West Nile Virus - Does it Exist?

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West Nile Virus (WNV) is one of the latest in a string of new disease-causing organisms that have been brought to the world's attention by science researchers and public health officials, most notably the US CDC (Centers for Disease Control). Although there is sometimes resistance to the protective measures recommended, there is rarely discussion of whether the threat really exists. If the claims of these officials that they know the answers are the legitimate targets of questioning, why not the claims that they have identified the problem?

Many recently discovered pathogens, most often viral, have exploded into the public consciousness, but then gradually faded from sight. Often a handful of cases in humans results in a massive reaction that seems out of all proportion to the danger. Avian flu resulted in the slaughter of most of the chickens in Hong Kong although there was no evidence that the first and most publicized death from this disease in humans, a 3 year old boy, had ever been in contact with an infected bird [Subbarao 1998]. Nipah disease similarly resulted in the slaughter of 890,000 pigs in Malaysia [MMWR 1999f]. Foot and Mouth disease caused the slaughter of 3,881,000 animals in England, although only 2,023 cases of this mild disease were found and there is believed to be virtually no risk to humans [DEFRA 2001]. Legionnaires' disease [MMWR 2000c], and Hantavirus [MMWR

1999e, MMWR 2000d] have followed a similar pattern of public panic followed by a steady decline in interest when the threat does not materialize, although not accompanied by the slaughter of animals.

Sometimes it is not the supposed animal vectors, but the current or potential future victims of the disease, who are the targets of public health attention. Thirteen occurrences of Swine Flu in February 1976, including one death, resulted in a recommendation to vaccinate virtually every American based on a prediction that the 1918 killer flu epidemic would be repeated towards the end of the year. When President Gerald Ford convened a meeting of top scientists in the United States including, in an uncharacteristically brilliant move, both arch-enemies Salk and Sabin, there was unanimous agreement that such a vaccination programme would be safe and effective. Manufacturing and insurance problems delayed the program so that, by the time of the expected epidemic only a fraction of Americans had been vaccinated. This was fortunate both because the epidemic did not appear (there was not a single case of Swine Flu after the original outbreak) but about 50 people died from the Guillain-Barré polio-like syndrome caused by the vaccine, and hundreds were injured [Silverstein 1981, Laitin 1997]. In the case of Hepatitis C and AIDS, the remedy is not a vaccine, but one of several highly toxic antiviral medications even though, in the case of Hepatitis C at least, there is increasing evidence that the virus is not very pathogenic [Cohen 1999, Seeff 2000 and Rodger 2000], while the medications are neither highly effective nor very safe.

The public health strategy with West Nile Virus has so far focused on the vectors - mosquitoes and birds, rather than on vaccines or therapy for the human victims. Opposition to the spraying of wide areas in the New York City epicenter has been based on the comparison between the few lives lost due to the virus so

far (7 in 1999, 1 in 2000), the millions of people placed at risk by the spraying of toxic pesticides, the inability of spraying to target only mosquitoes, the loss of predator species and the possibility of resistance developing.

While there has sometimes been criticism of the response of public health officials to a new disease outbreak, there has rarely been any discussion of the possibility that the existence of the pathogen, or its connection with the disease is unproven. Serious concerns over the existence of West Nile Virus or its causal role in disease would quickly put an end to attempts to eradicate its carriers, as well as research funding for tests, vaccines and pharmaceutical therapies.

Koch's Postulates

Koch's postulates are a statement of four logical rules for determining whether a pathogen exists and is the cause of a disease (e.g. [Cann 1997]). They must be satisfied before it can be accepted that a pathogen causes a disease. They state that:

1. The pathogen must be present in every case of the disease.

2. It must be isolated from the host and grown in vitro (culture).

3. The disease must be reproduced when a pure culture of the pathogen is inoculated into a healthy susceptible host.

4. The same agent must be isolated once again from the experimentally infected host.

Koch's postulates are merely a statement of the minimal evidence necessary to have confidence in the existence of a pathogen and its causal link to a disease. It is important to note that these postulates are not based on experimental evidence, but on simple logic.

They have not been satisfied for West Nile Virus.

