evolved a strategy to avoid DNA damage (Lyn 2013; Slezak 2012). Scientists looked at the fruit bat *Pteropus alecto* and the insectivorous bat *Myotis davidii* and found a concentration of genes in the DNA damage checkpoint (Zhang et al. 2013). They also found bats were missing a gene segment known to trigger extreme and potentially fatal cytokine storms (Lyn 2013), leading scientists to speculate that if we can manipulate the human immune response to be more like a bat immune response, we may have a better chance of surviving diseases that bats are immune to (Slezak 2012). Instead of characterizing bats as "pathogen

ferries on wings" (Grant 2014), we need to study them. We need to develop bat cell culture assays and bat-specific reagents to examine lymphocyte proliferation, antibody and cytokine synthesis, cell-mediated immune responses, and a host of other immunologic functions in bats that make them important reservoirs of emerging viruses (Calisher et al. 2006). We don't need batricide. We need intensified wildlife research.

Tissue Is the Issue

Necropsies of wildlife proved to be quite valuable during the Ebola virus outbreaks. Virus detection was 32.7% (18/55) for carcasses but only 0.2% (13/5309) for live-captured animals. An Animal Mortality Monitoring Network (AMMN) was established during the period 2001 to 2003 in northeast Gabon and in northwest Republic of Congo and collected data from hunters on animal morbidity/mortality. Over 60% of the 21 carcasses reported by hunters and tested were infected with the Ebola virus (Bisson et al. 2015). During outbreaks of WNV, raptors emerged as excellent sentinels for the virus. In one study, raptor admissions to rehabilitation clinics took place fourteen weeks earlier than other surveillance methods (Nemeth et al. 2007). When dealing with a previously unknown or a re-emerging zoonotic threat, the performance of systematic necropsies with tissue sampling for virus isolation and sequencing is critical (Olson et al. 2012).

Using animals as sentinels for human disease is in and of itself a strong justification for wildlife studies. However, emerging zoonoses can have devastating impacts on wild populations, and the negative impact this has on conservation is unfortunately often overlooked. Ape species that were abundant a decade ago have been decimated by Ebola virus. In 2006, 5000 gorillas died due to Ebola (Bermejo et al. 2006). Nonhuman primate numbers drastically declined in the Republic of Congo alone during the two-year Ebola outbreak from 2001 to 2003 (56% decline in gorillas and 89% decline in chimpanzees) (Leroy et al. 2004; Bisson et al. 2015). It is not known if these populations will recover.

Despite the ongoing threat of the next zoonotic disease outbreak and the demonstrated importance of such wildlife surveillance as an early warning system for disease emergence, no systematic wildlife surveillance program exists in most countries (Bisson et al. 2015).

West Nile Virus—What the Animal Studies Told Us in 1999

The WNV outbreak of 1999 is an excellent example of the powerful contributions animal studies can make to public health. Animal studies provided information on the breadth of species susceptible to WNV infection, which had an impact on surveillance efforts. Pathology studies on captive and free-ranging wildlife ultimately changed the tissue submission protocol for diagnostic testing. Veterinary serum banks provided insight into when the virus first appeared in New York City. The ability to follow infected captive wildlife over time provided the first indication of possible viral persistence. Many features and concerns about WNV pertinent to human health were identified by veterinary pathologists shortly after WNV's recognition.

In the immediate aftermath of the discovery of WNV in the United States, public health wanted to use birds as sentinels and launch dead bird surveillance. But which species of birds should be tested? The public health focus was solely on crows. However, histopathologic, virus isolation, and immunohistochemical (IHC) studies on the birds that had died at the Bronx Zoo in New York City indicated a broad species range of susceptibility to the virus (Steele et al. 2000). Bird species as diverse as bald eagles (*Haliaeetus leucocephalus*), snowy owls (*Bubo scandiacus*), and Chilean flamingoes (*Phoenicopterus chilensis*) all succumbed to the virus. The zoo was full of sentinel species. When New York City entered the second summer of WNV, the first warning came not from trapped mosquitoes nor from sentinel chickens but from an IHC-positive Guanay cormorant (*Phalacrocorax bougainvillii*) at the Bronx Zoo. The list of known susceptible bird species is now quite lengthy (Komar 2003), and species other than crows have been found to play an important role in transmission of WNV, illustrating the danger of too narrow a focus when dealing with a new disease.