How Virus Existence Should be Proven

A critical part of Koch's postulates is proving the existence of a pathogen before its association with a disease can be established. If West Nile Virus exists in biologically significant quantities, virus particles must be present in the body in large quantities. It should be able to separate them from body fluids such as blood through a process of filtration using ultra-fine pores that no other pathogen is small enough to pass through. This is how the first viruses were discovered [MMWR 1999d].

After filtration, a portion of the resulting material can be examined under an electron microscope to show that the sample is composed of particles of exactly the same size and shape, to an acceptably high degree of purity. The remainder of the sample, having been verified as pure, can be analyzed for its protein and DNA (or RNA, in the case of a retrovirus) constituents.

Isolating (or purifying) a virus is therefore an essential step in Koch's second and fourth postulates. Without this it is not possible to state that a virus is present in organisms that have the disease, it is not possible to know what the virus is composed of, and it is not possible to know whether it, and it alone, is sufficient to cause disease in another organism.

Isolation of West Nile Virus

The 1999 papers on West Nile Virus in the journal Science [Anderson 1999, Lanciotti 1999] claim that it has been isolated, but their frequent use of this word in no way corresponds to the normal definition of separating one thing (the purported virus) from everything else (including the cells that it might infect). The researchers instead abuse the term, disguising the fact that their evidence is non-specific and indirect, and that at no point during their research do they have any evidence that they had purified virus.

Co-Culturing

Viruses can only reproduce within cells, so a pure culture of virus on an inert medium is not possible. Instead a co-culture must be established, whereby (ideally) purified virus is added to a cell culture, both are allowed to grow, and then a larger quantity of virus is removed from the co-culture than was initially added.

The process used to establish a virus co-culture by both [Anderson 1999a] and [Lanciotti 1999] does *not* involve the addition of purified virus to the co-culture, nor extraction of pure virus from the co-culture.

[Anderson 1999a] established a culture by filtering ground up crow brains or mosquitoes through a 0.22 micrometer filter, even though flavivirus particles are about 0.04 micrometers in size [Lanciotti 1999]. The resulting material obviously contains much material that is of cellular origin, but was directly added to a cell culture after filtration without any further attempt at purification, and without attempting to validate that the material was already pure (which seems

extremely unlikely given the process used). Similarly, to establish Koch's fourth postulate, the putatively infected cell culture was centrifuged, without any attempt at separating the virus from the cell culture. The material that was analyzed included the cells in the culture as well as the virus.

The use of the word isolate is so prevalent in the Anderson paper that I attempted to clarify their use of it. Anderson replied to my query that "Viruses can only be isolated in cells" [Anderson 1999b]. This is obviously not true, because viruses must exist as particles outside cells in order to infect cells, and they must harness the machinery of a cell to produce more virus particles so that they can travel through the body to infect more cells. If West Nile Virus exists in the body of a person, a mosquito or a crow, or even in a co-culture, it must be possible to purify the particles, and separate them from all other organic materials.

[Lanciotti 1999] used similar 'Vero' cell cultures, but also claimed to culture the virus by inoculating "necropsy samples" from birds into chicken eggs. Again, there is no evidence that these samples had in any way been purified.

The method for determing whether a co-culture is producing virus is similarly non-specific. [Anderson 1999a] looked for cell death occurring, and for the ability for unpurified materials from these cell cultures to cause sickness in laboratory animals [Anderson 1999b, no details given]. Without purification, one can never be sure that the cytopathic effects are due to a virus, and not just due to exposure to foreign cellular constituents from a sick animal or person or due to an immune system reaction.

Antibodies

Antibodies are often used to show that a virus is present in a host, largely because they are quick, simple and cheap. Antibodies, however, persist after an active infection, and so cannot be taken as proof of an active infection. Even with virus isolation, it is impossible to prove that antibody reactions are specific and are immune from cross-reactions with other pathogens, or indeed with non-infectious conditions. Furthermore, one could only be sure that antibodies are even associated with the virus by injecting animals or humans with purified virus to ensure that the antibodies only arise afterwards. Even if antibodies are closely correlated with a disease they could be caused by processes resulting from the disease, they could be caused by opportunistic infections or they could be similar antibodies from another pathogen that cross-react.

[Anderson 1999a] admit that their West Nile antibodies are non-specific, because they used St. Louis Encephalitis antibodies to detect infections with West Nile Virus.

The use of antibodies to detect a virus then, at a minimum, requires that the virus has been purified, and even then can only be used as a less than perfect indicator of past infection, and can never be used as proof that Koch's first postulate has been established.

Antigens

Antigen testing is theoretically more direct than antibody testing, since an antigen is the constituent of a pathogen (usually a protein) that the body produces antibodies to. Detecting an antigen from a person suspected of having a disease can be an indication of the presence of current infection - but can only

be taken as proof if cross-reactions with other antigens are eliminated and if the antigens have been obtained from purified virus.

[Anderson 1999a] show that antigens are obtained merely by lysing cells (digesting the cell walls) resulting in all cellular constituents being obtained along with, possibly, virus particles that were within the cell. Such materials cannot properly be described as viral antigens, because this implies that they exclusively come from a virus. At best the virus would be a small part of these materials.

Detection of DNA or RNA

The detection of genetic material seems like another way to avoid the deficiencies of antibody testing since, as with antigens, it is more direct evidence. If the genome of a virus has been identified, a test that can reach into a sample and detect its presence or absence seems foolproof. Even here, however, there are significant problems. First of all, the ability to detect genetic material in sick animals or people that is not found in the healthy does not constitute proof that this material comes from a virus. It could come from the rearrangement of genetic material within the body, or as breakdown of cells resulting from the disease. Secondly, the Polymerase Chain Reaction (PCR), widely used as a genetic probe, is highly sensitive to false positive reactions as even a molecule of genetic material that matches trigger a chain reaction. Thirdly, genetic probes are only a small fraction of the genome, so cross-reactions with related viruses are possible.

The genome of WNV is claimed to be just over 11,000 nucleotides long [Lanciotti 1999], but there are no techniques to detect this directly unless purified viral

particles can be obtained. Consequently, without this, very short primers (21 or 23 bases in Anderson 1999a] are used to lock onto what is believed to be viral genetic material in non-purified materials . There is no proof that what is fished out of this cellular/viral stew came from a virus and not from within the cell. Even then the genome had to be reconstructed from six overlapping pieces [Lanciotti 1999].

The ability to send short primers into an unpurified mixture assumes that these gene sequences can uniquely identify a species of virus without error. But, this assumes a stability of the genome that may be unsupportable in healthy people, let alone in sick people where it can be expected that destruction of cells is occurring, resulting in damaged and rearranged genomes. As Dr. Mae-Wan Ho showed in [Ho 1998], the atomistic view of the genome is not warranted, and there are many processes in cells that result in significant modifications to the genome in response to its environment. It has also been noted that the human genome contains endogenous retroviral elements that can only be distinguished from infectious virus particles by the "presence of infectious, extracellular, membrane-enveloped particles in the retroviruses" [Boeke 1989]. Without purification it is simply not possible to tell whether one is dealing with a poorly understood cellular process or an infectious virus.

Proof of Causation

Even if the existence of a virus or other pathogen is shown in 100% of a sample of people with a particular illness, and in none of a sample of people without the illness (including a number of people with illnesses with similar symptoms or believed to be caused by similar pathogens), it does not prove that the pathogen causes the disease. Even if the first two of Koch's postulates had been satisfied

for WNV, it would still be necessary to expose an animal to purified virus and ensure that it acquired the same disease, and that virus could again be purified from it. Without starting with purified virus, it is impossible to perform the experiments necessary to establish this.

Even defining the symptoms of West Nile Encephalitis is not without confusion. The original outbreak in New York City was characterized by several cases of "severe, diffuse muscle weakness" that had not been found in previous outbreaks. [NYC 2001]

If not West Nile Virus, Then What?

It is difficult to say what has really caused the illnesses currently blamed on West Nile Virus if we accept that it does not exist or is not pathogenic. Lynn Gannett, a researcher from New York City, has found that all the people who died in 1999 were over 60 and all but two over 75. Another study showed that being diabetic increased the risk of death by more than 5 times [Nash ????].