In 1999, there was scant information on which mammalian species were susceptible to WNV infection. Many captive exotic mammals, as well as birds, were also neurologic with WNV. A serosurvey of the captive collections at the zoo facilities revealed rising titers in species as diverse as rhinoceros (*Rhinoceros unicornis*), snow leopards (*Panthera uncia*), and babirousa (*Babyrousa babyrussa*), A broad range of mammalian species are now known to be susceptible to WNV, including alligators (*Alligator mississippiensis*) (Nevarez et al. 2008), polar bears (*Ursus maritimus*) (Dutton et al. 2009), reindeer (*Rangifer tarandus*) (Palmar et al. 2004), harbor seals (*Phoca vitulina*) (Duncan et al. 2003), grey seals (*Halichoerus grypus*) (Duncan et al. 2003), killer whales (*Orcinus orca*) (St Leger et al. 2011), Barbary macaques (*Macaca sylvanus*) (Olberg et al. 2004), and psittacines (Palmieri et al. 2011). Urban sentinels like fox squirrels (*Sciurus niger*) (Root et al. 2006), Eastern chipmunks (*Tamias striatus*) (Platt et al. 2007), and Eastern cottontail rabbits (*Sylvagus floridanus*) (Tiawsirisup et al. 2005) have been found to develop viremias sufficient to infect mosquitoes and thus may play a role in the spread of WNV.

In addition to its extensive captive sentinel collection, the zoo had another unique resource. It had freezers filled with banked serum samples from some animals going back many years. The availability of serum banks allowed the US Army Medical Research Institute of Infectious Disease (USAMRIID) to establish the temporal sequence of WNV. By evaluating paired serum samples from Bronx Zoo elephants, they were able to demonstrate that WNV appeared for the first time in the captive collection in August 1999.

Too little emphasis is placed on the value of these veterinary biomaterials. As WNV swept across the United States, tremendous effort and manpower went into collecting dead birds for testing. Unfortunately, samples that tested negative for WNV were not banked for additional testing. After all of the effort and considerable expense that had been required to collect and test birds on a nationwide basis, only one diagnosis was pursued. Had tissues been banked, we could have tested those birds that were negative for WNV for other viruses such as highly pathogenic avian influenza, which later emerged as a major concern. This further underscores the need for a One Health approach to emerging zoonotic threats.

The launch of the dead bird surveillance program raised an important question as to which tissues should be collected for testing. Traditional literature suggested the primary target tissue for WNV was the brain. However, gross pathological examination, virus isolation, and IHC studies on the Bronx Zoo cases and wild crows indicated that many tissues could be positive in a WNV-infected bird. Working with USAMRIID, IHC and virus isolation were done on all of the tissues that had been collected at necropsy on the captive collection birds and wild crows. Of all of the tissues, only kidney was positive 100% of the time (Steele et al. 2000). A year later, after further studies, the New York State Department of Health amended its submission protocol

and requested batched tissues from each bird for testing, but many positive cases were probably missed during early surveillance efforts due to too narrow a case definition.

In the fall of 1999, the accepted dogma was that WNV was only transmitted through the bite of a mosquito. However, WNV-positive birds had dramatic enteric pathology and demonstrated abundant viral antigen on IHC staining of intestine and kidney, which suggests they were shedding large volumes of virus into the environment. This raised the question of whether alternative modes of transmission could be involved in the epidemiology of WNV. Aerosol transmission had been known to occur with WNV in a laboratory setting. If WNV-positive flamingoes were shedding virus into their pond, could other flamingoes be getting infected via ingestion of the virus via the fecal–oral route or were they being exposed via aerosol? There was also abundant viral antigen in ovarian and testicular tissue, which suggested vertical transmission was also a possibility. However, there was no federal or state funding available to do the necessary transmission studies. Ultimately, the Bronx Zoo, in a first-of-its-kind public-private partnership, donated $25,000 to the National Wildlife Health Center in Madison, Wisconsin, which had the necessary BL-3 level biosecurity to run the experimental studies. These studies confirmed that birds were acquiring WNV via aerosol as well as via the fecal–oral route (Komar et al. 2002), which had direct impact on how zoos handled suspect cases. This finding ultimately led to the creation of a rapid diagnostic test using oral and cloacal swabs that was adopted by many health departments (Komar et al. 2002; Stone et al. 2004). By 2002, patients had acquired WNV via transfusions and organ transplants (Charatan 2002; Cushing et al. 2004), babies had been born with WNV or became infected through breastfeeding (CDC 2002), and alternative modes of transmission were formally recognized in humans.

Another concern raised by the animal studies was the level of viremias in infected birds. WNV IHC indicated birds were "hot" in Department of Defense terminology. There was concern the birds could present a health risk to animal handlers. Veterinarians and wildlife rehabilitators treating and housing neurologic crows and raptors might be getting exposed to large volumes of virus. But it was generally assumed the viremias with this new strain of WNV would be low, like those that occur with St. Louis encephalitis. When viremia studies were performed, the results were astonishing, and they prompted the Centers for Disease Control and Prevention to announce its plans to do occupational hazard studies on veterinarians and bird handlers. In Smithsonian magazine, Dr. Thomas Monath, virologist extraordinaire, said, "I couldn't believe the incredible viremias these birds cook up . . . there's no precedent for it. There were from a trillion to ten trillion viral particles per milliter of blood. That's beyond no precedent. That's almost beyond belief. No self-respecting bird can gin up a viremia higher than 100,000 particles with St. Louis encephalitis (SLE) virus. So some birds are almost a billion-fold more infectious with West Nile than with SLE" (Hall 2003). Basic histopathology and IHC offered another preview into human pathology. One of the zoos' bird mortalities had demonstrated pathology and IHC staining of the anterior horn cells of the spinal cord consistent with poliomyelitis. The first human patients had presented with profound muscle paralysis that had not fit with the initial diagnosis of SLE. Poliomyelitis in patients with WNV neuroinvasive disease was first published in 2002 (Glass et al. 2002), but animal studies suggested its possibility in August 1999.

Many of the neurologic issues that later emerged as concerns in human patients were first seen in veterinary pathology studies. One of the more sobering and prescient histopathologic findings in zoo animals was the presence of ongoing inflammation of the brain weeks to months after initial infection with WNV. In 1999, WNV was believed to cause a mild febrile self-limiting illness in the majority of patients, with a small percentage developing neuroinvasive disease. However, a 1983 Russian publication (Pogodina et al. 1983) on primates inoculated with a number of strains of WNV found all were capable of producing long-term neurologic sequelae and viral persistence regardless of neurologic status of the animals. Was WNV-99 really self-limiting? What did the primate studies mean for us? With this in mind, pathologists at the Bronx Zoo intensively evaluated the brains of all known seropositive animals regardless of their clinical signs at the time of infection. Evaluation of a snow leopard *(Panthera uncia)* and a Greater Indian rhinoceros (*Rhinoceros unicornis)* that died 3 and 8 months after infection showed dramatic lymphoplasmacytic perivascular cuffing. An asymptomatic babirousa (*Babyrousa babyrussa*) died 10 months after seroconversion and also had evidence of ongoing inflammation, which suggested even subclinical infections might result in long-term central nervous system pathology and possible viral persistence. In 2004, it was recognized that WNV infection could result in a protracted convalescent period with long-term problems with memory, confusion, clinical depression, muscle weakness, tremors (Carson et al. 2006; Hall et al. 2008; Hughes et al. 2007; Klee et al. 2004; Sejvar 2007), and Parkinsonian-like disorders (Hughes et al. 2007) 18 months after infection. Sixty percent of encephalitic patients in a Houston study reported symptoms 5 years after infection (Voelker 2008). Patients with milder forms of illness are just as likely to suffer long-term health problems as encephalitis cases. Eighty-four percent of patients in a study on West Nile fever reported persistent fatigue, 59% had memory problems, and 49% had ongoing muscle weakness (Carson et al. 2006). It wasn't until 2006 that WNV was found in the brain and cerebrospinal fluid of a human patient 4 months after initial diagnosis (Penn et al. 2006). The hamster was established as a model for West Nile encephalitis in 2001 (Xiao et al. 2001). It was not until 2010 that a murine model of viral persistence was established (Appler et al. 2010). It showed that WNV persisted in the central nervous system and peripheral tissues for up to six months after infection in mice with subclinical infections. What does this mean for the estimated 1.2 million people with asymptomatic WNV infections in the United States and possible subclinical disease? Ironically, in 2005, a hamster study on WNV persistence found virus was shed in the urine up to 8 months after infection (Tesh et al. 2005), which shifted the focus back to the impact of WNV on the kidney. Viral RNA was detected in the urine of an encephalitic patient 8 days after symptom onset in 2005 (Tonry et al. 2005). A 2012 long-term study of patients in Houston found an association between neuroinvasive infection and the development of chronic kidney disease (Nolan et al. 2012). We may be seeing the tip of an iceberg in terms of long-term sequelae of WNV.

Conclusion

In today's world, it is impossible to separate public health, veterinary, wildlife, and ecosystem health studies along traditional lines. The One Health concept recognizes that the health of humans is inextricably linked to the health of animals and the environment (Morse et al. 2012).

The Emerging Pandemic Threats (EPT) program of United States Agency for International Development, which was launched in 2009, is an exciting example of recent synergy across human and animal health sectors. In the past, the sharp division between veterinary medicine and public health resulted in a failure to detect disease in sentinel animal populations in a timely

manner. EPT draws upon expertise from all health sectors in the hope that we may be able to detect zoonotic health threats prior to spillover into the human population. The predict portion of the project includes the University of California–Davis veterinary school, the Smithsonian Institution, and nongovernmental organizations such as Ecohealth Alliance and the Wildlife Conservation Society, which represent wildlife and ecosystem health. We need to continue to build these bridges and get back to what was recognized at the turn of the century: "Between human and animal medicine there is no dividing line—nor should there be" (Klauder 1958).

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