Jim West, a researcher with the NoSpray coalition in New York City, has focused his attention on the use of MTBE (Methyl Tertiary Butyl Ether) as a gasoline additive to reduce smog, noting that the majority of cases in birds and humans were in areas with high levels of pollution, where MTBE is used [West 2001].

Age, diabetes and air pollution are confounding factors. It is possible that they, by themselves, explain the illness of people testing positive for West Nile Virus.

Part of the perception of an expanding epidemic may be just a consequence of encouragement from public health workers to look in certain places and at certain times. The New York City Department of Health, for example, states that

"Suspected viral encephalitis cases must always be reported to the Health Department. To help us detect an outbreak as quickly as possible, however, from June through September (or until the first sustained frost) we ask physicians to immediately report any hospitalized cases with clinical evidence of viral encephalitis" and "Physicians should consider West Nile virus in the differential diagnosis of viral meningitis, especially in older patients presenting during the summer months", meaning that symptoms detected when mosquitoes are not active are less likely to be reported as cases, artificially increasing the correlation between cases called West Nile Encephalitis and the presence of mosquitoes. [NYC 2001]

The finding of infected birds, mosquitoes and human cases in Canada and Florida in 2001 could have been influenced by the greater intensity of the search. Without proof that birds, mosquitoes and humans uniformally tested negative for West Nile Virus in earlier years, it is impossible to say whether there is an epidemic caused by a spreading virus or an illusion caused by spreading expectations.

Conclusions

It is clear that West Nile Virus has never been purified, and that without purification not only is it impossible to say whether it is the cause of the disease that it is associated with, but it is impossible to say whether it even exists.

Even ignoring the lack of purification, scientists still cannot agree on what it is that they have found. One paper constructed a phylogenetic ('family') tree based on analysis of genetic material from the 'isolates') to claim that the 1999 New York virus was closest to Kunjin [Briese 1999]. This virus has never been known

to cause disease in humans, and so could hardly be used to create a public health alert and intense spraying programs. However, just two months later, the same researchers published three new genetic trees that showed that the New York virus was more closely related to West Nile Virus [Jia 1999], which is blamed for previous outbreaks of disease - justification to declare a public health emergency.

Even though [Anderson 1999a, Jia 1999 and Lanciotti 1999] show in five phylogentic trees that the New York isolates are more closely related to some West Nile isolates than Kunjin, all the trees also show that the New York isolates are *more* closely related to Kunjin than several other West Nile isolates, which brings the concept of West Nile virus as a species into question.

Even the size of the virus genome is subject to significant disagreement. [Lanciotti 1999] claim that it is 11,029 nucleotides long, while [Jia 1999] claim that it is only 10,945 nucleotides long.

There are significant financial interests that discourage questioning of the evidence for the existence of West Nile Virus too closely. Public Health officials and virologists get increased funding, status, attention and new facilities from being involved in a 'deadly' epidemic. Politicians can appear to be making tough decisions based on sound science. Pesticide companies can show that their products are essential to maintain good health. Biomedical researchers and companies may get government or stock market funding for the development of new drugs, tests, reagents and vaccines, and can anticipate enormous future profits. Even environmentalists are often satisfied with the argument that the emergence of new viruses is a consequence of globalization, overpopulation and disruption of ecosystems. There is something in the existence of West Nile Virus for almost everyone.

If people are injured by the pesticide spraying or by adverse reactions to vaccines or drugs, they will only be a few quiet voices that cannot be heard above the din of people struggling to be seen as leaders in the response to a public health emergency. A great danger is that the symptoms of pesticide poisoning (to combat mosquitoes that are believed to be the vector for West Nile Virus) will be similar enough to the rather vague symptoms of the disease itself, which are encephalopathy (inflammation of the brain), weakness and neuropathy (peripheral nerve damage, leading to symptoms such as numbness) [Briese 1999]). This could cause more cases and create calls for ever more intense spraying programs.

Opponents to 'public health' initiatives such as spraying mosquitoes with malathion should take a broader view. Not only should they consider the dangers of these interventions, but demand that the existence of West Nile as a pathogenic virus must be proven. They can argue that, not only are the interventions dangerous, but that they might be completely misguided, and are only delaying recognition of the true, underlying health problems.

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